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# AGRICULTURAL WASTE POTENTIAL OF TÜRKİYE

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**Abstract:** In Türkiye, agricultural activities are carried out on an area of 38482000 hectares. In Türkiye, where different climates are seen, animal husbandry is done intensively. Some of the animal and herbal products produced are consumed in the country and some are exported and consumed abroad. Many agricultural wastes are generated during animal and plant production activities. These wastes are fresh manures formed during animal production, wastes formed from grain and industrial plants during plant production, wastes formed during and after production in greenhouses, pruning wastes and wastes formed as a result of evisceration of hard-shelled fruits. In this study, agricultural wastes that can be obtained from these products were calculated based on the products with the highest production. According to the results of the study, in the light of 2022 data, the annual amount of manure that can be obtained from our livestock is 20.722 million tons on dry basis, 16.805 million tons of waste from grain and industrial plants, 393,048 thousand tons of waste generated during and after production in greenhouses, 3.045 million tons of pruning waste, wastes generated as a result of evisceration of hard-shelled fruits are 550.186 thousand tons and olive pulp (pomace) resulting from olive oil extraction is 1.630 million tons.

**Keywords:** Agricultural waste, Biomass, Livestock manure, Pruning waste, Greenhouse waste, Olive pulp

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## 1. Introduction

In Türkiye, agricultural activities are carried out on an area of 38482000 hectares. The area of 20194000 hectares, which corresponds to 52.48% of this area, is considered as the total cultivated area. 16510000 hectares of this area is planted area, 2960000 hectares is fallow land, 718000 hectares is vegetable garden area and 6000 hectares is ornamental plants area. There are perennial plants and trees on an area of 3671000 hectares, which corresponds to 9.54% of the total area where agricultural activities are carried out. 2385000 hectares of this area consists of other fruit, beverage and spice crops, 385000 hectares of vineyards and 901000 hectares of olive trees. It is considered as meadow pastureland on an area of 14617000 hectares, which corresponds to 37.98% of the total area where agricultural activities are carried out (TUIK, 2022).

If we look at the number of animals in Türkiye, according to the statistics of 2022, there are 17023791 bovine, 56265750 ovine and 361096026 poultry (TUIK, 2022).

While the activities in plant production are carried out at certain time periods during the year, production activities in animal husbandry continue throughout the year. There are many agricultural wastes generated during these activities, which can sometimes be evaluated and sometimes not adequately evaluated because they are formed suddenly and in large quantities. While some of the agricultural wastes can be evaluated directly without any treatment, some of them can be evaluated by going through some special processes. Some of these wastes can be used directly for animal feeding, and some of them can be directly used as fuel due to the thermal value they contain. Some of them are subjected to special processes and the thermal energy contained in them is evaluated by processes such as gasification and pyrolysis, they can be compressed and pressed into pellets or briquettes and used as fuel and can also be used as fertilizer to benefit from the high organic matter they contain. A large part of it is destroyed by burning directly or left to nature in a haphazard way, so no benefit can be obtained.

There is a need for farm scale or factory scale enterprises where these agricultural wastes can be subjected to these special processes. This requires a certain cost and investment. When the agricultural structure in Türkiye is examined, it is seen that the enterprises are small family enterprises. For this reason, the establishment and operation of such evaluation facilities in small family businesses is not considered very possible in practice.

In large enterprises where products are purchased and processed from the producer and their added value is increased, the establishment and operation of such waste recycling facilities is economically more convenient and more economical. It will be more beneficial to use the wastes generated during product processing for another purpose and to bring them into the economy. However, since such investments are not self-financing in a short time, they are not very attractive to establish and operate. In order to increase the number of such

enterprises, it is necessary to support the government and to make the establishment of facilities established for the evaluation of type wastes attractive. This will be an investment in both the country's economy and the environment.

## 2. Materials and Methods

In many studies, the methods used in the evaluation of agricultural wastes and the waste potentials that can be used in this method and the gains that can be obtained have been calculated. In this study, the amounts of all possible agricultural wastes that can be evaluated have been tried to be calculated.

The agricultural wastes that can be obtained are calculated in five different parts. These are dry matter-based manures that can be obtained from farm animals, post-harvest wastes that can be handled from grain and industrial plants, wastes from glass and plastic greenhouses formed in greenhouse production, pruning wastes, shell wastes formed during fruit extraction of hard crustaceans, and olive pomace (prina) formed during olive oil production.

### 2.1. Livestock Manure

Two different methods were used to calculate the manure potential. First, the total potential was found according to the calculation method used by Özcan et al (2011). Here, the calculation was made by classifying only the livestock as bovine, ovine and poultry. Based on the annual wet manure production of farm animals, then adding the dry matter content and usability rates, the annual usable manure production was calculated as 18.16 Mton/year. The details of the calculation are shown in Table 1.

In the second method, it was calculated separately for each farm animal by using the standards of the American Agricultural Engineers Association (ASAE, 2003). This standard is based on the manure produced by 1000 kg live weight. Live weights of each animal are given separately. The amount of manure that can be produced and produced according to the standards of the American Agricultural Engineers Association is 23.28 Mton/year on a dry basis. This method is more detailed than the first method. The details of the calculation are shown in Table 2.

**Table 1.** Annual obtainable manure potential (TUIK, 2022)

| 2022* | Bovine | Ovine | Poultry |
|---|---|---|---|
| Number of animals | 17023791 | 56265750 | 361096026 |
| Unit wet manure amount (ton/year) | 9.95 | 0.82 | 0.03 |
| Total amount of wet manure (ton/year) | 169386700 | 46137920 | 10832881 |
| Dry matter content (%) | 12.7 | 25 | 25 |
| Total amount of dry manure (ton/year) | 21512110 | 11534480 | 2708220 |
| Availability (%) | 65 | 13 | 99 |
| Usable total amount of dry manure (ton/year) | 13982870 | 1499482 | 2681138 |
| TOTAL (ton/year) | | | 18163494 |

**Table 2.** Manure potential according to american society of agricultural engineers standards (TUIK, 2022)

| Type of animal | Number of animals* | Live weight | Manure produced for 1000 kg live weight | Annual fresh manure (ton) | Barn retention rates (%) | Obtainable manure (ton) | Dry matter ratio (%) | Manure as obtainable dry matter (ton) |
|---|---|---|---|---|---|---|---|---|
| Cattle | 17023791 | 640 | 86 | 342001151.7 | 50 | 171000575.8 | 12 | 20520069.10 |
| Pig | 1648 | 61 | 84 | 3082.19 | 80 | 246.75 | 11 | 271.23 |
| Sheep | 44687888 | 27 | 40 | 17615965.45 | 13 | 2290075.51 | 11 | 251908.31 |
| Goat | 11577862 | 64 | 41 | 11088813.11 | 13 | 1441545.70 | 13 | 187400.94 |
| Türkiye | 3669726 | 6.8 | 47 | 428088.22 | 68 | 291099.99 | 12 | 34932.00 |
| Horse | 74359 | 450 | 51 | 622886.75 | 29 | 180637.16 | 15 | 27095.57 |
| Broiler | 251289799 | 0.9 | 85 | 7016639.41 | 99 | 6946473.02 | 22 | 1528224.06 |
| Laying hen | 109806327 | 1.8 | 64 | 4617136.44 | 99 | 4570965.07 | 16 | 73135.41 |
| TOTAL (ton) | | | | | | | | 2328125.63 |

The annual dry matter-based manure amount that can be obtained in the first method was calculated as 18.163494 million tons, and the annual dry matter-based manure amount that could be obtained in the second method was calculated as 23.28 Mton. The average of these two calculations is 20.72 Mton/year on dry matter basis. This average value will be accepted in this study.

### 2.2. Grain and Agricultural Products Processing Industry Wastes

In this section, six products that are produced the most in Türkiye are taken as basis. These products are wheat, barley, corn, sunflower, seed cotton and sugar beet. When we look at the literature, although there are different methods for the calculation of agricultural waste, the calculation method based on the harvest index will be used in this research (Mardikis et al., 2004; Polat, 2020). There is a relationship between the amount of agricultural waste and product yield, and therefore between the harvest index and the amount of product. The relationship between the harvest index and the amount of waste can be expressed as in the Equations 1, 2 and 3 given below (Polat, 2020).

$$HI = \frac{M_{ü}}{M_a + M_{ü}} \quad (1)$$

$$Ma = Mü \times (\frac{1}{HI} - 1) \quad (2)$$

$$Mk = Ma \times (1 - r_n) \quad (3)$$

In these equations, HI; Harvest index, Mü; Amount of product (tons), Ma; Amount of waste (tons), Mk; Dry matter amount of the waste (tons), rn; The moisture content is given as (%). It is not possible to obtain and use all of the agricultural wastes. The amount of waste generated should be multiplied by the percentage availability. In the calculations, the harvest indexes were accepted as follows. 0.37 for wheat (Önder et al., 2007; Deniz et al., 2010; Polat, 2020), 0.34 for barley (Deniz et al., 2010; Polat, 2020), 0.42 for corn (Taner et al., 2004; Polat, 2020), 0.35-0.42 for sunflower (Polat, 2020), on average 0.30 for cotton (Baydar and Kanber, 2012; Polat, 2020) and 0.90 for sugar beet (Polat, 2020).

The moisture rates of these products were accepted as follows; 15% for wheat and barley, 47% for corn, 15% for sunflower, 17% for cotton and 30% for sugar beet (Başçetinçelik et al., 2007; Polat, 2020).

The usability percentages for the products in question were accepted as follows; 14% for wheat, 15% for barley, 60% for sunflower and corn, 70% for cotton and 55% for sugar beet (Mardikis et al., 2004; Başçetinçelik et al., 2007; Polat, 2020).

With the help of the formulas given here, the amount of waste that can be obtained for wheat, barley, corn, sunflower, seed cotton and sugar beet is 16,805180 million tons on a dry basis, according to 2022 production data. Details of the calculation are given in Table 3.

**Table 3.** Waste potential from grain and industrial plants (TUIK, 2022)

| Product type | Produce amount* (ton) | Harvest Index | Humidity rates (%) | Availability percentages (%) | Total Waste (ton) | Dry Matter (ton) | Usable Dry Matter (ton) |
|---|---|---|---|---|---|---|---|
| Wheat | 19750000 | 0.37 | 0.15 | 0.14 | 33628378.38 | 28584121.62 | 4001777.03 |
| Barley | 8500000 | 0.34 | 0.15 | 0.15 | 16500000.00 | 14025000.00 | 2103750.00 |
| Sweetcorn | 8500000 | 0.42 | 0.47 | 0.6 | 11738095.24 | 6221190.48 | 3732714.29 |
| Sunflower | 2550000 | 0.35 | 0.15 | 0.6 | 4735714.29 | 4025357.14 | 2415214.29 |
| Cotton (raw) | 2750000 | 0.3 | 0.17 | 0.7 | 6416666.67 | 5325833.33 | 3728083.33 |
| Sugar beet | 19253962 | 0.9 | 0.3 | 0.55 | 2139329.11 | 1497530.38 | 823641.71 |
| TOTAL | | | | | | | 16805180.64 |

### 2.3. Greenhouse Wastes

In Türkiye, greenhouse production areas increase and support agricultural production. Greenhouses where greenhouse production is carried out are divided into two as glass and plastic covered greenhouses. Plastic greenhouses are divided into three in themselves as plastic greenhouse, low tunnel plastic greenhouse and high tunnel plastic greenhouse. According to 2022 data (TUIK, 2022), greenhouse production is carried out on a total area of 810881 decares. Glass greenhouse with an area of 59663 decares, which constitutes 7.35% of this area. It is a plastic greenhouse with an area of 471284 decares, constituting 58.12% of it. It is a high tunnel plastic greenhouse with an area of 110426 decares, constituting 13.62% of it, and a low tunnel plastic greenhouse with an area of 169538 decares, which constitutes 20.91%. Vegetables, fruits and ornamental plants are produced in greenhouses. Statistical values show that 8178089 tons of vegetables, 1151293 tons of fruit, 1357624870 branch cut flowers, 197860415 outdoor ornamental plants, 42387977 indoor ornamental plants and 1833300 bulbs were produced in 2022 (TUIK, 2022).

Wastes generated in greenhouse vegetable cultivation are divided into two as waste generated during production and waste generated during dismantling after production. In this section, wastes from tomato, pepper and eggplant plants, which are the most produced in greenhouse vegetable production, will be calculated. There is no scientific data on the amount of waste produced by other vegetables grown. In addition, no major problems have arisen regarding the waste generated by other products. In Türkiye, at the end of the tomato, pepper and eggplant growing season, the waste piles formed as a result of the dismantling wastes are reflected to the public from time to time and it is emphasized that it creates a problem.

In this calculation, the amount of waste in the study conducted by Bilgin et al. (2012) was taken as a basis. Waste amounts have been calculated separately for glass and plastic greenhouses where all three crops are grown. The amount of waste that can be obtained from glass greenhouses is 47836.84 tons annually on a dry basis, and the amount of waste that can be obtained from plastic greenhouses is 345211.21 tons on a dry basis annually. The total amount of waste that can be obtained from greenhouses on an annual dry basis is 393048.05 tons. Calculations related to these wastes are shown in Table 4.

### 2.4. Pruning Wastes

Pruning is the process of cutting the parts that are aging, drying, sick or preventing development and growth in plants. Pruning can be done for different purposes. For example, in fruit trees with low fruit yield, pruning is done to increase the yield, to make the fruits healthier, to prevent overgrowth, and to give the plants a certain form and preserve this form.

**Table 4.** Amount of waste that can be obtained in greenhouse production

| Glass greenhouse | | | | Plastic greenhouse | | |
|---|---|---|---|---|---|---|
| Product | Cultivation area (decare) | Waste amount* | Total waste (ton) | Cultivation area (decare) | Waste amount* | Total waste (ton) |
| Tomatoes | 30980 | 1.07 | 33148.60 | 227317 | 1.03 | 234136.51 |
| Pepper | 10629 | 0.93 | 9884.97 | 93946 | 0.93 | 87369.78 |
| Aubergine | 5521 | 0.87 | 4803.27 | 25218 | 0.94 | 23704.92 |
| TOTAL | | 47836.84 | | | 345211.21 | |
| GRAND TOTAL | | | | 393048.05 | | |

*Dry basis (ton/decare)

In this section, a calculation is made based on perennial fruit trees. Annual pruning waste amounts and usable pruning waste amounts were calculated by utilizing the number of fruit-bearing adult trees from (TUIK, 2022) agricultural statistics, pruning coefficients determined in the study conducted by Bilandzija et al. (2012) and usability rates determined by CEC (California Energy Commission) (Williams et al. , 2015; Sümer et al., 2016). Here, 14 types of trees were considered and total pruning waste was calculated based on the number of trees.

In addition, the pruning wastes of the grape plant, which has an important place in fruit production, were calculated based on the area. In the literature, two different values have been found in this regard as 3 tons (Arık, 2023) and 5 tons (Bekar, 2016) per hectare. This value varies depending on the type and age of the vineyards, and in the calculation in this section, it was taken as 5 tons per hectare.

According to the calculations, the annual amount of pruning waste that can be obtained from 14 types of trees is 3031.60 thousand tons, and the annual amount of pruning waste that can be obtained from vineyards is 13.46 thousand tons. Details about these are given in table 5 and table 6. The annual amount of pruning waste that can be obtained in total is 3045.06 thousand tons. It could not be added to this amount because there is no reliable data on pruning wastes from parks and gardens.

**Table 5.** Amount of pruning wastes

| Fruit tree | Number of trees bearing fruit | Pruning coefficient per tree (kg/year) | Pruning waste (ton/year) | Usable rate (%) | Usable pruning waste (ton/year) |
|---|---|---|---|---|---|
| Olive | 163035000 | 9.08 | 1480357.80 | 0.70 | 1036250.46 |
| Hazelnut | 395994000 | 3.05 | 1207781.70 | 0.70 | 845447.19 |
| Pistachio | 58144000 | 8.80 | 511667.20 | 0.70 | 358167.04 |
| Citrus | 48428000 | 5.30 | 256668.40 | 0.70 | 179667.88 |
| Apple | 75913000 | 2.34 | 177636.42 | 0.70 | 124345.49 |
| Cherry | 22200000 | 5.90 | 130980.00 | 0.70 | 91686.00 |
| Peach | 20416000 | 7.23 | 147607.68 | 0.70 | 103325.38 |
| Apricot | 19158000 | 5.79 | 110924.82 | 0.70 | 77647.37 |
| Plum | 9184000 | 7.34 | 67410.56 | 0.70 | 47187.39 |
| Fig | 10852000 | 4.58 | 49702.16 | 0.70 | 34791.51 |
| Almond | 13616000 | 5.81 | 79108.96 | 0.70 | 55376.27 |
| Cherry | 5611000 | 5.37 | 30131.07 | 0.70 | 21091.75 |
| Pear | 11554000 | 2.45 | 28307.30 | 0.70 | 19815.11 |
| Walnut | 15327000 | 3.43 | 52571.61 | 0.70 | 36800.13 |
| TOTAL | | | | | 3031598.98 |

**Table 6.** Pruning wastes from vineyards

| Fruit | Area (ha) | Pruning coefficient per hectare (ton /year) | Pruning waste (ton/year) | Usable rate (%) | Usable pruning waste (ton/year) |
|---|---|---|---|---|---|
| Grape | 3845 | 5 | 19225 | 0.70 | 13457.5 |

**2.5. Wastes from Some Hard Shell Fruits and Olive Oil Production**

In this section, the amount of wastes generated during evisceration of hazelnuts, walnuts, pistachios, almonds and apricot seed and olive oil production has been calculated. Since the evisceration processes of hazelnut, walnut, pistachio and apricot seed are fabricated, the shells obtained come out in large quantities and are pressed, briquetted or pelleted with this shell, which is largely evaluated as solid fuel and brought into the economy. In addition, these wastes can be used in the furniture industry. The rind ratio of the hard-shelled fruits here varies depending on the variety and climate characteristics. For this reason, average values were taken. There is scientific data on pistachio in the literature. The soft shell of the pistachio fruit with a moisture content of 6% constitutes 18.04% of the total weight, the hard shell constitutes 37.93%, and the interior constitutes 44.03% (Gezginç and Duman, 2004). The shell ratio in hazelnut was taken as 50%, in walnut

35-50% (on average 42.5%), in almond 50%, in apricot seed 75%. When we look at the availability rates, only 50% of the shelled walnuts are used in the pastry and dessert sector, so shells can be obtained at this rate. Since the other part is consumed at home, it is disposed of as household garbage and cannot be handled in practice. Since pistachios are mostly consumed as nuts, only 40% of the hard shells can be obtained. According to the data of 2022, the amount of waste that can be obtained in line with this information is 550186.6 tons. This waste is largely considered as solid fuel. The details of the calculation are given in Table 7 and Table 8.

**Table 7.** Hard shell fruits wastes

| Hard Shell Fruits | Annual production (ton) | Usable production (ton) | Shell ratio (%) | Waste amount (ton) | Obtainability (%) | Obtainable waste (ton) |
|---|---|---|---|---|---|---|
| Hazelnut | 684000 | 677844 | 50 | 338922 | 100 | 338922 |
| Walnut | 325000 | 317200 | 35-50 | 134810 | 50 | 67405 |
| Pistachios soft shell | 119355 | 116371 | 18.04 | 20993.3284 | 100 | 20993.33 |
| Pistachio hard shell | 119355 | 116371 | 37.93 | 44139.5203 | 40 | 17655.81 |
| Almond | 178000 | 174796 | 50 | 87398 | 100 | 87398 |

**Table 8.** Apricot seed wastes

| Fruit | Annual production (ton) | Usable production (ton) | Shell ratio (%) | Waste amount (ton) | Obtainability (%) | Obtainable waste (ton) |
|---|---|---|---|---|---|---|
| Apricot seed | 95000* | 23750 | 75 | 17812.5 | 100 | 17812.5 |

*2022 dried apricot production estimation. Approximately 25% of this apricot seed is produced.

**Table 9.** Olive pulp production

| Fruit | Annual production (ton) | Pulp rate (%) | Olive pomace amount (ton) | Obtainability (%) | Obtainable waste (ton) |
|---|---|---|---|---|---|
| Olive for oil | 2037783 | 80 | 1630226.4 | 100 | 1630226 |

Türkiye has an important place in world olive and olive oil production. Olive oil yield varies according to different varieties. One kg of olive oil is obtained by squeezing 3-8 kg of olives. When calculating in this section, it is assumed that 1 kg of olive oil and 4 kg of pulp are obtained from 5 kg of olives. In the light of this information, a total of 1630226 tons of olive pulp was produced in 2022. While some of the olive pomace is used for extraction of olive pomace oil, a large part is used as a fuel by drying. It is practically not possible to use it as animal feed. The details of the calculation are shown in Table 9.

## 3. Discussion and Conclusion

Türkiye is one of the rare countries in the world with a rich agricultural production pattern. Agricultural wastes generated during this production should be evaluated by bringing them into the country's economy. In the calculations, the data of 2022 were used. According to the results of this study, the amount of manure that can be obtained from livestock in Türkiye is approximately 20.7 million tons on a dry basis. The amount of waste that can be obtained from grain and some industrial plants is about 16.8 million tons on a dry basis, the amount of waste that can be obtained from greenhouses is about 393 thousand tons on a dry basis, the amount of waste that can be obtained from pruning waste is about 3 million tons, the amount of waste that can be obtained from hard shell fruits is about 550.2 thousand tons and the amount of waste generated during olive oil production is approximately 1.6 million tons. Apart from this, there are also wastes from agricultural activities for which there is no sufficient data. Domestic waste and sewage sludge are also organic waste. However, it cannot be said that it is exactly agricultural waste. In this study, agricultural wastes, which generate the most waste and have data about, were calculated. Domestic wastes and sewage sludge amounts are excluded from the calculation here.

In other studies, only a certain group of these wastes was handled. For example, Özcan et al. made calculations based on animal manure, some agricultural wastes, urban wastes and sewage sludge in their study on biogas production (Özcan et al., 2011). In his study, Polat used only wheat, barley, corn, sunflower, sugar beet and seed cotton production data while calculating the changes in the biowaste potential of Türkiye (Polat, 2020). In the biochar study of Sümer et al. (2016), calculations were made based on animal manure, some agricultural wastes and pruning wastes. Başçetinçelik et al. (2007) conducted a study by taking a large part of these wastes. In his study, Karaca (Karaca, 2015) determined Türkiye's annual product residue potential in terms of type, amount and mapping. The products used in the calculation in this study are wheat, barley, oats, sunflower, cotton, maize and groundnuts. In another study, Karaca (2018) determined the amount and energy value of biogas that can be produced from animal manure in Türkiye. In this study, biogas potential was calculated with the number of dairy cattle and laying hens taking into consideration, which have high availability manure. In the studies carried out, the number of animals and product amounts were taken as basis when calculating the amount of manure and crop residue. The values taken

change every year. Sometimes, the crop yield of the fields may change due to reasons such as unfavorable climatic conditions or the farmer changing his planting preference. There are also ups and downs in animal numbers over the years. For this reason, the statistical values taken as a basis in the studies differ from each other because they belong to different years. Although some of these calculations are detailed and some are superficial, they have not been made for all agricultural product wastes.

It is possible to obtain different additives used in the production of energy, fertilizer and composite materials from these wastes by using different methods. The biomass energy that can be obtained from these wastes, especially in Türkiye, which is dependent on foreign energy, is at a level that cannot be ignored. Independence in energy is of strategic importance for countries. Therefore, Türkiye, which is dependent on foreign energy for energy, should evaluate all energy resources in the most efficient way. For this, both the state and the private sector should establish facilities for the evaluation of these wastes. Legal arrangements should be made for the utilization of these wastes, and incentives should be increased by increasing the attractiveness of investments to be made to process these wastes. Evaluating agricultural wastes with more environmentally friendly and more efficient methods will be more beneficial for our environment, Türkiye and our world.

**Author Contributions**

The percentage of the author contributions is presented below. The author reviewed and approved the final version of the manuscript.

| | S.S. |
|---|---|
| C | 100 |
| D | 100 |
| S | 100 |
| DCP | 100 |
| DAI | 100 |
| L | 100 |
| W | 100 |
| CR | 100 |
| SR | 100 |
| PM | 100 |
| FA | 100 |

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

**Conflict of Interest**

The author declared that there is no conflict of interest.

**Ethical Consideration**

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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# PURIFICATION OF GLUTATHIONE REDUCTASE ENZYME FROM WHITING FISH GILL TISSUE AND INVESTIGATION OF METAL INHIBITION

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**Abstract:** Pollution in the seas basically accumulates in marine organisms. Heavy metal residues in aquatic ecosystems can pass through food and cause toxic effects and accumulations in human health. Glutathione reductase (GR), which is among the basic enzymes, has an important place in the suppression of stress in the cell. In this study, glutathione reductase enzyme from whiting fish gill tissue was partially purified for the first time in the literature and the effects of heavy metal compounds on enzyme activity were determined. The purification process was carried out in three stages as homogenate preparation, ammonium sulfate precipitation and also dialysis. In conclusion the study, optimum level pH 7.0, optimal substrate concentration 2 mM NADPH and optimum buffer 150 mM $KH_2PO_4$ were determined. After partial purification, the inhibition effects of $Cd^{2+}$, $Ni^{2+}$, $Zn^{2+}$ as heavy metal ions were investigated. The $IC_{50}$ levels of heavy metals were calculated as 20.17 µM, 33.7 µM and 59.31 µM, respectively.

**Keywords:** Enzyme inhibition, Purification, Glutathione reductase, Heavy metals, *Merlangius euxmus*

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## 1. Introduction

The whiting fish (*Merlangius euxmus*) is a member of the Gadidae family. Their body shapes whitare slender and elongated. All fins are soft and spineless. Colors may vary. Generally, the back is bluish or yellowish brown, but the abdomen is white or silver (Fisher, 1973). A whiting fish with an average length of 15-20 cm can reach up to 50 cm in length (Slastenenko, 1956). They are widely distributed in the European coastal regions of the Black Sea, Aegean Sea and Mediterranean (Ivanov, 1985). Whiting prefers muddy bottoms and coastal waters at depths up to 30-100 m.

Pollution in the seas basically accumulates in marine organisms. One of the important pollutants in the seas is heavy metals, which are among the most harmful elements. Heavy metal residues in aquatic ecosystems can pass into foods and cause toxic effects and accumulations in human health. For this reason, it passes to humans through the food chain (Tüzen, 2003). Unhealthy diet in humans causes oxidative stress in cells and reactive oxygen species that occur as a result of this stress damage cell components in the body. Reactive oxygen species that cause oxidation the main cause of cell damage and death and is well known to be associated with diseases such as cancer and cardiovascular disease (Valko et al., 2006).

Antioxidants can reduce the stress state caused by reactive oxygen species. Studies have focused on the role of antioxidants in treating and preventing diseases (Perry et al., 2002). Glutathione (GSH), a reducing molecule found in almost all eukaryotes, is effectively involved in many vital functions, including antioxidant defense, detoxification of metabolites, cell cycle regulation, gene expression, and immune function (Lue al., 2009).

Glutathione reductase (EC1.8.1.7; GR), which has an important place in the glutathione mechanism, is an important enzyme containing flavin adenine dinucleotide (FAD) and catalyzes oxidized glutathione (GSSG) to reduced glutathione (GSH) (Kocaoğlu et al., 2019).

GR is converted back to GSH by transferring an electron from NADPH to the disulfide bonds of GSSG. Therefore, this enzyme has an important role in the antioxidant defensive system together with GR. Nicotinamide Adenine Dinucleotide Phosphate (NADPH), which has a reduction potential against reactive oxygen species in the environment, is required for this reaction to take place in the mechanism. Therefore, NADPH protects against free radical damage (Sen et al., 2010). At the same time, GR plays an important role in the reduction and oxidation of intracellular GSH (Toribio et al., 1996). GSH and GR, the deficiency of which causes oxidative damage in cells,

causes many diseases such as cancer, Alzheimer's, diabetes, sickle cell anemia and Parkinson's (Wu et al, 2004).

The primary objective of this study, inhibition kinetics of heavy metals by making partial purification of the GR enzyme from the gill tissue of whiting (*Merlangius euxmus*) and due to its harmful effects on fish, as it has an important mechanism as a therapeutic approach for various diseases.

## 2. Materials and Methods

### 2.1. Chemicals

The chemicals used in the experiment process were purchased from Sigma-Aldrich and Merck.

### 2.2. Glutathione Reductase Enzyme Activity

Enzyme activity was measured in Shimadzu UV-1800 spectrophotometric device at 340 nm for 3 minutes, following the decrease in absorbance caused by the oxidation of NADPH (Calberg and Mannervik, 1985).

### 2.3. Preparation of Homogenate

Gill tissues were taken from whiting fish samples (Figures 1 and 2). 5.5 g of gill tissues were weighed and subjected to physical disintegration in liquid nitrogen in a mortar. After crushing, gill tissues were taken into a 50 ml falcon tube and 0.1 M $KH_2PO_4$ (pH7.6) buffer containing 1mM EDTA + 0.15 M KCl was added to it and made up to 35 ml. Then, centrifugation was performed at +4 °C at 15000 rpm for 60 minutes. After centrifugation, the supernatant and precipitate were separated from the filter paper by filtration and the enzyme activity was examined.

**Figure 1.** Whiting (*Merlangius euxmus*).

### 2.4. Ammonium Sulphate Precipitation and Dialysis Process

One of the methods used to increase the concentration of the protein of interest in the protein purification process is to separate that protein from the proteins of interest. The solubility of proteins is related to the distribution of hydrophilic and hydrophobic parts of that protein. Thanks to these features, precipitation is done in accordance with our purpose. The most preferred of these precipitation processes is precipitation with high salt concentrations. For the homogenate prepared from the gill tissue of whiting, precipitation processes were carried out at different intervals of 0-20%, 20-40%, 40-60% during the ammonium sulfate treatment. For this process, solid $(NH_4)_2SO_4$ was added piece by piece and transferred to the homogenate in ice with the help of magnetic stirrer. As a result of the precipitation, the range in which the enzyme was active was determined by reaching saturation in the range of 40-60%. The precipitate was dissolved in 150 mM $KH_2PO_4$ (pH 7.0) buffer. After the interval obtained, dialysis was done to desalinate the protein sample. Dialysis was performed for 3 hours in 15 mM $KH_2PO_4$ (pH 7.0) buffer.

**Figure 2.** Whiting gill tissue.

### 2.5. Characterization Study of GR Enzyme from Whiting Gill Tissue

#### 2.5.1. Determination of optimum ionic strength

In order to determine the optimum ionic strength for the activity of the GR enzyme from whiting gill tissue, solutions of $KH_2PO_4$ buffer were prepared at 50, 100, 150, 200, 300, 400, 500, 600, 700, 800 mM concentrations and their activity measurements were carried out. As a result of the measurements, the optimum level ionic strength was determined in 150 mM $KH_2PO_4$ buffer.

#### 2.5.2. Determination of optimum pH values

As a result of the optimization of the $KH_2PO_4$ buffer, pH optimization was performed at the concentration with the highest activity. It was prepared for optimization at pH values of 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9 in 150 mM $KH_2PO_4$ buffer and activity measurements were examined. As a result of the measurements, 150 mM $KH_2PO_4$ buffer was determined as the optimum ionic strength.

#### 2.5.3. Determination of optimum amount of substrate

For the determination of the optimum substrate amount, the activity was determined using the optimum pH and optimum buffer determined from whiting gill tissue and 50, 60, 70, 80, 90 and 100 µl NADPH as substrate. As a

result of the measurements, the optimum substrate of the GR enzyme of the gill tissue was determined as 100 µl using 100 mM $KH_2PO_4$ (pH 7.0) buffer.

**2.6. *In vitro* Enzyme Inhibition**

Using heavy metals at various concentrations, their inhibition effects on the GR enzyme of whiting gill tissue were evaluated. A heavy metal-free assay was used as a control (100% activity). Effects of different heavy metal constituents of $Ni(NO_3)_2$ , $Cd(NO_3)_2$ and $ZnCl_2$ heavy metals on the GR enzyme activity of gill tissue were measured spectrophotometrically. Using typical polynomial regression software, a graph of inhibitory concentration versus percent activity was plotted for each heavy metal. Heavy metal concentrations ($IC_{50}$) that inhibit enzyme activity by 50% were determined and shown in Table 1.

**Table 1.** Whiting (*Merlangius euxmus*) GR enzyme inhibition data with heavy metal components

| Metal components | $IC_{50}$ (µM) |
|---|---|
| $Cd^{2+}$ | 20.17 |
| $Ni^{2+}$ | 33.7 |
| $Zn^{2+}$ | 59.31 |

## 3. Results and Discussion

GSH is a tripeptide composed of glutamic acid, glycine and cysteine, especially produced in the liver and many tissues. Moreover, GSH is a powerful intracellular antioxidant and has a protective effect on antioxidant vitamins C and E (Blokhina et al., 2003). Besides all this, GSH, which is responsible for amino acid transport, peroxide metabolism, bone and muscle integrity, regulation of many enzyme functions, is effective in DNA synthesis and repair of damaged parts, and its deficiency leads to cell death (Macmillan and Cruthirds, 2001). GR is one of the essential enzymes of the antioxidant system. GR is an enzyme that maintains proper function and is required in humans, encoded by the GSR gene.It plays an important role in the prevention of cellular oxidative stress (Angelucci et al., 2008).It is a low molecular weight thiol that protects the organism from the harmful effects of intracellular oxidized molecules (Angelucci et al., 2008). It catalyzes the reduction of oxidized glutathione in the presence of NADPH (Williams et al. 1976). In the NADPH-dependent reaction with GSSG, glutathione reductase acts as a reducing agent that reduces the activity of GSH (Carlberg et al., 1985).The most important issue in the reaction catalyzed by the enzyme is the maintenance of the GSH/GSSG ratio in the cellular environment (Toribio et al., 1996). In addition, for this reason, it helps to maintain important cellular functions such as detoxification of reactive oxygen species (ROS) (Çakmak et al., 2011; Şentürk and Şentürk 2020).

Heavy metal accumulation, which is one of the biggest environmental pollution problems in the world, is exposed to serious accumulation in seas, lakes and streams. As a result of this negative effect, heavy metals have a toxic effect on fish, which is an important food source for human life. As a result of heavy metals, which cause accumulation on fish as a result of their intake of food, they cause inhibition of antioxidant enzymes in the cell and some problems occur in living organisms due to oxidative damage. The GR enzyme, which has an important place among antioxidant enzymes, constitutes a defense mechanism against the damage caused by oxidative stress.

In this experiment carried out, the glutathione reductase enzyme was partially purified from whiting fish gill tissue. After determining their characteristic properties, heavy metal inhibition kinetics were investigated. After partial purification process, homogenate preparation, ammonium sulfate precipitation process, dialysis, determination of characteristic properties, the study was completed by determining the inhibition kinetics with heavy metal application.

The purification process was first performed by the homogenate preparation process. The prepared gill tissue homogenates were precipitated between 0-100% ammonium sulphate. In the precipitation process, it was determined that the GR enzyme precipitated in the range of 40-60%. Erat (2002) determined the ammonium sulfate range for GR enzyme from bovine and human erythrocytes as 30-70%, Acan and Tezcan (1989) determined the ammonium sulfate range of GR enzyme for sheep brain as 35-55%, Ulusu et al. (2005) found it in the range of 0-60% from sheep liver. After ammonium sulfate precipitation, dialysis was performed to remove undesirable ions in the environment. Işık and Soydan (2023a) found the ammonium sulfate range of GR enzyme from gill tissue of Scorpion fish is between 60-80%.

In another study by Işık and Soydan (2023b), the ammonium sulfate range of the GR enzyme from the muscle tissue of Scorpion fish was found to be in the range of 60-80%.

$Ni^{2+}$, $Zn^{2+}$ and $Cd^{2+}$ heavy metals were applied on the partially purified enzyme. The $IC_{50}$ values of applied heavy metals were calculated as 33.7 µM, 59.31 µM, and 20.17 µM, respectively. Graphs of $IC_{50}$ values are given in Figure 3-5 and Table 1.

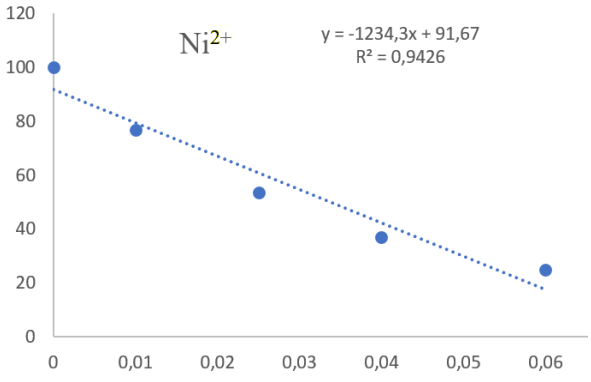


**Figure 3.** Effect of [$Ni^{2+}$] GR enzyme activity from whiting fish gill tissue.

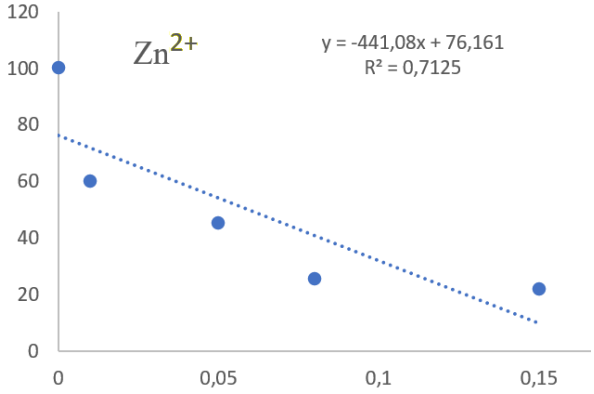


**Figure 4.** Effect of [$Zn^{2+}$] GR enzyme activity from whiting fish gill tissue.

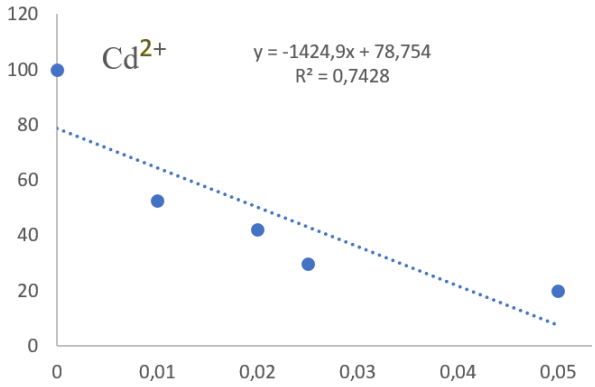


**Figure 5.** Effect of [$Cd^{2+}$] GR enzyme activity from whiting fish gill tissue.

The characterization process was carried out in the study. The optimum pH value of the GR enzyme was found to be 7.0, the optimum substrate amount was 100 µl NADPH (2 mM) and the optimum buffer concentration was 150 mM $KH_2PO_4$.In the literature, the optimum level ionic strength was found to be 435 mM phosphate buffer for bovine erythrocyte GR enzyme and 50 mM Tris for sheep liver (Erat, 2002; Ulusu et al., 2005).In studies conducted with different species, it has been determined that the optimum pH of GR is in the range of 6.5-8.5 (Açan, 1990; Ogus and Ozer, 1998; Özer and Öğüs, 1991; Willmore and Storey, 2007; Tekman et al., 2008).

The damage caused by heavy metals to the environment and especially the excessive exposure of marine organisms show the importance of the study. Because aquatic organisms are exposed to heavy metals, they pass on to humans through food intake. As a result, it causes many diseases by leaving harmful effects on people (Çoban et al., 2007).

Ekinci et al. (2011) investigated the inhibition interaction of GR enzyme isolated from the liver of rainbow trout with heavy metals $Co^{2+}$, $Zn^{2+}$, $Ca^{2+}$, $Fe^{2+}$, $Mn^{2+}$, $Cr^{2+}$, $Sn^{2+}$ and $Mg^{2+}$. The $IC_{50}$ values of heavy metals were found as 42.2 µM, 63.1 µM, 357 µM, 486 µM, 508 µM, 592 µM, and 657 µM, respectively. In a study by Tekman et al. (2008), the inhibition kinetics of heavy metals $Cd^{2+}$, $Cu^{2+}$, $Pb^{2+}$, $Hg^{2+}$, $Fe^{3+}$ and $Al^{3+}$ applied on the GR enzyme purified from the liver of rainbow trout (*Oncorhynchus mykiss*) were investigated.$IC_{50}$ values were found as 65.5 µM, 82 µM, 122 µM, 509 µM, 797 µM and 804 µM, respectively. Temel and Çiftçi (2017) determined the inhibition effects of $Ni^{2+}$, $Zn^{2+}$, $Pb^{2+}$, $Hg^{2+}$, $Ag^{+}$ and $Al^{3+}$ heavy metals on the enzyme in the study conducted on the GR enzyme purified from chicken kidney. The $IC_{50}$ values were found to be 337 µM, 191 µM, 168 µM, 187 µM, and 289 µM, respectively. Işık and Soydan (2023) investigated the interaction of GR enzyme isolated from muscle tissue of scorpion fish with $Mn^{2+}$, $Cd^{2+}$, $Ni^{2+}$, and $Cr^{3+}$ heavy metals. The $IC_{50}$ values of heavy metals were found as 2.4 µM, 30 µM, 135 µM and 206 µM, respectively.

## 4. Conclusion

As a result, in this study, it was determined that the GR enzyme of whiting gill tissue has an inhibitory feature with metal ions even at low concentrations. GR plays an important role in the antioxidant defense system. Inhibition of various heavy metals has a negative effect on the organism. Therefore, it is a factor in the emergence of many pathological disorders. The GR enzyme, which is partially purified from the whiting fish gill tissue, keeps the vitally important GSH/GSSG ratio under control. Care should be exercised in the use of these heavy metals, as they inhibit the GR enzyme and disrupt the balance, and their use should be kept under control. This study was carried out for the first time in the literature in terms of partial purification, characterization and determination of kinetic properties of GR enzyme from whiting fish gill tissue. The findings of our study will contribute to antioxidant enzyme studies.

### Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

| | S.B.D. | K.I. | B.M. | E.S. | D.E. |
|---|---|---|---|---|---|
| C | 10 | 10 | 20 | 30 | 30 |
| D | 100 | | | | |
| S | | 100 | | | |
| DCP | | | 50 | 50 | |
| DAI | | | | | 100 |
| L | 20 | 20 | 20 | 20 | 20 |
| W | 20 | 20 | 20 | 20 | 20 |
| CR | 20 | 20 | 20 | 20 | 20 |
| SR | 20 | 20 | 20 | 20 | 20 |
| PM | 20 | 20 | 20 | 20 | 20 |
| FA | 20 | 20 | 20 | 20 | 20 |

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

### Conflict of Interest

The authors declared that there is no conflict of interest.

**Ethical Consideration**

Ethics committee approval is not required because of this study used commercially caught and sold whiting fish as experimental material.

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# THE EFFECT OF OHMIC HEATING ON THE QUALITY PROPERTIES OF COUSCOUS DURING COOKING

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**Abstract:** Couscous has been widely eaten around the world because it has a low glycemic index, is low in fat, and is simple to prepare. However, it should be cooked before consumption. Therefore, novel heating methods, such as ohmic heating, can be used to cook couscous. This study aimed to investigate the potential use of ohmic heating at a voltage gradient of 17 V/cm to cook couscous and compare it with the conventional cooking method. To determine the effect of ohmic heating and conventional methods on the quality properties (color, texture profile analysis, cooking loss, moisture content, and weight increase (%)) of couscous, samples were cooked in a 0.1% salt solution. The samples were analyzed at different cooking times (4, 8, 12, and 16 min). The results obtained in the present study revealed that the total color difference also increased with an increase in cooking time. In addition, similar trends were observed for cooking loss, moisture content, and weight gain. Furthermore, the couscous samples treated with ohmic heating and conventional heating methods were completely cooked after 12 minutes. Overall, compared to the conventional cooking method, the ohmic heating process did not induce any negative effects on the quality parameters of couscous.

**Keywords:** Couscous, Quality, Ohmic heating, Cooking, Texture profile analysis

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## 1. Introduction

Couscous, often called kusksi or kseksu, is a Middle Eastern and northwestern African dish consisting of small steamed granulated durum wheat semolina (Yüksel et al., 2017). Couscous is an essential food source that is highly preferred in the cuisines of Algeria, Tunisia, Morocco, Libya, and other North African countries (Yüksel et al., 2017; Benayad et al., 2021). It began to appear in French and European cuisine in the early twentieth century through the French colonial empire. Nowadays, it has been widely consumed worldwide in various ways such as in salads and meals due to its cheapness, low glycemic index, low fat, and simplicity of preparation (Bellocq et al., 2018). However, dried couscous must be cooked before consumption.

There are several methods to cook couscous based on the culture of people in each region (Jittanit et al., 2017; Benayad et al., 2021). Since couscous is cooked in a similar way to cooking pasta and rice, it has already been cooked with an electric heater, gas heater, and similar heating methods. In the conventional method of cooking couscous, couscous is taken into a bowl, and then water and salt are added to it and left to boil (Yüksel et al., 2017; Cankurtaran and Bilgiçli, 2021). It is cooked in boiling water including salt until it reaches the desired softness and then drained. Nevertheless, during conventional heating, too many heat losses occurred due to indirect heating (Jittanit et al., 2017). In addition, the physicochemical properties and eating quality of cooked couscous could be influenced by the cooking methods and utensils as reported for rice or pasta in the previous studies (Jittanit et al., 2017; Ding et al., 2021). Therefore, new approaches are needed to reduce energy losses and not affect the physicochemical properties and eating quality of cooked couscous.

Many approaches, such as ohmic, infrared, microwave, and radiofrequency, for heating have been developed so far (Jittanit et al., 2017; Gómez-López et al., 2021; Goksu et al., 2022). Among these heating methods, ohmic heating (OH) called Joule heating, electrical resistance heating, or electroconductive heating has gotten more attention from researchers due to its high electrothermal efficiency, simple structure, easy control of equipment, uniform, and rapid heating (Ding et al., 2021; Goksu et al., 2022). In addition, the absence of waste products and the very low operating costs appear as advantages of the ohmic system (Ding et al., 2021). Hence, there are several

applications of ohmic heating, such as extraction, evaporation, cooking, and thawing (Jittanit et al., 2017; Parmar et al., 2018; Angel-Rendon et al., 2019; Gavahian et al., 2019; Makroo et al., 2020; Sabanci and Icier, 2020; Cevik and Icier, 2020; Alcantara-Zavala and Figueroa-Cardenas, 2022). Currently, ohmic heating has been used to cook grains and pasta in some studies where it was reported that there were no significant differences in the textural properties of the foods treated with the ohmic and conventional cooking treatments (Jittanit et al., 2017; Gavahian et al., 2019; Rokhbin et al., 2021; Goksu et al., 2022).

Therefore, the aim of this study was to investigate the effects of applying ohmic heating on the quality parameters including color, texture profile analysis, moisture content, weight increase (%), and cooking loss of the dried couscous during the cooking period.

## 2. Materials and Methods

### 2.1. Materials

The dried couscous (Filiz, Bolu, Türkiye) used in the study was purchased from a local market. After the couscous came to the laboratory, they were packed in 10 g packages with a vacuum packaging machine (Lipovak, MV-20/30, Türkiye) in order not to absorb moisture and stored in a dark and cool environment. Before each cooking process, the vacuum packages were opened, and the cooking process was immediately begun.

### 2.2 Ohmic Cooking System

The ohmic heating system used in the present study consists of a heating cell, electrodes, a T-type thermocouple (Cole Parmer, UK), a custom-made microprocessor, a computer, and a voltage-regulated variac (0-360 V: 50 Hz). The electrodes used in the experiment were stainless steel and their dimensions were 150x10x1 mm (Figure 1). The ohmic heating cells were made of polyoxymethylene, and their dimensions were 60x60x10 mm. The temperature, the current, and the voltage values were recorded with the microprocessor per second intervals.

### 2.3 Cooking Procedure

Ten grams of couscous and 100 ml of 0.1% NaCl solution were filled into the ohmic heating cell. Later, couscous samples were cooked at a voltage gradient (17 V/cm) and cooking times (0, 4, 8, 12, and 16 min). The temperature of the solution was initially about 23 °C, and then the ohmic system was turned on to heat samples in the solution. After that, the cooking process started when the temperature of the solution reached the boiling point. The cooked couscous samples were filtered through a strainer to separate the solid and liquid parts. The solid part was quickly cooled with cold water and then wiped with a napkin. Afterward, these couscous samples were used for the analysis of color, texture, and moisture content. The liquid part remaining from the couscous cooking process was also used to measure the total soluble solid content (TSSC) as reported in Section 2.6. In addition, before and after the ohmic heating process, the weight of the solid and liquid parts in the heating cell was weighed and recorded.

For conventional cooking, an adjustable (Awox, Lotus, Türkiye) heater used as a heat source was used to cook couscous samples. The temperature was measured with a T-type thermocouple and the amount of energy consumed by the heater was measured with a power meter (UNİ-T, UT230B-EU, China) throughout the cooking process. The cooking time for the conventional heating was 12 min because the cooking time of couscous samples treated with OH was 12 min due to the absence of an opaque white center, which is used for that the cooking process is completed (Bayram, 2006). The experiments were repeated in triplicate.

### 2.5 Moisture Content

A moisture analyzer (Radwag-MA-110R, Poland) was used to measure the moisture content of couscous samples cooked with the ohmic system at 17 V/cm and conventional heating methods.

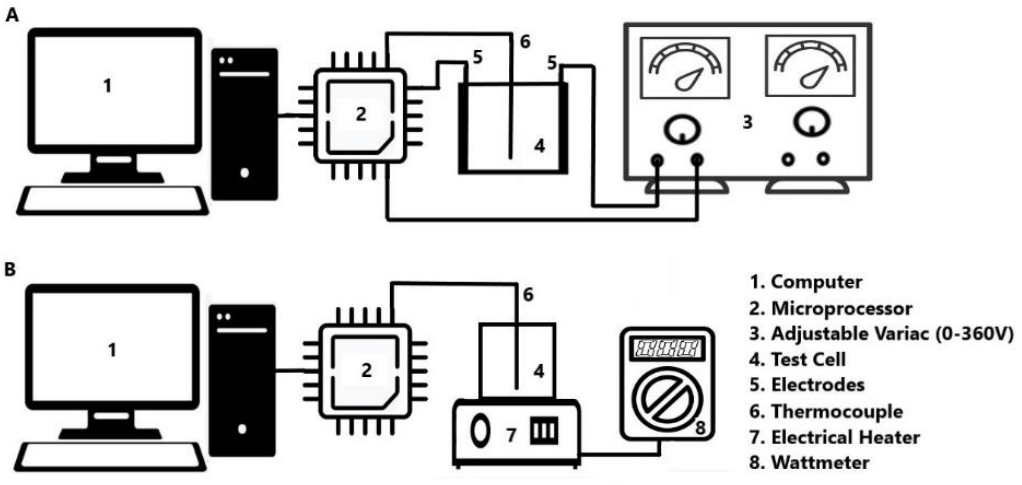


**Figure 1.** Schematic representation of (A) Ohmic cooking (B) Conventional cooking system.

**2.6 Cooking Loss**

A digital refractometer (Hanna, HI 96801, Romania) was used to determine the total soluble solid content (TSSC) that passed into the water during the cooking process and TSSC values were measured when the water was cooled to room temperature (~23 °C). TSSC values were expressed in ˚Brix.

**2.7 Analysis of Percent Changes in Weight Increase (WI %)**

The method reported by Demir et al. (2010) was followed with minor modifications. Ten grams of couscous were cooked in 100 ml distilled water including 0.1% NaCl for each cooking time (0, 4, 8, 12, and 16 min). Then, distilled water was used to wash the cooked samples. After that, the samples were drained for 2 min and allowed to dry on paper for 2 min. Later, weight increase (WI) was calculated by using equation 1.

$$WI(\%) = 100\,\frac{(b-a)}{a} \quad (1)$$

where, $a$ and $b$ are the weight of raw couscous and the weight of cooked couscous, respectively.

**2.8 Texture Profile Analysis and Color Parameters of Cooked Couscous**

In general, the procedure of Goksu et al. (2022) was followed with minor modification for determining Texture Profile Analysis (TPA) and color parameters. The cooked couscous samples (1.0 ± 0.1 g) were used to determine the TPA including hardness, adhesiveness, cohesiveness, springiness, gumminess, and chewiness of the samples at room temperature (23±1 °C). A Texture Analyzer (TA.XT.Plus, Stable Micro Systems, Surrey, UK) interfaced with the computer software Texture Exponent (version 6.1.16.0) was used to manage the texture analysis. A cylindrical aluminum probe of 31 mm was used to compress the samples to 80 % strain for two cycles with the test conditions: 1 mm/s of pre-test and post-test, and 0.5 mm/s of the test. The trigger force and time between the cycles used in TPA were 5 g and 5 s, respectively. The presented TPA results were an average of twenty measurements.

The color values of couscous samples prepared for each time (0, 4, 8, 12, and 16 min) were determined by measuring the CIE L* (100 = white; 0 = black), a* (+, red; -, green) and b* (+, yellow; -, blue) values using a Chroma Meter (Konica Minolta, CR-400, Japan) calibrated with a white ceramic as reference. An average of twenty measurements were used for the color parameters of the samples. Then, the hue angle (h, equation 2), chroma or saturation index (C, equation 3), and total color change ($\Delta E$, equation 4) were calculated as

$$h = tan^{-1}(\frac{b^*}{a^*}) \quad (2)$$

$$C = \sqrt{(a^*)^2 + (b^*)^2} \quad (3)$$

$$\Delta E = \sqrt{(L_0^* - L_t^*)^2 + (a_0^* - a_t^*)^2 + (b_0^* - b_t^*)^2} \quad (4)$$

herein, $L_0^*$, $a_0^*$, and $b_0^*$ represent the color values of dried couscous, and $L_t^*$, $a_t^*$, and $b_t^*$ represent the color values of cooked couscous at a specific cooking time.

**2.9 Data Analysis**

Results were indicated as the mean and standard deviation. The data were compared by using the SPSS (version 20.0, 2011, IBM, USA) software to perform the statistical analysis. The one-way analysis of variance (ANOVA) using Duncan`s multiple range test and student`s t-test were separately used to display the differences in samples cooked with OH for different cooking times (0, 4, 8, 12, and 16 min) and samples cooked with OH and conventional heating method for a constant cooking time (12 min), respectively. Statistical significances were demonstrated at the $P<0.05$ levels.

## 3. Results and Discussion

**3.1. Analysis of Color Parameters**

Color is one of the crucial outward aspects of the food matrix because it not only affects consumer acceptability but also it is used for process controlling (Sadika Tuly et al., 2021). The cooking process influences the degree of color formation due to the caramelization reactions progress (Yüksel et al., 2018). It was reported that the higher lightness (L*) and yellowness (b*) values are more desirable for couscous, bulgur, pasta, and semolina products due to consumer acceptance (Yüksel et al., 2017). The color values of the couscous treated with the ohmic heating are presented in Table 1. The L*-value (60.20±1.43) of the samples cooked with conventional heat treatment for 12 min was not different from that for the samples cooked with OH for 16 min and 12 min ($P>0.05$), but it was significantly different from that for the samples cooked with OH for 0, 4, and 8 min. As expected, the lowest L*-value was observed for the samples cooked for 0 min, which is significantly different from this value for 8 min. In addition, the L*-value of the samples cooked for 4 min was not significantly different from that for 0, and 8 min. In addition, the lightness value of the couscous samples treated with OH for 16 min increased from an initial value of 57.68±0.81 to a value of 60.49±1.40 during the cooking process (Table 1). This increase in lightness may be due to water absorption. Wang et al. (2010) reported that the changes in the lightness value of potato starch noodles were related to the water content. Similarly, Hatcher et al. (1999) found that the amount of water absorbed by noodles affected their lightness value.

The highest b-value was found for the couscous samples cooked with OH for 0 min ($P<0.05$). This was followed by 4 min, 8 min, and 12 min ($P<0.05$). The lowest b-value was measured for the samples cooked for 16 min ($P<0.05$), which was very close to the b-value (15.41±0.33) obtained from the samples treated with conventional heat treatment ($P>0.05$). In addition, the increase in the cooking time up to 12 min caused significant changes in the a-values ($P<0.05$) while the a-values of couscous samples cooked for 12 min and 16 min were not different from each other ($P>0.05$).

**Table 1.** The color parameters, including L*, a*, b*, C (Chrome), ΔE (Total color change), and BI (Browning index) of couscous cooked with the ohmic heating (OH) at different cooking times

| Heat treatment | The cooking time (min) | L | a | b | C | BI | ΔE |
|---|---|---|---|---|---|---|---|
| Ohmic heating | 0.00 | 57.68[a] (0.81)* | -2.09[a] (0.23)* | 21.22[a] (1.41)* | 21.32[a] (1.38)* | 41.70[a] (3.96)* | 9.05[a] (1.15)* |
| | 4.00 | 57.94[a, b] (1.03)* | -2.51[b] (0.14)* | 18.81[b] (0.74)* | 18.97[b] (0.74)* | 34.79[b] (1.92)* | 10.69[b] (0.84)* |
| | 8.00 | 58.45[b] (0.89)* | -2.73[c] (0.13)* | 17.32[c] (0.45)* | 17.53[c] (0.43)* | 30.59[c] (1.35)* | 11.65[c] (0.59)* |
| | 12.00 | 59.83[c] (1.04)* | -3.00[d,e] (0.14)* | 16.64[d] (0.37)* | 16.91[d] (0.38)* | 27.84[d] (0.78)* | 11.72[c] (0.60)* |
| | 16.00 | 60.49[c] (1.40)* | -2.98[d] (0.20)* | 15.96[e] (0.36)* | 16.23[e] (0.38)* | 26.04[e] (0.96)* | 12.14[c,d] (0.63)* |
| Conventional heating | 12.00 | 60.20[c] (1.47) | -3.12[e] (0.15) | 15.41[e] (0.34) | 15.72[e] (0.36) | 24.82[e] (0.39) | 12.76[d] (0.76) |

* Standard deviation, [a, b]= the values in a column with the same lowercase letter are not significantly different (P>0.05).

Furthermore, there was no distinct difference in a-values among the samples cooked with conventional heat treatment (-3.12±0.15) and OH for 12 min, but the a-values of the samples treated with conventional heat treatment were significantly different from those of other samples cooked with OH for 0, 4, 8, and 16 min (P<0.05). According to these results obtained from the present study, yellowness (b*) and redness (a*) values of couscous samples reduced with increasing cooking time (Table 1). These results were in agreement with the results of Cocci et al. (2008) and Gull et al. (2015). This decrease in these values during cooking is probably because of the quantity of the carotenoid pigment and enzymatic reactions (Islas-Rubio et al., 2014). Yilmaz (2019) stated that total carotenoid content (TCC) was significantly reduced during bulgur production due to the strong effect of exposure to heat, light, and hydroperoxide.

As observed for b* and a* values, chrome (C) and browning index (BI) values decreased throughout the increase in cooking time (Table 1). The C and BI values of samples treated with OH reduced from an initial value (0 min) of 21.32±1.38 and 41.70±3.96 to a value of 16.2±0.38 and 26.04±0.96, respectively, at the end of the cooking (16 min). There were significant differences in the C and BI values among the samples cooked with OH for 0, 4, 8, 12, and 16 min. and the conventional heat treatment whereas no significant difference was observed between OH for 16 min and the conventional heat treatment. In addition, the total color difference (ΔE) increased when the cooking time was increased (Table 1). It was found that the ΔE values of samples treated with OH for 8, 12, and 16 min were not significantly different (P>0.05) from each other, but they were significantly different (P<0.05) from that for samples treated with OH for 0 and 4 min. Moreover, the ΔE value (12.76±0.54) of samples cooked with conventional heat treatment was like this value for the sample cooked with OH for 16 min. (P>0.05), but it was significantly different from those values for other samples cooked with OH for 0, 4, 8, and 12 min. Based on our results, the changes in *C*, *BI*, and ΔE values were directly related to moisture content (Lefkir et al., 2017).

**3.2 Analysis of Texture Profile Analysis (TPA)**

The texture profile analysis of the couscous cooked with ohmic heating at 17 V/cm is given in Table 2. The textural attributes including hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience of the couscous samples changed during the cooking process. The hardness, gumminess, and chewiness values of samples cooked with OH for 0 and 4 min were significantly different from each other and other samples cooked with OH for 8, 12, and 16 min and conventional heating (P<0.05) whereas there were no significant differences (P>0.05) between samples cooked with OH for 8, 12, and 16 min and conventional heating. This result may be due to the fact that the It was reported that the hardness of noodles was related to the compositions of the starch, gluten, and dietary fiber (Zou et al., 2021).

There were also significant differences (P<0.05) in adhesiveness values of samples treated with OH for 0, 4, and 8 min, but these values were not significantly different (P>0.05) among samples cooked with OH for 12 and 16 min and conventional heating. These results showed that the surface of the cooked couscous was not changed after the cooking time was equal to 12 min and over because the adhesiveness commonly attributed to the surface of the cooked couscous (Jittanit et al., 2017). Similarly, in a study, it was reported that the adhesiveness of spaghetti cooked with conventional heating for 15, 18, and 21 min was not significantly different (Sozer and Kaya, 2003). Furthermore, the springiness values of all cooked samples were not different (P>0.05) because springiness was not affected by the cooking time (Bello et al., 2006). Finally, the results of this study revealed that all the textural properties except the cohesiveness of samples cooked with OH for 12 and 16 min and conventional heat treatment were not significantly different (P>0.05).

**Table 2.** Texture profile analysis (TPA) parameters (H: Hardness; A: Adhesiveness; S: Springiness; C: Cohesiveness; G: Gumminess; CH: Chewiness; R: Resilience) of couscous cooked with the ohmic heating (OH) at different cooking times

| Heat Treatment | The cooking time (min) | H (g) | A (g.sec) | S (%) | C (%) | G | CH | R (%) |
|---|---|---|---|---|---|---|---|---|
| Ohmic heating | 0.00 | 30684$^{a}$ (5073)* | -1171$^{a}$ (320)* | 0.80$^{a}$ (0.08)* | 0.67$^{a}$ (0.03)* | 20652$^{a}$ (4199)* | 16724$^{a}$ (4451)* | 0.52$^{a}$ (0.05)* |
| | 4.00 | 14967$^{b}$ (2073)* | -871$^{b}$ (174)* | 0.58$^{b}$ (0.05)* | 0.55$^{b}$ (0.03)* | 8232$^{b}$ (1589)* | 4839$^{b}$ (1190)* | 0.32$^{b}$ (0.04)* |
| | 8.00 | 7866$^{c}$ (931)* | -376$^{c}$ (86)* | 0.54$^{b}$ (0.04)* | 0.48$^{c}$ (0.02)* | 3774$^{c}$ (560)* | 2037$^{c}$ (402)* | 0.21$^{c,d}$ (0.01)* |
| | 12.00 | 6220$^{c}$ (803)* | -156$^{d}$ (40)* | 0.54$^{b}$ (0.04)* | 0.47$^{c}$ (0.03)* | 2899$^{c}$ (459)* | 1561$^{c}$ (305)* | 0.21$^{c}$ (0.02)* |
| | 16.00 | 5035$^{c}$ (567)* | -96$^{d}$ (29)* | 0.51$^{b}$ (0.06)* | 0.44$^{d}$ (0.04)* | 2199$^{c}$ (166)* | 1126$^{c}$ (164)* | 0.21$^{c,d}$ (0.03)* |
| Conventional heating | 12.00 | 5135$^{c}$ (548)* | -117$^{d}$ (39.87)* | 0.51$^{b}$ (0.03)* | 0.42$^{d}$ (0.01)* | 2139$^{c}$ (195)* | 1088$^{c}$ (138)* | 0.19$^{d}$ (0.01)* |

* Standard deviation, $^{a, b, c, d}$= the values in a column with the same lowercase letter are not significantly different (P>0.05).

**Table 3.** The moisture content (%), total soluble solid content (°brix), and weight increase (%) of couscous cooked with the ohmic heating at different cooking times

| Heat treatment | The cooking time (min) | TSSC (°brix) | Moisture content (%) | Weight increase (%) |
|---|---|---|---|---|
| Ohmic heating | 0.00 | 0.27$^{a}$ (0.06)* | 35.20$^{a}$ (2.66)* | 5.65$^{a}$ (1.03)* |
| | 4.00 | 0.60$^{b}$ (0.01)* | 47.73$^{b}$ (1.13)* | 10.45$^{b}$ (0.28)* |
| | 8.00 | 0.97$^{c}$ (0.06)* | 55.94$^{c}$ (1.47)* | 13.79$^{c}$ (0.67)* |
| | 12.00 | 1.30$^{d}$ (0.01)* | 59.86$^{d}$ (1.05)* | 16.14$^{d}$ (0.52)* |
| | 16.00 | 1.77$^{e}$ (0.06)* | 63.89$^{e}$ (1.04)* | 18.44$^{e}$ (0.46)* |
| Conventional heating | 12.00 | 1.20$^{d}$ (0.10)* | 64.42$^{e}$ (0.67)* | 19.23$^{e}$ (1.06)* |

* Standard deviation, $^{a, b, c, d, e}$= the values in a column with the same lowercase letter are not significantly different (P>0.05).

**3.3 The Cooking Loss**

This term was defined as the number of solids dissolving in the water throughout the cooking process and it can be used as an indicator of couscous structural integrity throughout the cooking process. The cooking loss is also important for consumer acceptance (Song et al., 2013). Hence, a low cooking loss is a sign of a high-quality product (Turgut et al., 2021). The total soluble solid content (TSSC) values passing into the boiling water during the different cooking times (4, 8, 12, and 16 min) were presented in Table 3.

In the present study, the highest cooking loss was measured for the couscous samples cooked with OH for 16 min (P<0.05). This was followed by the conventional heat treatment (12 min) and the OH for 12 min. The differences in cooking loss values of samples are because of the change in cooking times of couscous samples (Turgut et al., 2021). Likewise, Sobota et al. (2013) reported that the cooking loss of spaghetti increased with the cooking time.

**3.4 Moisture Content and Weight Increase**

During cooking, the amount of water absorbed by couscous is an important factor demonstrating the cooking quality of couscous as presented in Table 3. Since deficient water absorption may result in couscous with a hard and coarse texture and an overabundance of water absorption generally forms very soft and stick couscous. The highest water absorption was calculated for the couscous samples treated with conventional heat treatment (P<0.05). The quantity of water absorbed by the samples cooked with OH for 16 min and conventional heat treatments were not significantly different (P>0.05). This result was likely because these couscous samples were exposed to high temperatures for longer periods as seen in Table 3. It was reported that water absorption was related to the temperature of the cooking medium and degree of starch gelatinization (Cunningham et al., 2007). Turgut et al. (2021) pointed out that a relatively low-level gelatinization occurred for the samples exposed to temperatures lower than 70 °C.

The percentage in weight increase displayed a similar trend to the moisture content (Table 3). Although the highest cooking loss took place in the samples treated with OH for 16 min (P<0.05), the percentage of a weight

increase of the samples cooked with OH for 16 min and conventional heat treatments were not significantly different (P>0.05).

## 4. Conclusion

In addition, the color and textural properties of couscous samples were not significantly different from the conventional heat treatment if a suitable cooking time was selected for OH at 17 V/cm as found in the present study. However, the total soluble solids content (TSSC), remaining in the boiling water after the cooking process was done, had a linear relation with the cooking time when the ohmic heating process was used. Therefore, the cooking time should be reduced by increasing the voltage gradient as reported in our previous study (Goksu et al., 2022). Thus, it is needed to find an appropriate voltage gradient for the OH treatment to reduce the TSSC in the boiling water during the cooking process of dried couscous before consumption.

**Author Contributions**

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

| | B.O. | A.G. | E.I. | S.S. |
|---|---|---|---|---|
| C | 30 | 30 | 10 | 30 |
| D | 25 | 25 | 25 | 25 |
| S | | | | 100 |
| DCP | 25 | 25 | 25 | 25 |
| DAI | 25 | 25 | 25 | 25 |
| L | 30 | 30 | 20 | 20 |
| W | 30 | 20 | 20 | 30 |
| CR | 25 | 25 | 25 | 25 |
| SR | 25 | 25 | 25 | 25 |
| PM | 25 | 25 | 25 | 25 |
| FA | 25 | 25 | 25 | 25 |

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

**Conflict of Interest**

The authors declared that there is no conflict of interest.

**Ethical Consideration**

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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# PHENOLOGICAL AND POMOLOGICAL CHARACTERISTICS OF *Rosa canina* L. SPECIES CULTIVATED AND NATURALLY DISTRIBUTED IN AMASYA PROVINCE

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**Abstract:** The aim of this study was to investigate the phenological and pomological characteristics of *R. canina* species cultivated and naturally distributed in Amasya province. *Rosa canina* is one of the important plants distributed worldwide and used in food, medicine, raw materials, and landscaping. The research was carried out in 2022 using cultivated and naturally growing rosehip plants and their fruits in Suluova (Bayırlı Village and Yüzbeyi Village) and Taşova (Esençay Village and Kırkharman Village) districts of Amasya province. The phenological characteristics of the species were observed between April and December. The height (cm), average crown width (cm), and number of branches were measured from north-south and east-west directions of the natural and cultivated rosehip species; the number of fruits and fruit weight of the fruits sampled from 10 individuals were measured by weighing them on a precision balance. The thorniness of the natural and cultivated rosehip plants selected in each location in the study was scored as Very; 3, Moderate; 7, Less; 10. According to the findings, the most significant difference between naturally grown rosehip and cultivated rosehip plants in terms of phenological periods is the ripening time of the fruits. Plant height varied between 180.10±11.94 - 78.00±8.45 in cultivated *R. canina* species and between 119.50±56.34 and 89.00±50.43 in wild species. Crown width ranged between 288.90±12.35 cm and 89.40±2.23 cm in cultivated individuals and between 146.10±7.38 cm and 123.20±7.30 cm in wild species. The number of branches was found between 14.70±4.39 and 6.20±1.22 in cultivated individuals and between 11.20±4.36 and 8.70±5.81 in wild individuals. The study, it was observed that the thorniness was low or moderate in all cultivated rosehip plants. Fruit weight, fruit diameter, and fruit length ranged between 2.29±0.18 and 1.97±0.28; 14.27±0.64 and 12.93±0.59; 20.27±0.80 and 20.10±1.42 in wild *R. canina* individuals, respectively. In cultivated *R. canina* species, fruit weight, fruit diameter, and fruit length ranged from 3.48±0.12 to 2.61±0.28; 17.21±0.85 to 15.53±0.97; 26.65±0.85 to 20.16±0.72, respectively. Fruit weight and fruit diameter of cultivated and wild rose hips were statistically different in different locations.

**Keywords:** *Rosa canina* L., Phenology, Fruit characteristics, Culture, Wild, Amasya

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## 1. Introduction

Türkiye, which is rich in biodiversity, is among the leading countries in the world in terms of accessibility to medicinal and aromatic plants that can cure diseases. One of these plants is *Rosa canina* species. It is known that rosehip plant, which is an important medical and economic plant, has been used by many civilizations for thousands of years. Rosehip has a wide distribution almost worldwide (Çelik, 2007). There are 27 *Rosa* spp. species in Türkiye. It also grows in provinces with different climate and soil characteristics, such as Erzincan, Erzurum, Bitlis, Van, and Hakkâri in the Western Black Sea region and Eastern Anatolia, where the harsh continental climate prevails (Güleryüz and Ercişli, 1996). Among rosehip species, *R. canina* is one of the most suitable species for processing in terms of its distribution and fruit characteristics (Anşin and Kılınç, 1996; Öz, 2016). The fruits of rosehip species are very important criteria in selection studies (Arslan et. al., 1996).

Although the local name of the species is commonly known as rosehips, it is also known by different names such as Yabangülü, Civil, Gül burnu, Gül elması, Şillan, Asker gülü, and Deligül. These plants are resistant to harsh environmental conditions (rocky and sloping terrain, poor soiland lack of water). It is a plant that can grow in a wide altitude range of 30-2500 m, especially on rocky slopes in forest openings. In our country, it grows both in cultivated form and in natural environment under different geographical conditions (Yılmaz and Ercişli, 2011; Öz, 2016; Tolekova et al., 2020). As a result of the expanding product range, it is seen that our low-income farmers can obtain an important source of income from rosehip cultivation (Encü, 2015).

When rosehip oil is analyzed, 97 different chemicals; organic acids, saturated fatty acids, natural sugars, phenolic substances, carotenoids, etc. are found (Nowak 2005; Ahmad and Anwar 2016). Rosehip is used as a

valuable raw material in the food and pharmaceutical industries in many countries. It is used in the production of baby foods, tea, jam, marmalade, rose water, rose oil, and fruit juice and in the enrichment of various fruit and vegetable juices in terms of vitamins (Güneş and Şen, 2001a; Uggla et al., 2003; Engin and Boz, 2019).

Thanks to the abundant amount of ascorbic acid it contains, rosehip significantly increases body resistance against flu, colds, colds, and febrile diseases (Güleryüz and Ercişli, 1996). It is known to be effective against diabetes and used as a strengthening agent. Its seeds are used due to its soothing effect (Baytop, 2004). It is also known that it is frequently used in the treatment of diabetes, kidney disorders, inflammation, cancer, heart diseases, colds, psoriasis, stomach pain, hypertension, ulcer and asthma diseases, and stomach and intestinal gases (User, 1967; Chrubasik et al., 2008; Ghazghazi et al., 2012; Oyedemi, et al., 2016).

Especially the recent studies reveal that it can inhibit the proliferation of cancer cells and can be used in cancer treatment (Cagle et al., 2012). In addition, Önal and Oruç (2012) reported in a study that it can be used in fabric dyeing and as a source of natural raw materials in the field of textiles.

Studies conducted in experimental animals stated that rosehip seeds can be added to the foods of dieting people because they reduce cholesterol and triglyceride levels (Öz, 2016). It has been reported that *R. canina* has a strong antimicrobial effect against certain microorganisms (Horváth et al., 2012; Rovná et al., 2020).

*R. canina* fruits have an important place among forest secondary products. Rosehip plant has a very important place in protecting and forming biodiversity. Thanks to its roots that go deep and spread to the surface and its wide crown, it both increases the soil's water retention capacity, prevents soil erosion and provides habitat for other plants and animals (Yılmaz, 1996). It is also important for wildlife to not shed its leaves until the fall. While its fruits and leaves provide food for some animals, its shrub form provides shelter for some animals. It is important to ensure that rose hips, which are so ecologically and economically valuable, spread to larger areas through planting and cultivation. It is also very important to spread the rosehip plant through planting and planting after breeding in order to produce better quality rosehip fruits (Karakaya, 2016).

The aim of this study was to investigate the phenological and pomological characteristics of *R. canina* species which are cultivated and naturally distributed in Amasya province.

## 2. Material and Methods

### 2.1. *Rosa canina* L. (Rosehip)

Rosehip plant, which belongs to the Rosaceae family, is included in the genus *Rosa*. The rosehip plant, which has an upright shrub or climbing form, is a perennial plant that can reach 3.5 m in height, its root goes quite deep, and its branches are densely structured. The stem and branches are curved back, and most species are thorny. The thorns are hook-shaped. The leaves are about 3 cm long; they consist of 5.7 leaflets and usually have saw-toothed margins. Leaves are egg-shaped or elliptical and usually bluish or dull green. Flowers are solitary or 2-15 of them together in an umbellate raceme and have an erselic flower structure (Çelik, 2007). Fruits of *R. canina* species are round or egg-shaped, 1-3 cm long, fleshy, bright red (Figure 1). Flowering time is in May-June and July. Sepals fall off after flowering. Fruits ripen in late summer or fall.

**Figure 1.** Flowers and fruit of the *R. canina* plant.

### 2.2. Characteristics of the Research Area and Collection of Samples

The research was conducted in 2022 in Suluova (Bayırlı Village and Yüzbeyi Village) and Taşova (Esençay Village and Kırkharman Village) districts of Amasya province using cultivated and naturally grown rosehip plants and fruits (Figure 2). Producers were interviewed for cultivated plants. Accordingly, it was determined that the seedlings of the cultivated plants were purchased and planted from Tokat province in March 2015 within the scope of a project of the Provincial Directorate of Agriculture. Ten individuals were randomly selected in the field, taking care to ensure that the cultivated plants were of the same age. As for the naturally grown rosehip plants, 10 individuals were randomly selected from the

wild rosehip plants located at a maximum distance of 50 m from the mentioned rosehip fields. Each sample was identified by the name of the village from which it was taken. In addition, naturally grown rosehip plants were referred to as 'wild' and cultivated rosehip plants were referred to as 'cultivated' (Figures 3 and 4).

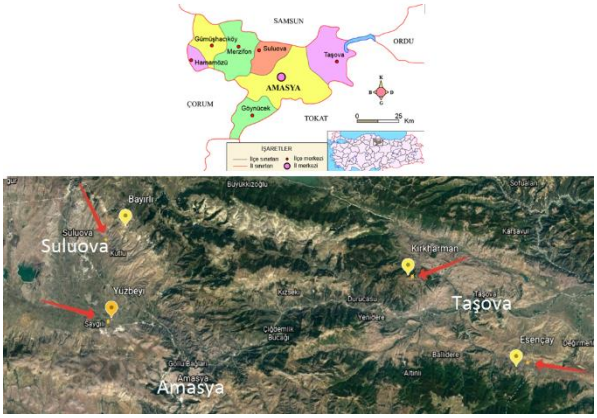


**Figure 2.** Map of the study areas (Map used from Google maps).

**Figure 3.** Rosehip plant cultivated in Bayırlı village and dried fruits.

**Figure 4.** Rosehip plant and fruits growing naturally in Kırkharman village.

### 2.3. Climatic Characteristics of the Research Area

Located in the interior of the Central Black Sea region, Amasya is in a transition zone between the continental climate and the Black Sea climate. This situation causes the transition climate between the two climates to prevail in Amasya. While winters are not as cold as the continental climate, they are not as mild as the Black Sea climate. Summers are not as rainy as the Black Sea climate and not as dry as the continental climate. Since 1937, the average annual precipitation in the provincial center was 436.7 mm, 458.3 mm in Suluova and 400.0 mm in Taşova. The annual average temperature is 13.6 °C and the annual average relative humidity is 61%.

In 2022, the highest average temperature in Suluova district, where Bayırlı and Yüzbeyi villages are located, was 29 °C in August. The lowest average temperature was 0 °C in February. When we look at the precipitation data, Suluova received the highest precipitation in April with 56 mm. The lowest precipitation was 5 mm in August. In May, when the rosehip plant is thought to need water the most, 46 mm of precipitation fell (Url1).

According to the precipitation and temperature values of Taşova district, where Kırkharman and Esençay villages are located, the highest average temperature was 29 °C in July and August. The lowest average temperature was 2 °C in January and February. When precipitation data is analyzed, the highest precipitation in 2022 was 52 mm in March and December, while the lowest precipitation was

5 mm in August. It was observed that 34 mm of precipitation fell in May, when the water demand is thought to be the highest (Url 2). The total amount of precipitation received by Suluova and Taşova districts in 2022 was measured as 411 mm and 438 mm, respectively.

**2.4. Phenological Observations**

The phenological characteristics of the species were observed monthly from April to December 2022. The necessary measurements were made with a ruler, and the observations and measurements were recorded in the observation table. Phenological development periods were determined according to Leon-Bertiller (1982). Phenological data were presented by giving maximum and minimum limits.

**2.5. Morphological and Pomological features**

The height (cm), average crown width (cm), and number of branches were measured from north-south and east-west directions of natural and cultivated rosehip species. Fruit weight was determined by measuring healthy fruits from 10 different plants on a precision balance and averaging them (Baloğlu and Bilir, 2020). In addition, fruit width was obtained by measuring the widest part of the fruit diameter, and fruit length was obtained by measuring the length between the top and bottom of the fruit (Sağır, 2010). Fruit length and fruit diameter measurements were made with calipers.

**2.6. Thorniness in rosehip plants**

*R. canina* is a thorny shrub. Thorniness is of great importance especially during rosehip harvesting. In the research, the thorniness of the natural and cultivated rosehip plants selected in each location was rated as Very; 3, Moderate; 7, Less; 10. The thorniness of the species was determined by examining other rosehip plants with the naked eye and comparing them (Güleryüz and Ercişli, 1996).

**2.7. Statistical Analysis**

SPSS 22 package program was used for statistical analysis of the data obtained. Tukey's multiple comparison test was used to determine the reasons for the differences between naturally grown and cultivated rosehip plants in different locations and One-Way Analysis of Variance (ANOVA) was used to evaluate the differences of both species.

## 3. Results and Discussion

**3.1. Phenological Findings**

Phenological observations of rosehip plants in the determined locations are given in Table 1.

In terms of phenological periods, the most prominent difference between naturally grown rose hips and cultivated rose hips is the ripening time of the fruits. While the fruits of wild wild rose hips ripen in September, the fruits of cultivated rose hips, especially in Suluova, ripen in early August. Another difference occurs at the end of the dormancy period and the opening of the first leaf. Again, in rosehip plants cultivated in Suluova, dormancy ends in April and the emergence of the first leaf, which is the first step of vegetative development, occurs. Apart from this, dormancy ends in May and the first leaf emergence occurs in May in natural rosehip plants in all locations examined and in cultivated rosehip plants cultivated in Taşova (Table 1).

**Table 1.** Phenological developmental stages of rosehip plants (Leon-Bertiller, 1982)

| Phase | Months | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Bayırlı Natural | Bayırlı Culture | Yüzbeyi Natural | Yüzbeyi Culture | Kırkharman Natural | Kırkharman Culture | Esençay Natural | Esençay Culture |
| Emergence of the first leaf | May | May | May | May | May | May | May | May |
| Early vegetative development | May | May | May | May | May | May | May | May |
| Intermediate vegetative development | May | May | May | May | May | May | May | May |
| Late vegetative development | May | May | May | May | May | May | May | May |
| Flower Period | | | | | | | | |
| Development of the flower axis | May | May | May | May | May | May | May | May |
| Bud growth | May | May | May | May | May | May | May | May |
| Ripening of flowers | June | June | June | June | June | June | June | June |
| Fruit Period | | | | | | | | |
| Fruits are green | July August | July | July August | July | July | July | July | July |
| Fruit ripening | September | August | September | August | September | September | September | September |
| Vegetative stagnation | November | November | November | November | November | November | November | November |
| Onset of Aging | November | November | November | November | November | November | November | November |
| Old age (defoliation) | December | December | December | December | December | December | December | December |
| Dormansi | January February March April | January February March | January February March April | January February March | January February March April | January February March April | January February March April | January February March April |

In other studies, it was reported that the flowering of *R. canina* lasted from late April to May and early June (Gyan and Woodel 1987; Türkben et al., 1999; Ekincialp, 2007). On the other hand, Kutbay and Kılınç (1996) reported that flowering time was between 5th-7th months. They reported that it was between 5th and 7th months. Dölek (2008) reported that the flowering period is in the 5th and 6th months, fruit ripening is in the 9th month, and the species sheds its leaves at the end of November. Fruit ripening is between August and September (Ekincialp, 2007). Different phenological data were obtained in the studies of other researchers (Kühn, 1992; Yılmaz, 1996; Güneş et al., 2017)

Although the phenological characteristics of *R. canina* species in the study area are in accordance with the findings of other researchers, the phenological characteristics of the species may vary according to climate, soil, and environmental conditions.

**3.2. Morphological and Pomological Findings**

Plant height varied between 180.10±11.94 -78.00±8.45c in cultivated *R. canina* species and between 119.50±56.34 and 89.00±50.43 in wild species (Table 2). It has been reported that the height of *R. canina* species varies between 2 and 3.5 m (Ercişli 2005; Demir and Özcan 2001; Javid et al., 2021; Dölek 2008).

Plant height is an important factor in increasing the plant's fruit yield. First of all, when we compare the naturally grown and cultivated rosehip plants, it is seen that there is a difference according to the localities. In Yüzbeyi and Bayırlı villages, cultivated rosehip plants were significantly superior to naturally grown rosehip plants. In Esençay and Kırkharman villages, on the other hand, the height of cultivated plants lagged behind naturally grown rosehip plants in terms of height (Table 2).

It is thought that this situation is due to the fact that the farmers producing rose hips in Esençay and Kırkharman do not pay the necessary attention to plant care. In the naturally grown rosehip plants, it is seen that Yüzbeyi and Bayırlı villages located within the Suluova district border are superior in terms of height. It is assumed that the reason for this situation may be due to the climatic differences of the two districts.

When we examined the plant height of rose hips according to natural and cultivated cultivation independent of localities, it was determined that cultivated rose hips plants were superior to naturally grown rose hips plants. While cultivated rosehip plants have the advantage of plant care such as watering the plant, meeting fertilizer needs, and controlling weeds, naturally grown rosehip plants lack these advantages. However, it was observed that cultivated rosehip plants do not have the same degree of plant care in every location.

Crown width was found between 288.90±12.35 cm and 89.40±2.23 cm in cultivated individuals and between 146.10±7.38 cm and 123.20±7.30 cm in wild species.

Crown widths of 3-year-old *R. canina* species distributed in Erzican were reported to be between 30-330 cm (Kızılcı, 2005), which is consistent with our study. Dölek (2008) reported crown width as minimum 1.2 m and maximum 3.9 m in his study conducted in Amasya. Crown width is the most important indicator of how wide the plant can spread. Fruit yield increases in plants that spread over a wide area. When we evaluated the crown width in terms of locations, it was determined that the widest crown width was found in rosehip plants cultivated in Yüzbeyi village (Table 2). Similarly, the crown width of cultivated rosehip plants grown in Bayırlı village, which is also located in the same district, ranked second. No significant difference was found between the crown lengths of naturally grown rosehip plants on the basis of locations.

The number of branches ranged from 14.70±4.39 to 6.20±1.22 in cultivated individuals and from 11.20±4.36 to 8.70±5.81 in wild individuals (Table 2).

When the number of branches of rosehip plants was examined, it was seen that the plants with the highest number of branches were cultivated rosehip plants in Bayırlı village. In other localities, rosehip plants cultivated in Yüzbeyi village ranked second.

**Table 2.** Plant height, crown width and number of branches of *R. canina* species according to localities and Tukey HSD Results (Cultivated and Natural values in the same column with different letters are different from each other at P<0.05 level)

| Locality | n | | Plant height (cm) | Plant height (cm) | Plant height (cm) | Plant height (cm) |
|---|---|---|---|---|---|---|
| Yüzbeyi | 10 | Wild | 104.50±67.28$^{a}$ | 134.40±67.65$^{a}$ | 8.70±5.81$^{a}$ | Very Spiny |
| Esençay | 10 | Wild | 90.40±43.79$^{a}$ | 123.20±73.02$^{a}$ | 10.10±4.88$^{a}$ | Very Spiny |
| Bayırlı | 10 | Wild | 119.50±56.34$^{a}$ | 146.10±73.87$^{a}$ | 11.20±4.36$^{a}$ | Very Spiny |
| Kırkharman | 10 | Wild | 89.00±50.43$^{a}$ | 128.90±86.63$^{a}$ | 10.00±5.73$^{a}$ | Very Spiny |
| Yüzbeyi | 10 | Culture | 154.30±9.32$^{b}$ | 288.90±123.59$^{a}$ | 11.00±5.20$^{a}$ | Less prickly |
| Esençay | 10 | Culture | 80.90±12.48$^{c}$ | 89.40±22.35$^{b}$ | 6.20±1.22$^{c}$ | Medium prickly |
| Bayırlı | 10 | Culture | 180.10±11.94$^{a}$ | 261.50±88.06$^{a}$ | 14.70±4.39$^{a}$ | Less prickly |
| Kırkharman | 10 | Culture | 78.00±8.45$^{c}$ | 97.20±22.83$^{b}$ | 8.00±2.16$^{b}$ | Medium prickly |

It is especially noteworthy that rosehip plants cultivated in Esençay have the lowest number of branches in the graph. In terms of the number of branches in rosehip plants, plant care comes to the forefront again. In the observations we have made, it is revealed that the cultivated rosehip plants in Bayırlı village, which are in the best condition in terms of plant care, show better development in every aspect, while the cultivated rosehip plants in Esençay village, which are very inadequate in terms of plant care, cannot show sufficient development. It is seen that there is no significant difference in terms of the number of branches in rosehip plants growing naturally in different localities. Baloğlu and Bilir (2020) found the average number of branches to be 17 in a study conducted in Burdur.

The thorniness of the plant is among the important factors in the breeding studies of rosehip species (Ercişli, 1996; Güneş and Şen, 2001b; Çelik, 2007; Akkuş, 2016). In the study, it was observed that the thorniness of all cultivated rosehip plants was "low" or "medium" (Table 2). Especially the rosehip plants cultivated in Suluova were found to have very "low" thorniness. Thorniness was noted as "very" in all samples examined in naturally grown rosehip plants. Low thorniness appears to be a great advantage that will facilitate the collection of rosehip plants, especially during harvesting. Yıldız " identified 4 types of rose hips (*R. canina*) species as less thorny, 4 types as medium thorny and 3 types as very thorny out of 11 types (Güneş et al., 2017). Akkuş (2016) recorded *R. canina* species as low, medium and very spiny. A study conducted in Hakkari province reported thorniness as very thorny in 11 genotypes, medium in 35 genotypes, and low in 4 genotypes (Ekincialp and Kazankaya 2012). In a study conducted in and around Bolu province, Özen (2013) found that half of the 9 different genotypes studied were less spiny.

In wild *R. canina* individuals, fruit weight, fruit diameter, and fruit length varied between 2.29±0.18 and 1.97±0.28; 14.27±0.64 and 12.93±0.59; 20.27±0.80 and 20.10±1.42, respectively. In cultivated *R. canina* species, fruit weight, fruit diameter, and fruit length ranged from 3.48±0.12 to 2.61±0.28; 17.21±0.85 to 15.53±0.97; 26.65±0.85 to 20.16±0.72, respectively. Fruit weight and fruit diameter of cultivated and wild rose hips were statistically different in different locations.

When Table 3 is examined, it is seen that the highest fruit weight is in the fruits of cultivated rosehip plants grown in Bayırlı village, but the fruit weights of cultivated rosehip plants in all locations are higher than the weight of naturally grown rosehip fruits. Producers' preference for improved rosehip plants while selecting the seedlings to be cultivated plays an important role in this situation. When the fruit diameter values were analyzed, it was observed that a situation similar to fruit weight emerged (Table 3). The diameters of cultivated rosehip fruits were superior to those of naturally growing rosehip plants in all localities. Among the cultivated rosehip fruits, the diameter of the rosehip fruits growing in Bayırlı village reached the highest level, again suggesting that the adequate plant care was carried out. When we evaluated *R. canina* species in terms of fruit length; the lengths of the fruits collected from cultivated rosehip plants in Yüzbeyi and Bayırlı villages were the highest (Table 3).

Based on these data, we can conclude that cultivated *R. canina* species have larger fruit weight, diameter, and length than wild species. This difference can be explained by cultivated plants growing in better conditions and having more nutrient resources.

Kazankaya et al. (2001) determined that the fruit diameter was between 10.80 mm-17.06 mm and the fruit length was between 17.86 mm-29.50 mm in their study on rosehip plants growing naturally in Adilcevaz. In a study conducted in Nizharadze (1971) found that the fruit length was 19.3 mm. This study also supports the values we determined especially in the natural environment. In a study conducted in Tokat, fruit weight was reported as 2.15-2.90 g (Güneş et al., 2017).

Again, Özen (2013) reported that the fruit lengths of rosehip genotypes taken in the study conducted in the central district of Bolu varied between 13.28 mm-25.37 mm and fruit diameter between 9.33 mm-15.88 mm, which is in parallel with the findings obtained in our study.

**Table 3.** Results of fruit weight, fruit diameter, fruit length, and multiple comparison test (Tukey HSD) of *R. canina* species according to localities (Wild and cultivated values in the same column with different letters are different from each other at P<0.05 level)

| Locality | n | | Fruit weight (g) | Fruit diameter (mm) | Fruit length (mm) |
|---|---|---|---|---|---|
| Yüzbeyi | 10 | Wild | 2.29±0.18$^{a}$ | 14.27±0.64$^{a}$ | 20.27±0.80$^{a}$ |
| Esençay | 10 | Wild | 2.05±0.08$^{b}$ | 13.28±0.52$^{b}$ | 20.16±0.72$^{a}$ |
| Bayırlı | 10 | Wild | 1.97±0.28$^{b}$ | 13.14±0.40$^{b}$ | 20.16±0.72$^{a}$ |
| Kırkharman | 10 | Wild | 1.99±0.15$^{b}$ | 12.93±0.59$^{b}$ | 20.10±1.42$^{a}$ |
| Yüzbeyi | 10 | Culture | 3.21±0.13$^{b}$ | 15.71±0.56$^{b}$ | 26.65±0.85$^{a}$ |
| Esençay | 10 | Culture | 2.61±0.28$^{c}$ | 16.34±1.18$^{a}$ | 20.16±0.72$^{a}$ |
| Bayırlı | 10 | Culture | 3.48±0.12$^{a}$ | 17.21±0.85$^{a}$ | 26.38±0.82$^{a}$ |
| Kırkharman | 10 | Culture | 2.77±0.22$^{c}$ | 15.53±0.97$^{b}$ | 23.28±1.70$^{b}$ |

**Table 4.** ANOVA test table of rosehip plants according to the locality where it grows and whether it is wild or cultivated (**P<0.01, *P<0.05)

| | Parameters | F Value | P Value |
|---|---|---|---|
| Locality | Plant height | 13.686 | 0.00** |
| | Crown length | 10.987 | 0.00** |
| | Number of branches | 4.321 | 0.01** |
| | Fruit weight | 24.860 | 0.00** |
| | Fruit diameter | 5.821 | 0.00** |
| | Fruit length | 34.097 | 0.00** |
| Wild* Culture | Plant height | 6.405 | 0.01** |
| | Crown length | 8.903 | 0.00** |
| | Number of branches | 0.001 | 0.98ÖD |
| | Fruit weight | 447.314 | 0.00** |
| | Fruit diameter | 271.739 | 0.00** |
| | Fruit length | 276.844 | 0.00** |
| Locality *Wild *culture | Plant height | 4.589 | 0.01** |
| | Crown length | 8.209 | 0.00** |
| | Number of branches | 3.020 | 0.04* |
| | Fruit weight | 20.612 | 0.00** |
| | Fruit diameter | 10.322 | 0.00** |
| | Fruit length | 27.037 | 0.00** |

In a study conducted in Siirt, fruit widths ranged between 10.08-15.63 mm and fruit lengths ranged between 15.00-24.55 mm in the samples taken in the first year, and fruit widths ranged between 10.12 mm-15.36 mm and fruit lengths ranged between 17.40 mm-25.29 mm in the samples taken in the second year (Yörük, 2006). In a study conducted in Ordu province, fruit weight was reported as 1.22-3.47 g, fruit width 10.2-16.9 mm, fruit length 13.2-25.2 mm (İpek and Balta 2020).

Although these results are in parallel with our findings, the lower limit of the given values is close to the values of rosehip fruits grown in the natural environment in our study, while the upper limit of the given values is close to the values of the fruits of cultivated rosehip plants in our study.

**3.3. ANOVA Results**

The following findings were found in the results of one-way analysis of variance (ANOVA) according to the localities where rosehip plants were grown. Differences in plant height, crown width and number of branches were significant according to the localities. In rosehip fruits, differences in fruit weight, fruit diameter and fruit length were significant (Table 4).

In the one-way analysis of variance (ANOVA) results according to whether the rosehip plants were wild or cultivated, it was observed that the difference in plant height and crown width were significant, while the difference in the number of branches was not significant. In rosehip fruits, differences in fruit weight, fruit diameter, and fruit length were significant.

## 4. Conclusion

In this study we conducted in Suluova and Taşova districts of Amasya province; when we first compare the morphological characteristics of cultivated rosehip plants and naturally grown rosehip plants, it is seen that there is an advantage in favor of rosehip plants cultivated by producers especially in terms of the length of rosehip plants. As a result of a five-year development process, cultivated rosehip plants were found to be taller than naturally grown rosehip plants. A similar situation is also observed in crown length.

When compared in terms of fruit size, it was observed that the average fruit width of naturally grown rosehip plants was 10 mm and the average fruit length was 17 mm, while the average fruit width of cultivated rosehip plants was 16.5 mm and the average fruit length was 22.75 mm. In terms of fruit weight, it was determined that the fruit weights of cultivated rosehip plants were higher especially in Yüzbeyi and Bayırlı villages. Higher fruit weight will provide a great advantage for the producers, increasing the total annual yield.

Although the wild rosehip plant grows widely in our country and its nutritional value has been better understood especially in recent years, there has not been enough progress in cultivation and breeding studies. Although there is a high demand for rosehip seedlings from both public and private nurseries, it is insufficient to meet the need. In order to meet the demand for quality rosehip seedlings, it should be ensured to select and breed the best types from the wild rosehip population by selection and then to meet the demand by multiplying these types in nurseries (Güneş and Şen, 2001a).

Its aesthetic and economic value will make it possible to further increase the areas of use of rose hips. In addition, the establishment of a rosehip processing industry in the regions where it is produced intensively will further strengthen the economic value of rosehip. Today, rose hips are produced and cultivated by using rosehip seedlings produced by breeding through selection from

rosehip plants that grow naturally, albeit in limited areas, and there are small-scale industrial organizations that process the rosehip fruits produced (Koçan, 2010). Rosehip fruits are rich in many vitamins and minerals. It is one of the richest fruits especially in terms of vitamin C. Studies have concluded that 1 kg of rosehip fruit contains approximately 500 mg of vitamin C (Arslan et al., 1996). Due to these features, expanding the cultivated cultivation of rose hips is very important.

Our study is a rare study in terms of revealing the difference between naturally grown rose hips and cultivated rose hips. In similar studies to be conducted in the future, the soil structure, the amount of water needed and the irrigation period of rosehip can be investigated.

**Author Contributions**

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

| | F.D. | D.D.K. |
|---|---|---|
| C | 50 | 50 |
| D | 50 | 50 |
| S | 50 | 50 |
| DCP | 60 | 40 |
| DAI | 40 | 60 |
| L | 50 | 50 |
| W | 50 | 50 |
| CR | 50 | 50 |
| SR | | 100 |
| PM | 10 | 90 |

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management.

**Conflict of Interest**

The authors declared that there is no conflict of interest.

**Ethical Consideration**

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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# THE USABILITY OF EXTRACTS OF WALNUT GREEN OUTER SHELL FOR IN VITRO SURFACE STERILIZATION OF ROSEHIP (*Rosa canina* L.) PLANT

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**Abstract:** In this study, it was aimed to determine the efficiency ratios of extracts obtained from walnut outer green shell at different concentrations in order to provide surface sterilization in in vitro propagation of rosehip plant. Axillary buds taken in June were used as explants. After surface sterilization of the explants with walnut outer shell extract, they were cultured in MS0 nutrient medium. The lowest contamination rate on the 5th and 7th days was observed in the control and 20K groups. In addition, the highest number of shoots per explant was obtained in the 20K group. Uncontaminated explants were subcultured to MS0 medium containing 1.0 mg /L IBA + 1.0 mg /L BAP and 1.0 mg/L BAP + 1.0 mg/L NAA at the end of the 7th day. Rooting was not observed in the explants due to browning. In the in vitro propagation of plants, extract (20K) obtained from oven-dried walnut shell can be used instead of chemical sterilant.

**Keywords:** *Rosa canina* L., Walnut shell extract, Tissue culture, Antimicrobial activity, Antifungal activity

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## 1. Introduction

*Rosa canina L*. (Rosaceae) is a wild-growing shrub that is used not only for its nutritive and therapeutic properties (Wegg and Townsley, 1983; Kazaz et al., 2009) but also as a rootstock for garden roses (Khosh-Khui and Sink, 1982; Balaj and Zogaj, 2011). *Rosa canina L.*can be propagated by seeds although the germination percentage is low, but vegetative propagation is a better method to preserve the desired traits and propagate selected wild genotypes. (Pawłowski et al., 2020). Many types of roses are difficult to root, traditional propagation methods are too slow, time consuming and tiring. Tissue culture is becoming traditional more important as an alternative to plant propagation methods (Roberts and Schum, 2003). Compared to traditional plant propagation methods, micropropagation has advantages such as propagation of elite clones, production of large numbers of plants from the selected genotype, production of pathogen-free plants, and propagation of plants throughout the year (Kavand et al., 2011; Pahnekolayi et al., 2014). Although clonal micropropagation of roses is common, the developed protocols are not universally applicable to all species within the *Rosa L*. genus due to high heterozygosity and polyploidy. Consequently, separate micropropagation techniques need to be developed for each genotype (Bhat, 1992; Pati et al., 2006; Canli and Kazaz, 2009; Shirdel et al., 2013).

In vitro protocols offer a way to shorten growth cycles, and plant propagation from axillary buds has proven to be a widely applicable method that ensures high growth rates and maintains clonal fidelity (Ngezahayo and Liu, 2014). The initial stage of micropropagation is influenced by various parameters, and it has been found that buds from softwood trunks are more sensitive than those from hardwood (Mederos and Enriquez, 1987). Significant differences in shoot proliferation rates have been observed in different *R. hybrida* cultivars, depending on the position of the node on the stem (Bressan et al., 1982). The performance of nodal segments is much better than the shoot tips (Horn, 1992). Cultivation of *Rosa canina L*. by non-traditional techniques offers many advantages resulting from its opportunities to improve, protect and maintain a plant of food and pharmaceutical value (Van der Mark et al., 1990). It is critical to prevent microbial contamination of plant tissue cultures or for successful micropropagation. Epiphytic and endophytic organisms can cause severe losses in micropropagated plants at all stages of growth (Cassells, 1991). The expected contamination rate is generally very low when using young plants or seedlings grown in a greenhouse. However, during the propagation of selected wild genotypes, the contamination rate is expected to increase as well as the presence of pathogens, and care must be taken during the sterilization phase (Marković et al.,

2021). Causes of contamination in plant tissue cultures include acute contamination caused by ineffective superficial sterilization and contamination caused by microorganisms hidden in the explant or by microorganisms established during subculture. In addition, chronic contamination can occur naturally after a long sterile culture period (Babaoğlu et al., 2002). Walnut shell were found 13 different phenolic compounds such as juglone, chlorogenic acid, caffeic acid and gallic acid. The phenolic compound is found in all parts of the juglone walnut and is known for its antimicrobial effect (Stampar et al., 2006). It is also one of the oldest examples of allelopathy with its inhibitory effect on walnut plant species. The chemical responsible for walnut allelopathy is juglone (5-hydroxy-1,4 naphthoquinone) and its inhibitory effect on plant species is one of the earliest examples of allelopathy (Davis, 1928; Rice, 1984). Juglone has been isolated from various plants within the walnut family (*Juglandaceae*), including *J. nigra* and *J. regia* (Daglish, 1950; Prataviera et al., 1983). Walnut leaf extracts are used in allelopathic research due to their juglone content and antimicrobial activity (Clark et al., 1990, Dama et al., 1998, Tan et al., 2012). The antioxidant potential of the aqueous extracts of 6 walnut cultivars grown in Portugal (Franquette, Lara, Marbot, Mayette, Mellanaise and Parisienne) was determined. It was determined that they have a concentration-dependent antioxidant capacity, the lowest in the Parisienne variety (Pereira et al., 2008). In addition, antimicrobial capacities against gram positive *(Bacillus cereus, Bacillus subtilis, Staphylococcus aureus*) and gram negative bacteria (*Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae*) and fungi (*Candida albicans, Cryptococcus neoformans*) were determined. In addition to the antimicrobial activities of all extracts, it was revealed that the effect of each walnut variety extract was different according to the tested microorganism (Pereira et al., 2008). In our study aims to determine the antimicrobial and antifungal efficacy of the walnut's green outer shell extract against contaminants that develop in tissue culture media, as well as to evaluate its regeneration capability. While there are numerous studies assessing the antimicrobial and antifungal effects of the walnut's outer green shell, this study holds significance as it pioneers the use of natural sterilization materials in in vitro studies, thereby minimizing chemical usage.

## 2. Materials and Methods

### 2.1. Plant Material

Axillary bud explants of the rosehip plant (*Rosa canina L.*) were utilized for in vitro regeneration in our study. The explants were collected from plants growing in nature in June 2022. Walnut outer green shells were acquired during the 2021 harvest. Some of these shells were subjected to drying in an oven at 45°C, while others were sun-dried. Oven-dried walnut green outer shells were weighed 10 g (10K), 15 g (15K) and 20 g (20K) and put into separate containers. Sun-dried walnut green outer shells were weighed 10 g (10Y), 15 g (15Y) and 20 g (20Y) and put into separate containers. The samples was added 50 ml ethanol. The extracts were subjected to a 3-hour hot water bath at 60°C, and the particulates were subsequently filtered out using Whatman No. 4 filter paper. The ethanol used as the solvent was removed using a rotary evaporator, and the crude extracts were obtained.

### 2.2. Sterilization

Two different surface sterilization methods were applied. Control group surface sterilization method; All explants were washed under tap water and then treated with 70% ethanol within a sterile cabinet for 5 minutes. For surface sterilization of explants they were treated with a 3% NaOCl (sodium hypochlorite) solution for 10 minutes, and then the explants (15 explants) were rinsed three times with bi-distilled water. These explants, with excess water removed using sterile blotting paper, were cultured in MS0 medium with one explant per test tube (Figure 1).

**Figure 1.** Explant sterilization with walnut outer green shell extract and control group.

Herbal surface sterilization method; All explants were washed under tap water and then treated with 70 % ethanol within a sterile cabinet for 5 minutes. For surface sterilization of explants, 10µl of each of the solvents named 10K, 15K, 20K and 10Y, 15Y, 20Y was added separately to 100 ml of bidistilled water. Sterilization mixture at 6 different concentrations was obtained. 15 explants in the sterilization mixture of each concentration were sterilized at 10 minutes. Excess water on the explants was removed using sterile filter paper and the explants were then transferred in MS0 medium with one explant in each test tube.

### 2.3. Preparation of MS0 Culture Medium

1000 ml bidistilled water were added 4.4 g of MS (PhytoTech, M519), 6.5 g agar (Condalab), 30 g sucrose (Merc) and its pH was adjusted to 5.7. This mixture was sterilized in an autoclave at 121 °C for 21 minutes. Then, this mixture mixture was then divided into tubes, 30 ml for each tube.

### 2.4. Culture of Explants

Each of the explants, which were previously sterilized in mixtures of different concentrations were transferred to MS0 culture medium in tubes.

**2.5. Culture Conditions and Analysis**

The explants were maintained in a climate cabinet at 23±2 °C with a light intensity of 3500 lux under a 16-hour light and 8-hour dark photoperiod. Contamination rates of the explants were assessed on the 2th, 5th, and 7th days in all treatment groups. At the conclusion of the 7th day, explants from all treatments were subcultured into MS medium containing 1.0 mg/L BAP + 1.0 mg/L IBA and 1.0 mg/L BAP + 1.0 mg/L NAA. The obtained results were subjected to analysis of variance in the Minitab 12.0 program. The determination of differences between the means was used to Tukey's multiple comparison test (Genç and Soysal, 2018).

## 3. Results and Discussion

The contamination rate and the number of shoots per explant by sterilization methods are given in Table 1. In all treatments, no contamination was observed in the explants on the 2th day of culture. On the 5th and 7th days, the best results were in the 20K and control groups ($P<0.01$). The best results in terms of the number of shoots per explant were determined in the 20K group. Compared to the control group, it can be said that walnut outer green shell extracts, which are prepared by drying in the sun and in an oven, result in significant sterilization during the sterilization phase. Browning was observed in subcultured explants and rooting was not achieved.

It is thought that different contamination agents developed in the culture medium and contamination that may occur during culturing affect this rate. Explants without contamination at the end of the 7th day were subcultured to MS medium containing 1.0 mg/L IBA + 1.0 mg/L BAP and 1.0 mg/L NAA + 1.0 mg/L BAP. Browning was observed in subcultured explants and rooting was not achieved. According to Vijaya et al. (1991), BAP was reported as the most effective growth regulator in stimulating shoot proliferation. Due to the browning and contamination of the explants, an evaluation could not be made in terms of the effectiveness of the two different auxins used in our study. Zapata et al. (1999), reported that meristem have inductive properties, so it is not necessary to use growth regulators in the shoot medium. The shoot numbers per explant indicated in our study were obtained in MS0 medium. Ambros et al. (2016), in their study of *Rosa canina L*. axillary buds, in contrast to the treatment of $AgNO_3$ with $HgCl_2$ approximately 98.0% of axillary explants reported that not contamination and the explants retained 59.6% of viability. In our study, the number of shoots per explant caused a decrease in many treatment groups due to the increase in contamination. Also, Ambros et al. (2016), reported that *Rosa canina L*. axillary buds exhibited low viability due to a high level of browning in axillary meristems collected in April-May, and explants isolated from leaf axils in July-August did not undergo development. They reported that the best results were obtained in the late vegetation period in September-October. It can be said that the reason for the low number of shoots per explant obtained in our study is due to the fact that the explants were obtained from June.

**Table 1.** Percentage contamination rates and number of shoots per explant on the 2th, 5th and 7th days of explants in the treatment carried out in June

| Treatments | 2nd | 5th | 7th | Number of shoots per explant |
|---|---|---|---|---|
| 10 Y | 0 | 21.97b | 32.58b | 0,57b |
| 10K | 0 | 21.97b | 32.58b | 0,50c |
| 15 Y | 0 | 40.98d | 68.44c | 0,14d |
| 15K | 0 | 21.97b | 21.97a | 0,43c |
| 20Y | 0 | 32.58c | 40.98b | 0,10d |
| 20K | 0 | 0.00a | 21.97a | 0,86a |
| Control | 0 | 0.00a | 21.93a | 0,43c |

The difference between treatment in the same column is significant ($P<0.01$).

Khorrami et al. (2018), investigated the potential of walnut green shell in the production of silver nanoparticles. AgNPs have been reported to exhibit antibacterial activity against standard strains of both Gram-positive and Gram-negative bacteria. In our study, sterilization was treatment with walnut outer green shell extracts for surface sterilization in plant tissue culture. As a result, it has been observed that sun-dried walnut outer shell solvent and oven-dried walnut outer shell solvent can be applied as a sterilization protocol.

Adebomojo and AbdulRahaman (2020), used biosynthesized nanosilver for the surface sterilization of Ocimum seeds and tissues in their study and evaluated its effects on callus formation. They concluded that there was no adverse effect on explant viability and callus formation, and that it could be used as an antimicrobial agent in surface disinfection, thus expanding the limits of potential treatmentof biosynthesized nanosilver in tissue culture. In our study, surface sterilization was achieved with walnut outer green shell extracts in order to ensure surface sterilization in plant tissue culture.

At the same time, no negative effects on explant viability were observed. Kocaçalışkan and Terzi (2001), investigated the allelopathic effects of walnut (*Juglans regia L*.) juglone and leaf extracts on seed germination and seedling. Juglone and different proportions of diluted

and undiluted walnut leaf extract were used. They reported that there was a positive correlation between the effects of juglone and the extracts. Seed germination was less affected than root and shoot development in all species. Cosmulescu et al. (2011), determined the amount of juglone in the leaves and green shell of five walnut varieties. It was observed that juglone was dominant in the green shell. They reported that the outer green shell and leaves of the walnut represent the most important source of walnut phenolics. In our study, no negative effects of extracts on shoot regeneration were observed. In addition, it was observed that the contamination rate was not high in the explants in the treatment group called 20 K and the number of shoots per explant was higher. The number of shoots per explant increased and decreased as a result of contamination and browning.

## 4. Conclusion

Contamination is a major problem in in vitro propagation of plants. Because contamination adversely affects the development of the plant in the culture medium and causes its death. The prevent contamination in the culture medium is used to more chemical agents. These chemical agents in some cases may adversely affect the development of the plant in the culture medium. In order to minimize these negative effects are researches carried out for the determination of herbal-based sterilization agents. In this study, walnut green outer shell was used as a sterilization agent in the in vitro propagation of the rosehip plant. The results revealed that walnut green outer shell can be used instead of chemical agents in sterilization.

**Author Contributions**

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

| | E.Ş. | B.A. | A.K. |
|---|---|---|---|
| C | 70 | 20 | 10 |
| D | 80 | 10 | 10 |
| S | 30 | 40 | 30 |
| DCP | 90 | 10 | |
| DAI | 100 | | |
| L | 80 | 20 | |
| W | 80 | 10 | 10 |
| CR | 80 | 10 | 10 |
| SR | 80 | 10 | 10 |
| PM | 70 | 20 | 10 |
| FA | 80 | 10 | 10 |

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

**Conflict of Interest**

The authors declared that there is no conflict of interest.

**Ethical Consideration**

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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Research Article
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# EFFICIENCY OF DNA EXTRACTIONS METHODS FROM PIGEONS AND COCKATIELS FEATHERS

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**Abstract:** This study aimed to compare different DNA extraction methods to achieve higher amounts and purity levels from molted feathers of pigeons (*Columba livia* f. *domestica*) and cockatiels (*Nymphicus hollandicus*). We evaluated 226 animals consisting of 202 pigeons and 24 cockatiels for these purposes. We performed three commercially available DNA extraction kits to isolate DNA from the feather samples. These kits were compared regarding DNA yield and quality depending on the different applications made during the isolation. DNA concentration (ng/mL) and absorbance ratio (260/280) were measured using a Nanodrop spectrophotometer. Kruskal-Wallis test with the Dunn's post hoc comparison was performed for the statistical comparisons. The mean DNA concentration was the highest in isolation with the kit C. Among three commercial kits, statistically significant differences were observed concerning nucleic acid concentration (ng/µL) ($P<0.001$). Also, the best 260/280 nm ratio absorbance was obtained with the kit B, while the lowest purity was obtained from kit C. Moreover, the concentration and purity of DNA were detected as higher in cockatiels than in pigeons, and the significant differences were determined between birds based on spectrometric measurements ($P<0.001$). In conclusion, the reported findings in this study may be helpful for the DNA extraction from the feather samples collected non-invasively in the field for genetic analysis in birds.

**Keywords:** Feather, DNA extraction, Pigeon, Cockatiel, DNA quality, Spectrophotometer

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## 1. Introduction

In avian study, the accurate extraction of DNA is vital for a wide range of studies, including population genetics, phylogenetics, and conservation biology. Reliable DNA extraction methods enable researchers to obtain high-quality genetic material that can be used for various downstream applications. The choice of appropriate extraction protocols is particularly critical when comparing different bird species, as variations in feather structure and composition can affect the efficiency and quality of DNA extraction (Taberlet and Bouvet, 1991; Bello et al., 2001; Freedman et al., 2008; Adam, Scharff and Honarmand, 2014). Traditionally, obtaining genetic information required invasive methods, such as capturing birds and collecting blood or tissue samples. However, these methods can be stressful for birds and time-consuming for researchers. Non-invasive sampling methods have emerged as a promising alternative, allowing scientists to extract DNA without physically handling the birds (Ellegren, 1991; Sacchi et al., 2004; Horváth et al., 2005; Presti et al., 2013; Zemanova, 2021). Pigeons (*Columba livia*) and cockatiels (*Nymphicus hollandicus*) are two avian species commonly studied due to their diverse genetic backgrounds and population dynamics of bird species. However, there is a lack of standardized protocols for extracting DNA from feathers in these species. Hence, it is imperative to assess and contrast different DNA extraction techniques designed specifically for pigeons and cockatiels (Yılmaz and Boz., 2012; Grindol, 1998). Pigeons and cockatiels, in particular, have long been favored for their unique qualities. Pigeon breeding is an ancient practice that has been refined over generations. The domestic pigeon has been bred for different purposes for 6,000 years or more. More than 800 breeds have been described since it was domesticated. They are bred for their beauty in appearance, ability to fly and navigation, and meat. Pigeons can be grouped as diver, tumbler, reeler, spinner, fleet flyer, high flyer, mail, ornamental and passerine according to their breeding purposes (Yılmaz and Boz, 2012). By selecting pigeons with desired traits such as feather patterns or flight capabilities, breeders have been able to create a wide variety of pigeon breeds. Cockatiels, on the other hand, are beloved for their intelligence and ability to mimic human speech. Breeding parrots requires a deep understanding of their complex social structures and behavioral patterns. By pairing parrots with compatible personalities and ensuring optimal living conditions, breeders aim to produce healthy and well-adjusted offspring. This not only enhances the welfare of the birds but also contributes to the

conservation efforts of endangered parrot species (Grindol, 1998; Banaszewska et al., 2015).

Commercial DNA extraction kits provide reagents and spin column filters to isolate DNA from feather samples. Kits often use lysis buffers and Proteinase K to break down feather material, then DNA is bound to a silica membrane spin column and washed. Kits can be more expensive but convenient, avoiding toxic chemicals and providing high-quality, concentrated DNA. Kits designed specifically for isolating DNA from hair, feathers, or other keratinized materials tend to work best for feather samples (Şentürk et al., 2023).

Although the use of shed feathers is preferred because it is difficult to take blood and tissue samples in birds, the keratin structure of the feathers complicates the process. However, determining the practical method of DNA isolation is essential for the continuity of genetic analysis. In this context, this study aims to compare the effectiveness of different DNA isolation methods from the molted feathers of birds, including pigeons and cockatiels.

## 2. Materials and Methods

### 2.1. Sample Collection

In this study, naturally fallen feathers in a cage from the wing and tail parts of the birds were used, and they were placed in tubes with the help of ethanol-sterilized forceps for DNA analysis. Pigeon feathers typically contain an expected range of 10-30 nanograms of DNA per feather, while cockatiel feathers contain approximately 5-15 nanograms. Thus, at least 3-5 feathers per bird were used to obtain enough high-quality DNA for the designed study. Samples in each tube were labeled with the bird's identity, feather type, and collection date and stored at +4°C until DNA isolation. The critical point to note here is that the earlier the DNA is isolated, the better results can be obtained since the yield and quality of DNA may decrease over time.

### 2.2. DNA Extraction

Feather samples were obtained from individual breeders to isolate genomic DNA. Two hundred twenty-six birds consisting of 202 pigeons and 24 cockatiels were chosen randomly and used in this study. Under sterile conditions, feathers are placed on the petri dish to cut each sample. Before applying commercial isolation kits, the DNA-containing part of two different areas for feathers, which are the basal tip of the calamus and blood clot from the superior umbilicus (Figure 1), were cut with the help of a scalpel and divided into small pieces (Horváth et al., 2005). Three commercial DNA extraction kits were used to isolate DNA from feather samples, following each kit protocol based on manufacturer instructions.


**Figure 1.** Different sampling areas for feathers are shown: (A) barbs; (B) blood clot from the superior umbilicus; (C) calamus and basal tip of the calamus.

### 2.3. Statistical Analysis

Statistical analysis was performed by GraphPad Prism 9 (Graph-Pad Software, La Jolla, USA). The Anderson-Darling test was utilized to assess the normality of the data. Kruskal-Wallis with Dunn's post hoc multiple comparison tests were performed to determine differences between groups, with $P<0.05$ considered statistically significant.

## 3. Results and Discussion

The use of noninvasive techniques has increased greatly over the past decades as the development of molecular methods has facilitated the use of noninvasive tissues to sample genetic material from natural populations. DNA sexing in birds can be done on a variety of easily accessible non-invasive samples, such as feces, feathers, or buccal swabs. Due to the feather shafts' ability to shield DNA molecules from damaging factors like UV rays, hydrolysis, frequent freezing and thawing, and bacteria, molted feathers are potentially a valuable source of DNA. The relationship between the selected extraction kits with regard to nucleic acid concentration

was presented in Table 1 respectively. The mean DNA concentration was the highest in isolation with the kit C, followed by kit A and kit B results. The difference between these three kits was found to be statistically significant for the amount (Figure 2A) and purity (Figure 2B). Means, standard errors, minimum and maximum values for nucleic acid concentration (ng/µL) based on isolation with different commercial kits are presented in Table 1.

**Table 1.** Means, standard errors (SE), minimum (min) and maximum (max) values for nucleic acid concentration (ng/µL) based on the isolation with the different commercial kits

| Isolation kit | Mean±SE | CV | Min | Max |
|---|---|---|---|---|
| Kit A | 16.81± 3.33[b] | 201,30 | 1.00 | 331.80 |
| Kit B | 5,74±0.95[c] | 159,16 | 0.40 | 58.10 |
| Kit C | 24.01± 5.76[a] | 131,37 | 1.30 | 108.50 |

[a,b,c]Means with different superscripts are different (P<0.001). CV refers to coefficient of variation.

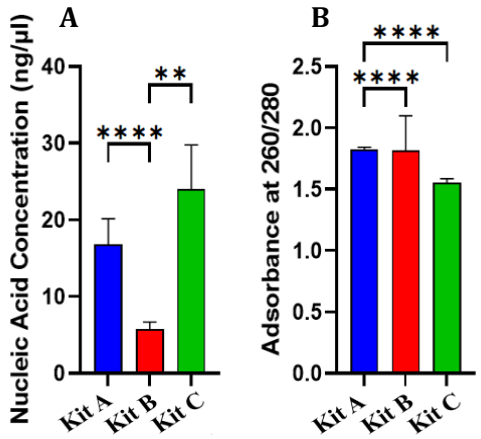


**Figure 2.** The comparison among three commercially available DNA isolation kits regarding amount and purity of the DNA samples. (A) The analysis on nucleic acid concentration, ng/µL. (B) The analysis on purity of the samples, absorbance at 260/280. The statistical analysis was performed using Kruskal Wallis with the Dunn's post hoc comparison. *P<0.05; **P<0.01; ***P<0.001; ****P<0.00001.

The ratio between the absorbance of the sample at the wavelength of 260 and 280 nm is used to assess DNA purity and integrity. A ratio of about 1.8 is generally accepted as "pure" for DNA. If the ratio is lower than 1.6, it may indicate the presence of phenol or other contaminants that absorb strongly at or near 280 nm. Higher ratios can indicate that DNA has contaminated isolated proteins (William et al., 1997). The impacts of the selected extraction kits, bird species, anatomic region, and incubation alteration on the absorbance ratio of 260/280 nm were presented in Tables 2, 3, 4, 5, 6, 7 and 8, respectively. In this study, significant differences in DNA purity were observed among the studied three kits (Figure 2B). The ideal 260/280 ratio value was obtained with the kit B, while the lowest value was obtained from kit C. On the other hand, DNA concentration was the highest in isolation with the kit C, followed by kit A and kit B results. The difference between these three kits was found to be statistically significant (P<0.001). Table 2 shows the means, standard errors, minimum and maximum values for the absorbance ratio of 260/280 nm based on the isolation with the different commercial kits.

**Table 2.** Means, standard errors (SE), minimum (min) and maximum (max) values for the absorbance ratio of 260/280 nm based on the isolation with the different commercial kits

| Isolation kit | Mean±SE | CV | Min | Max |
|---|---|---|---|---|
| Kit A | 1.82±0.02[b] | 11,62 | 1.24 | 2.32 |
| Kit B | 1.82±0.28[a] | 148,46 | 0.10 | 20.85 |
| Kit C | 1.55±0.03[c] | 12,09 | 1.14 | 1.93 |

[a,b,c]Means with different superscripts are different (P<0.001). CV refers to coefficient of variation.

**Table 3.** Means, standard errors (SE), minimum (min) and maximum (max) values for nucleic acid concentration (ng/µL) based on bird species

| Species | Mean±SE | CV | Min | Max |
|---|---|---|---|---|
| Pigeon | 11.04±1.20[b] | 152.61 | 0.40 | 108.50 |
| Cockatiel | 31.5 ±13.3[a] | 206.64 | 6.5 | 331.8 |

[a,b]Means with different superscripts are different (P<0.001). CV refers to coefficient of variation.

**Table 4.** Means, standard errors (SE), minimum (min) and maximum (max) values for the absorbance ratio of 260/280 nm based on bird species

| Species | Mean±SE | CV | Min | Max |
|---|---|---|---|---|
| Pigeon | 1.78±0.13[b] | 103.29 | 0.10 | 20.85 |
| Cockatiel | 1.85±0.02[a] | 6.23 | 1.49 | 2.000 |

[a,b]Means with different superscripts are different (P<0.001). CV refers to coefficient of variation.

**Table 5.** Means, standard errors (SE), minimum (min) and maximum (max) values for nucleic acid concentration (ng/µL) based on anatomic region

| Anatomic Region | Mean±SE | CV | Min | Max |
|---|---|---|---|---|
| Only the calamus | 12.34±2.04[b] | 219,68 | 0.4 | 331.80 |
| With barbs | 16,26±3.73[a] | 162,25 | 0.5 | 108.50 |

[a,b]Means with different superscripts are different (P<0.001). CV refers to coefficient of variation.

**Table 6.** Means, standard errors (SE), minimum (min) and maximum (max) values for the absorbance ratio of 260/280 nm based on anatomic region

| Anatomic Region | Mean±SE | CV | Min | Max |
|---|---|---|---|---|
| Only the calamus | 1.782±0.098 | 72,68 | 0.1 | 15.970 |
| With barbs | 1.795±0.396 | 155,98 | 0.1 | 20.850 |

CV refers to coefficient of variation.

**Table 7.** Means, standard errors (SE), minimum (min) and maximum (max) values for the nucleic acid concentration (ng/µL) based on incubation time

| Incubation Time | Mean | CV | Min | Max |
|---|---|---|---|---|
| 1 Hour | 13.64 ±1.9[c] | 39.69 | 5.30 | 22.50 |
| 2 Hour | 18.66 ±4.56[b] | 208.87 | 2.30 | 331.80 |
| 3 Hour | 10.48±1.74[d] | 182.47 | 0.40 | 108.50 |
| 4 Hour | 22.8±10.5[a] | 122.01 | 8.3 | 85.3 |
| Overnight | 5.11±1.29[e] | 104.04 | 1.40 | 22.30 |

[a,b,c,d,e]Means with different superscripts are different (P<0.001). CV refers to coefficient of variation.

**Table 8.** Means, standard errors (SE), minimum (min) and maximum (max) values for the absorbance ratio of 260/280 nm based on incubation time

| Incubation Time | Mean | CV | Min | Max |
|---|---|---|---|---|
| 1 Hour | 1.943±0.048[a] | 6.93 | 1.69 | 2.12 |
| 2 Hour | 1.791±0.019[e] | 9.15 | 1.37 | 2.20 |
| 3 Hour | 1.763±0.215[c] | 134.30 | 0.10 | 20.85 |
| 4 Hour | 1.879±0.067[b] | 9.75 | 1.51 | 2.10 |
| Overnight | 1.802±0.099[d] | 22.59 | 1.07 | 2.42 |

[a,b,c,d,e]Means with different superscripts are different (P<0.001). CV refers to coefficient of variation.

When studying large bird species, using feathers instead of blood as a source for genomic DNA reduces stress on the bird and makes sampling easier. In the study by Bello et al. in 2001, it was emphasized that lysis temperature and incubation times differ depending on feather size. In this study, regardless of the size of the cockatiel and pigeon feathers examined, the calamus parts were cut and the incubation time and lysis temperature appropriate to the procedures were applied. The study noted a substantial influence of cockatiel feathers being longer than pigeon feathers on concentration and purity (Figure 3A). In addition, when the 260/280 nm absorbance ratio was compared according to bird species, it was found that DNA purity was better in cockatiels than in pigeons (Figure 3B). The difference between birds was also statistically significant (P<0.001). Means, standard errors, coefficient of variation, minimum and maximum values for nucleic acid concentration (ng/µL) and absorbance ratio of 260/280 nm based on bird species are presented Table 3 and Table 4.

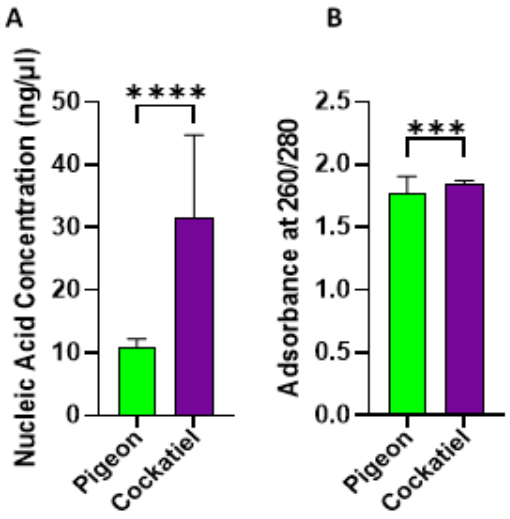


**Figure 3.** Comparison between the sampled species regarding amount and purity of the DNA samples. (A) The analysis on nucleic acid concentration, ng/µL. (B) The analysis on purity of the samples, absorbance at 260/280. The statistical analysis was performed using Mann-Whitney U test. **P<0.001; P<0.0001.

Several studies reported that the quality and quantity of DNA obtained from non-invasive samples can vary significantly, requiring optimization of extraction techniques and the development of standardized protocols (Avanus and Koenhemsi, 2018; Şentürk et al., 2023). In the study conducted by De Volo et al. in 2008, the effect of feather size on DNA yield was examined and as a result, it was stated that large feathers had higher DNA yield than small feathers, but no significant difference was observed between feather size and DNA amplification. In addition, it has been determined that the use of the superior umbilicus part of the bird feathers in addition to the calamus parts will increase the DNA concentration obtained by approximately two times. In this study, we observed that there is a significant relationship with DNA concentration when we included the superior umbilicus part of both cockatiel feathers and pigeon feathers, in addition to the calamus parts ($P<0.01$) (Figure 4A). However, no significant relationship was detected with the 260/280 nm absorbance ratio ($P>0.05$) (Figure 4B).

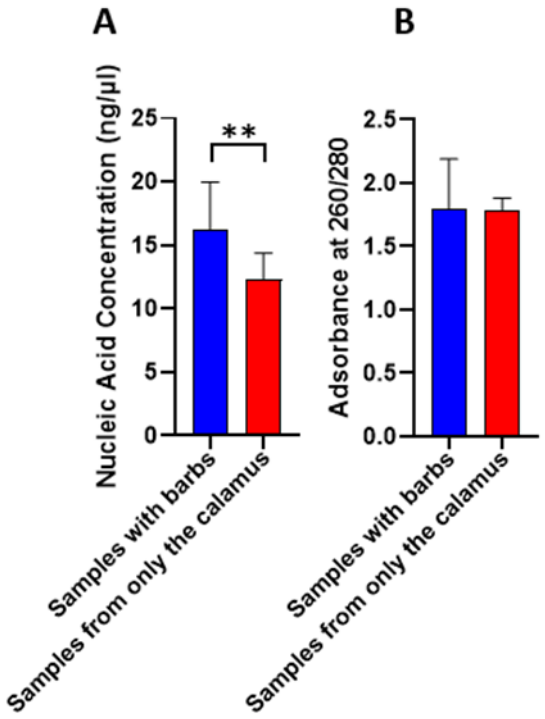


**Figure 4.** Comparison of structural features of sampled feathers regarding amount and purity of the DNA samples. (A) The analysis on nucleic acid concentration, ng/µL. (B) The analysis on purity of the samples, absorbance at 260/280. The statistical analysis was performed using Mann-Whitney U test. **P<0.01.

For nucleic acid content (ng/µL) and absorbance ratio of 260/280 nm based on anatomic region, means, standard errors, coefficients of variation, minimum and maximum values are shown in Tables 5 and 6. Furthermore, this study investigated the impact of variations in incubation times during the kit procedures on nucleic acid concentration and the 260/280 absorbance value. Means, standard errors, coefficient of variation, minimum and maximum values for the DNA concentration and absorbance ratio of 260/280 nm based on incubation time are investigated table 7 and table 8. In this context, significant results were observed for both DNA concentration and purity. The highest purified DNA amount was observed for 4h incubation (Figure 5A). The present results indicated that the most desirable 260/280 absorbance values were observed after a 2-hour incubation period (Figure 5B).

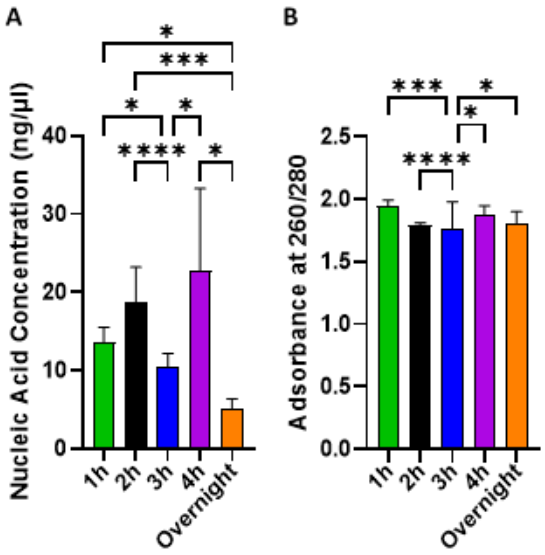


**Figure 5.** The comparison among various incubation applications regarding amount and purity of the DNA samples. (A) The analysis on nucleic acid concentration, ng/µL. (B) The analysis on purity of the samples, absorbance at 260/280. The statistical analysis was performed using Kruskal Wallis with the Dunn's post hoc comparison. *P<0.05; ***P<0.001; ****P<0.00001.

In DNA samples, particularly in routine studies, purity takes precedence over quantity. Hence, DNA isolation kits are more suitable for routine applications, where speedy results are desired. DNA purity is a critical factor regardless of the scenario, as samples with impurities outside the desired range pose challenges for subsequent processing and lead to lower success rates. Various factors, including the amount of proteinase K used in the procedure, the degree of agitation during incubation, and the temperature and duration of incubation, can influence the results. Therefore, it is essential to gather data on these factors to optimize the methodology. In this study, we found that a 2-hour incubation period yields the desired level of purity. It is worth noting that differences may arise due to individual practices, application specifics, and sample characteristics. The feather structures of birds, sourced from diverse regions and displaying distinct characteristics, can exhibit variations not only between species but also among individual birds (Figure 6). Nevertheless, this study can serve as a valuable reference for future research.

**Figure 6.** Various feather structures of birds from distinct body regions in the present study, each possessing its unique characteristics.

When comparing the results of this study with the commonly used standard phenol-chloroform isolation method, it becomes evident that although the standard method is cost-effective and provides higher genomic DNA yields, it is a complex and time-consuming process. Commercial DNA isolation kits are designed to provide a standardized and reproducible DNA isolation protocol and consistent results between experiments. This eliminates the need for manual optimization and reduces the possibility of human error (Silva et al., 2020; Şentürk et al., 2023; Sakyi et al., 2023). This study delves into the commonly employed methods and their adaptations for achieving the desired levels of DNA yield and purity during the isolation process from bird feathers. Methodological investigations hold significant value in genetic research, serving to optimize techniques and address challenges that may arise during their implementation, offering potential solutions. These findings are particularly invaluable for small-sized bird species prone to stress.

## 4. Conclusion

DNA isolation is the most essential step in genetic analysis. High quantity and quality DNA samples are indispensable for successful genetic analysis. Therefore, the determination of DNA isolation methods is of great importance. The structure of the tissue to be isolated also affects the quality of the isolated DNA sample. Some methods need to be modified in keratin-rich tissues, such as feathers. This study details the effects of three different methods and the effects of modifications on DNA quality and quantity based on commercially available DNA isolation kits. As a result, it may be thought that the results obtained will shed light on a wide range of studies in the fields of bird molecular genetics, population genetics, and phylogenetics.

**Author Contributions**

The percentage of the author contributions is presented below. The author reviewed and approved the final version of the manuscript.

| | Ö.Ç | N.Ş. | S.A. |
|---|---|---|---|
| C | 50 | | 50 |
| D | 50 | | 50 |
| S | 40 | 20 | 40 |
| DCP | 40 | 30 | 30 |
| DAI | 20 | 40 | 40 |
| L | 30 | 40 | 30 |
| W | 40 | 30 | 30 |
| CR | 30 | 30 | 40 |
| SR | 30 | 30 | 40 |

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision

**Conflict of Interest**

The author declared that there is no conflict of interest.

**Ethical Consideration**

Ethics committee approval was not required for this study because of there was no study on live animals or humans.

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# DETERMINANTS OF POULTRY EGG FARMERS' PARTICIPATION IN LIVESTOCK INSURANCE IN RIVERS STATE, NIGERIA

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**Abstract:** Poultry farming is exposed to several hazards caused by epidemic diseases, climate change and marketing among others which lead to losses of revenue. Yet, majority of poultry farmers do not neither shared nor transferred their hazards rightly. In the light of this, this study examined the determinants of poultry egg farmers' participation in livestock insurance in Rivers State, Nigeria. Primary data were obtained with the aid of questionnaire and interview schedules from 120 farmers drawn through multistage sampling procedure. Descriptive statistics and Probit regression model were used to analyze the data. Results shows the mean age of the poultry egg farmers to be 45.21 years, years spent in formal education mean of 14.87 years, mean household size of five (5), and stock size of 1721 birds were obtained. 60.8% were aware of livestock insurance and about 35% of the farmers insured their farms. Probit regression result shows that access to credit facilities, stock size and household size were statistically significant determining the poultry egg farmers' participation in livestock insurance scheme. Poor agricultural extension service delivery and delay in indemnity payment among others were constraints encountered in participating livestock insurance. The study recommends that extension agents in collaboration with the insurance company providers should educate poultry farmers on livestock insurance role in risk management. Also, insurance companies should endeavor to keep terms of contractual arrangements not to delay in indemnity payment to the farmers.

**Keywords:** Indemnity, Livestock insurance, Poultry farmers, Participation, Premium, Probit model

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## 1. Introduction

Livestock is made up of 8.89% agricultural sector in 2015 (NBS, 2016). The Nigeria's livestock population consists of 16.3 million cattle; 40.8 million goats; 27 million sheep; 3.7 million pigs and 151 million poultry (Nasiru et al., 2012), Nigeria's livestock population projected to be about 53.6 million cattle; 207.8 million goats; 78.2 million sheep; 21.1 million pigs and 1.3 billion poultry by 2050 (Sasu, 2022). Going by the former figure, poultry alone constitutes about 63.2% of the total livestock population in Nigeria, signifying the dominance of poultry sector in the livestock industry; since majority can practice it easily. Poultry can be described as birds of economic value to man which provide meat and eggs. Poultry production plays a vital role in rural livelihoods and food security is enormous. The poultry farming enterprise provides employment opportunity for the teaming population, thereby serving as a source of income to the people. Also, it provides a good source of animal protein in meat and eggs which have a high nutritional value (Nasiru et al., 2012). Egg is an excellent source of iron, zinc and vitamin A, all of which are essential for health, growth and well-being of people; egg is a complete protein with excellent quality (Tijani et al., 2006).

Poultry farming business are usually confronted with many risks and uncertainties; some are natural hazard like floods, drought, fire outbreak, diseases, pest attacks and theft among others. Since, the poultry egg farmers cannot foresee the possibility of occurrence of any of these hazards and cannot bear these risks and uncertainties alone, the farmer is faced with the choice of transferring or sharing the risks involved in poultry egg production. In a situation like this, insurance remains the only option to assist the farmers to go back to business. In general, insurance is a form of risk management used to hedge against a contingent loss. Therefore, poultry egg farmers underwrite livestock insurance in order to mitigate or assuage the bad effects of risks. Agricultural insurance is an economic constituent of farm management practices designed to lessen the adverse consequence of natural adversity on poultry egg farmers' incomes through the payment of indemnity (Ajieh, 2010). The National Agricultural Extension and Research Liaison Services (NAERLS) identified the following as the benefits of agricultural insurance to farmers: (i) it protects farmers against financial disaster after suffering any of the insured risks for which indemnity (compensation) is paid; (ii) it empowers the farmers to

obtain farm credit. Given that, insurance guarantees protection against crop and/or livestock failure, the insured farmer has greater confidence in obtaining loans; (iii) it facilitates better planning and project implementation since there is a high level assurance for continuity in business; (iv) it serves as an assurance to banks and other financial institutions who grant loan for agricultural purposes that loans given will be repaid; and (v) it build farmers confidence in using new technologies and making greater investments in agriculture according to NAERLS (1991) as cited in Akinrinola and Okunola (2014).

Despite the benefits accrue to insured farms; Nigerian farmers are reluctant about taking an insurance policy. This can be traced to the less than satisfactory image of the insurance industry regarding loss compensations and this problem has created mixed feelings towards Agricultural insurance by prospective farmers and hence, the farmers become reluctant in participating in an insurance cover and also considering the very low incomes, the small sizes of holdings aimed at subsistence production, large scale ignorance and poverty and the adverse view of other people's experiences with activities of insurance companies in other sectors, peasant poultry egg farmers are generally reluctant to patronize the insurance market, let alone paying a small amount in the form of premiums in exchange for their farm risks (Olubiyo et al., 2009). Hence, poultry industry in Nigeria continually suffered a great deal of losses, which has affected both poultry egg farmers and consumers.

Despite the existence of insurance services rendered by Nigerian Agricultural Insurance Corporation (NAIC) and other private insurance firms in Nigeria, there has been a low level of participation of farmers buying insurance premium. In view of this, there is need to examine the determinants of poultry egg farmers participation in livestock insurance in Rivers State Nigeria.

The specific objectives of the study are to:

a) Describe the socio-economic characteristics and management practices of the poultry egg farmers in the study area;
b) Determine the factors affecting poultry egg farmers participation in livestock insurance in the study area;
c) Identify the constraints in insuring poultry egg farms in the study area.

## 2. Material and Methods

The study was carried out in Rivers State Nigeria. The State lies between longitude 7°00'E and Latitude 5°70'N, and covers an area of 11,077km2. Rivers State shared boundary with Imo, Abia and Anambra States towards north side, Akwa/Ibom State to the east side, Bayelsa State to the west side, and Atlantic Ocean on the south side. The population of Rivers State was 5,198,716 according to the National Population Commission of 2006 (NBS, 2011). Livestock reared in the State include poultry, pig, goats and fish. The poultry bird reared includes laying bird for eggs, broilers, cockerels and turkeys.

Multistage sampling was used to select the poultry egg farmers for the study. In the first stage, simple random sampling was used to select six Local Government Areas (LGAs). Secondly, four communities in each of the six LGAs were randomly selected to give twenty-four. Thirdly, five poultry egg farmers were purposefully selected from each of the communities, making one hundred and twenty (120) poultry egg farmers that form the sample size for the study. Data were analyzed using descriptive statistics, and Probit Regression model. The above model is estimated using Probit Regression estimation procedure in Gun Regression, Econometrics and Time-series Library (GRETL).

### 2.1. Model Specification

Probit regression model was used to determine the factors affecting poultry egg farmers' participation in livestock insurance. The model was used by Dhanireddy (2010) and Adeyonu et al. (2016), the implicit of the model is expressed as:

$$y_i = \beta_0 + \sum_{i=1}^{N} \beta_i X_i + e_i \quad (1)$$

Probability expression as;

$$P(Y = 1/X_1, X_2, \dots X_n) = \varphi(\beta_0 + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_n X_n) \quad (2)$$

here, P= Probability of insured poultry egg farms, Y= 1 means yes and 0 otherwise, φ= Cumulative distribution function of the standard normal distribution, and β= Parameter estimates.

The explicit form model specified as:

$$y_i = \beta_0 + \sum_{i=1}^{10} \beta_i X_i + e_i \quad (3)$$

where; Y= Probability of insured poultry egg farms (1 means yes and 0 otherwise), $\beta_0$= constant, $\beta_1$ to $\beta_{10}$= coefficients of the parameter estimates, $X_1$ to $X_{10}$= explanatory variables.

The a priori expectations of the explanatory variables are presented in Table 1.

## 3. Results and Discussion

Result shows the maximum and minimum age of the poultry egg famers to be 65 years and 21 years respectively, with mean of 45.21 years Table 2. This implies that the farmers were strong enough to be engaged in poultry enterprise. This result agrees with Akintunde (2015), and Adeyonu et al., (2016) who obtained a mean of 45.5 years and 43.63 years respectively among poultry farmers' who participated in Insurance Scheme in Southwest and Oyo State Nigeria respectively. A mean of 14.87 years was obtained as the years spent to attain the level of education. This indicates that the poultry egg farmers were well educated in the study area; this could make the farmers to understand the benefit of insuring their poultry farms.

**Table 1.** The a priori expectations of the explanatory variables used in determine the factors affecting poultry egg farmers' participation in livestock insurance

| Key | Variables | Description | Measurement | Expected sign |
|---|---|---|---|---|
| $X_1$ | Age | Continuous | Years | - |
| $X_2$ | Gender | Categorical | 1 if male, 0 otherwise | +/- |
| $X_3$ | Marital status | Categorical | 1 if married, 0 otherwise | +/- |
| $X_4$ | Household size | Continuous | Number | +/- |
| $X_5$ | Farmer association | Categorical | 1 if yes, 0 otherwise | + |
| $X_6$ | Farming experience | Continuous | Years | +/- |
| $X_7$ | Stock size | Continuous | Number | + |
| $X_8$ | Extension contact | Categorical | 1 if yes, 0 otherwise | + |
| $X_9$ | Years of formal education | Continuous | Years | + |
| $X_{10}$ | Access to credit | Categorical | 1 if yes, 0 otherwise | + |

**Table 2.** Socioeconomic characteristics and management practices of the poultry egg farmers in the study area

| Variables | Mean | Std. Dev. | Min | Max |
|---|---|---|---|---|
| Age, years | 45.21 | 10.38 | 21 | 65 |
| Educational level | 14.87 | 3.8 | 2 | 18 |
| Household size | 5 | 1.72 | 1 | 9 |
| Farm distance to homestead (km) | 5.14 | 5.94 | 0 | 29.00 |
| Stock size (number) | 1721.00 | 1284.68 | 250.00 | 8000.00 |
| Amount pay on land rent/yr (₦) | 175862.07 | 72457.73 | 50,000.00 | 350,000.00 |
| Labour cost/month (₦) | 31,880.73 | 32,626.31 | 10,000.00 | 260,000.00 |

This result is similar to that of Adeyonu et al., (2016) who obtained a mean of 13.46 years spent schooling among poultry farmers' who were willing to participate in National Agricultural Insurance Scheme in Oyo State, Nigeria.

A mean of five (5) persons was obtained as household size. This implies that the farmers have moderate household size. This result is similar to Akintunde (2015) who obtained a mean of five (5) persons among poultry farmers in Southwest Nigeria. 5.14km was obtained as the farm distance to the homestead of the farmers. This implies that the farmers were closed to the farms. The stock size shows that most poultry egg farmers are small-scale to medium scale with mean of 1721 birds. This result agrees with Oduwaiye et al., (2017) who recorded a mean of 1320 birds among poultry farmers in Kwara State Nigeria. Mean of ₦175,862.07 and ₦31,880.73 was obtained for amount spent on rent/lease of poultry land and labour cost respectively.

Table 3 shows that majority (60.8%) of the poultry egg farmers were aware of livestock insurance, while about 35% actually insured their poultry farms. This indicates that majority of the farmers do not insure their farms; this could be as a result that most of the farms were small-scale poultry farms. This result is similar to Akintunde (2015) who recorded 59.6% farmers that were aware of livestock insurance, while only 11.9% insured their farms among poultry farmers in Southwest Nigeria.

Table 4 shows the premium paid per annul on insuring poultry egg farm in the study area. About 42.9% pay between ₦51,000.00 – ₦100,000.00 per annul, while large scale farms pay above ₦210,000.00 were 7.1%. This implies that poultry egg farmers pay premium per year according to the number of laying birds stocked.

**Table 3.** Awareness and participation of poultry egg farmers in livestock/poultry insurance

| Variables | n | % |
|---|---|---|
| Awareness of livestock insurance | | |
| Yes | 73 | 60.8 |
| No | 47 | 39.2 |
| Insured poultry farm | | |
| Yes | 42 | 35.0 |
| No | 78 | 65.0 |
| Total | 120 | 100.0 |

**Table 4.** Premiums pay per annual on poultry farms

| Amount (₦) | n | % |
|---|---|---|
| 50,000 and below | 6 | 14.3 |
| 50,001 – 100,000 | 18 | 42.9 |
| 100,001 – 150,000 | 9 | 21.4 |
| 150,001 – 200,000 | 6 | 14.3 |
| 200,001 and above | 3 | 7.1 |
| Total | 42 | 100.0 |

Table 5 shows the Probit regression results of determinants factors affecting poultry egg farmers' participation in livestock insurance in the study area. A McFadden R-squared value ranging from 0.2 to 0.4 indicates very good model fit. Hence, a McFadden R-squared of 0.351 was obtained, which indicates that the regression model is a good fit of the data. The Log likelihood value was significant which also, validated the fitness of the model with likelihood ratio Chi-square (10)

of 27.4047 which is statistically significant at 1% level (0.0022). Error is normally distributed test statistic Chi-square (2) of 2.1588 with p-value of 0.3398 was obtained; this shows that the test for normality of residual (Null hypothesis) error is normally distributed since the p-value is greater than the level of significant, with 91.7% cases correctly predicted in the model as well as 0.301 mean of independent variables captured in the model. This indicates that, overall, the regression model statistically significantly predicts the outcome variables. This implies that poultry egg farmers who transferred or shared their risks and uncertainties hazards with livestock insurance company is beneficial in getting indemnity in the study area. Almost all the variables included in the model satisfied the a priori expectation as presented in Table 5, however, access to credit facilities, stock size and household size were statistically significant variables as related to the determinants of poultry egg farmers' participation in livestock insurance scheme in the study area.

Table 5 shows that access to credit with coefficient of 1.0706 was statistically significant at 0.011% directly related with the probability of the poultry decision to share losses with livestock insurance. This implies that poultry egg farmers that have access to credit facility are more prone to participate in sharing their losses with livestock insurance companies than other farmers who do not have access to credit. This was observed from the response of the poultry egg farmers that insured their farms that accessing loans from banks and insurance companies is better simplified and expedited when the farmers have insurance certificate, hence, such farmers assented to livestock insurance scheme so as to increase in intensity to loans accessibility. This finding agrees with Dhanireddy (2010) and Adeyonu et al. (2016) who noted in their studied that access to credit facilities facilitate the decision of farmers to participate in the insurance scheme were directly positively related. However, it contradicted the report of Akintunde (2015) who showed no significant relationship.

Another important and strong determinant of poultry farmers' decision to participate in livestock insurance is poultry egg farmers' stock size. This stock size is positively correlated and statistically significant at 0.0 levels with poultry egg farmers' participation in the livestock insurance scheme. This implication of this is that poultry egg farmers who invested more or have high stock size in have a higher probability of insuring their poultry egg farms than other poultry farmers with lower stock size. This is logical and reasonable for the reason that, the poultry egg farmers that has invested huge financial resource will tend to insure the poultry farm to avert a condition whereby the entire investment both material and finance will go down the drain in case of any tragedy which is common with the poultry production business. Furthermore, generally the large scale poultry farmers do have access to credit facilities and the poultry farmers have to insure their poultry farms as one of the criteria of obtaining the loans from banks and other formal financial institutions. The finding agrees with report of Akintunde (2015) and Adeyonu et al. (2016) who reported that the level of stock size invested by the poultry farmers' is directly correlated with the farmers' decision to participate in the livestock insurance scheme.

Lastly, household size coefficient of −0.2630 was obtained which is negatively connected with the probability that the poultry egg farmers will participate in livestock insurance scheme at 0.05 level of significant. This indicates that poultry egg farmers who have less household size or the other are more likely not to participate in livestock insurance scheme in comparison with other poultry farmers who have large household size.

**Table 5.** Probit regression results of determinants factors affecting poultry egg farmers' participation in livestock insurance in the study area

| Variables | Coefficient | Std. Error | z | P-value |
|---|---|---|---|---|
| Constant | −1.1502 | 1.1504 | −0.9998 | 0.3174 |
| Age ($X_1$) | −0.0027 | 0.0273 | −0.0976 | 0.9222 |
| Gender ($X_2$) | −0.0308 | 0.4177 | −0.0738 | 0.9411 |
| Marital Status ($X_3$) | 0.1555 | 0.5194 | 0.2994 | 0.7646 |
| Household size ($X_4$) | −0.2630 | 0.1115 | −2.3590 | 0.0183** |
| Farmer association ($X_5$) | 0.3495 | 0.4883 | 0.7158 | 0.4741 |
| Farming experience ($X_6$) | 0.0422 | 0.0393 | 1.0730 | 0.2834 |
| Stock size ($X_7$) | 0.0003 | 0.0001 | 2.1000 | 0.0357** |
| Extension contact ($X_8$) | 0.4755 | 0.5247 | 0.9062 | 0.3648 |
| Years of formal education ($X_9$) | −0.0446 | 0.0358 | −1.2470 | 0.2123 |
| Access to credit ($X_{10}$) | 1.0706 | 0.4118 | 2.6000 | 0.0093*** |
| Mean dependent var | 0.1000 | S.D. dependent var | | 0.3013 |
| McFadden R-squared | 0.3513 | Adjusted R-squared | | 0.0693 |
| Log-likelihood | −25.3076 | Akaike criterion | | 72.6152 |
| Schwarz criterion | 103.2776 | Hannan-Quinn | | 85.0673 |

**Table 6.** Constraints of poultry egg farmers in insuring the poultry farms in the study area

| Constraints | SA | | A | | D | | SD | | DK | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variables | F | % | F | % | F | % | F | % | F | % | Mean | Rank |
| Poor agricultural extension service delivery | 35 | 29.2 | 19 | 15.8 | 29 | 24.2 | 31 | 25.8 | 6 | 5 | 2.6 | 1st |
| Illiteracy of the poultry farmer | 36 | 30 | 36 | 30 | 23 | 19.2 | 23 | 19.2 | 2 | 1.7 | 2.3 | 2nd |
| Inadequate government policies to empower poultry farmers | 46 | 38.3 | 52 | 43.3 | 8 | 6.7 | 3 | 2.5 | 11 | 9.2 | 2.0 | 3rd |
| Delay in indemnity payment | 37 | 30.8 | 63 | 52.5 | 8 | 6.7 | 7 | 5.8 | 5 | 4.2 | 2.0 | 3rd |
| Land ownership problem | 59 | 49.2 | 41 | 34.2 | 16 | 13.3 | 1 | 0.8 | 3 | 2.5 | 1.7 | 4th |
| High cost of poultry farmland | 72 | 60 | 37 | 30.8 | 7 | 5.8 | 2 | 1.7 | 2 | 1.7 | 1.5 | 5th |

SA= Strongly Agreed, A= agreed, D= disagreed, SD= strongly disagreed, DK= don't know, F= frequency, %= Percentage.

Table 6 shows the constraints encountered by the poultry egg farmers in participating in livestock insurance. Poor agricultural extension service delivery with mean of 2.6 ranks first. This implies that the poultry farmers in the study area do not have adequate access to extension agents. Illiteracy of the poultry farmers with mean of 2.3 ranked second, inadequate government policies to empower poultry farmers and delay in indemnity payment ranked third with mean of 2.0. This result is similar to the finding of Ajieh (2010) among poultry farmers' response to agricultural insurance in delta State Nigeria.

## 4. Conclusion

This study concludes that the poultry egg farmers were in their productive age, with more years in formal education, and majority of the farmers were aware of livestock insurance. Access to credit, stock size and household size were statistically significant determining the poultry egg farmers' participation in livestock insurance scheme. Poor agricultural extension service delivery, illiterate education of the poultry farmers and inadequate government policies to empower poultry farmers were constraints encountered in participating livestock insurance in the study area.

The study therefore, recommends that:

Extension agents in collaboration with the insurance company providers should create awareness and educate the livestock insurance company role in risk management should be incorporated in their package for outreach to poultry farmers especially.

Government should make livestock insurance more affordable to poultry egg farmers by increasing the present level of subsidy granted for agricultural insurance cover.

Insurance companies should endeavour to keep terms of contractual arrangements so as to delay in indemnity payment to the farmers allays the fears of the poultry egg farmers.

A special loan scheme for poultry farmers should be established by government to enable the farmers cope with the financial requirement involved in taking an agricultural insurance cover.

**Author Contributions**

The percentage of the author contributions is presented below. The author reviewed and approved the final version of the manuscript.

| | A.R.A. |
|---|---|
| C | 100 |
| D | 100 |
| S | 100 |
| DCP | 100 |
| DAI | 100 |
| L | 100 |
| W | 100 |
| CR | 100 |
| SR | 100 |
| PM | 100 |
| FA | 100 |

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

**Conflict of Interest**

The author declared that there is no conflict of interest.

**Ethical Consideration**

Permissions were obtained from the University of Port Harcourt Ethics committee (protocol code: 2022/23 and date: February 23, 2022).

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Research Article
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# GENOTYPIC EFFECTS OF B-CASEIN IN MILK COMPOSITION IN JERSEY COWS

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**Abstract:** The aim of this study was to investigate the relationship between the β-casein CSN2 genotypes (A1A1, A1A2, A2A2) and the biochemical characters and fatty acid composition of milk. Twenty-three milk samples from Jersey cows from the same herd from a farm in Hungary were studied. Animals were grouped according to β-casein genotype variants A1A1, A1A2 and A2A2. A1A1 milk had a significantly higher content of monounsaturated fatty acids (P<0.001) and a lower content of saturated fatty acids (<0.001). A2A2 milk had a higher content of polyunsaturated fatty acids (P<0.001) in milk. Moreover, the three varieties of milk show no significant difference for the composition of the polyunsaturated between CSN2 genotypes A1A1, A1A2 and A2A2. Also, no significant differences were observed in physicochemical composition of the milk. Accordingly, selective selection of genotypes with preferred qualities can improve milk and dairy products. In conclusion the fatty acid content the milk could be influenced by CSN2 genotypes A1A1, A1A2 and A2A2.

**Keywords:** A1 milk, A2 milk, Beta-casein, Fatty acid

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## 1. Introduction

The composition of milk from different mammal species and in particular bovine such as lipid protein, lactose, fatty acids, and nitrogen fractions, were influenced by several factors, including nutrition, age, stage of lactation, breed and genetic variation (Baer, 1991; Carroll et al., 2006). Several research confirms that bovine milk is a nutritionally valuable food. Indeed, caseins and whey milk proteins constitute more than 95% of all proteins (Ivanković et al., 2021). Caseins are one of the major proteins in cow's milk, which includes four forms: alpha s1 casein (39-46% of total caseins), alpha s2 casein (8-11%), beta-casein (25-35%) and kappa casein (8- 15%) (Roginski et al., 2003; Ivanković et al., 2021). Research was focused on the genetic polymorphism of proteins and their great interest, because of its relations with the, the physicochemical composition, the quality and other important traits. Milk casein is encoded by genes CSN1S1, CSN2, CSN1S2 and CSN3 located on chromosome 6 with 250 Kb of length (Ferretti et al., 1990; Ahmed et al., 2017). The Beta-casein protein is encoded by the CSN2 gene which is 8.5 kb in length, and contains five exons and eight introns (Bonsing et al., 1988). Beta-casein includes 12 known genetic variants (A1, A2, A3, B, C, D, E, F, G, H1, H2, I; (Farrel et al., 2004; Cui et al., 2012). The A1 and A2 variants represent the most common protein types of beta-casein which differ in the presence of the amino acid histidine (CAT) at position 67 in milk A1 and proline (CCT) in milk A2, due to a single nucleotide difference in the sequence of the exon VII bovine CSN2 gene at position 8101 (Bonsing et al., 1988). This genetic modification has a direct effect on the proteolytic digestion of the primary protein structure of the caseins, which allows the production of different peptides. Indeed, the enzymatic digestion of variants of β-casein A1 lead to the formation of the peptide B-casomorphin

which is characterized by significant opioid activity. This peptide can seep into the bloodstream more easily and cause various health problems, such as gastrointestinal disorders, insulin-dependent diabetes, atherosclerosis, ischemic heart disease and sudden infant death syndrome (Vougiouklaki et al., 2020).

Nowadays, the choices of the consumers have changed, they have become based not only on the nutritional aspects and organoleptic qualities of food, but also on products known to promote for a good health and prevent disease. In this regard, although milk represents an important element in the human diet all over the world, a new debate on the type of milk is followed by the heterozygous A1A2 genotype and the homozygous A2A2 and A1A1. Studies have shown that A2 milk has a beneficial effect on health than A1 milk, which can cause several diseases. Therefore, this milk is marketed as a healthier choice than A1 milk (Kumar et al., 2017)

Although the evaluation of the genetic variation of the genes encoding for the β-casein protein has been widely tackled by researchers. But, the study of the correlation between the biochemical composition of milk (DM, pH, proteins, fat, lactose, acidity) and the sensory traits (appearance, taste, odor and color of milk) and the CSN2 gene of β-casein has been rarely studied (Samoré et al., 2012). Also, studies that accurately correlate genetic polymorphism with milk fatty acid composition are limited (De Vitte et al., 2022).

This study aims to determine the link between milk protein genotypes (A1A1, A1A2 and A2A2) and biochemical traits of milk.

## 2. Materials and Methods

### 2.1. Sampling

The raw milk samples from Jersey cows collected from a local single farm in Hódmezővásárhely, Hungary, in order to see its physico-chemical characteristics for further investigation. Jersey dairy cow is selected as the study subject because they had the highest conception rates (59.6%) and higher percentages of cows pregnant in 75 d (78.1%) in Hungary (Washburn et al., 2002). All the cows at lactation times used in this study are from the same herd and were kept under the same housing and feeding conditions. The animals were classified according to the β-casein genotype variants A1A1, A1A2 and A2A2. 23 samples of 120 ml of milk from the previously chosen cows were collected in sterile polypropylene plastic bottle, then transported to the laboratory to be analyzes (Xiao et al., 2022). It is important to emphasize that the collection of samples from the Sampling stations fully comply with the recommended hygiene and asepsis rules in microbiology. Indeed, during the collection of cow's milk by breeders, washing and rinsing the teats of the udders of the cows with water mixed with the chlorine, followed by the elimination of the first milk jets, are carried out before each milking, therefore before the recovery of the sample (Mayer et al., 2021). For fatty acids analysis, the samples were frozen until use.

### 2.2. Physico-Chemical Properties

The physico-chemical properties of the milk were determined using the LactoScope FT-A infrared (FTIR) mid-infrared milk analyzer (model FT 400, Delta Dairy Analyzer, Budapest, Hungary). The concentrations of fat, protein, dry substance and lactose and also the density, in g/ml, were determined. The samples were stored at − 20 °C for further analysis (DiGiacomo et al., 2022).

### 2.3. Titrable Acidity

To measure Dornic acidity of milk we append a drop of alcoholic solution of 1% phenolphthalein as an indicator of the color change point in 1 mL of milk. 0.01 mL of sodium hydroxide (NaOH) were added, until the sample changed color from white to light pink and the color change was maintained. Titratable acidity was expressed in Dornic acidity (°D) as described by (Vázquez-Román et al., 2013).

### 2.4. Quantitative Analysis of Fatty Acids, Fatty Acids Extraction

The extraction of fatty acid was carried out following the conventional method of (De Jong and Badings, 1990) with a slight modification. Milk samples were homogenized by shaking with 10.75 mL of sulfuric acid (18%) and 1 mL of amyl alcohol. After centrifugation at 5000 rpm for 10 min, a volume of 50 µl of the supernatant was returned to a round bottom flask then mixed with 2 mL sodium methoxide at a concentration of 25%. The mixture was incubated for 40 min at 95°C in a water bath. Afterwards, 200 µl of methanolic sulfuric acid at a concentration of 3% was added until the coloring of the solution was observed. A second incubation of 5 min at 95 °C in water bath was performed. After liquid cooling, 4 mL of saturated NaCl and 1 mL of concentrated hexane was added. Samples were stored at −20 °C until analysis.

### 2.5. Capillary Gas Chromatography with Flame Ionization Detector: GC-FID

Before analysis, Samples were thawed at in room temperature, then filtered using 0.2 µm pore diameter (MiniSart Syringe Filter, Satorius, Goettingen, Germany), and a 1 µL filtered sample was subsequently kept in 2 mL GC vials for analysis step. Fatty acid content and profile were determined by gas chromatography (Nexis GC-2030, Shimadzu Scientific Instruments Inc., Kyoto, Japan) equipped with a polyethylene glycol column (ZB-WAX; 30 m × 0.25 mm inner diameter, 0.25 µm film thickness; Zebron, Phenomenex, CA, USA), and flame ionization detector. Helium was used as carrier and make-up gas. The run time per sample was 8.71 min. The oven temperature was programmed at 145 °C for 3 min and then increased from 145 °C to 245 °C at 16.6 °C/min. The injector and the flame ionization detector were maintained at 220 and 250 °C. The gas flows were 24, 32 and 200 ml/min for Helium. A standard curve was made using a mixture of volatile fatty acids from Sigma Aldrich (St. Louis, MO, USA) (Barnsteiner et al., 2011; Eisenstecken et al., 2021).

### 2.6. Statistical Analysis

All data were expressed as mean ± standard Error (SE). Variance analysis (one-way ANOVA) of the experimental data was done using Origin Pro 8.0 software (OriginLab Corporation, MA, USA), using Tukey's test, at a significance level of 95% (P<0.05). In addition, correlation analysis was used to investigate the relationship between the different genotypic b-casein and the biological activities using SPSS software (SPSS, version 23.0, USA).

## 3. Results and Discussion

The physicochemical composition in the milk of Partial Least Squares (PLS, %), protein (%), lactose (%), pH (%), Solids (%), SNF (Solids Not Fat, %), Conductivity (%) and FFD (Freezing Point Depression, %) was investigated using LactoScope FT-A and the result was given in Table1. The data shows No significant difference between all samples (P<0.05) (Table 1 and Supplementary Table 2). According to our results, the different cow's genotypes affect the appearance, taste or smell of milk, supported by the presence of significant differences (P<0.05) in chemical composition of lactose, and pH.

These results are consistent with the work of Nguyen et al. (2019) and De Vitte et al. (2022) which showed no significant (P<0.05) difference between the CSN2 genotypes (A1A1, A1A2, A2A2) and the composition of the milk. While the data of Albarella et al. (2020) finding that A2A2 milk had a higher protein and higher total solids than A1A1 milk. While for the percentage of lactose no significant difference between the different genotypes was observed (Samoré et al., 2012). Regarding A1A2 samples showed a slightly higher percentage of lactose, solids, FFD and fat content than the samples of the other two genotypes. For the A2A2 genotype milk samples show significantly (P<0.05) higher values in total protein

### 3.1. Titrable Acidity

In our study, titrable acidity results reported in Table 1, were not significantly correlated with β-casein genotypes (A1A1, A1A2, A2A2).

### 3.2. Quantitative Analysis of Fatty Acids

Several studies grant a correlation between genetic variation and protein and fat content and milk production (Samoré et al., 2012). However, studies that accurately correlate genetic polymorphism with milk fatty acid composition are limited (De Vitte et al., 2022).

### 3.3. Saturated Fatty Acids in Milk

The results of the monounsaturated fatty acid composition in milk fat (% of total fat) are presented in Table 3 and Supplementary Table 4. The saturated fatty acid content of the majority of genotype samples A1A1 milk was significantly higher (P<0.05) than that of A2A2 and A1A2. Except for saturated fatty acids Undecylic acid, Tridecylic acid, Margaric acid, and Behenic acid which are significantly higher in A2A2 gene type milk. In present research, saturated fatty acid was significantly correlated with β-casein genotypes (A1A1, A1A2, A2A2) expected the data of Undecylic acid. These results are similar to the study performed by De Vitte et al. (2022) which found that genetic polymophism plays an important role in fatty acid composition in milk. Indeed, following their studies on the comparison between saturated fatty acids, they concluded that undecyl acid (11:0) (P<0.001), tridecanoic acid (13:0) (P<0.01), Myristic acid (14:0) (P<0.001), pentadecanoic acid (15:0) (P<0.001), palmitic acid (16:0) (P<0.001) and behenic acid (22:0)) (P<0.01) of A1A2 milk was significantly higher than those of A1A1 and A2A2 milks.

### 3.4. Monounsaturated Fatty Acids in Milk

The composition of monounsaturated fatty acids in milk fat (% of total fat) according to genotype are reported in Table 5 and Supplementary Table 6. The monounsaturated fatty acid content of milk A2A2 was significantly higher (P<0.05) than of A1A1 and A1A2, with a content of Tetradecenoic acid, Palmitoleic acid and cis-Vaccenic acid of A1A1 milk significantly higher (P<0.05) than that of A1A2 and A1A1 milk. But, the Oleic acid content (P<0.05) of A1A2 milk was significantly higher than that of A1A1 and A2A2 milks. De Vitte et al. (2022), correlates the monounsaturated fatty acid content significantly with the different genotypic combination of milk β-casein genotypes (A1A1, A1A2, A2A2). On the other hand, Perna et al. (2016) reported in their study that the significant difference between the contents of monosaturated fatty acids are linked to the different allelic combinations of αS1-, β- and κ-casein loci, BB-A2A2-AB.

**Table 1.** Characterization of raw milk

| | A2 | A1A2 | A1 | Sig |
|---|---|---|---|---|
| Fat Partial Least Squares (PLS) | 5.32±0.92 | 5.04±0.96 | 4.92±1.63 | 0.791 |
| Protein | 3.71±0.35 | 3.52±0.47 | 3.38±0.15 | 0.13 |
| Lactose | 4.70±0.23 | 4.69±0.25 | 5.02±0.23 | 0.047 |
| Solids | 14.47±1.08 | 13.93±1.18 | 13.98±1.59 | 1.03 |
| Solids Not Fat (SNF) | 9.12±0.39 | 8.93±0.39 | 9.11±0.09 | 0.135 |
| Conductivity | 6.02±0.45 | 6.45±0.58 | 5.7±0.11 | 0.179 |
| Freezing Point Depression (FPD) | 506.462±6.53 | 514.546±7.47 | 510.15±0.92 | 37.319 |
| pH | 6.485±0.02 | 6.486±0.05 | 6.45±0.09 | 0.003 |
| Fat | 5.363±0.96 | 5.056±0.98 | 4.945±1.61 | 0.809 |
| Titrable acidity TA (°D) | 37.77±4.37 | 36.933±4.50 | 39±10.60 | 16.73 |

Note: Data are expressed as mean ± standard Error of three replicates.

**Table 3.** Saturated fatty acid composition

| | | A2 | A1A2 | A1 | Sig. |
|---|---|---|---|---|---|
| Butyric acid | C:4 | 1.451±0.169 | 1.342±0.258 | 1.381±0.007 | 0.025 |
| Caproic acid | C:6 | 1.692±0.186 | 1.524±0.262 | 1.672±0.118 | 0.031 |
| Caprylic acid | C:8 | 1.039±0.147 | 0.948±0.176 | 1.106±0.060 | 0.019 |
| Capric acid | C:10 | 2.463±0.393 | 2.253±0.529 | 2.709±0.021 | 0.137 |
| Undecylic acid | C:11 | 0.053±0.016 | 0.059±0.036 | 0.065±0.014 | 0.001 |
| Lauric acid | C:12 | 3.268±0.449 | 3.015±0.822 | 3.566±0.143 | 0.179 |
| Tridecylic acid | C:13 | 0.113±0.017 | 0.105±0.038 | 0.124±0.005 | 0.001 |
| Myristic acid | C:14 | 11.521±0.742 | 10.822±1.481 | 11.978±0.540 | 0.489 |
| Pentadecylic acid | C:15 | 1.228±0.127 | 1.112±0.359 | 1.238±0.125 | 0.014 |
| Palmitic acid | C:16 | 33.983±0.349 | 32.043±4.091 | 32.228±1.416 | 0.943 |
| Margaric acid | C:17 | 0.628±0.095 | 0.656±3.485 | 0.574±0.017 | 0.008 |
| Stearic acid | C:18 | 14.840±1.678 | 13.950±0.090 | 15.027±1.152 | 2.501 |
| Arachidic acid | C:20 | 0.217±0.021 | 0.191±0.047 | 0.191±0.014 | 0.002 |
| Behenic acid | C:22 | 0.076±0.014 | 0.065±0.020 | 0.054±0.017 | 0.001 |

Note: Data are expressed as mean ± standard Error of three replicates.

**Table 5.** Fatty acid composition

| Samples | | | A1 | A1A2 | A1 | Sig. |
|---|---|---|---|---|---|---|
| Monoun saturated fatty acid | Tetradecenoicacid | C14:1 | 0.688±0.105 | 0.685±0.259 | 0.745±0.000 | 0.010 |
| | Palmitoleicacid | C16:1 | 1.148±0.186 | 1.308±0.287 | 1.123±0.000 | 0.031 |
| | Oleicacid | C18:1n9c | 19.992±2.287 | 23.595±3.298 | 20.785±1.126 | 4.651 |
| | cis-Vaccenicacid | C18:1n7 | 2.950±0.934 | 3.205±0.804 | 3.453±0.012 | 0.775 |
| Polyun saturated fatty acid | Linoleicacid | C18:2n6c | 1.591±0.305 | 1.811±0.307 | 1.311±0.025 | 0.083 |
| | γ-Linolenic | C18:3n6 | 0.028±0.012 | 0.027±0.010 | 0.016±0.000 | 0.227 |
| | α-Linolenic | C18:3n3 | 0.288±0.076 | 0.313±0.061 | 0.262±0.000 | 0.005 |
| | CLA | CLA | 0.605±0.160 | 0.789±0.315 | 0.832±0.000 | 0.023 |
| | Homo-γ-Linolenic | (C20:3n6) | 0.044±0.011 | 0.040±0.018 | 0.038±0.000 | 0.021 |
| | Arachidonic | (C20:4n6) | 0.027±0.033 | 0.041±0.044 | 0.086±0.000 | 0.001 |
| | EPA | (C20:5n3) | 0.032±0.008 | 0.044±0.021 | 0.025±0.000 | 0.139 |
| | Dpan-6 | (C22:5n3) | 0.064±0.014 | 0.065±0.025 | 0.080±0.000 | 0.020 |

**Table 7.** Matrix of correlation between samples and milk composition

| | | Fat (PLS) | Protein | Lactose | Solids | SNF | Conductivity | (FPD) | pH | Fat(0101) |
|---|---|---|---|---|---|---|---|---|---|---|
| r | FatPLS | 1.000 | 0.352 | -0.144 | 0.944 | 0.287 | -0.420 | 0.207 | -0.524 | 0.999 |
| | Protein | 0.352 | 1.000 | -0.447 | 0.585 | 0.799* | -0.085 | -0.118 | -0.150 | 0.393 |
| | Lactose | -0.144 | -0.447 | 1.000 | -0.086 | 0.180 | -0.697 | 0.137 | -0.223 | -0.168 |
| | Solids | 0.944 | 0.585 | -0.086 | 1.000 | 0.583* | -0.532 | 0.153 | -0.548 | 0.953 |
| | SNF | 0.287 | 0.799 | 0.180 | 0.583 | 1.000 | -0.560 | -0.036 | -0.312 | 0.315 |
| | Conductivity | -0.420 | -0.085 | -0.697 | -0.532 | -0.560 | 1.000 | 0.197 | 0.477 | -0.410 |
| | FPD | 0.207 | -0.118 | 0.137 | 0.153 | -0.036 | 0.197 | 1.000 | -0.155 | 0.201 |
| | pH | -0.524 | -0.150 | -0.223 | -0.548 | -0.312 | 0.477 | -0.155 | 1.000 | -0.510 |
| | Fat(0101) | 0.999 | 0.393 | -0.168 | 0.953 | 0.315 | -0.410 | 0.201 | -0.510 | 1.000 |
| Sig. | FatPLS | | 0.050 | 0.256 | 0.000 | 0.092 | 0.023 | 0.171 | 0.005 | 0.000 |
| | Protein | 0.050 | | 0.016 | 0.002 | 0.000 | 0.349 | 0.296 | 0.247 | 0.032 |
| | Lactose | 0.256 | 0.016 | | 0.349 | 0.206 | 0.000 | 0.266 | 0.153 | 0.222 |
| | Solids | 0.000 | 0.002 | 0.349 | | 0.002 | 0.004 | 0.243 | 0.003 | 0.000 |
| | SNF | 0.092 | 0.000 | 0.206 | 0.002 | | 0.003 | 0.436 | 0.074 | 0.071 |
| | Conductivity | 0.023 | 0.349 | 0.000 | 0.004 | 0.003 | | 0.184 | 0.011 | 0.026 |
| | FPD | 0.171 | 0.296 | 0.266 | 0.243 | 0.436 | 0.184 | | 0.241 | 0.179 |
| | pH | 0.005 | 0.247 | 0.153 | 0.003 | 0.074 | 0.011 | 0.241 | | 0.006 |
| | Fat(0101) | 0.000 | 0.032 | 0.222 | 0.000 | 0.071 | 0.026 | 0.179 | 0.006 | |

PLS= partial least squares, SNF= solids no tfat, FPD= freezing point depression, r=correlation coefficient.

### 3.5. Polyunsaturated Fatty Acids in Milk

The composition of polyunsaturated fatty acids in milk fat (% of total fat) is reported in Table 5. The results of the polyunsaturated fatty acid content showed that there is no significant difference between the three varieties. Perna et al. (2016) and De Vitte et al. (2022) found that the A1A1, A1A2 and A2A2 genotypes showed a significant difference in polyunsaturated fatty acids in

the milk samples tested. But in their studies, they reported that A2A2 and A1A2 milk had the lowest percentage of polyunsaturated fatty acid and essentially α-linolenic acid, linoleic acid, eicosapentaenoic acid, docosahexaenoic acid and therefore n-6 PUFA in milk.

## 4. Conclusion

Our results suggest that there is no influence of β-casein variants (A1A1, A1A2 and A2A2) on the physicochemical composition of milk in fat (%), protein (%), lactose (%), pH (%), Solids (%), SNF (%), Conductivity (%) and FFD (%). On the other hand, this genetic variation influences the fatty acid composition. Briefly, A1A1 milk had a higher content of saturated fatty acids and a lower content of monounsaturated fatty acids in milk fat. Whereas, A2A2 milk was higher in polyunsaturated fatty acid in milk fat. However, due to the limited literature available and the relatively small sample size of this study, which is limited to a single farm, which may affect generalizability, further research with larger sample sizes is recommended.

**Author Contributions**

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

| | L.B.F | A.H. | V.T. | A.S. | K.S.L | F.A. | E.M. |
|---|---|---|---|---|---|---|---|
| C | 20 | 10 | | 50 | | 20 | |
| D | 30 | 30 | 30 | | 10 | | |
| S | | | | 30 | | 30 | 40 |
| DCP | 40 | 40 | 10 | | 10 | | |
| DAI | 20 | 10 | | 30 | | 20 | 20 |
| L | 40 | 40 | 10 | | 10 | | |
| W | 40 | 30 | 10 | 20 | | | |
| CR | | | | 30 | | 40 | 30 |
| SR | 50 | | 50 | | | | |
| PM | 10 | | | 20 | | 40 | 30 |
| FA | | | 10 | 40 | 10 | | 40 |

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

**Conflict of Interest**

The authors declared that there is no conflict of interest.

**Ethical Consideration**

Ethics committee approval was not required for this study because of there was no study on animals or humans (the data was taken from the farm, there was no experimental application on the animals).

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Research Article
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# CULTURE- AND POLYMERASE CHAIN REACTION-BASED DETECTION OF *Flavobacterium psychrophilum* IN NATURALLY INFECTED RAINBOW TROUT (*Oncorhynchus mykiss* walbaum, 1792) FROM TROUT FARMS

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**Abstract:** The present study aimed to detect *Flavobacterium psychrophilum* in fish samples collected from rainbow trout (*Oncorhynchus mykiss* Walbaum 1792) farms in the Southeastern Anatolia region of Türkiye by means of bacteriological culture and polymerase chain reaction and to investigate the antibiotic susceptibility of the causative bacteria. A total of 40 trout farms located in Diyarbakır, Adıyaman, Şanlıurfa, and Batman provinces were visited, and 1200 samples were examined. During January and February 30 fish with an average live weight of 200–250 g were collected from each farm. Samples were obtained from the liver, spleen, kidneys, and tissues following macroscopic laboratory examination of the specimens. Antibiotic treatment is the treatment of choice owing to the lack of an effective vaccine in the control of the disease. Therefore, it is important to rapidly identify the bacterial species and investigate its susceptibility to antibiotics. In this study, the causative bacteria were detected in 5 out of 40 farms. The causative bacteria infected the liver, kidney, and tissues. The sensitivity of Enrofloxacin (5 microgram (µg)), Florfenicol (30µg), Neomycin (5µg), Amoxicillin (25µg), Oxytetracycline (30µg), Erythromycin (10µg), Gentamycin (5µg), Streptomycin (5µg) and Nalidixic acid (10µg) were defined at chancing ratios. In conclusion, these bacteria were detected in regional farms, which should minimize the stress factors by avoiding overstocking and following the required hygiene rules.

**Keywords:** Antibiotic susceptibility, Bacterial cold-water disease, *Flavobacterium psychrophilum*

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## 1. Introduction

In recent years, the production quantity, species diversity, and economic value of international aquaculture have increasingly improved. Rainbow trout (*Oncorhynchus mykiss*) is an important salmonidae species farmed worldwide (FAO, 2020). In Türkiye, the rainbow trout production has become a pioneer in the aquaculture sector. Trout is the most important fish species with an output of 127,905 tons (TUİK, 2021). Bacterial-origin fish diseases are one of the factors associated with serious economic losses in aquaculture farms (Özcan, 2022). The severity of the disease varies by the age and species of the fish. Nevertheless, many infections in fish can be successfully treated. However, adverse environmental conditions complicate the efforts or may even make it impossible to protect fish from disease and to take control measures (Buckley et al., 1998). Cold water disease (*Flavobacterium psychrophilum*) is among the primary bacterial diseases in rainbow trout that are associated with economic losses (Austin and Austin 2016). *F. psychrophilum* occurs in the natural flora of the skin, mucous, fin, gill, and operculum of trout (Nematollahi et al., 2003). The causative factor is a Gram negative, thin, long (0.3–0.75 µm × 1.5–7.5 µm) bacillus with a varying size and morphology depending on its reproductive period (Holt et al., 2012). The virulence of the bacteria gradually increases, and a change in environmental factors leads to infection. The bacteria are rapidly transmitted between fish through

water and contact; therefore, they may cause extremely high mortality in the rainbow trout stock in the aquaculture farms, resulting in significant economic losses (Cipriano and Holt, 2005). The mortality rate varies between 30% and 50% when the water temperature drops below 10°C in pools with a high stock ratio, low circulation, low oxygen, and impaired water quality and without hygiene measures in place. Stress-related factors may also play an effective role in the development and spread of the disease. In addition, bacteria can reproduce in water for up to 4 months (FAO, 2020). The causative factor is an infectious and fatal bacterial disease characterized by lesions of various sizes on the dorsal and caudal fins and on the body surface of the fish, bleeding on the skin, exophthalmos, paleness in the gills and liver, swelling of the abdomen, yellow content covering the intestine, and spleen and liver enlargement (Cipriano and Holt, 2005). Antibiotic treatment is the treatment of choice owing to the lack of an effective vaccine for the control of the disease. Therefore, it is important to rapidly identify the bacterial species and investigate its susceptibility to antibiotics. In the present study, samples were collected from separate aquaculture farms in each province to detect *F. psychrophilum* in trout farming facilities located at Diyarbakır, Şanlıurfa, Adıyaman, and Batman provinces of Türkiye. Cold chain transport was used to transfer the samples to the laboratory. After macroscopic examination, culture and polymerase chain reaction (PCR) was used for bacterial identification, followed by the examination of antibiotic susceptibility.

## 2. Materials and Methods

In the present study, samples were collected from 40 different commercial rainbow trout farms located in Diyarbakır, Adıyaman, Şanlıurfa, and Batman provinces in southeastern Türkiye, between January and February 2021. Thirty fish with an average live weight of 200–250 g, which were insensitive to feed, immobile, floating on the water surface, and showing signs of disease, including a darkened color, were collected from each farm. The samples were transferred on ice cubes to the laboratory of the Department of Aquaculture and Fish Diseases, Faculty of Veterinary Medicine, Dicle University. The 1200 samples were first macroscopically examined in the laboratory, followed by dissection and examination of the internal organs. Liver, kidney, and tissue samples were collected from the dissected samples and cultivated on a medium to detect bacteria. Anacker-Ordal agar (AO) was employed for bacterial isolation for 5–7 days at 18°C, and the dominant uniform bacterial colonies were purified by streaking onto the AO plates 3 times. The methods used in the identification were slow and time-consuming owing to the difficulties associated with *F. psychrophilum* culture and the need for a prolonged incubation period (3–4 days) (WIklund et al., 2000). Fluorescence polyclonal antibodies and enzyme linked immunosorbent assay techniques are also used to identify the bacteria in the host organism (Rangdale and Way 1995). However, there is a requirement for faster and more sensitive genetic techniques to identify a lower count of *F. psychrophilum* in fish and environmental samples and to determine the epidemiology of the causative factor. PCR, a molecular genetic method, is a recommended technique and has found widespread use for diagnostic purposes (Rocha et al., 2017). The disease usually has a poor prognosis and is associated with high mortality rates. Therefore, there is a requirement for accurate and rapid diagnostic methods to fight the disease. PCR gives fast and accurate results. Nyztech *Flavobacterium psychrophilum* Real Time PCR Kit (Catalog No: MD01331) was used for identification. The Kirby–Bauer Disc Diffusion method, Mueller–Hinton medium, and Bauer et al., 1966 was used for testing antibiotic susceptibility, and the assessment was made by the procedures suggested by the CLSI (2004), Ruangpan and Tendencia (2004), and BDC (2011).

## 3. Results

The present study used culturing to isolate and identify *F. psychrophilum* in suspected trout samples collected from 40 different rainbow trout (*Oncorhynchus mykiss*) aquaculture farms in Diyarbakır, Batman, Şanlıurfa, and Adıyaman provinces in the southeastern Türkiye. The general clinical picture of the cultivated rainbow trout samples included darkening of their color, paleness of the liver, abdominal swelling, splenic enlargement, bleeding in the fins, wear on the dorsal and adipose fins, bleeding in the jaw, exophthalmos, bleeding in the kidney, and congestion in the liver (Figure 1 and 2).

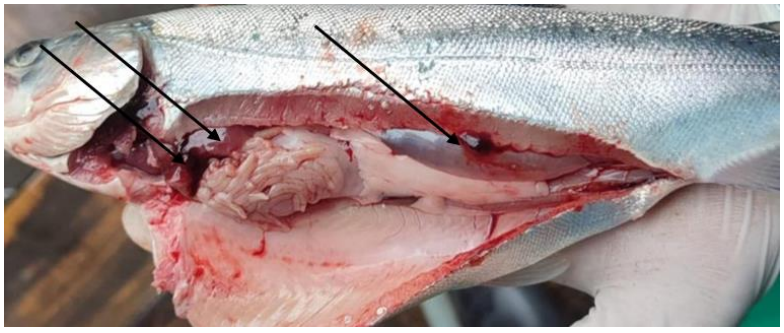

**Figure 1.** Examination upon autopsy indicated bleeding in the internal organs, paleness of the liver and splenic enlargement.

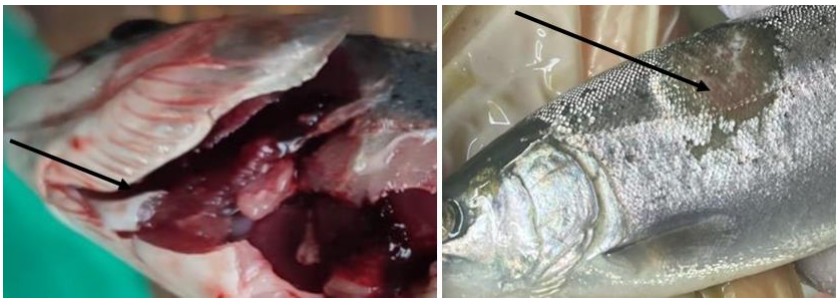

**Figure 2.** Melting and petechial hemorrhages in the gills, ulcer formation in the dorsal region.

In the present study, the causative bacteria were identified in 5 out of 40 farms upon examination of 1200 samples. The causative bacteria infected the liver, kidney, and tissues. The trout farms in the southeastern Anatolia region were contaminated by *F. psychrophilum* at a rate that ranged from 0% to 100%, and the causative bacteria developed different levels of resistance to antimicrobials. In the present study, the bacteria were isolated at a higher rate in farms with excessive water and overstocking. The antibiogram found that the causative bacteria were susceptible to enrofloxacin, florfenicol amoxicillin, and oxytetracycline (Table 1).

**Table 1.** Antimicrobial susceptibility of *Flavobacterium psychrophilum*

| Antimicrobial agent | Isolate 1 | Isolate 2 | Isolate 3 |
|---|---|---|---|
| Enrofloxacine (5µ) | S | S | S |
| Florfenicol (30µ) | S | S | S |
| Neomycine (5µ) | R | R | R |
| Amoxicillin (25µ) | S | S | S |
| Oxytetracycline (30µ) | S | S | S |
| Erythromycin (10µ) | R | R | R |
| Gentamycine (5µ) | R | S | R |
| Streptomycine (5µ) | R | R | R |
| Nalidixic acid (10µ) | R | R | R |

S= susceptible, R= resistant

## 4. Discussion

The morbidity rates were considerably high in the intensive aquaculture farms owing to the increased output. High stocking density and changes in water quality might result in a higher prevalence of bacterial diseases. Today, cold water disease is an enzootic disease in many countries, is associated with severe fish mortality in rainbow trout farms worldwide (Ersoy *et al.,* 2018). *Flavobacterium psychrophilum* grows in the natural flora of the skin, mucous, fin, gill, and operculum of trout (Nematollahi et al., 2003). The virulence of the causative factor may increase and changes in environmental factors, including lower water temperatures, poor quality of water, high stock density, and poor maintenance and feeding may lead to infection. The bacteria are rapidly transmitted between fish through water and contact horizontally, leading to extremely high mortality in rainbow trout stock in the aquaculture farms, resulting in significant economic losses (Cipriano and Holt 2005). The clinical manifestations of *F. psychrophilum* infections in rainbow trout included anorexia, lethargy, darkening of the skin, ascites, bilateral exophthalmos, and periocular bleeding (Yıldırım and Özer 2010). The clinical findings in the present study included the fish immobility, decreased feed consumption, and irregular floating and were consistent with those of previous reports. Many fish had an eroded epidermis. The erosions were transformed into ulcers penetrating deep muscle layers in certain locations (Figure 2). The presence and distribution of bacteria in those ulcers were indicative of the fact that the skin acted as an entry port. Previous studies reported signs of anemia-related paleness, hyperemia, and petechial bleeding in the gills of infected fish (Nematollahi et al., 2003). As a matter of fact, degeneration and eruption were observed in the gills (Figure 2) in the present study. In addition, the spread of infection from these gill lesions might have been a factor that increased the severity of the disease. Furthermore, there was hyperemia in the primary lamella and edema in the secondary lamella in the gills along with degeneration and eruption in the epithelium. The abundant foreign particles in the gills indicated water pollution, which was interpreted as a factor that increased the severity of the disease and disease-associated mortality rate. A 2018, study by Ersoy et al. on the identification of *F. psychrophilum* in rainbow trout samples collected from 14 different farms in the Mediterranean region reported a general clinical picture, including darkening of color, paleness of the liver,

abdominal swelling, splenic enlargement, bleeding in the fins, bleeding in the jaw, exophthalmos, bleeding in the kidney, and hepatic congestion. The macroscopic findings of another study by Epikmen et al. dated 2020 are consistent with the results of the present study. Dead fish were collected from the farms and included in the study. They demonstrated abdominal bloating owing to fluid accumulation, exophthalmos, darkening of the skin color, and few fish demonstrated the presence of white-colored lesions on the back and caudal fins, with the caudal fin completely destroyed along with the appearance of radiuses. The liver and kidneys of the fish were pale, and the fish demonstrated splenic enlargement in cases of advanced disease.

As a matter of fact, these results are consistent with those previously reported in the relevant literature (Wiklund et al., 2000). The antibiogram found that the *F. psychrophilum* strains were susceptible to enrofloxacin, florfenicol amoxicillin, and oxytetracycline and were resistant to neomycin, erythromycin, gentamicin, nalidixic acid, and streptomycin (Table 1). Durmaz et al. (2012) reported that all the strains were susceptible to oxytetracycline and enrofloxacin, and that the antibiotic susceptibility profiles of the strains were varied. Another study by Ersoy et al. reported in 2018 that the strains were susceptible to trimethoprim/sulfametoxazol, clindamycin, ampicillin, tetracycline, enrofloxacin, chloramphenicol, oxytetracycline, fluorophenicol, and tobramycin. These results are consistent with those of the present study.

## 5. Conclusion

In conclusion, the present study isolated and identified *F. psychrophilum*, the psychrophillic causative bacteria associated with morbidity and heavy economic losses in rainbow trout farms in Türkiye and in many countries across the world, using culturing. Pathogen positive was found in 150 of the samples examined. Among the tested antibiotics, Enrofloxacin (5µ), Florfenicol (30µ), Amoxicillin (25µ) and Oxytetracycline (30µ) were found to be sensitive to the pathogen. However, the isolation of the bacteria is difficult and time consuming. Therefore, PCR, a molecular technique that provides rapid results in the diagnosis of bacterial diseases, was used for the identification of *F. psychrophilum.* Thus, major economic losses suffered by aquaculture farms can be prevented by timely treatment as cultured *F. psychrophilum* can be diagnosed over a relatively short period of time using PCR. Further, the bacteria were identified in trout farms across the southeastern Anatolia region, and it was concluded that more attention should be paid to hygiene and that high stock density should be avoided.

**Author Contributions**

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

| | F.Ö. | N.B | M.K | K.A | N.K | N.Ö. | B.Ç. |
|---|---|---|---|---|---|---|---|
| C | 40 | 10 | 10 | 10 | 10 | 10 | 10 |
| D | 40 | 10 | 10 | 10 | 10 | 10 | 10 |
| S | 40 | 10 | 10 | 10 | 10 | 10 | 10 |
| DCP | 40 | 10 | 10 | 10 | 10 | 10 | 10 |
| DAI | 40 | 10 | 10 | 10 | 10 | 10 | 10 |
| L | 40 | 10 | 10 | 10 | 10 | 10 | 10 |
| W | 40 | 10 | 10 | 10 | 10 | 10 | 10 |
| CR | 40 | 10 | 10 | 10 | 10 | 10 | 10 |
| SR | 40 | 10 | 10 | 10 | 10 | 10 | 10 |
| PM | 40 | 10 | 10 | 10 | 10 | 10 | 10 |
| FA | 40 | 10 | 10 | 10 | 10 | 10 | 10 |

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

**Conflict of Interest**

The authors declared that there is no conflict of interest.

**Ethical Consideration**

Ethics committee approval is not required because of this study used rainbow trout obtained from commercial farms as experimental material.

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Research Article
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# BREEDING OF AKKARAMAN SHEEP IN TOKAT

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**Abstract:** This study was carried out in Akkaraman sheep breed in Tokat province, which is within the scope of The National Sheep and Goat Breeding Project. In this study, some reproductive traits of Akkaraman sheep and live weights of lambs were determined. In this study, the data of 28797 sheep and 25983 lambs between the years 2017-2021 were used. The average birth weight was 4.14 kg in the 5-year period. The birth weight according to the years was determined as 4.31, 3.85, 4.13, 3.92 and 4.25 kg, respectively. Mean birth weights vary according to year, maternal age, birth type and sex (P<0.01). The average weaning weight was determined as 30.85 kg. Average weaning weights vary according to year, maternal age, birth type and sex (P<0.01). Lambing rate was found to be 85% on average. Litter size is 0.89. Fecudity was 1.05. Infertility rate was determined as 15 %. The average survival rate was determined as 93%. As a result, it has been revealed that there have been improvements in terms of live weight and fertility in herds over the years.

**Keywords:** Sheep, Akkaraman sheep, Live weight, Fertility, Breeding, Tokat

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## 1. Introduction

Sheep is one of the first domesticated animals and the domestication process took place in Central Anatolia (Zeder, 2008). Sheep breeding is carried out in a wide area in the world (Zeder, 2008). There are 40 sheep breeds in our country (FAO, 2017) and there are 31 million sheep (TUIK, 2017). It has been revealed that native breeds adapted to their region are resistant to diseases and can high production in poor environmental conditions (Hoffmann, 2013). Although there are local sheep breeds in different regions of Türkiye (FAO, 2017), Akkaraman breed constitutes about half of the total sheep population (Akçapınar, 2000). Akkaraman sheep is a fat-tailed domestic breed that is resistant to diseases and has a high adaptation to bad environmental conditions (Akcapinar, 2000). There are types named as Kangal, Şavak and Karakaş in Akkaraman breed.

Until recently, there was no systematically applied breeding program in the Akkaraman breed. For this reason, selection was made according to morphological characteristics (Ceyhan et al., 2019). Sheep is generally carried out under poor pasture conditions in Türkiye. For this reason, the genetic potential of animals is insufficient (Biçer et al., 2019). The inclusion of the Akkaraman breed in the national breeding program initiated a selection practice based on yield records.

Birth weight is one of the parameters that determine the survival rate of lambs. Weaning weight affects the fattening performance. Birth and infertility rates in Akkaraman sheep was 86% (Başpınar, 1985) and 4.7% (Güney, 1979), respectively. Fecundity in Akkaraman breed was 1.95 (Güney, 1979). Birth and weaning weight in Akkaraman sheep was 4.71 and 23.69 kg, respectively (Özbey et al., 2000). It was aimed to determine the some fertility traits in Akkaraman sheep and live weights of lambs from birth to weaning within the scope of the breeding project carried out in Tokat.

## 2. Materials and Methods

In this study, data belonging to the national sheep and goat breeding project supported by General Directorate of Agricultural Research and Policies (TAGEM) were used. In this context were kept yield records of 28870 sheep and 25603 lambs between the years 2017-2021 in Tokat province. Sex, maternal number, maternal age, date of birth, type of birth, weaning weight (90th day) and birth weight of the lambs were recorded. The numbers of the animal material used within the scope of the project are given in Table 1.

The formulas below were used to determine fertility levels;

Lambing rate: (ewes lambed/ ewes mated) × 100

Infertility rate: infertile ewes/ ewes exposed × 100

Fecundity: lambs born/ ewes mated

Litter size: lambs born/ number of lambing ewes

Survival rate (%) (90th day): number of living lambs/number of lambs born x 100

Lambs were weaned at 90 days of age. Estimation of weaning weights;

Estimate weight (90th): Lamb live weight – b x (lamb's age at weigh– 90)

b: regression co-efficient

The data were subjected to analysis of variance in Minitab 13.0 program. Means were subjected to the Tukey multiple comparison test.

## 3. Results

Birth weight, weaning weight and daily live weight gain by year, maternal age, birth type and sex are given in Table 2. The highest birth weight was reached in 2017 and 2021 (P<0.01). It was determined that lambs obtained from 2-year-old mothers who gave their first birth had the lowest birth weight (P<0.01). It was determined that single lambs had higher birth weights than twin lambs (P<0.01). Male lambs have higher birth weights than female lambs (P<0.01).

Weaning weight was determined as 30.85 kg on average. In terms of weaning weight, single were higher than twins and males were higher than females (P<0.01). The daily live weight gain reached the highest level as 227.9 kg in 2021 (P<0.01). It has been determined that the daily live weight gain is higher in males than females and single than twin (P<0.01).

Some reproductive traits and survival rate in Akkaraman sheep are given in Table 3. The highest lambing rate was determined as 89 %. In this study, fecundity was 0.89. In this study, litter size was 1.05. The survival rate of the lambs on the 90th day was determined as 93 %.

**Table 1.** Number of animals by year

| Year | Number of sheep | Number of lambing sheep | Number of lambs born | Number of lambs at weaning |
|---|---|---|---|---|
| 2017 | 5900 | 4374 | 5042 | 4308 |
| 2018 | 5670 | 4986 | 4308 | 4273 |
| 2019 | 5670 | 4944 | 5679 | 5397 |
| 2020 | 5815 | 5178 | 5660 | 5206 |
| 2021 | 5815 | 5025 | 4914 | 4596 |
| Total | 28870 | 24507 | 25603 | 23708 |

**Table 2.** Birth, weaning weights and average daily weight gain by year, maternal age, birth type and sex

| | Birth Weight (kg) | | Weaning weight (kg) | | Average Daily Weight Gain (gr) | |
|---|---|---|---|---|---|---|
| | n | $\bar{X}$±Sx | n | $\bar{X}$±Sx | n | $\bar{X}$±Sx |
| Avarage | 28797 | 4,14±0,03 | 25983 | 30,85±0,6 | 25983 | 239,3±6,04 |
| Year | ** | ** | | ** | | |
| 2017 | 6790 | 4,31±0,02$^{a}$ | 4240 | 30,05±0,7$^{a}$ | 4240 | 242,7±8,9$^{a}$ |
| 2018 | 5779 | 3,85±0,04$^{b}$ | 5315 | 31,12±0,8$^{b}$ | 5315 | 243,1±7,4$^{a}$ |
| 2019 | 5679 | 4,13±0,03$^{c}$ | 5397 | 31,60±0,9$^{b}$ | 5397 | 215,8±6,9$^{b}$ |
| 2020 | 5635 | 3,92±0,02$^{d}$ | 5435 | 27,52±0,6$^{c}$ | 5435 | 215,6±8,1$^{b}$ |
| 2021 | 4914 | 4,25±0,04$^{a}$ | 4596 | 31,05±06$^{b}$ | 4596 | 227,9±6,7$^{c}$ |
| Maternal Age | ** | ** | | ** | | |
| 2 | 5530 | 3,92±0,02$^{a}$ | 4234 | 31,39±0,6$^{a}$ | 4234 | 232,1±5,2$^{a}$ |
| 3 | 6396 | 4,21±0,04$^{b}$ | 6181 | 32,87±0,6$^{b}$ | 6181 | 218,8±6,1$^{b}$ |
| 4 | 6947 | 4,39±0,03$^{b}$ | 6745 | 30,05±0.7$^{c}$ | 6745 | 256,7±7,9$^{c}$ |
| 5 | 6919 | 4,37±0,05$^{b}$ | 5754 | 30,99±0,8$^{a}$ | 5754 | 260,3±7,5$^{c}$ |
| 6 | 3005 | 4,18±0,05$^{b}$ | 3069 | 31,37±0,6$^{a}$ | 3069 | 217,8±6,6$^{b}$ |
| Birth type | ** | ** | | ** | | |
| Single | 14464 | 4,21±0,02$^{a}$ | 20800 | 30,27±0,2$^{a}$ | 20800 | 239,55±6,9$^{a}$ |
| Twin | 5369 | 3,60±0,03$^{b}$ | 5113 | 29,05±0,3$^{b}$ | 5113 | 228,10±6,5$^{b}$ |
| Sex | ** | ** | | ** | | |
| Male | 14464 | 4,10±0,04$^{a}$ | 13541 | 30,98±0,4$^{a}$ | 13541 | 231,24±5,3$^{a}$ |
| Female | 14333 | 3,38±0,04$^{b}$ | 12442 | 28,15±0,6$^{b}$ | 12442 | 210,6±5,1$^{b}$ |

** The differences between the averages shown with different letters in the same column are very significant (P<0.01).

**Table 3.** Some reproductive traits and survival rates.

| Year | Lambing Rate (%) | Fecundity | Litter Size | Infertility Rate (%) | Survival Rate (90th) (%) |
|---|---|---|---|---|---|
| 2017 | 74 | 0.85 | 1.15 | 26 | 85 |
| 2018 | 87 | 0.76 | 0.86 | 13 | 99 |
| 2019 | 88 | 1.00 | 1.15 | 12 | 95 |
| 2020 | 89 | 0.97 | 1.09 | 11 | 92 |
| 2021 | 86 | 0.85 | 0.98 | 14 | 94 |

## 4. Discussion

Environmental factors such as maternal age, birth type, sex and gestational feeding period also affect birth weight (Akçapınar, 2000). In a study, it was determined that the birth weight of Akkaraman lambs was 4.71 kg (Özbey et al., 2000). The birth weight obtained in the study by Özbey et al., (2000) is similar to the birth weight obtained in this study.

There is also the effect of the mother on the weaning weight along with the feeding. In a study, it was determined that the weaning weight (90th day) of Akkaraman lambs was 23.69 kg (Özbey et al., 2000). The weaning weight obtained in the study by Özbey et al., (2000) was lower to the weaning weight obtained in this study. In the study conducted in Akkaraman breed determined that the average daily weight gain was determined 0.220 kg (Mis ve Öztürk, 2018). This result is similar to the daily weight gain obtained in our study. In another study, the average weight gain in male lambs was found to be 0,171 kg (Turkmen ve Cak, 2021).

In the study conducted determined that the survival rate ranged between 80 % and 100 % in Akkaraman sheep (Tekerli et al., 2002). The 90th day survival rates obtained in this study are an acceptable range for Akkaraman breed. Acceptable values for infertility rate are between 5 % and 8 % (Kaymakçı and Taşkın, 1997). The infertility rate was above the expected values. It is thought that the high infertility rate is due to environmental factors. In the study conducted by Demiral and İşcan (2012) was determined between 1.00 and 1.29. The low fertility rate is due to the high infertility rate.

In another study, litter size was determined as 1.33 (Tekerli et. al., 2002). The reason for this low rate is due to the lower rate of twin births. In order to increase this rate, it is necessary to perform flushing before mating. It can be said that the survival rate determined in this study is above the average survival rate rates determined in other studies (Tekerli et al., 2022) for Akkaraman lambs. High survival rate indicates that the maintenance and feeding conditions are sufficient for lambs until the 90th day.

## 5. Conclusion

It has been determined that the birth weight and daily weight gain have varied over the years with the applied breeding program. However, there was variation in weaning weight. It is seen that the infertility rate is well above the accepted value. The reason for this situation is thought to be due to environmental conditions. Therefore, environmental factors (especially pasture) should be improved on a herd basis in order to reduce the infertility rate.

**Author Contributions**

The percentage of the author contributions is presented below. The author reviewed and approved the final version of the manuscript.

| | E.Ş. |
|---|---|
| C | 100 |
| D | 100 |
| S | 100 |
| DCP | 100 |
| DAI | 100 |
| L | 100 |
| W | 100 |
| CR | 100 |
| SR | 100 |
| PM | 100 |
| FA | 100 |

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

**Conflict of Interest**

The author declared that there is no conflict of interest.

**Ethical Consideration**

Ethics committee approval was not required for this study because of there was no study on animals or humans. The data used in the study were obtained from the "National Project of Ovine Breeding in Public" courtesy of TAGEM.

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Research Article
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# ESTIMATION OF CROP WATER REQUIREMENT FOR TOMATO PLANT IN AFGOYE-SOMALIA, USING CROPWAT 8.0 MODEL

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**Abstract:** Crop water requirement or crop evapotranspiration is a vital parameter for irrigation, and it is necessary to determine the quantity of water to be applied for irrigation and develop an effective irrigation schedule. CROPWAT 8.0, decision-support computer software developed by the United Nations Food Agriculture Organization, is used to calculate crop water requirements. In this paper, the CROPWAT 8.0 model was used to estimate the water requirements of tomato crops in Afgoye. The model estimated that the reference evapotranspiration throughout the year reaches 1927.6 mm and the daily reference evapotranspiration is 5.29 mm. The total annual rainfall reaches 584.0 mm with an effective rainfall of 511.1 mm. The total crop evapotranspiration during the growing period was estimated at 678.2 mm and the total irrigation amount was calculated as 452.3 mm with an effective rainfall of 230.6 mm. During the growing period, the net and gross irrigation reaches 392.9 and 561.3 mm respectively. Field experiments should be conducted in the same season and cropping patterns to validate the accuracy of the crop water requirement prediction.

**Keywords:** CROPWAT 8.0, Tomato, Crop water requirement, Irrigation scheduling

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## 1. Introduction

Water resources are becoming scarce, particularly in arid and semi-arid climate zones, owing to their potential uses in various sectors. On the other hand, the demand for food, fiber, and forage is increasing due to the increase in the world population, which is predicted to reach 10 billion in the mid-century (Halimi and Tefera, 2019). This has led to the enhancement of agricultural production to meet the needs of large populations and the requirement of fresh water by water users is continually increasing (Eshete et al., 2020). In developing countries, more than 70% of the available freshwater is used for irrigation to achieve optimal agricultural production (FAO, 2017; Michelon et al., 2020; Solangi et al., 2022). Irrigation is an artificial means of supplying water to crops and sometimes supplementing rainwater, where rainfall alone cannot support crop growth and optimal yield production (Waller and Yitayew, 2015). As some regions of the world are facing severe water scarcity, appropriate irrigation practices are the only way to improve the exploitation of available water and increase or avoid causing a reduction in agricultural production. Irrigation engineers, agronomists, and researchers have conducted several studies and continue to focus on irrigation and its governing factors, including plants, soil, water, and atmospheric or climatic characteristics (Liu et al., 2021; Tong et al., 2022; Yerli et al., 2022). Investigating the relationship between plants and water while considering the physical characteristics of the soil and climate enables researchers to boost their understanding of irrigation, which leads to the efficient use of water and saves a significant amount of water that can be used for other purposes. More than 90% of the water used for irrigation or rainwater is lost through evapotranspiration, and a small portion of the water supplied is utilized by crops to carry out metabolic activities for growth and production. (Sterling, 2005) Therefore, understanding crop water requirements, referred to as evapotranspiration, is vital for irrigation. Evapotranspiration is the combination of evaporation, the quantity of water vaporized by solar energy from a bare soil surface or open water body, and transpiration, the amount of water lost by the plant through its leaf stomata (Allen et al., 1998). Climate and micrometeorological characteristics, plant variety, and soil moisture influence evapotranspiration rates. Considering all these factors, evapotranspiration can be used as a tool to minimize water loss resulting from poor irrigation and agronomic practices. According to the Köppen climate classification method, Somalia is characterized by an arid and semi-arid climate zone, and precipitation is high in semi-arid climate regions, mainly in southern regions, while potential evapotranspiration is high in northern Somalia. The country was severely affected by recurrent droughts resulting from erratic rainfall in both the northern and southern regions, lower-level river streams in riverine agricultural lands, and the dry running of boreholes and shallow wells in many

inland regions. Therefore, the quantity of water that would be used for irrigation and the amount of rainfall that would be utilized for rainfed farming are both below average and cannot support crop production. According to SWALIM and FAO, the paper on Somali Climate, there is a higher imbalance in water in Somalia: the annual potential evapotranspiration (PET) exceeds the incoming precipitation, indicating that crops experience water stress at their canopy development stages (Muchiri, 2007). Therefore, crop irrigation is necessary during critical water-stress situations.

In Somalia, tomatoes are grown under irrigation regimes, rainfall, and water recessions resulting from floods in the Juba and Shebelle regions. Tomatoes are consumed as soup with sauces, staple dish cereals, or starchy foods by households in agricultural villages and urban areas. The local cherry, Shalambood, Roma VF San Marzano, and Moneymaker varieties are the main tomato cultivars used for growth in Somalia. These tomato cultivars are selectively grown in different climate zones, considering their tolerance to drought and their resistance to pests, insects, and diseases (Abukar, 2004). There are many other recently imported exotic varieties; however, there is no information on their adaptability and production. In the country, there is no available literature and studies focused on the ETc of tomatoes or other vegetables and cereals, except for a few observational studies and reviews (Basnyat, 2007; Ibrahim et al., 2020). Similarly, there is no available literature that focuses on irrigation schedules and crop water requirements. Therefore, this paper aimed to estimate the crop and irrigation water requirements of Tomatoes at Afgoye and develop an irrigation schedule in the 'Xagaa' (summer) season by using the FAO CROPWAT 8.0 model version. The CROPWAT 8.0 model is an irrigation software developed by FAO for quantifying crop water requirements using the Penman-Monteith method. This model can also be utilized for projecting future irrigation water demand (Sunil et al., 2021; Mana et al., 2023).

## 2. Materials and Methods

### 2.1. Study Area

Afgoye is located 30 km (18 miles) in western Mogadishu, the Somali capital city. It is well known for its alluvial soil and the Shebelle River, which runs at the center of the town. The study area lies at a latitude of 2° 15'N and a longitude of 45°15E, with an altitude of 83 m. The climate of the town is semi-arid; the annual maximum, minimum, and mean temperatures were recorded as 32.7 °C, 22.1 °C, and 27.3 °C, respectively, and the annual rainfall is 584 mm (Muchiri, 2007). The highest rainfall season occurs from April to Jun and the short rainfall season occurs in October and November (Musei et al., 2021). The soil is black-clayed, and crops such as bananas, cereals (mainly maize), sesame, citrus, and vegetables are grown. Most crops are irrigation-dependent, rainfall is a supplement to irrigation, and it sometimes becomes an alternative when the level of the river's flow is low and irrigation cannot be supported, particularly in farms located far away from the river.

### 2.2. CROPWAT 8.0 Model Description

The CROPWATT 8.0, irrigation software developed by the Department of Land and Water Resource Management of FAO, is the model used for computing the reference evapotranspiration (ETo), crop water requirements, and irrigation water requirements. According to the FAO, the model also enables the development of irrigation schedules for various management conditions and determines the water supply scheme for different cropping patterns. The model requires meteorological data that is, minimum and maximum temperatures, average relative humidity, wind speed at 2 m in height, and daylight hours, to quantify radiation and reference evapotranspiration. The model also requires rainfall data, crop data such as root depth, the coefficient of crop evapotranspiration ($K_c$), and soil data for determining crop and irrigation water requirements and crop evapotranspiration. This model with CLIMWAT 2.0 database can also be used when local meteorological data are limited (https://www.fao.org/land-water). CROPWATT 8.0, which was calibrated by comparing the daily prediction of evapotranspiration with Class A pan evaporation and evapotranspiration measured with gauges in the United States, indicates that evapotranspiration computed with CROPWAT 8.0, is reliable.

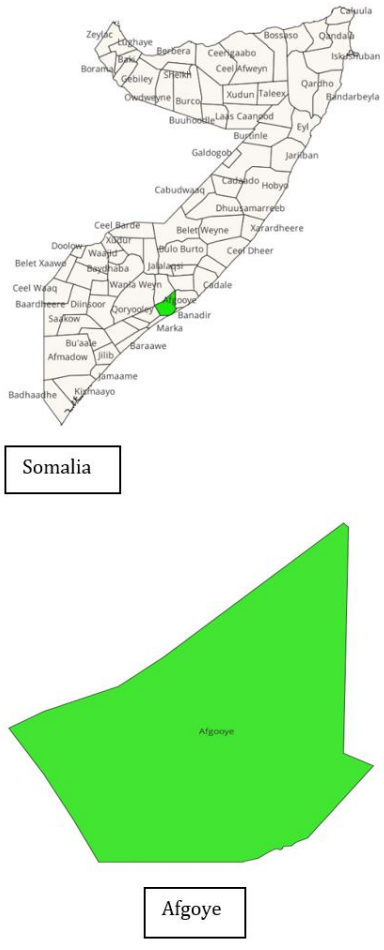


**Figure 1.** Study area.

CROPWAT 8.0 is also a good decision-making support system for farmers to evaluate irrigation practices in both irrigation and rain-fed farming (Trivedi et al., 2018). Although there are no ready data in meteorological stations in the study area, the climate, soil, and crop data used in this study were E from the FAO CLIMWAT database in between 1975 to 2006). CROPWAT 8.0, was developed from two FAO publications on drainage and irrigation series: FAO-56 "Crop Evapotranspiration-Guidelines for Computing Water Requirement" and FAO-33 "Yield Response to Water" (Allen et al., 1998; Doorenbos et al., 1980).

**2.3. Reference Evapotranspiration (ETo)**

The CROPWAT program first calculates ETo using the FAO Penman-Monteith method (Allen et al., 1998). This method was developed from a combination of the Penman and Monteith methods and can be applied at various locations. FAO Penman-Monteith method had become one of the equation methods used by researchers, and irrigation engineers to determine irrigation schedules and also predicting for future crop and irrigation water demand (Equation 1).

$$ET_O = \frac{0.408\Delta\,(R_n - G) + \gamma\,\frac{900}{T+273}u_n(e_s - e_a)}{\Delta + \gamma(1 + 0.34u_n)} \quad (1)$$

Where $ET_o$ is; reference evapotranspiration (mm/day), $R_n$; net radiation at crop surface (MJ $m^{-2}$ $day^{-1}$), G; soil heat flux density (MJ $m^{-2}$ $day^{-1}$) mean air temperature with 2 m height, $u_2$; wind speed at 2 m height (m $s^{-1}$), $e_s$; saturation vapor pressure, $e_a$; actual vapor pressure (kPa), $e_s$-$e_a$; saturation vapor pressure deficit (kPa) Δ; slope vapor pressure curve (kPa °$C^{-1}$), γ; psychometric constant (kPa °$C^{-1}$). This method is however not applicable for quantifying crop evapotranspiration ($ET_c$) of plant grown in greenhouse, so some researcher had indicated that they used machine learning model for ET prediction (Ge et al., 2022).

Reference evapotranspiration ($ET_o$) is sometimes interchanged to reference crop evapotranspiration and it is the rate of water evaporated from hypothetical references crop with an assumed crop height of 0.12 m, the surface resistance of 70 sec $m^{-1,}$ and an albedo of 0.23 closely matching evapotranspiration rate from dense green-grass of uniform height, vigorously growing, well irrigated, and completely shading the entire soil surface. The reference crop is not exposed to water stress and is free of diseases (Irmak and Haman, 2003). In the experiments, the researchers use grass and alfalfa crops as references, as the two crops entirely cover the ground surface. In this study, we quantified ETo using climate parameters, including minimum and maximum temperature, relative humidity, wind speed, daily sunlight duration, and solar radiation.

To compute the crop evapotranspiration, the crop coefficient (Kc) obtained from the FAO crop coefficient data and ETo was used. $ET_o$ is an input parameter used to quantify crop evapotranspiration when the coefficient of crops ($K_c$) in different stages is known (Equation 2).

$$ETc = ETo \times Kc \quad (2)$$

Where ETc is crop evapotranspiration and $K_c$ is coefficient crop evapotranspiration.

**2.4. The Crop Coefficient (Kc)**

Crop Coefficient (Kc) is the ratio of $ET_c$ to $ET_o$, which integrates the effect of characteristics that distinguish a specific crop's water use from reference evapotranspiration. $K_c$ is used to calculate $ET_c$. and sometimes use to partition $ET_c$ into soil evaporation and plant transpiration. Kc is affected by crop varieties, growth stages, and climate and soil characteristics (Kang et al., 2003). CROPWAT requires the Kc of the crop in different stages. The $K_c$ of tomato was updated from the FAO CLIMWAT database. The crop water requirement is the amount of water applied to compensate for the water lost through evapotranspiration and that from cropped fields (Ewaid et al., 2019)

## 3. Results and Discussion

**3.1. Reference Evapotranspiration (ETo), Rainfall, and Effective Rainfall**

The average daily $ET_o$ throughout the year and the amount of rainfall and effective rainfall is given in Table 1, annual daily $ET_o$ was calculated as 5.2 mm/day with a total of 1927.6 mm, the total rainfall is 584 mm and the total amount of effective rainfall is 511.4 mm. The highest ETo rates, range from 6.47, 6.28, and 6.04 mm in March, February, and January, respectively; both actual crop evapotranspiration and reference evapotranspiration were high due to higher temperature and lower relative humidity. Furthermore, the rainfall is low and ranges from 2 to 10 mm from January to March. The highest average rainfall changes from 62, 121, and 39 mm from October to December and 91, 94, and 64 mm from April to June accordingly and usually occurs during 'Deyir' (autumn) and 'Gu' (spring) seasons and that is why the rainfall in Somalia characterized by bio-model (figure 1). In Somalia, rainfall is the only climatic parameter that indicates seasonal weather changes (Muchiri, 2007). Wind speed, which is also high in dry seasons, contributes to an increase in the ETo rate. There is not enough literature focused on evapotranspiration except for evapotranspiration, which is available in the Global FAO Database, the FAO data contains the average monthly Potential Evapotranspiration (PET). According to the FAO Global database, the highest PET in Afgoye was recorded as 184.3 mm in March and the lowest PET was determined as 118.5 mm in June (Basnyat 2007; Muchiri, 2007).

As Figure 2 illustrates, rainfall and effective rainfall are not significantly different, and both are approximately equal at the 'Jilaal' (December to March) and 'Xagaa' (Jun to September) seasons and also indicate the bimodality of the rainfall i.e higher in rainy. FAO and SWALIM have also reported rainfall bimodality (Muchuri, 2007).

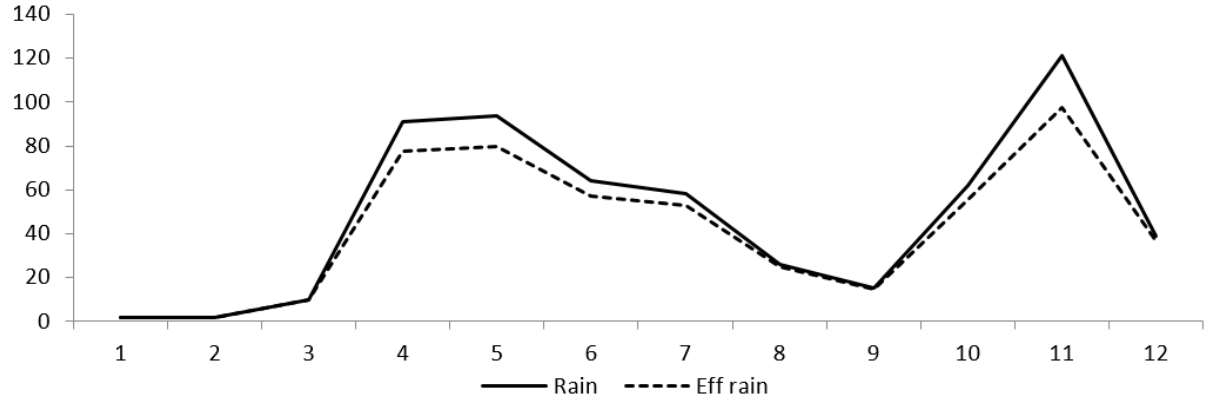


**Figure 2.** The rainfall and effective rainfall (mm).

**Table 1.** Minimum and Maximum temperatures, relative humidity (%), wind speed (km/h), sun (Hours), Rad (MJ/m²/day), rainfall, and effective rainfall

| Month | Min Temp (°C) | Max Temp (°C) | Humidity (%) | Wind (km/day) | Sun (hours) | Rad (MJ/m²/day) | $ET_o$ (mm/day) | Rainfall (mm) | Effective rainfall (mm) |
|---|---|---|---|---|---|---|---|---|---|
| January | 21.6 | 33.5 | 65 | 346 | 7.9 | 20.6 | 6.04 | 2 | 2 |
| February | 21.8 | 34.1 | 70 | 363 | 9.2 | 23.5 | 6.28 | 2 | 2 |
| March | 23 | 35 | 69 | 328 | 9 | 23.6 | 6.47 | 10 | 9.8 |
| April | 23.5 | 34.3 | 71 | 216 | 7.5 | 20.8 | 5.37 | 91 | 77.8 |
| May | 23.1 | 32.8 | 76 | 216 | 6.4 | 18.3 | 4.61 | 94 | 79.9 |
| June | 22.6 | 31.2 | 79 | 259 | 6.2 | 17.3 | 4.22 | 64 | 57.4 |
| July | 21.5 | 30.5 | 75 | 259 | 7.9 | 20.1 | 4.71 | 58 | 52.6 |
| August | 21.5 | 31.1 | 75 | 268 | 8.1 | 21.2 | 4.95 | 26 | 24.9 |
| September | 21.7 | 32 | 71 | 268 | 8.5 | 22.5 | 5.5 | 15 | 14.6 |
| October | 22 | 32.2 | 72 | 242 | 7.5 | 20.8 | 5.18 | 62 | 55.8 |
| November | 21.8 | 32.3 | 67 | 181 | 6.8 | 19 | 4.78 | 121 | 97.6 |
| December | 21.6 | 33 | 66 | 277 | 6.6 | 18.4 | 5.32 | 39 | 36.6 |
| Total | | | | | | | | 584 | 511.1 |
| Average | 22.1 | 32.7 | 71 | 269 | 7.6 | 20.5 | 5.29 | | |

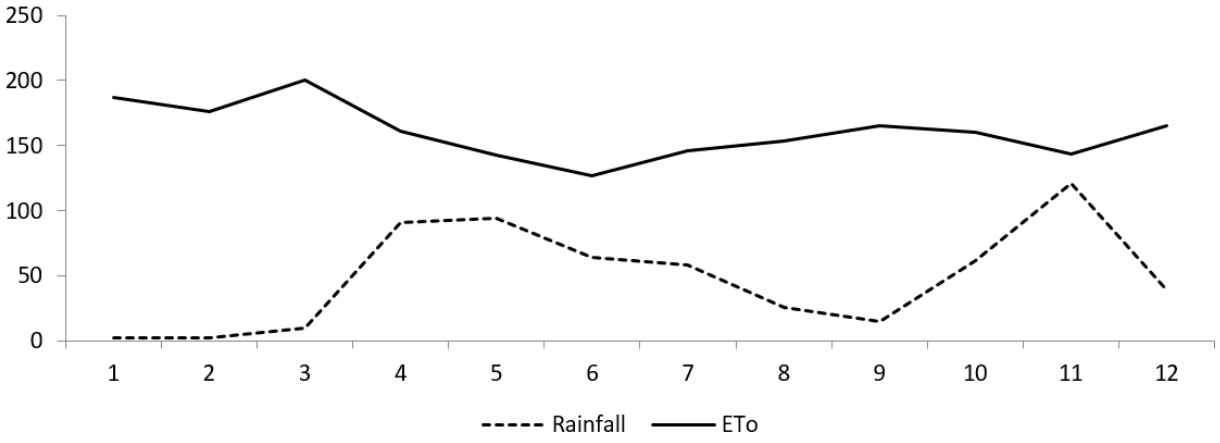


**Figure 3.** Rainfall and ETo (mm).

Figure 3 illustrates that rainfall cannot provide the amount of water required by reference crops i.e. hypothetical green grass or/ and alfalfa plant, the rainfall cannot cover the water requirement of a certain crop to meet its potential evapotranspiration.

### 3.2. Crop Coefficient, Irrigation, and Crop Water Requirement

Table 2 presents the crop and irrigation water requirements, crop coefficients, and effective rainfall values. At the initial stages, the daily average of tomato

water use (ETc) was estimated to be very low, at 2.73, 2.83, and 2.88 mm/day. However, during the development stage, the tomato ETc ranged from 2.93 to 5.26 mm/day, indicating a rapid increase in crop evapotranspiration with the growth of the crop." In the middle stage, the crop water slightly increases from 6.05 to 6.19 mm/day; nevertheless, the tomato $ET_c$ starts to decrease in this stage reaching 5.29 mm, In the final stage, the tomato $ET_c$ experienced a rapid reduction reaching 4.01 mm, which was attributed to senescence and fall of tomato leaves. The crop coefficient of tomato, which is used for quantifying tomato crop water use, is also low at the initial stage and starts to promptly increase at the development and middle stages. At the initial stage, the lowest and highest tomato Kc was 0.6 and 1.15 in the middle stage, and Kc increased with the growth of the crop. Furthermore, the CROPWATT model also provides $ET_c$ in every 10 days.

During the growing period of the crop, as can be seen in Table 2. The average $ET_c$ in mm per decade also matches the one estimated as mm per day, illustrating that it increases with the development of crop stages. During the entire growing period, the total tomato $ET_c$ was estimated as 678.2 mm which is higher than the aforementioned total annual rainfall. The model calculates rain and irrigation water required in decade per millimeter during the growing period, the lowest irrigation is 2.7 mm in the first ten days after planting and rapidly increases reaching 59 mm in the middle stage, and the irrigation decreases in the final stages. The tomato plant growing Afgoye from summer to autumn requires 451.3 an irrigation amount which is supplemented by 230.6 mm of effective rainwater that falls during this period, indicating that the irrigation amount is also lower than $ET_c$ (Figure 4 and Table 3). However, the amount of water loses through $ET_c$ is slightly lower than the total amount of irrigation and rainwater, indicating that the largest portion of water applied to plants as irrigation and rainwater is lost through evapotranspiration. Therefore, some plants that are not resistant to water stress cannot be grown in rainfed areas far from the river.

In Kenya, Maing et al. (2020) reported that the total tomato crop evapotranspiration reached 437.2 mm/dec, which lower than the amount found in this study. However, the growing period and the climate characteristics of their study area differ from this study area (Afgoye). On the other hand, the study area mentioned by the cited researchers receives more rainfall, indicating that the irrigation amount needed to supplement the rainfall is significantly lower comparing to Afgoye where the irrigation amount required is higher than the total amount the effective rains. At Kabete in Kenya Karuku et al. (2014) stated that tomato water requirement in this area reaches 456.5 mm/dec. In some area in Ethiopia, high tomato water requirement was reported (Desta et al., 2017).

The total mean of gross irrigation is estimated as 561.3 mm, while the total mean net irrigation reaches roughly 393.0 mm, demonstrating that irrigation efficiency reaches 70%. Net irrigation (NIR) is the quantity of water required by the crop to meet its evapotranspiration or the quantity of water applied as irrigation to reach field capacity, while gross irrigation is the total amount of water applied to cropping fields. Net irrigation is governed by climatic characteristics, soil types, and cropping patterns (Ewaid et al., 2019). Traditional irrigation practices are mainly used in Somalia, particularly Afgooye and other riverine farming, and river water conveyed by a canal distanced from the river encourages the loss of a significant amount of water through both evaporation and evapotranspiration.

**Table 2.** Tomato crop water requirement (initial, development, middle, and later stages)

| Month | Decade | Stage | $K_c$ | $ET_c$ (mm/day) | $ET_c$ (mm/dec) | Eff rain (mm/dec) | Irr. Req. (mm/dec) |
|---|---|---|---|---|---|---|---|
| Jul | 1 | Init | 0.6 | 2.73 | 2.7 | 1.9 | 2.7 |
| Jul | 2 | Init | 0.6 | 2.83 | 28.3 | 18.6 | 9.7 |
| Jul | 3 | Init | 0.6 | 2.88 | 31.6 | 15.2 | 16.5 |
| Aug | 1 | Deve | 0.6 | 2.94 | 29.4 | 10.9 | 18.6 |
| Aug | 2 | Deve | 0.7 | 3.48 | 34.8 | 7.5 | 27.3 |
| Aug | 3 | Deve | 0.85 | 4.35 | 47.8 | 6.6 | 41.2 |
| Sep | 1 | Deve | 0.99 | 5.26 | 52.6 | 4.4 | 48.2 |
| Sep | 2 | Mid | 1.12 | 6.15 | 61.5 | 2.5 | 59 |
| Sep | 3 | Mid | 1.15 | 6.19 | 61.9 | 7.9 | 54 |
| Oct | 1 | Mid | 1.15 | 6.06 | 60.6 | 14 | 46.6 |
| Oct | 2 | Mid | 1.15 | 5.94 | 59.4 | 18.6 | 40.8 |
| Oct | 3 | Mid | 1.15 | 5.79 | 63.6 | 23.3 | 40.4 |
| Nov | 1 | Late | 1.09 | 5.29 | 52.9 | 31.1 | 21.8 |
| Nov | 2 | Late | 0.97 | 4.54 | 45.4 | 37.4 | 8 |
| Nov | 3 | Late | 0.85 | 4.15 | 41.5 | 29 | 12.5 |
| Dec | 1 | Late | 0.78 | 4.01 | 4 | 1.8 | 4 |
| | | | | | 678.2 | 230.6 | 451.3 |

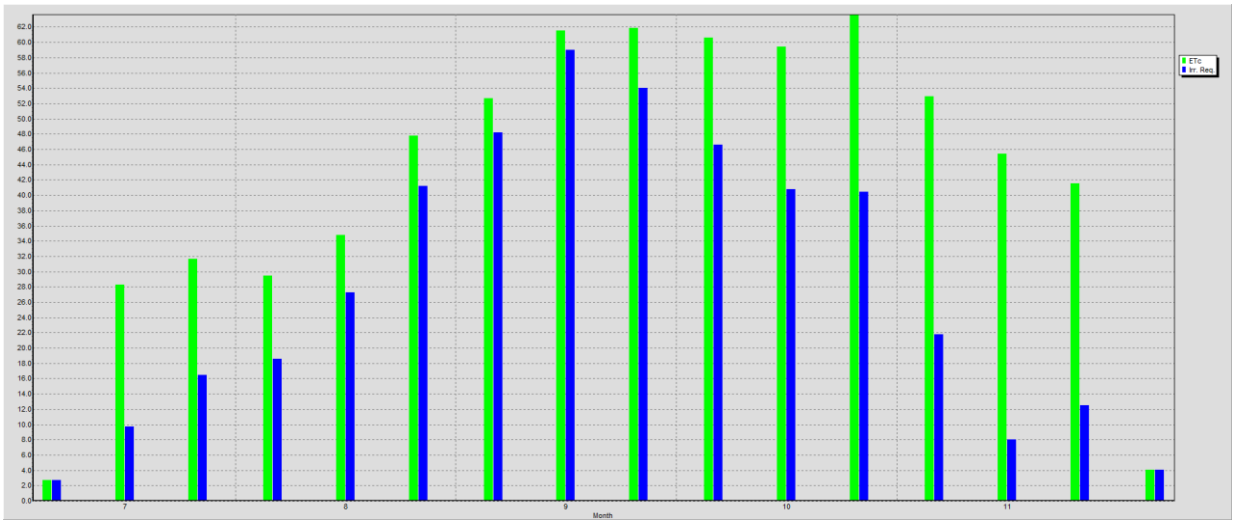


**Figure 4.** ETc and Irrigation requirement.

**Table 3.** Irrigation schedule

| Date | Day | Stage | Rain (mm) | Ks | Depl (%) | Net Irr (mm) | Deficit (mm) | Loss (mm) | Gr. Irr (mm) | Flow (l/s/ha) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1-Aug | 23 | Init | 0 | 1 | 31 | 30.4 | 0 | 0 | 43.4 | 0.22 |
| 21-Aug | 43 | Dev | 0 | 1 | 35 | 49.2 | 0 | 0 | 70.3 | 0.41 |
| 6-Sep | 59 | Dev | 0 | 1 | 37 | 66 | 0 | 0 | 94.3 | 0.68 |
| 20-Sep | 73 | Mid | 0 | 1 | 40 | 80.3 | 0 | 0 | 114.7 | 0.95 |
| 6-Oct | 89 | Mid | 0 | 1 | 41 | 82.3 | 0 | 0 | 117.5 | 0.85 |
| 26-Oct | 109 | Mid | 0 | 1 | 42 | 84.8 | 0 | 0 | 121.2 | 0.7 |
| 1-Dec | End | End | 0 | 1 | 24 | | | | | |
| | | | | | | 392.9 | | | 561.3 mm | |

Using an advanced irrigation systems and exposing the tomato to deficit irrigation can however, increase water use efficiency, as well as crop such soluble solids (Lu et al., 2019). Therefore, it is necessary to use appropriate irrigation practices to increase the efficiency of irrigation water fruit qualities.

## 4. Conclusion

CROPWAT 8.0 is employed to quantify $ET_o$, effective rainfall, crop water requirement ($ET_c$), and irrigation water demand of tomatoes. Climate, soil, and crop data were obtained from the FAO CLIMATWAT 2.0. Irrigation demand of tomato through its whole growing period was estimated as 451.3 mm and the effective rainfall to supplement the tomato crop water requirement was determined as 230.6 mm and the total tomato evapotranspiration reached 678.2 mm, the net and gross irrigation were estimated at 392.9 and 561.3 mm respectively. The results were not obtained from field experiments but were estimated using computer software and secondary data. Therefore, it is necessary to conduct an experimental study to validate or evaluate the accuracy of these findings and/or whether the model overestimates crop water requirements.

Afgoye is an agriculture zone characterized by a semi-arid climate; the irrigation crop water requirement is high, and sometimes there is not enough rainfall and river flow which can support the growth of many staple crops, therefore, it is required to conduct experimental studies to validate the CROPWAT model and determine an effective irrigation technique which improves water use efficiency with optimal crop production.

**Author Contributions**

The percentage of the author contributions is presented below. The author reviewed and approved the final version of the manuscript.

| | Y.M.I. |
|---|---|
| C | 100 |
| D | 100 |
| S | 100 |
| DCP | 100 |
| DAI | 100 |
| L | 100 |
| W | 100 |
| CR | 100 |
| SR | 100 |
| PM | 100 |
| FA | 100 |

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

**Conflict of Interest**

The author declared that there is no conflict of interest.

**Ethical Consideration**

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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# THE EFFECTS OF DIFFERENT CROP LOADS ON YIELD, QUALITY, AND SUGAR FRACTIONS IN EARLY SWEET (*Vitis vinifera* L) TABLE GRAPE

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**Abstract:** The quality of the grapes taken from the vines varies depending on many factors. Grape quality is one of the critical determining factors in the crop load left on the vine. The aim of this study was to investigate the effects of three different crop load levels (36 (T1), 75 (T2), and 105 (T3) bud vine$^{-1}$) on yield, quality, and sugar fractions of Early Sweet (Vitis vinifera L.) table grape variety grown in Alaşehir district of Manisa/Türkiye. As two years average, the heaviest clusters, berry weight, and soluble solid content (733.0g, 4.41g, 18.05%) were determined in T1 crop load level while the lowest weight clusters and berry weight (580.7g, 388g, 17.42%) were obtained from T3 crop load level. The opposite of these findings was observed in titratable acidity values. In the research; the highest amount of table grapes per vine was obtained at T2 treatment in both years. The mean total glucose values for both years varied between 45.70% (T1), 45% (T2), and 37.90% (T3), respectively. Fructose content ranged between 41.50% (T1) and 41% (T3), and sorbitol content was 2.17% (T1), 2.05% (T2), and 2.17% (T3). Galactose content was negligible in all crop load treatments and ranged between 0.54% and 0.56%. The result is also T2 treatment (75 bud vine$^{-1}$) can be recommended to 'Early Sweet' grape growers as the most effective treatment that provides the highest amount of marketable grapes in terms of yield-quality balance.

**Keywords:** Early sweet grape, Crop load, Grape quality, Sugar fraction

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## 1. Introduction

Grapes are one of the most consumed grapefruits in the world. The history of viticulture dates back to 5000 BC. The Anatolian region called Asia Minor is the homeland of the grapevine (Uzun and Bayır, 2008; Senthilkumar et al., 2015). In the world, 7,085,350 hectares of vineyard area are used for fresh grape production. Türkiye has 393.420,000 hectares of vineyards and 3.650,000 million tons of fresh grape production (Anonymous, 2022). The most crucial region in Türkiye in terms of both vineyard area and grape production is the Aegean region. This region alone has 31-38% of the vineyard area and accounts for 50% of the grape production.

Pruning is the most important cultural practice in viticulture. It is the only way to maintain and increase productivity in vineyard farming, to maintain vitality, and to ensure the balance between development and yield (Winkler et al., 1974). The crop load of the vine can be regulated by the number of buds left on the vine during winter pruning or by cluster thinning during the growth period (Pehlivan and Uzun, 2001). In this respect, winter pruning is the primary process determining crop load yield and grape quality. Determining the optimal pruning and crop load for a grape variety's region is crucial for sustainable viticulture (Benavente et al., 2014). Various studies have been carried out in our country and in the world to determine the appropriate pruning levels of different grape varieties (Dardeniz and Kısmalı, 2005; Söyler et al., 2020). Early Sweet is the earliest commercial, seedless, white grape cultivar, excellent eating quality, with a muscat flavor. This grape cultivar is highly desired and famous worldwide. In this respect, the cultivation area in our region has tended to increase in recent years. However, it has been observed that growers perform different pruning procedures on this cultivar and face problems such as not achieving the desired quality.

High sugar concentration in grapes is particularly desirable and is a critical component of edible eating quality, defining ripeness and harvest time. This parameter is often used to evaluate grape berry quality in literature (Gehan et al., 2020). Prior research indicates that grape berries contain significant amounts of glucose and fructose, with their values being similar to each other. Additionally, the glucose and fructose content and proportions can vary widely among grape berries, depending on factors like the grape variety used and the growing conditions of the vineyards (Petrisor et al.,

2019). The content and composition of sugar have a significant influence on the taste, color, and other nutritional components of grapes.

The crop load given to the vines is the most important factor affecting the quality of the grapes, sugar content, sugar-acid balance, and ripening time (Sabır et al., 2010; Ashraf and Farag, 2022). The content and composition of sugar have a great influence on the taste, color, and other nutritional components of grapes. Sugar is an important nutrient in grapes and a sign of ripeness (Gehan et al., 2020).

This study aimed to investigate the effect of different levels of crop load on yield, quality, and sugar fractions in Early Sweet (*Vitis vinifera* L.) table grapes.

## 2. Materials and Methods

### 2.1. Plant Material and Study Site

The research was carried out at the grapevine of Early Sweet (*Vitis vinifera* L.) in the Alaşehir-Manisa/Türkiye in 2021-2022 years. The climate in this region is semi-arid with hot dry summers and cold rainy winters. The average yearly temperature is 18.0 $^0$C and the total amount of annual rainfall is about 635 mm. Early Sweet's vines are highly vigorous when planted on their roots, 6 years old. The planting distances were 3.1 m between the rows and 1.6 m on the rows and vines were trained onto a Y trellis system. A drip irrigation system was used, and the soil structure of these vineyards is the loamy alluvial soil, and the routine cultural processing such as soil management, and fertilizers.

### 2.2. Applications

Winter pruning was carried out in mid-February in both years. The trial design was performed as four treatments:

T1 - 10 buds (3 Long-cane, 2 spurs-cane with 3 buds each), a total of 36 buds per vine,

T2 – 12 buds (5 long, 5 spurs with 3 buds each), a total of 75 buds per vine,

T3 – 15 buds (6 long, 5 spurs with 3 buds each), a total of 105 buds per vine.

### 2.3. Methods of Analysis

At harvest (June 25, when SSC reached 16 °Brix), vines in each treatment plot were weighed to determine fresh grape yield per vine (kg vine$^{-1}$). Five clusters (g) per vine were randomly selected and weighed on a digital balance.

For each cluster, 12 berries were randomly sampled from the shoulder, middle, and tail. Fresh fruits were weighed using a digital balance to determine fruit weight (g). The soluble solid content (SSC) of juices was determined as % using a handheld temperature-compensated refractometer (Atago Pal-1, Japan). The titratable acidity (TA) by titrating 10 mL juice with 0.1 N NaOH to pH 8.1 was expressed as g tartaric acid L$^{-1}$. The pH of berry juices was determined with a pH meter (Mettler Toledo MP220, Zurich, Switzerland).

Marketable table grapes, the amount of marketable table grapes was determined according to Turkish Standards 101 table grape standard (Anonymous, 2002). The percentage of overall yield was calculated

### 2.4. Sugar Fractions of Analysis

For sugar fractions of analysis, we followed the methods described by Melgarejo et al. (2000). One hundred g of berries were crushed with a Heidolph SilentCrusher M (Germany). Seven grams of samples that contained skin and pulp of berries were homogenized with a homogenizator (A-10 Analytical Mill, Tekmar Ohio, USA) after the addition of 50 mL 0.009 N $H_2SO4$. We centrifuged 1 milliliter of whole fruit extract at 10,000 revolutions per minute for 2 minutes at 4$^o$C. Then we passed the supernatants through a SEP-PAK C18 cartridge. HPLC readings were taken using a µbondapak-NH2 column along with 85% acetonitrile as the liquid phase, and a refractive index detector (IR). We performed chromatographic separation on an Agilent 1100 series HPLC using a DAD detector (Agilent, Waldbronn, Germany). Using the sugar fractions, we calculated the sugar contents, and the results are expressed as percent.

### 2.5. Statistical Analysis

The experiment was laid out according to completely randomized blocks with three replicates and each replication had six vines. The data collected underwent statistical analysis using the SPSS statistical software package (version 20.0; SPSS Inc., Chicago, IL, USA). Differences between means were evaluated through ANOVA analysis of variance and determined by the Duncan multiple comparison test ($P<0.05$).

## 3. Results and Discussion

The effects of different levels of crop load left at pruning on the yield of fresh grapes and average cluster and berry weight, percentage of marketable table grapes, SSC (%), titratable acidity (g 100 mL$^{-1}$), and SSC/TA ripening index were found to be statistically significant in both years ($P<0.05$). However, pH was found statistically non-significant in 2021 and 2022. As a result of the variance analysis, three distinct groups were identified (Table 1).

### 3.1. Yield

Yield includes the total amount of fresh grapes. The highest yield was obtained from 105 buds vine$^{-1}$, a group (T3) while the lowest yield was 36 buds vine$^{-1}$, c group (T1) in both years. After analyzing the effect of crop load left on the vine during pruning, it was discovered that the vine productivity increased as the crop load left on the vine increased. In other words, when the total number of buds left per vine in winter pruning increased from 36 to 105 buds in both years, the yield increased by about 49.4%. These results are similar to the results reported in previous studies by Harikanth et al. (2015), Kumar et al. (2017), and Popović et al. (2023) that the yield of fresh grapes increases with an increasing number of buds left in pruning.

### 3.2. Cluster Weight

Average cluster weight, is a very important parameter for the quality of table grapes. The Average cluster weight was found between 508.20 and 740.0 g.

**Table 1.** The effects of different crop load levels on yield and quality properties

| | Treatment, 2021 | | | Treatment, 2022 | | |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T1 | T2 | 73 |
| Yield, kg vine$^{-1}$ | 20.2±2.21c* | 28.75±2.50b | 41.50±3.0 a | 22.1±2.00 c | 27.25±2.20 b | 42.10±2.50 a |
| Cluster weight g | 750.5±25.8 a | 704±30.0b | 580.2±30.3 c | 725.5±24.10a | 770±28.0 b | 581.20±28.3c |
| Berry weight g | 4.40±0.20 **a | 4.26±0.15b | 3.86±0.12 c | 4.42±0.15 a | 4.25±0.15 b | 3.90±0.12 c |
| TSS % | 18.10±1.45 a | 17.90±2.10b | 17.50±1.90 c | 18.00±1.45 a | 17.75±.1.90 b | 17.35±1.90 c |
| TA g tartaric acid 100$^{-1}$ ml | 0.61±0.10 a | 0.64±0.12b | 0.66±0.10 c | 0.60±0.10 c | 0.63±0.12 ab | 0.66±0.10 a |
| TSS/TA | 29.6±1.80 a | 27.9±1.42b | 26.5±1.51 bc | 30.00±1.60 a | 27.73±1.25 b | 26.28±1.32 c |
| pH | 3.88±0.1ns*** | 3.87±0.15ns | 3.80±0.10 ns | 3.75±0.12 ns | 3.70±0.15 ns | 3.68±0.10 ns |
| M. grape**** % | 79.1 b | 86.9 a | 53.6 c | 76.9 b | 88.0 a | 47.5 c |

*a, b= Mean values (means ± SEM**) followed by the same letter in each rows are not significantly different (P>0.05), **SEM: standard error of mean, ***ns= non-significant, T1= 36 buds vine$^{-1}$, T2= 75 buds vine$^{-1}$, T3= 105 buds vine$^{-1}$, ****Markatable grape= total fresh yield (kg)/table grapesx100.

As two years average, the heaviest clusters and berry weight (733.0 g) were determined in treatment T1 (36 buds vine$^{-1}$, a group) while the lowest weight clusters (580.7 g) were obtained in treatment T3 (105 buds vine$^{-1}$, c group) (Table 1). Previous studies (Fawzi et al., 2010; Pehlivan and Uzun, 2015; Popović et al., 2023) in similar research in different grape varieties are also consistent with the assertion that excessive crop load reduces cluster weight.

**3.3. Berry Weight**

The berry weight was found between 3.86 and 4.42 g**.** As the number of pruned buds increased, the number of clusters increased, and cluster weight and berry weight decreased in the grapevine. As a two-year average, the highest berry weight was observed in T1 (4.41 g, a group) and followed by T2 (4.25 g, b group) and T3 (3.88 g, c group), respectively (Table 1). This result is convenient for Zhu-mie et al. (2010), Gil et al. (2013), Benavent et al. (2014), and Popović et al. (2020).

**3.4. SSC (%)**

As the number of buds left per vine increased, the SSC (%) values decreased although the yield increased. Increased exposure of fruit to light has been associated with increased accumulation of soluble solids. The highest SSC (%) value (18.05±1.45, a group) was observed in the T1 treatment, and the lowest value (17.42±1.67, c group) was obtained from the T3 treatment. Somkuwar and Ramteke (2010), Kök et al. (2013), and Söyler et al. (2020) reported that the more the severity of pruning, the lower the percentage of berry drop and fruit increased and acidity decreased when pruning severity increased in Thompson Seedless cultivar. This is consistent with our findings.

**3.5. TA**

The Titratable acidity was found between 0.61 and 0.66 g 100 ml$^{-1}$. The highest TA value (0.66±0.10, a group) occurred in the T3 treatment and the lowest value (0.60±0.10, c group) was determined in the T1 treatment (Table 1). According to Gaser et al. (2017) and Gehan et al. (2020), an increase in crop load results in a higher yield of fresh grapes and acidity values.

**3.6. SSC/TA**

The effect on the SSC/TA ratio in the juice was similar in both seasons studied. Maturation is delayed as the level of buds left in pruning increases. Because the increase in yield causes an increase in acidity and a decrease in SSC. Also, the highest SSC/TA ratio value (29.80) was found in the T1, and the lowest value (26.65) was obtained in the T3 (Table 1). Studies on different grape varieties yielded similar results by Fawzi et al. (2010), Ashraf and Farag (2022), and Popović et al. (2023). Maturation of table grape (Vitis vinifera L.) extends from a period of almost 40 days from véraison to harvest. During this phase of fruit development, the most significant physiological changes occur, allowing for the accumulation of sugar, acid, phenolic compounds, and an increase in weight.

**3.7. Marketable Table Grapes**

When the effect of different crop load levels on marketable table grapes was analyzed (Table 1), the highest amount of table grapes was obtained from T2 (86.9%, 88%) in both years. This was followed by T1 (79.1%, 76.9%) and T3 (53.6%, 47.5%) treatment levels, respectively. This result showed the importance of correct crop load in winter pruning.

**3.8. Sugar Fraction**

The effects of different levels of crop load left during pruning on α-Glucose (%), β-Glucose (%), Total Glucose (%), Sorbitol (%), and Glucose/Fructose were found statistically significant in both years (P<0.05). However, it was found statistically non-significant when Fructose (%) and Galactose (%) were considered (P>0.05) (Table 2).

Grapes contain sugar, which is a crucial nutrient and a sign of ripeness (Petrisor and Chirecanu, 2019). The sugar in grapes is mainly made up of glucose and fructose (Zhang et al. 2021). In analyzing fresh grape samples, glucose, α, and β anomers were determined separately. The total amount of glucose was calculated by adding up α-glucose and β-glucose. As presented in Table 2. total glucose was higher than fructose for T1 and T2 treatment samples and vice versa for T3. The mean total glucose values for both years varied between 45.70 % (T1), 45 % (T2), and 37.90 % (T3), respectively.

**Table 2.** The effects of different crop load levels on sugar fraction

| | Fructose (%) | | | α-Glucose (%) | | | β-Glucose (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2021 | 2022 | Mean | 2021 | 2022 | Mean | 2021 | 2022 | Mean |
| T1 | 41.80 | 41.20 | 41.50 | 17.00 a | 17.20 a | 17.10 a | 29.60 a | 27.60 a | 28.60 a |
| T2 | 41.25 | 40.75 | 41.00 | 16.40 b | 16.80 ab | 16.60 b | 28.80 b | 28.00 a | 28.40 a |
| T3 | 41.50 | 40.50 | 41.00 | 14.30 c | 14.00 c | 14.15 c | 23.40 c | 24.10 b | 23.75 b |
| LSD $_{0.05}$ | ns | ns | ns | | | | | | |

| | Sorbitol (%) | | | Galactose (%) | | | Total Glucose (%) | Glucose/Fructose |
|---|---|---|---|---|---|---|---|---|
| | 2021 | 2022 | Mean | 2021 | 2022 | Mean | | Mean |
| T1 | 2.15 a* | 2.20 a | 2.17 a | 0.54 | 0.54 | 0.54 | 45.70 a | 1.101 a |
| T2 | 2.00 b | 2.10 b | 2.05 a | 0.56 | 0.54 | 0.55 | 45.00 ab | 1.097 b |
| T3 | 2.00 b | 2.15 ab | 2.07 b | 0.55 | 0.53 | 0.54 | 43..90 c | 0.924 c |
| LSD $_{0.05}$ | | | | ns** | ns | ns | | |

*a, b= Mean values (means ± SEM**) followed by the same letter in each rows are not significantly different (P>0.05), **SEM: standard error of mean, ***ns= non-significant, T1= 36 buds vine$^{-1}$, T2= 75 buds vine$^{-1}$, T3= 105 buds vine$^{-1}$.

Fructose content ranged between 41.50 % (T1) and 41 % (T3), and sorbitol contents were 2.17 % (T1), 2.05 % (T2), and 2.17 % (T3). Galactose content was negligible in all of the different crop load treatments and it ranged between 0.54 % and 0.56 % (Table 2). It has been observed that grapes accumulate sugar primarily as glucose and fructose, according to Davies (1996) and Zhang et al. (2021). During harvest, fructose and glucose are found near each other, which aligns with the findings of this study. Additionally, the concentration of other sugars in grape berries tends to be relatively low. They typically undergo hydrolysis and become reducing sugars during transportation from the grape leaves to the berries. The findings are comparable to those of Abd El-Ghany (2006), Gaser et al. (2017), and Coelho et al. (2018). They observed that vines with longer pruning (overload) units had lower total chlorophyll leaf content and sugar accumulation compared to vines with shorter pruning (underload) units.

As a result, these other sugars may only be present in small amounts, Petrisor and Chirecanu (2019) also found that the content of sucrose and other sugars was very low (or even undetectable) in all grape varieties and that the glucose/fructose ratio in grape berries ranged from 1.0 to 1.06 with some varieties having fructose content slightly higher than glucose.

## 4. Conclusion

Pruning (crop load) is considered one of the most important vinicultural practices for grape production. Furthermore, pruning severity (bud load) is extremely important to obtain optimum yield and quality for sustainable viticulture in any grape variety.

Our research discovered that increasing the crop load (number of buds per vine) during winter pruning led to higher fresh grape yield. However, we also observed a decrease in grape quality characteristics and an increase in non-standard products and we found that the loss for table grapes ranged from 33.1% to 40.5%.

According to the results of present study, T2 treatment (12 buds-5 cane-long, 3 buds 5 cane-spurs; total 75 bud vine$^{-1}$) can be recommended to 'Early Sweet' table grape growers as the most effective treatment that provides the highest amount of marketable table grapes with yield-quality balance.

**Author Contributions**

The percentage of the author contributions is presented below. The author reviewed and approved the final version of the manuscript.

| | H.Ç. |
|---|---|
| C | 100 |
| D | 100 |
| S | 100 |
| DCP | 100 |
| DAI | 100 |
| L | 100 |
| W | 100 |
| CR | 100 |
| SR | 100 |
| PM | 100 |
| FA | 100 |

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

**Conflict of Interest**

The author declared that there is no conflict of interest.

**Ethical Consideration**

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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# INVESTIGATION OF THE EFFECTS OF SOME PLANT ACTIVATORS AGAINST VERTICILLIUM WILT (*Verticillium dahliae* Kleb.) ON COTTON

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**Abstract:** Wilt disease caused by *Verticillium dahliae* Kleb. is one of the stress factors affecting yield and fiber quality traits in cotton cultivation. Plant activators provide resistance by stimulating genes that activate the resistance mechanism in the plant. The aim of this study is to determine the effect of plant activators such as auxiGRO, Green Miracle, Maxicrop, ProAct Plus, and Sojall Vitanal against *Verticillium* wilt under both *in vitro* and *in vivo* conditions. Firstly, the effect of various concentrations of plant activators (0, 1, 5, 25, 100, 250, and 500 ppm) on mycelial growth of two fungal isolates of *V. dahliae* (PHCVd3-non-defoliating pathotype and PHCVd47-defoliating pathotype) in potato dextrose agar (PDA) media was investigated *in vitro*. The effect of plant activators on *V. dahliae* was determined in tolerant cotton plants (cv Carmen) and susceptible cotton plants (cv Acala SJ2) in two different ways seed coating and foliar application *in vivo*. *In vitro* experiments were carried out with three replicates, and *in vivo* experiments were with five replicates depending on a completely randomized plot design. 500 ppm dose of all plant activators inhibited the mycelial growth of both isolates by approximately 90%. The lowest disease index (DI) against PHCVd3 was determined as 1.43 in the tolerant cv Carmen with seed coating of auxiGRO. The lowest DI against PHCVd47 was found in Sojall Vitanal and ProAct Plus at 2.09 and 2.12, respectively. The lowest DI against both isolates was found as 1.42 and 2.18 in cv Carmen by foliar application of ProAct Plus, respectively. Plant activators did not show any inhibitory effect on disease severity against both isolates in cv Acala SJ2. The combination of tolerant cultivar + plant activators can be suggested against *Verticillium* wilt disease as an alternative control.

**Keywords:** *Verticillium dahliae*, Plant activators, Disease index, Alternative control, Cotton

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## 1. Introduction

Cotton is a leading source of natural fiber, cotton seed oil, and livestock feed in more than 80 countries, covering an area of ~35 million hectares (Jans et al., 2021). Upland cotton (*Gossypium hirsutum* L.) ranks first in world production and accounts for 90% of world cotton production (Chen et al., 2007). Therefore, 99.5% of Türkiye's cotton is *G. hirsutum* L. species cotton (Gürel et al., 2000). Türkiye is the sixth largest cotton producer in the world after China, India, USA, Brazil, and Pakistan (USDA, 2021). In Türkiye, cotton is grown in 477.000 hectares in 4 main regions, yielding 2.2 million tons of seed cotton yield (TSI, 2021).

The soil-borne fungus *Verticillium dahliae* causes *Verticillium* wilt, one of the most important diseases of cotton (Klosterman et al., 2009). The fungus infects more than 400 plant species, including cotton (Berlanger and Powelson, 2000). *Verticillium* wilt results in a yield loss of around 10-35% worldwide (Song et al., 2020). Nowadays, places where cotton is grown have both defoliating and non-defoliating pathotypes of the disease (Bejarano-Alcazar et al., 1995). The fungus survives in the soil as microsclerotia. Microsclerotia form in the dead plant, thus increasing the inoculum potential for future years (Huisman and Ashworth, 1976). Pathogen blocks the movement of water and other minerals from the root to the leaves and tissues. Then it causes wilting, desiccation, reduced photosynthesis, shedding of small bolls, and changes in yield and fiber quality characteristics, starting with the lower leaves (Agrios, 2005). Today, an effective chemical control against *Verticillium* wilt has not been developed yet. In this context, alternative control methods are needed in the control against *V. dahliae*.

The use of plant activators for sustainable agriculture is increasing day by day. The development and practical use of new substances that stimulate the physiological activity of plants have gained momentum (Akbudak and Tezcan, 2006). These substances are a source of plant

growth regulators, organic osmolytes, amino acids, and macro and micronutrients (Khan et al., 2009). Plant activators are environmentally friendly and non-toxic preparations that increase plant growth by positively affecting the morphological and physiological properties of plants (Kunicki et al., 2010), strengthen the natural defense mechanisms of plants, and increase the resistance of plants to abiotic and biotic stress factors (Bulgari et al., 2015). Hcm1 containing harpin protein suppressed the growth of *V. dahliae* and *Fusarium oxysporum*, at the same time Hcm1 can activate innate immunity and prevent *Verticillium* and *Fusarium* wilt in cotton (Zhang et al., 2016). Green Miracle plant activator is a long-chain fatty acid-based new-generation stress alleviator for improving the plant health (Bursalioglu and Aki, 2018). The study aims to determine the effect of some plant activators against two fungal isolates of *V. dahliae* (PHCVd3-non-defoliating pathotype and PHCVd47-defoliating pathotype) under both *in vitro* and *in vivo* conditions.

## 2. Materials and Methods

### 2.1. Plant Materials, Plant Activators and Fungal Pathogen

Cotton cultivars (cv) tolerant Carmen (*Gossypium hirsutum* L.), and susceptible Acala SJ2 (*G. hirsutum* L.) were used as plant material (Bolek et al., 2005; Erdoğan et al., 2014). Plant activators such as auxiGRO (30 g/100 l water), Green Miracle (200 ml/100 l water), Maxicrop (30 g/100 l water), ProAct Plus (10 g/100 l water), Sojall Vitanal (60 ml/100 l water) licensed in many cultivated plants including cotton were used in the study. Pure cultures of *V. dahliae* (PHCVd3 isolate-non-defoliating pathotype; PHCVd47 isolate-defoliating pathotype) were provided by Prof. Dr. Şener KURT (Hatay Mustafa Kemal University/Türkiye). Pathogen isolates were grown in the dark at 24±1°C for 14 days and after that subcultured on potato dextrose agar (39 g/l, PDA-Difco) media.

### 2.2. *In-Vitro* Studies

#### 2.2.1. Determination of the effects of plant activators on mycelial growth of isolates of *V. dahliae*

A variable concentration of plant activators (1, 5, 25, 100, 250, and 500 ppm) was added to the sterilized PDA medium before being dispensed in 25 ml portions into sterilized Petri plates (90 mm). PDA medium with plant activators was kept at room temperature for 24 hours. A single 5-diameter- mycelium disc taken from the leading growth edge of 7-day-old cultures of both pathotypes of *V. dahliae* grown on PDA was placed in the center of a Petri dish containing plant activator + PDA. As a control, a disc of *V. dahliae* was grown on a PDA plate. The radius of each fungal colony was measured after a 14-day incubation at 24±1 °C in the dark. The relative growth inhibition was expressed as a percentage [(control–treatment)/control x 100] (Deans and Svoboda, 1990). This experiment was performed using a fully randomized parcel design with three replicates and was replicated twice.

### 2.3. *In Vivo* Trials

#### 2.3.1. Determination of the effects of seed coating applications of plant activators against *V. dahliae*

The effects of five plant activators against *Verticillium* wilt were tested in a plant growth room on two cultivars of cotton plants (cvs Carmen and Acala SJ2). The coated with plant activators and control seeds were each planted into 10-cm diameter plastic pots containing an autoclaved soil–sand–peat (1:1:1) mixture. Then, when the cotton seedlings reached the cotyledon stage, thinning was performed, and one seedling was left in each pot. To determine the susceptibility of plant activators-coated cotton cultivars to *V. dahliae* (Erdoğan et al., 2014) two-week-old spores cultured in broth medium (0.01 g $FeSO_4.7H_2O$, 0.5 g $MgSO_4.7H_2O$, 2 g $NaNO_3$, 1 g $K_2HPO_4$, 0.5 g KCl, and 7.5 g sucrose, 1 l sterile distilled water) cultured isolates of PHCVd3 and PHCVd47 were filtered through 2 layers of cheesecloth and mycelium and pieces of agar were removed from the suspension and then the spore concentration was adjusted to $4 \times 10^6$ spores/ml using a Thoma slide in the light microscope (Leica) and used for the inoculation of cotton plants. The plants were transplanted into new plastic pots with spore solution (10 ml) when they reached the six-true-leaf stage. Plants were incubated at 24±1ºC, with 12 hours light / 12 hours dark conditions. Control plants were applied with sterile water. 30-35 days after inoculation, disease severity was assessed for each plant on a 0-to-5 rating scale according to the percentage of foliage affected by acropetal chlorosis, necrosis, wilt, and/or defoliation (0 = no symptoms, 1 = chlorosis in the lower leaves, 2 = moderate (30-50% of the leaves) wilt with severe chlorosis, 3 = moderate wilting and necrosis, 4 = severe (more than 50% of leaves) wilting and necrosis, 5 = dead plant) (Tsror et al., 2001). The pot experiment was performed with five replicates in a fully randomized parcel design.

#### 2.3.2. Determination of the effects of foliar applications of plant activators against *V. dahliae*

Plants of Carmen and Acala SJ2 were grown in the plant growth room until the six-true-leaf stage. As in the seed coating application, *V. dahliae* isolates were obtained and spore concentrations were adjusted. Pathogen isolates were applied according to the conidia suspension technique. Recommended doses of plant activators were sprayed on the leaves of cotton plants as a first application one day after pathogen application. The second application was sprayed 7 days after the first application and the third application was sprayed 14 days later. Control plants were sprayed with sterile water. 30-35 days after inoculation, disease severity was assessed for each plant on a 0-to-5 rating scale. The experiment was performed with five replicates in a fully randomized parcel design. The disease index (DI) value was determined according to the method described by Karman's (1971) formula (Equation 1)

$$DI= (ax0) + (bx1) + (cx2) + (dx3) + (ex4) + (fx5)/M \quad (1)$$

where a, b, c, d, e and f are the plant numbers with degrees 0, 1, 2, 3, 4, and 5, respectively, and M is the overall plant number.

**2.4. Statistical Analysis**

The data were subjected to analysis of variance (ANOVA) using JMP software version 13 (SAS Institute Inc., Cary, NC, USA). Statistical software and the means were grouped using the LSD (0.01) test (Genç and Soysal, 2018).

## 3. Results and Discussion

**3.1. The Effects of Plant Activators on Mycelial Growth of Isolates of *V. dahliae***

The plant activator doses were found to be significant according to the statistical analysis results (P≤0.01) of the *in vitro* experiment. In the high dose (500 ppm) application of Maxicrop, the lowest mycelial diameter (MD) was found at 1. 00 mm and 2.83 mm, respectively; the highest effect was determined at 96.80%, and 91.00% respectively, against PHCVd3 and PHCVd47 isolates. In the high dose of Sojall Vitanal, the lowest MD was found at 1.00 mm and 3.83 mm against PHCVd3 and PHCVd47 isolates, respectively; the highest effect was detected at 96.70% against PHCVd3 and 87.80% against PHCVd47, respectively. In the high dose of Green Miracle, the lowest MD was determined at 1.08 mm and 3.42 mm, respectively; the highest inhibition rate was measured at 96.50% and 89.20%, respectively against both isolates. In the high dose of ProAct Plus, the lowest MD was found at 1.08 mm and 4.00 mm, respectively; the highest effect was determined at 96.50% and 87.30%, respectively against both isolates. In the high dose of auxiGRO, the lowest MD was found at 1.42 mm and 4.58 mm, respectively, and the highest effect was found at 95.60% and 85.50%, respectively against both isolates (Table 1).

In a similar study, Yildirim and Yapici (2007) reported that the harpin protein was 76% effective at 1000 µg/ml concentration and showed a high effect even at low doses against the gray mold caused by *Botrytis cinerea* in strawberries. Zhang et al. (2016) stated that Hcm1-containing harpin protein suppresses the growth of *V. dahliae* and *Fusarium oxysporum in vitro*, and that Hcm1 can activate innate immunity. Aysan et al. (2019) have reported that salicylic acid (SA) and Fosetyl-Al at concentrations above 700 µg/ml *in vitro* suppressed mycelial growth of strawberry black root rot caused by *R. solani*, plant activators such as Acibenzolar-S-Methyl (A-S-M), Messenger, ISR-2000, Crop-Set did not inhibit mycelial growth of the pathogen.

Our results are not similar to Şahbaz and Akgül (2016) found that the plant activators Fosetyl-al, SA, A-S-M + Metalaxyl-m and ISR-2000 did not inhibition against mycelial growth of *F. oxysporum* f. sp. *vasinfectum* and *V. dahliae in vitro*.

**3.2. The Effects of Seed Coating Applications of Plant Activators on *V. dahliae***

Application x cultivar interaction was found to be significant according to the statistical analysis results (P≤0.01) of the *in vivo* experiment. Disease index (DI) values of PHCVd47 isolate were higher than PHCVd3 isolate in tolerant cv Carmen and susceptible cv Acala SJ2. The lowest DI value against the PHCVd3 isolate was obtained from the plant activator of auxiGRO (1.43) in the tolerant cotton plant (cv Carmen) (Figure 1), followed by the plant activator of Green Miracle (1.50) and Sojall Vitanal (1.54). The lowest DI value against the PHCVd3 isolate was found in the plant activator Maxicrop (2.92) in the susceptible cotton plant (cv Acala SJ2). The lowest DI value against the PHCVd47 isolate was obtained from the plant activators of Sojall Vitanal (2.09) and ProAct Plus (2.12) in cv Carmen (Figure 1), and these activators were statistically in the same group. The lowest DI value was found in the plant activator Maxicrop (3.28) in cv Acala SJ2 (Table 2).

According to our results, plant activators containing GABA + L-Glutamic acid, *Lactobacillus acidophilus*, and harpin protein active substances showed the highest effect against both pathotypes of the pathogen. Harpin protein activates the natural resistance mechanism of plants against diseases and pests and increases the resistance of the plant against pathogen attack (Strobel et al., 1996). Kinnersley and Turani (2000) reported that GABA increased the plant's resistance to disease as it reduced stress against the pathogen. *L. acidophilus* has been reported to increase plant resistance through induced systemic resistance (Anonymous, 2017). Our results are in agreement with those of Tosun et al. (2003) reported that applied SA and harpin alone and together with fungicide (Agrifos 400), then infected the plants with *Phytophthora infestans* and found SA 47%, harpin 55%, Agrifos 400 88% suppressed the disease. High efficacy results have been obtained in Messenger Gold (MG), MG + ERS-T22 Planter Box + Bordeaux mixture, and MG + Bordeaux mixture applications. Application of MG and in combination with MG suppressed the *Verticillium* wilt disease (Arici and Demirtas, 2019).

**Table 1.** Inhibitory effect of plant activators on mycelial growth of isolates of *V. dahliae*

| Plant activators | Dose (ppm) | PHCVd3 isolate | | PHCVd47 isolate | |
|---|---|---|---|---|---|
| | | MG (mm) | MGI | MG (mm) | MGI |
| | 1 | 23.75 b* | 25.40 | 28.25 b | 10.80 |
| | 5 | 19.67 c | 38.20 | 23.75 c | 25.00 |
| | 25 | 14.75 d | 53.70 | 19.83 d | 37.40 |
| auxiGRO | 100 | 10.17 e | 68.10 | 14.83 e | 53.20 |
| | 250 | 5.50 f | 82.70 | 9.75 f | 69.20 |
| | 500 | 1.42 g | 95.60 | 4.58 g | 85.50 |
| | Control | 31.83 a | 0.00 | 31.67 a | 0.00 |
| $F_{application}$ | | | ** | | ** |
| $CD_{(p=0.01)}$ | | | 6.6 | | 3.3 |
| | 1 | 25.50 b | 21.40 | 27.33 b | 13.70 |
| | 5 | 17.58 c | 43.50 | 19.58 c | 38.20 |
| | 25 | 13.92 d | 55.40 | 18.50 c | 41.60 |
| Green Miracle | 100 | 8.75 e | 71.90 | 11.75 d | 62.90 |
| | 250 | 4.42 f | 85.80 | 7.33 e | 76.80 |
| | 500 | 1.08 g | 96.50 | 3.42 f | 89.20 |
| | Control | 31.17 a | 0.00 | 31.50 a | 0.00 |
| $F_{application}$ | | | ** | | ** |
| $CD_{(p=0.01)}$ | | | 6.7 | | 3.9 |
| | 1 | 24.08 b | 22.80 | 26.00 b | 17.00 |
| | 5 | 16.08 c | 48.50 | 21.33 c | 31.70 |
| | 25 | 11.08 d | 64.50 | 13.00 d | 58.30 |
| Maxicrop | 100 | 6.92 e | 77.90 | 8.83 e | 71.70 |
| | 250 | 4.00 f | 87.20 | 5.33 f | 82.90 |
| | 500 | 1.00 g | 96.80 | 2.83 g | 91.00 |
| | Control | 31.17 a | 0.00 | 31.17 a | 0.00 |
| $F_{application}$ | | | ** | | ** |
| $CD_{(p=0.01)}$ | | | 9.7 | | 3.6 |
| | 1 | 23.92 b | 23.10 | 27.58 b | 12.10 |
| | 5 | 15.67 c | 49.60 | 23.42 c | 25.70 |
| | 25 | 12.08 d | 61.10 | 18.67 d | 40.60 |
| ProAct Plus | 100 | 9.33 e | 70.00 | 13.58 e | 56.80 |
| | 250 | 5.00 f | 83.90 | 7.17 f | 77.10 |
| | 500 | 1.08 g | 96.50 | 4.00 g | 87.30 |
| | Control | 31.08 a | 0.00 | 31.42 a | 0.00 |
| $F_{application}$ | | | ** | | ** |
| $CD_{(p=0.01)}$ | | | 8.1 | | 3.3 |
| | 1 | 23.58 b | 22.50 | 27.42 b | 12.10 |
| | 5 | 17.08 c | 43.80 | 22.17 c | 28.90 |
| | 25 | 11.50 d | 62.20 | 17.92 d | 41.90 |
| Sojall Vitanal | 100 | 8.50 e | 72.00 | 14.08 e | 54.70 |
| | 250 | 3.50 f | 88.50 | 8.58 f | 72.50 |
| | 500 | 1.00 g | 96.70 | 3.83 g | 87.80 |
| | Control | 30.42 a | 0.00 | 31.17 a | 0.00 |
| $F_{application}$ | | | ** | | ** |
| $CD_{(p=0.01)}$ | | | 6,9 | | 2.6 |

*Mean values followed by different letters within the column are significantly different according to LSD Test, **(P≤0.01), MG= mycelial growth, MGI= mycelial growth inhibition (%), CD= critical difference.

### 3.3. The Effects of Foliar Applications of Plant Activators on *V. dahliae*

The statistical analysis of the pot experiments data revealed that application x cultivar interaction differences were significant (P≤0.01). DI values of PHCVd3 and PHCVd47 isolates were lower in tolerant cotton plants (cv Carmen) than susceptible cotton plants (cv Acala SJ2). The lowest DI value against PHCVd3 isolate was found in the application of ProAct Plus (1.42) in tolerant cotton plants (cv Carmen) (Figure 2). The lowest DI value was determined in susceptible cotton plants (cv Acala SJ2) with Maxicrop (3.02) and Green Miracle (3.07). The lowest DI value against PHCVd47 isolate was detected in the application of ProAct Plus

(2.18) in tolerant cv Carmen (Figure 2). The lowest DI value was found in susceptible cv Acala SJ2 with Maxicrop (3.29) (Table 3).

The highest effect of plant activators sprayed on leaves was again determined in plant activators with GABA + L-Glutamic acid, *L. acidophilus*, and harpin protein active ingredient. In a similar study, Bishnoi and Pavyavula (2004) reported that Harpin and A-S-M activators reduced the severity of leaf blight by 8-12% in tomato cultivars and did not affect scab of canola in canola cultivars. Delisoy and Altınok (2019) determined that auxiGro reduced disease development by 49.25%, Crop-Set by 41.80% and, ISR-2000 by 35.82% compared to positive control. Tuğlu (2019) reported that *L. acidophilus* yeast extract and Benzoic acid, A-S-M + Metalaxyl-M and harpin protein reduced the severity of hazelnut powdery mildew disease by 49.26%, 55.83%, and 33.34%, respectively.

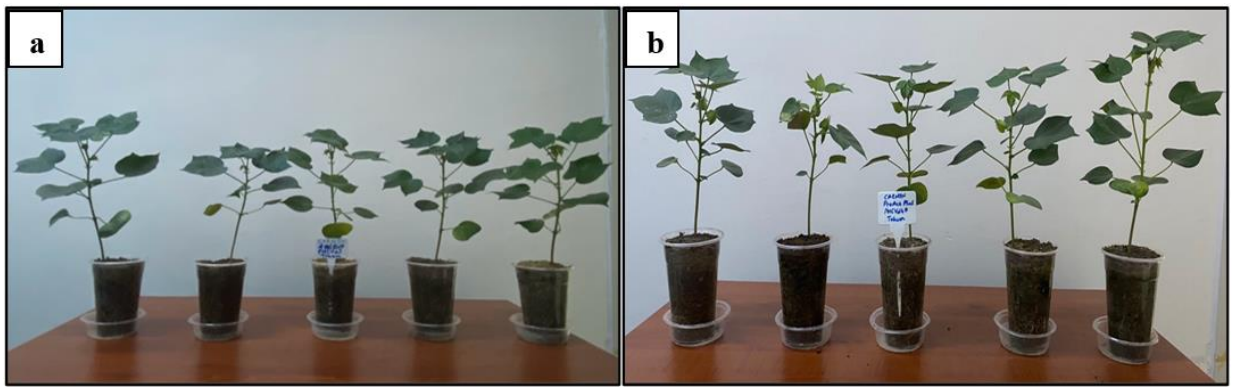


**Figure 1.** (a) Effect of auxiGRO against PHCVd3 isolate in the seed coating tolerant Carmen cultivar, (b) Effect of ProAct Plus against PHCVd47 isolate in the seed coating tolerant Carmen cultivar.

**Table 2.** Disease index values in cotton cultivars seed coated with plant activators after PHCVd3 and PHCVd47 inoculation

| Plant activators | Carmen cultivar | | Acala SJ2 cultivar | |
|---|---|---|---|---|
| | PHCVd3 DI | PHCVd47 DI | PHCVd3 DI | PHCVd47 DI |
| auxiGRO | 1.43 c* | 2.20 ab | 3.08 bc | 3.54 b |
| Green Miracle | 1.50 bc | 2.17 ab | 3.00 bc | 3.50 bc |
| Maxicrop | 1.59 b | 2.21 ab | 2.92 c | 3.28 c |
| ProAct Plus | 1.58 b | 2.12 b | 3.20 b | 3.60 b |
| Sojall Vitanal | 1.54 bc | 2.09 b | 3.12 b | 3.67 b |
| Control | 1.76 a | 2.32 a | 3.61 a | 4.20 a |
| $F_{cultivar\ x\ apllication}$ | ** | ** | ** | ** |
| $CD_{(P=0.01)}$ | 5.6 | 5.5 | 4.9 | 4.9 |

*Mean values followed by different letters within the column are significantly different according to LSD Test, **P≤0.01, DI= Diseases index, CD= Critical difference.

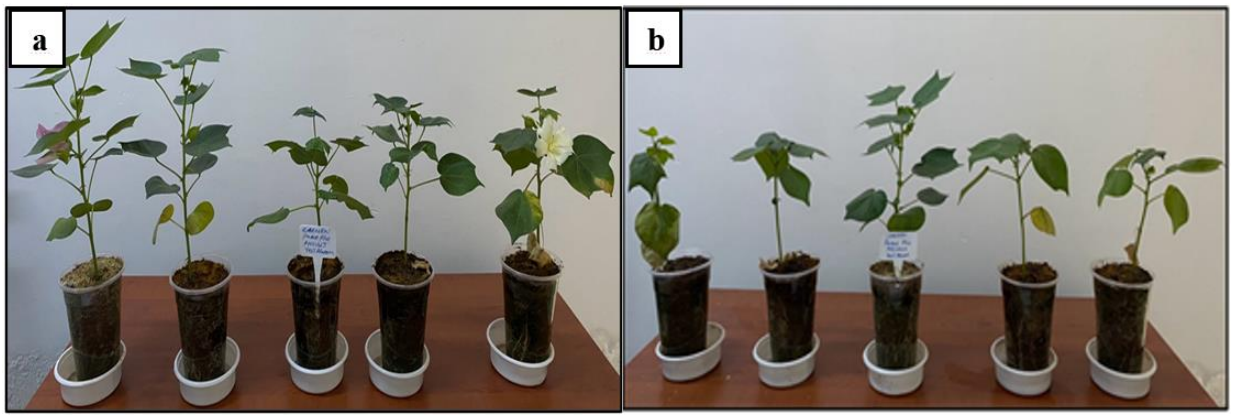


**Figure 2.** (a) Effect of ProAct Plus against PHCVd3 isolate, (b) PHCVd47 isolate in the foliar application tolerant Carmen cultivar.

**Table 3.** Disease index values in cotton cultivars foliar applicated with plant activators after PHCVd3 and PHCVd47 inoculation

| Plant activators | Carmen cultivar | | Acala SJ2 cultivar | |
|---|---|---|---|---|
| | PHCVd3 DI | PHCVd47 DI | PHCVd3 DI | PHCVd47 DI |
| auxiGRO | 1.50 c* | 2.25 b | 3.12 cd | 3.58 c |
| Green Miracle | 1.57 bc | 2.22 bc | 3.07 d | 3.54 c |
| Maxicrop | 1.64 b | 2.25 b | 3.02 d | 3.29 d |
| ProAct Plus | 1.42 d | 2.18 c | 3.25 b | 3.64 bc |
| Sojall Vitanal | 1.51 c | 2.23 bc | 3.19 bc | 3.72 b |
| Control | 1.80 a | 2.35 a | 3.66 a | 4.35 a |
| $F_{\text{cultivar x apllication}}$ | ** | ** | ** | ** |
| $CD_{(P=0.01)}$ | 6.7 | 2.2 | 2.4 | 2.4 |

*Mean values followed by different letters within the column are significantly different according to LSD Test, **P≤0.01, DI= Diseases index, CD= Critical difference.

## 4. Conclusion

High doses of plant activators MaxiCrop, Sojall Vitanal, ProAct Plus, Green Miracle and auxiGRO inhibited the mycelial growth of isolates of PHCVd3 and PHCVd47 by approximately 90%. AuxiGRO against PHCVd3 isolate, Sojall Vitanal and ProAct Plus against PHCVd47 isolate in tolerant cotton cultivar (Carmen) with seed coating with plant activators, and ProAct Plus plant activator against both isolates in foliar application tolerant cv Carmen was found promising. Plant activators coated on seeds and sprayed on leaves did not reduce disease severity against both pathotypes in susceptible cotton cultivar (Acala SJ2). In this context, the combination of tolerant cultivar + plant activators can be suggested against *Verticillium* wilt disease as an alternative control, which is the best alternative within the scope of integrated control. However, we need detailed studies related to assessing the licensed doses of plant activators in cotton cultivars and *Verticillium* wilt under field conditions.

**Author Contributions**

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

| | Z.S. | O.E. |
|---|---|---|
| C | 50 | 50 |
| D | 50 | 50 |
| S | | 100 |
| DCP | 50 | 50 |
| DAI | | 100 |
| L | 50 | 50 |
| W | | 100 |
| CR | 50 | 50 |
| SR | | 100 |

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision.

**Conflict of Interest**

The authors declared that there is no conflict of interest.

**Ethical Consideration**

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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# COMPARISON OF GOOSE BREEDING ACTIVITIES IN UŞAK AND AFYONKARAHISAR PROVINCES

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**Abstract:** This study was carried out in order to reveal the current situation of goose breeding in extensive and semi-intensive conditions in Afyonkarahisar and Uşak provinces and to determine the important problems encountered in breeding. The material of the study; consists of a total of 200 survey data, 125 from the villages of the Merkez, Bolvadin, Sinanpaşa, Sultandağı, Çobanlar, and İhsaniye districts of Afyonkarahisar province, 75 from the Central, Banaz, Sivaslı, and Karahallı districts of the Uşak province. According to the research findings, it has been determined that more than half of the producers in Afyonkarahisar and Uşak have an average of 1-10 years of goose breeding. It has been determined that the number of breeding male geese per farm is 1-5, and the average number of breeding female geese is 3-20 (M/F: 1/3-5/20). It has been determined that 64% of the goose shelters in Afyonkarahisar and 50.6% in Uşak are made of briquettes or bricks. In conclusion, it can be said that the main problems of the producers are feed costs, inadequacies in care and feeding, breeding with low-yielding domestic breeds, difficulties in the supply of breeding animals, and problems in marketing. Expanding the scope of the goose incentive will provide an opportunity to prevent losses in our goose stock and to increase our goose presence again in the future.

**Keywords:** Goose, Breeding, Problems, Solution proposals

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## 1. Introduction

In general, goose breeding is concentrated in areas with cold climatic conditions. According to FAO's 2022 data, more than 87.20% of goose production in the world is made in the Asian continent, while approximately 98% of the production in the continent takes place in China. After the Asian continent, approximately 6.70% of goose production is in the African continent, in countries such as Mozambique, Egypt, and Madagascar, and 5.30% in the European continent, Poland, Romania, Hungary, and Türkiye, etc. taking place in countries. Türkiye accounts for about 5% of the European goose production. The American continent, on the other hand, meets only 0.20% of the world goose production (Akin, 2022; FAO, 2022). As in the whole world, the share of goose breeding in total poultry production in Türkiye is quite low, less than 1%. The low egg production of the geese and the long slaughtering period have a significant effect on this situation. In addition, hot and dry climatic conditions make cultivation impossible (Şengül and Yeter, 2020). Despite the mentioned negativities, in recent years, goose breeding has been increasing its importance among alternative livestock activities that attract attention in Türkiye as well as all over the world. Goose breeding is mostly produced for meat in line with the demands of consumers, liver, and feathers are in demand in European countries. In Türkiye, goose breeding is common in rural areas at the level of small family businesses and consists of 10-15 geese flocks (Boz, 2015; Akin, 2022; Akin, 2023).

Although goose breeding is carried out in all regions in Türkiye, in the North East Anatolia Region where cold climatic conditions exist; it is concentrated around Kars, Ardahan, Erzurum, Ağrı, and Muş. Goose breeding is common in Samsun and Çorum in the Central and Western Black Sea Regions, Yozgat and Kırşehir in Central Anatolia, Isparta in the Mediterranean, and Kütahya, Afyonkarahisar, and Uşak in the Central Aegean (İşgüzar and Pingel, 2003; Saatçi, 2008; Çelik and Bozkurt, 2009; Tilki et al., 2011; Yakan et al., 2012; Boz, 2015; Akin and Çelen 2020; Akin, 2022). Since geese have higher grazing abilities compared to other poultry species, they can consume weeds and they can resist difficult environmental conditions, goose breeding is done more in cold and rural areas than in other regions. Production is carried out in the form of grazing on open pastures under extensive conditions and is carried out by small-scale familial enterprises consisting of 10-15 heads of geese. In familial farms, the production of domestic goose breeds, which are usually divided into black, white, gray, and tawny varieties, is common (Selçuk et al., 1983; İşgüzar and Pingel, 2003; Boz et al., 2014). With the increase in demand for goose meat in recent years, the number of commercial enterprises producing semi-

intensive and intensive production with a capacity of 100-1,000 head of goose is increasing (Akin, 2022). Generally, goose breeding is carried out in order to meet the animal protein needs of the family, and the leftover production is sold in local markets and contributes to the family economy.

The mentioned provinces are very suitable for goose breeding in terms of climatic conditions and draw attention as an important livestock activity in rural areas. In the Aegean Region, as in other provinces, the traditional extensive production system has been adopted. The geese are grazed in the pasture for up to 1-1.5 months before slaughter, and they are fed with grains such as corn, wheat, and barley, as well as bread and food scraps as supplementary feeding. It has been observed that the use of factory feed is at very low levels (Akin, 2022; Akin, 2023). As in all livestock activities in Türkiye, feed costs are the biggest problem in sustainable livestock breeding. In addition, as a result of the loss of qualifications of many agricultural lands, livestock activities become increasingly difficult and producers have to withdraw from the sector.

According to TUIK 2022 data, there has been a decrease in all livestock activities and product amounts in Türkiye compared to the previous year. According to 2021, laying hen production from 120 million to 110 million, broiler production from 270 million to 251 million, turkey production from 4.7 million to 3.6 million, goose production from 1.4 million to 1.3 million, and duck presence from 500 thousand, It was announced that this number decreased to 400 thousand (Akin, 2023; TUIK, 2023a; TUIK, 2023b).The presence of geese in regions in Türkiye between the years 2013-2022 is shown in Table 1. The presence of geese in the Aegean Region is in Table 2, and the presence of geese at the district level of Afyonkarahisar and Uşak provinces is in Table 3 and Table 4 (TUIK, 2023a). According to the data for 2022 in Türkiye, the North Anatolian Region is in the 1st place with the number of geese exceeding 690 thousand, and it constitutes approximately 50% of the goose population of Türkiye. Afterward, Central Anatolia ranks 2nd with more than 121 thousand geese and 9% of the total production, and South East Anatolia ranks 3rd with nearly 104 thousand geese and meets 8% of the total production.

The Aegean Region, on the other hand, has a share of 6% in the total production with the number of geese approaching 85 thousand. The geese presence in the region continued to increase periodically every year, from 68,000 in the first 5 years. While the goose population of the region increased by 40% to 96,000 in 2017, it increased from 102,000 to 104,000 by 2020 in the second 5-year period, and then decreased to 85,000 at the end of 2022, with a decrease of 18% compared to 2021. Afyonkarahisar, Kütahya, and Uşak have an important place in goose breeding in the Aegean Region. In the first 5-year period covering the years 2013-2017, Afyonkarahisar ranked first in the region with around 30,000 geese, and the share of geese in the region (SGR) was around 40%. As of 2017, Kütahya ranked first with a goose production exceeding 44,000 (SGR 45%). On the other hand, Uşak doubled the number of geese (SGR 4%) from 3.000 as of 2017 and exceeded 6.000. In the second 5-year period covering the years 2018-2022, Kütahya decreased from 42,000 geese to 33,000 as of 2022, while Afyonkarahisar decreased from 32,000 to 21,000. In this period, Uşak increased from 9,000 units to 23,000 units as of 2020 (SGR 22%), then decreased to 18,000 units (SGR 18%), and then to 12,000 units by 2022 (SGR 14%).

In Afyonkarahisar, goose breeding is concentrated in İhsaniye, Sinanpaşa, Merkez, Bolvadin, Sultandağı, and Çobanlar districts. In the last 10 years, covering the years 2013-2022, 5 districts met 95% of the total goose production. In Uşak, Merkez and Banaz are the districts with the highest production in the province, and Uşak province constitutes 95% of goose production. This study; has tried to present information about the existence and share of geese in Afyonkarahisar and Uşak provinces in the Aegean Region, demographic characteristics of breeders in both provinces, comparison of goose breeding activities, problems of breeders, and solutions to their problems.

## 2. Materials and Methods

The study was formed from the survey data conducted with the goose breeders in the villages of Bolvadin, Çobanlar, İhsaniye, Merkez, Sinanpaşa, and Sultandağı districts of Afyonkarahisar province, and the goose breeders in the villages of Banaz, Karahallı, Merkez and Sivaslı districts of Uşak, according to the data of TUIK for the year 2022, in February-May 2023. The questionnaire forms used in the study were prepared by making use of the previously arranged questionnaires on zootechnics and agricultural management (Alkan and Eren, 2019; Şengül and Yeter, 2020; Akin, 2023). While determining the sample size of the study, a grouped one-stage random probability sampling method based on population ratios was used (Şengül and Yeter, 2020). In determining the sample size, the following formula (equation 1), which was used in limited societies as reported by Karasar (1994), was used (Akin, 2023).

$$n=(z^2*N*p*q)/(N*d^2+z^2*p*q) \quad (1)$$

here; n: Sample volume, z: "Z" table value corresponding to 95% significance level, N: Number of main masses, p: The probability of occurrence of the investigated event in the main mass is taken as 50%, q: The probability that the investigated event will not occur (1-p), d: Accepted margin of error (In this study, margin of error was taken as 5%).

According to the equation; It was determined that a survey should be conducted with 125 enterprises in Afyonkarahisar and 75 enterprises in Uşak, and one-on-one interviews were conducted with the enterprises. 15 surveys were conducted in Bolvadin, 50 surveys in İhsaniye, 15 surveys in the Center, 35 surveys in

Sinanpaşa, and 5 surveys each in Çobanlar and Sultandağı. Since almost all of the production in Uşak is in the Center and Banaz districts, 45 surveys were conducted in the Center, 20 in Banaz, and 5 each in Karahallı and Sivaslı. In the study, the average number, gender, age, breeding characteristics of the geese, feeding of the geese, egg production, the reason for the goose breeding, infrastructure opportunities, shelters, slaughter time and slaughter age, marketing methods of goose products, as well as the advantages and disadvantages of goose breeding were investigated. The data of the study were evaluated in the SPSS 16.0 package program and expressed as descriptive statistics and percentage values.

**Table 1.** Türkiye geese production amounts 2013-2022 (Akin, 2023; TUIK, 2023a)

| Region | Years | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2013 | 2014 | 2015 | 2016 | 2017 | 2018 | 2019 | 2020 | 2021 | 2022 |
| İstanbul TR1 | 3013 | 3025 | 2571 | 2428 | 2072 | 2177 | 3455 | 8294 | 3390 | 6552 |
| West Marmara | 32596 | 36130 | 37164 | 37997 | 39883 | 41478 | 41621 | 41207 | 42791 | 40558 |
| Aegean TR3 | 68666 | 72463 | 73410 | 76791 | 96340 | 102739 | 104784 | 104239 | 101654 | 84886 |
| East | 30960 | 29966 | 30791 | 31227 | 36289 | 41837 | 48652 | 59079 | 63973 | 53367 |
| West | 22189 | 25210 | 25934 | 28292 | 33336 | 35023 | 37879 | 44737 | 45050 | 38992 |
| Mediterranean TR6 | 17102 | 15776 | 17858 | 18937 | 29328 | 37041 | 45800 | 47211 | 48903 | 40510 |
| Middle Anatolia TR7 | 52026 | 50332 | 52845 | 59704 | 67849 | 74354 | 82343 | 98065 | 130936 | 121132 |
| West Black Sea TR8 | 51584 | 59210 | 66749 | 71027 | 85407 | 143037 | 116671 | 123381 | 115582 | 102275 |
| East Black Sea TR9 | 891 | 1325 | 962 | 1281 | 1636 | 2385 | 6869 | 11189 | 10556 | 10253 |
| Northeast Anatolia TRA | 297818 | 432142 | 366648 | 426678 | 388849 | 403425 | 471099 | 474022 | 668351 | 690692 |
| Middle East Anatolia TRB | 52026 | 50332 | 52845 | 59704 | 67849 | 74354 | 82343 | 98065 | 130936 | 92447 |
| Southeast Anatolia TRC | 67819 | 63506 | 57431 | 58467 | 74119 | 74664 | 73518 | 162800 | 105566 | 103843 |
| Total | 755286 | 911990 | 850694 | 933353 | 978384 | 1080190 | 1157049 | 1373960 | 1477569 | 1385507 |

**Table 2.** Aegean Region geese production amounts 2013-2022 (Akin, 2023; TUIK, 2023a)

| Aegean Region | Years | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2013 | 2014 | 2015 | 2016 | 2017 | 2018 | 2019 | 2020 | 2021 | 2022 |
| Afyonkarahi | 30944 | 32130 | 32130 | 33086 | 29568 | 32534 | 34835 | 27743 | 30460 | 21407 |
| Aydın | 2374 | 2674 | 2717 | 2836 | 3214 | 4330 | 3125 | 3135 | 3037 | 3032 |
| Denizli | 1837 | 2822 | 2531 | 3455 | 4683 | 4723 | 5421 | 5201 | 5676 | 5537 |
| İzmir | 1979 | 2641 | 2953 | 3522 | 4030 | 4554 | 4515 | 4862 | 4412 | 4041 |
| Kütahya | 23940 | 24675 | 24738 | 25087 | 44427 | 42211 | 42321 | 33742 | 34394 | 33539 |
| Manisa | 1327 | 1455 | 1594 | 1421 | 1732 | 2011 | 2109 | 3261 | 2703 | 2680 |
| Muğla | 2835 | 3055 | 3217 | 3169 | 2656 | 3526 | 3518 | 3099 | 2307 | 2338 |
| Uşak | 3430 | 3011 | 3720 | 4215 | 6020 | 8850 | 8940 | 23196 | 18665 | 12312 |
| Total | 68666 | 72463 | 73410 | 76791 | 96340 | 102739 | 104784 | 104239 | 101654 | 84886 |

**Table 3.** Goose production amounts of Afyonkarahisar Province and its districts 2013-2022 (TUIK, 2023a)

| Afyonkarahisar Districts | Years | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2013 | 2014 | 2015 | 2016 | 2017 | 2018 | 2019 | 2020 | 2021 | 2022 |
| Merkez | 10200 | 10404 | 10405 | 10716 | 6600 | 8000 | 11549 | 6700 | 8500 | 1500 |
| Bayat | 360 | 357 | 357 | 368 | 450 | 440 | 420 | 445 | 483 | 495 |
| Başmakçı | 660 | 673 | 673 | 693 | 768 | 756 | 750 | 505 | 480 | 110 |
| Bolvadin | 1335 | 1377 | 1377 | 1418 | 1852 | 2445 | 2100 | 1865 | 1902 | 1845 |
| Dazkırı | 230 | 235 | 234 | 242 | 333 | 205 | 182 | 181 | 165 | 100 |
| Dinar | 2850 | 2907 | 2907 | 2994 | 1414 | 1410 | 1110 | 1116 | 955 | 990 |
| Emirdağ | 340 | 357 | 356 | 368 | 360 | 361 | 375 | 355 | 315 | 250 |
| Evciler | 600 | 612 | 613 | 630 | 440 | 365 | 345 | 260 | 215 | 164 |
| Hocalar | 99 | 102 | 102 | 105 | 150 | 150 | 158 | 50 | 260 | 250 |
| Kızılören | 240 | 214 | 213 | 220 | 200 | 220 | 210 | 150 | 120 | 100 |
| Sandıklı | 1500 | 1530 | 1530 | 1576 | 1790 | 1850 | 1725 | 665 | 670 | 638 |
| Sinanpaşa | 2750 | 2805 | 2805 | 2889 | 4000 | 4000 | 3956 | 4100 | 4200 | 4395 |
| Sultandağı | 430 | 306 | 307 | 315 | 252 | 315 | 350 | 841 | 916 | 1035 |
| Çay | 1000 | 1020 | 1020 | 1051 | 600 | 700 | 725 | 2005 | 1979 | 300 |
| Çobanlar | 3400 | 3417 | 3417 | 3520 | 1040 | 1200 | 950 | 1100 | 1050 | 1000 |
| İhsaniye | 3800 | 4488 | 4487 | 4623 | 6103 | 6805 | 6850 | 6000 | 7000 | 7500 |
| İscehisar | 800 | 816 | 817 | 840 | 2700 | 2800 | 2600 | 900 | 850 | 350 |
| Şuhut | 350 | 510 | 510 | 518 | 516 | 512 | 480 | 505 | 400 | 385 |
| Total | 30944 | 32130 | 32130 | 33086 | 29568 | 32534 | 34835 | 27743 | 30460 | 21407 |

**Table 4.** Goose production amounts of Uşak Province and its districts 2013-2022 (TUIK, 2023a)

| Uşak Districts | Years | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2013 | 2014 | 2015 | 2016 | 2017 | 2018 | 2019 | 2020 | 2021 | 2022 |
| Merkez | 2500 | 2200 | 2500 | 2950 | 4230 | 5000 | 5000 | 14656 | 13180 | 8285 |
| Banaz | 700 | 565 | 930 | 1000 | 250 | 2750 | 3000 | 7162 | 4664 | 3385 |
| Eşme | 125 | 141 | 120 | 110 | 800 | 110 | 140 | 160 | 120 | 0* |
| Karahallı | 20 | 35 | 85 | 70 | 250 | 210 | 180 | 407 | 156 | 202 |
| Ulubey | 0* | 0* | 0* | 0* | 200 | 250 | 105 | 299 | 130 | 0* |
| Sivaslı | 85 | 70 | 85 | 85 | 300 | 530 | 515 | 512 | 415 | 440 |
| Total | 3430 | 3011 | 3720 | 4215 | 6030 | 8850 | 8940 | 23196 | 18665 | 12312 |

*The number of geese in the relevant year for the Şaphane District was stated as "0" by TUIK.

## 3. Results and Discussion

The socio-demographic characteristics of the breeders who participated in the survey in the study area are shown in Table 5, the reasons for breeding goose, the breeding period, the presence of geese, and their desire to increase are shown in Table 6. When the socio-demographic characteristics of the breeders participating in the survey were examined in both provinces, 15.2% and 22.7% of the goose breeders in Afyonkarahisar and Uşak were aged between 18-39, while the rate of those aged 40-59 was 52.0% and 22.7%. It was determined as 64.0. These results showed that goose breeding is done by young populations in both provinces and it is promising for the future of goose breeding. Similarly, Akin (2023) stated that goose breeding is done by the young population in Kütahya province. Boz et al., (2014), 58% of breeders are 40-59 years old and 23% are 20-39 years old, Demir et al., (2013) mean age is 41.9, Alkan and Eren (2019), 49.67% of them are 40-59 years old, 30.46% are 60-80 years old, Şengül and Yeter (2020) stated that 42.8% are younger than 40 years old, 26.7% are 50 years old and over the age stated. While the proportion of households with 1-6 persons in the goose breeders was determined as 83.2% and 88%, respectively, 55.2% and 29.3% of the education level were primary school, 17.6% and 24.7% were secondary school, 19.2 of them and 45.3 of them were high school and university. In previous studies, number of households and education level; In Kütahya, 87.2% have 1-6 people, 72.8% are primary school-secondary schools, in Ağrı 56.29% are 4-6 people, 48.34% are primary school, in Yozgat breeders are It was stated that 86% of them consisted of 1-6 people, 75.5% of them were at primary-secondary school, 89.5% in Muş was at primary school-secondary school, and 75% in Ardahan was at primary school level (Demir et al., 2013; Boz et al., 2014; Alkan and Eren, 2019; Şengül and Yeter, 2020; Akin, 2023).

**Table 5.** The socio-demographic characteristics of the goose breeders

| Age | Family (n) | R.F. (%) | Education | Family (n) | R.F. (%) | Number of individuals | Family (n) | R.F. (%) |
|---|---|---|---|---|---|---|---|---|
| Afyonkarahisar | | | | | | | | |
| 18-39 | 19 | 15.2 | Illiterate | 10 | 8.0 | 1-3 | 34 | 27.2 |
| 40-59 | 65 | 52.0 | Primary | 69 | 55.2 | 4-6 | 70 | 56.0 |
| 60-80 | 33 | 26.4 | Secondary | 22 | 17.6 | ≥7 | 21 | 16.8 |
| >80 | 8 | 6.4 | High | 17 | 13.6 | - | | |
| - | - | - | University | 7 | 5.6 | - | | |
| Total | 125 | | | 125 | | | | |
| Uşak | | | | | | | | |
| 18-39 | 17 | 22.7 | Illiterate | 2 | 2.7 | 1-3 | 22 | 29.3 |
| 40-59 | 48 | 64.0 | Primary | 22 | 29.3 | 4-6 | 44 | 58.7 |
| 60-80 | 7 | 9.3 | Secondary | 17 | 24.7 | ≥7 | 9 | 12.0 |
| >80 | 3 | 4.0 | High | 27 | 36 | - | | |
| - | - | - | University | 7 | 9.3 | - | | |
| Total | 75 | | | 75 | | | | |

n= number of families surveyed, RF= relative frequency.

**Table 6.** Distribution of goose producers according to their breeder's activities in Afyonkarahisar and Uşak Provinces

| Breeding Reason | Family (n) | SIF (%) | Family (n) | SIF (%) |
|---|---|---|---|---|
| | Afyonkarahisar | | Uşak | |
| Addition to Livelihood | 44 | 35.2 | 32 | 42.7 |
| Meat Need-Consumption Habit | 70 | 56.0 | 38 | 50.7 |
| Hobby | 7 | 5.6 | 2 | 2.7 |
| No other income | 4 | 3.2 | 3 | 4.0 |
| Breeding Times (year) | | | | |
| 0-5 | 14 | 11.2 | 14 | 18.7 |
| 6-10 | 54 | 43.2 | 29 | 38.7 |
| 11-20 | 34 | 27.2 | 20 | 26.7 |
| 21-30 | 15 | 12.0 | 7 | 9.3 |
| >30 | 8 | 6.4 | 5 | 6.7 |
| Number of geese (number) | | | | |
| 1-10 | 27 | 21.6 | 15 | 20.0 |
| 11-20 | 65 | 52.0 | 10 | 13.3 |
| 21-50 | 22 | 17.6 | 41 | 54.7 |
| 51-100 | 9 | 7.2 | 7 | 9.3 |
| >100 | 2 | 1.6 | 2 | 2.7 |
| Desire to increase the presence of goose (number) | | | | |
| No | 7 | 5.6 | 15 | 20.0 |
| Yes (11-20) | 23 | 18.4 | 10 | 13.3 |
| Yes (21-50) | 46 | 36.8 | 41 | 54.7 |
| Yes (51-100) | 40 | 32.0 | 7 | 9.3 |
| Yes (>100) | 9 | 7.2 | 2 | 2.7 |
| Person Responsible for Care and Feeding | | | | |
| Myself | 66 | 52.8 | 63 | 84.0 |
| Wife/husband | 35 | 28.0 | 3 | 4.0 |
| Mother/Father | 8 | 6.4 | 2 | 2.7 |
| Kids and the whole family | 15 | 12.0 | 7 | 9.3 |
| Goose herder | 1 | 0.8 | 0 | 0.0 |
| Poultry Presence Other than Goose | | | | |
| None | 8 | 6.4 | 2 | 2.7 |
| Hen | 88 | 70.4 | 59 | 78.7 |
| Turkey | 10 | 8.0 | 5 | 6.7 |
| Duck | 14 | 11.2 | 7 | 9.3 |
| Quail. partridge. and other | 5 | 4.0 | 2 | 2.7 |

n= number of families surveyed, SIF= share in investigated family.

While 56% of the breeders in Afyonkarahisar stated that 50.7% of the breeders in Uşak carried out goose breeding in order to meet the meat needs of the family, the ratio of those who stated that they did not contribute to their livelihood and had no other income was 38.4% and 46.7%. 54.4% of goose breeders in Afyonkarahisar and 57.4% in Uşak stated that they have been playing an active role in goose production for 1-10 years, and according to this result, goose breeding is a relatively new alternative livestock activity in Afyonkarahisar and Uşak compared to other provinces can be said to be Akin (2023) stated that the rate of those who have been engaged in breeding activities for 1-10 years in the province of Kütahya is 64%.

In a study conducted by Şengül and Yeter (2020), the average rearing period in Muş was 17 years and 38.2% of the respondents stated that this period was 20 years or more. stated that they were engaged in aquaculture in order to obtain While this period was reported as 18.6 years in Ardahan, 79.3% of them stated that goose breeding is an important source of income, 48% of the breeders in Yozgat have been breeding geese for less than 10 years and 85.5% It has been reported that they do breeding as a consumption habit, 63.58% of them have been breeding geese for 1-10 years and 64.9% of them are producing as a consumption habit (Demir et al., 2013; Boz et al., 2014; Alkan and Eren, 2019). 52.8% of the breeders in Afyonkarahisar and 84.0% of the breeders in Uşak reported that they were interested in the care and management of geese, and 52.0% in both cities reported that they raised an average of 11-20 geese in a year. 70.4% in Afyonkarahisar and 78.7% in Uşak stated that they raised chickens other than geese, 94.4% and 80.0% stated that they wanted to increase the number of geese. Alkan and Eren (2019) stated that 71.52% of non-goose hens were raised and 85.43% of them were goose breeding in addition to other livestock activities. 73.51% of the breeders stated that they wanted to increase the presence of geese and that woman and children generally took an active role in the care and feeding of geese. In the study conducted in Kütahya, it was stated that 65.6% of the breeders themselves took care of the care and management of geese, an average of 11-20 geese were raised in a year, while 73.6% of them raised chickens other than geese. In this study, it was also stated that 88.0% of the breeders wanted to increase the number of geese (Akin, 2023). In Afyonkarahisar and Uşak, 60.8% and 73.4% of the breeders, respectively, keep an average of 1-5/3-20 male/female (M/F) breeder digs, while 28.9% and 17%, 3 of them reported that they do not have breeding geese. 57.6% and 80.0% of the breeders, respectively, reported that they obtained the gosling broody/hatching, and 89.6% and 90.7% of them raised domestic goose breeds. It was determined that 56.8% of them in Afyonkarahisar and 56.0% of them in Uşak preferred variegated and white varieties. The ratio of breeders who do not make supplementary feeding is 16.0% and 8.0% in these two provinces. Among the breeders, those who make supplementary feeding, respectively; 36.8% and 36.0% preferred corn, 20.8% and 26.7% preferred wheat, 18.4% and 18.72% preferred barley, while the others preferred bread and food scraps they stated that they used. In these two provinces, 88.8% and 96.0% of the goslings are taken to the pasture within the first two weeks. While the proportion of those who did not use any equipment was 18.4% in Afyonkarahisar and 10.7% in Uşak, the ratio of those who stated that they used at least one piece of equipment was 81.6% and 89.3%. Alkan and Eren (2019) stated in their study in Ağrı that breeders keep 4-6 breeding gooses in their hands, and the goslings and breeders are obtained by hatching. He explained that almost all of the breeders in Ağrı prefer the domestic goose breed and that the variegated variety is more popular among the domestic goose varieties. The researchers stated that in this study, the goslings were released to the pasture after an average of 2-3 weeks.

In the study conducted in Ardahan, it was stated that the geese were generally fed on pasture and that 88.8% of the people used barley for supplementary feeding, while in the study conducted in Yozgat, wheat, barley, and corn were preferred as supplementary feeding, and also bread and food scraps were evaluated in feeding (Demir et al., 2013; Boz et al., 2014). While 70.4% of the breeders in Afyonkarahisar and 85.3% of the breeders in Uşak stated that they get an average of 1-15 eggs from a goose in a year, the rate of those who stated that they received 26+ eggs among the breeders was 4.8% and 2.7% detected. As in the study conducted in Kütahya, it was observed that breeders who stated that they received a high number of eggs in these two provinces preferred high-yielding breeds such as Chinese, Linda, and Mast (Akin, 2023). In these two provinces, 88.8% and 96.0% of the goslings are taken to the pasture within the first two weeks. While the proportion of those who did not use any equipment was 18.4% in Afyonkarahisar and 10.7% in Uşak, the ratio of those who stated that they used at least one piece of equipment was 81.6% and 89.3%. Alkan and Eren (2019) stated in their study in Ağrı that breeders keep 4-6 breeding gooses in their hands, and the goslings and breeders are obtained by hatching. He explained that almost all of the breeders in Ağrı prefer the domestic goose breed and that the variegated variety is more popular among the domestic goose varieties. The researchers stated that in this study, the goslings were released to the pasture after an average of 2-3 weeks. In the study conducted in Ardahan, it was stated that the geese were generally fed on pasture and that 88.8% of the people used barley for supplementary feeding, while in the study conducted in Yozgat, wheat, barley, and corn were preferred as supplementary feeding, and also bread and food scraps were evaluated in feeding (Demir et al., 2013; Boz et al., 2014). While 70.4% of the breeders in Afyonkarahisar and 85.3% of the breeders in Uşak stated that they get an average of 1-15 eggs from a goose in a year, the rate of those who stated that they received 26+

eggs among the breeders was 4.8% and 2.7% detected. As in the study conducted in Kütahya, it was observed that breeders who stated that they received a high number of eggs in these two provinces preferred high-yielding breeds such as Chinese, Linda, and Mast (Akin, 2023).

The number of broody/chick, breeder geese, breeder supply and selection, keeping time in breeder, and breeder egg price of both provinces are shown in Table 7. While 57.6% of the breeders incubated an average of 21-50 eggs in Afyonkarahisar, it was determined that the number of chicks hatched was 11-30, and the hatchability was found to be 55-60% in Afyonkarahisar, as in the study carried out in Kütahya. The rate of those who stated that they put an average of 1-30 eggs in the incubation in Uşak was determined as 59.7%, and the number of chicks hatched was found to be 1-20. It has been observed that the hatchability of Uşak province is at the level of 60-65%. The ratio of those who provide breeding geese from their own resources was determined as 76.3% in Afyonkarahisar, 74.2% in Uşak, and 21.7% and 19.4% from neighbors and local animal markets. 53.6% of breeders in Afyonkarahisar and 45.1% in Uşak stated that they consider body size and egg production in the selection of breeding goose.

The rate of those who chose randomly was 26.8% and 43.5%, respectively. While the rate of those who keep breeding geese for 1-6 years is 93.8% in Afyonkarahisar, and 95.2% in Uşak, the rate of those who keep the breeder geese above 7-8+ was found to be 6.2% and 4.8%. While the rate of producers who stated that the prices of breeding eggs were between 20-40 TL on average, was 79.2% in Afyonkarahisar and 80.0% in Uşak, the rate of those who said they did not buy or sell eggs was 12.8% and 10.7%.

**Table 7.** Number of hatching eggs and chicks, number of breeding geese, breeding geese supply and selection, period of keeping in breeding and breeding egg price

| Hatching egg (E) / Chick (C) | Family (n) | SIF (%) | Family (n) | SIF (%) |
|---|---|---|---|---|
| | Afyonkarahisar | | Uşak | |
| 1-20 E / 0-10 C | 32 | 25.6 | 28 | 45.2 |
| 21-30 E / 11-20 C | 26 | 20.8 | 9 | 14.5 |
| 31-50 E / 21-30 C | 46 | 36.8 | 17 | 27.4 |
| 51-100 E / 31-70 C | 16 | 12.8 | 8 | 12.9 |
| >100 E / >70 C | 5 | 4.0 | 0 | 0.0 |
| Number of breeding geese (M/F) | | | | |
| Not has breeder geese | 28 | 28.9 | 13 | 17.3 |
| 1-3 M / 3-10 F | 40 | 41.2 | 38 | 50.7 |
| 4-5 M / 11-20 F | 19 | 19.6 | 17 | 22.7 |
| 6-10 M / 21-50 F | 9 | 9.3 | 7 | 9.3 |
| >10 M / >50 F | 1 | 1.0 | 0 | 0.0 |
| Breeding geese supply | | | | |
| From own resources | 74 | 76.3 | 46 | 74.2 |
| Neighbors | 15 | 15.5 | 7 | 11.3 |
| Animal markets | 6 | 6.2 | 5 | 8.1 |
| Other provinces | 2 | 2.1 | 4 | 6.5 |
| Breeding selection | | | | |
| Randomly | 26 | 26.8 | 27 | 43.5 |
| Size/Body | 20 | 20.6 | 17 | 27.4 |
| Egg yield | 32 | 33.0 | 11 | 17.7 |
| Feather color | 12 | 12.4 | 4 | 6.5 |
| Race | 7 | 7.2 | 3 | 4.8 |
| Period of keeping in breeding (year) | | | | |
| 1-2 | 7 | 7.2 | 16 | 25.8 |
| 3-4 | 35 | 36.1 | 34 | 54.8 |
| 5-6 | 49 | 50.5 | 9 | 14.5 |
| ≥7-8 | 6 | 6.2 | 3 | 4.8 |
| Breeding egg price (TL) | | | | |
| No buying or selling | 16 | 12.8 | 8 | 10.7 |
| 20-30 | 39 | 31.2 | 18 | 24.0 |
| 31-40 | 60 | 48.0 | 42 | 56.0 |
| >40 | 10 | 8.0 | 7 | 9.3 |

n= number of families surveyed, SIF= share in investigated family.

Boz et al., (2014) stated that the average egg production is 11, the number of chicks obtained from hatching is 8, the hatchability is 73%, the average retention period of the breeders is 2 years, and the breeder male/female ratio is 1/3. In a study conducted in Kırşehir, the average number of eggs per farm was 53.13, and the number of chicks was 45.11. The breeder male/female ratio was reported as 1.14/4.83, and the period of keeping in breeders was 2-12 years, while the rate of those who gave priority to body size in the selection of breeders was reported as 35%. It is said that 30% of the breeders care about egg production in the first place in the selection of breeders (Taşkın et al., 2017). Slaughter time, slaughter age, live and carcass weight, feather plucking method, feather usage situation, and place of sale, type, and price of goose are shown in Table 8. 52.0% of the producers in Afyonkarahisar and 48.0% in Uşak stated that the slaughtering process was done in December-January. In Afyonkarahisar, 42.4% of the breeders stated that the geese were slaughtered when they were 13-15 months old, 60.8% were 4-7 kg live weight, and 51.2% stated that they obtained an average of 3-5 kg of carcass. In Uşak, 58.7% of them stated that they slaughtered geese at the age of 10-12 months, 71.3% of them 4-7 kg of live weight, and 69.4% of them obtained an average of 3-5 kg of carcass. While those who preferred the wet plucking method were 72.8% in Afyonkarahisar and 65.3% in Uşak, the proportion of those who said they discarded goose feathers without making any use of them was 80.8% and 86.7%. In the study conducted in Yozgat, it was stated that geese were slaughtered in October, November, and December, while some breeders carried out slaughter in January-February. In this study, it was observed that the slaughter age was 8 months and the carcass weight was 3.7 kg on average, and 96% of the breeders preferred wet plucking to remove the feathers. The rate of those who use goose feathers in making quilts and pillows was found to be only 2.5%. Researchers have stated that 77% of breeders consume geese fresh without waiting (Boz et al., 2014). In order for the goose feathers, which are extremely valuable and have high economic value, to be evaluated, it is urgently necessary to bring feathers to the economy by establishing various organizations affiliated with the Municipality, Ministry of Agriculture and Forestry, and feather collecting units.

92.4% of the goose breeders in Afyonkarahisar and 89.3% in Uşak sell the geese they produce as live or carcasses. The rate of those who sell to neighbors and local markets in the village is 89.8% in Afyonkarahisar, 86.7% in Uşak, the rate of those who state that they earn 300-500TL from an average live goose in Afyonkarahisar is 79.6%, while in Uşak it is 300-450. The rate of those who stated that they earned TL income was 82.7%. While 50.4% of breeders in Afyonkarahisar and 58.7% in Uşak state that they see goose breeding as a profitable business and will continue, 33.6% and 28.0% do not see it as a profitable business. They stated that he would continue because of the habit. 41.6% of the respondents in Afyonkarahisar, 57.3% in Uşak fried goose meat, 14.4% and 12.0% boiled it, 8.8% and 9%, 3 of them stated that they prefer to consume it by using it in local dishes. Şengül and Yeter (2020) stated that in Muş, 55.2% of live geese are generally sold in the city center and 44.8% in villages, while Taşkın et al., (2017) stated that the highest sales by breeders are in local markets (% 40), it was stated that it was then made to the merchant (25%) and the immediate environment (15%).

"Do the geese have a special shelter, is disinfection applied?" to the question; 85.6% of the breeders in Afyonkarahisar for shelter and 68.8% for disinfection, in Uşak 82.0% and 57.3% answered "Yes". In Afyonkarahisar and Uşak, 92.0% of the breeders struggle with their own means in adverse conditions such as disease, 8.0% in Afyonkarahisar receive support from Veterinarians and Agriculture Organizations, 8.0% in Uşak only indicated that they received veterinary support. In general, it was observed that the losses occurred in the first week after hatching (17.6% in Afyonkarahisar, 12.0% in Uşak). Şengül and Yeter (2020) In Muş, goose shelters are 50 m2 in size on average, and the shelters are made of briquettes, wood, etc. stated that it was made of materials. While 67% of the breeders reported that they did not take any precautions against diseases, he stated that very few of the geese died. Boz et al., (2014) reported that breeders kept the geese in the same shelter as other animals, 61.5% did not apply any disinfection, and 98.5% stated that their animals never got sick. 50.4% of the breeders in Afyonkarahisar and 26.7% in Uşak evaluated the fact that geese are compatible with pasture and more resistant to diseases than other poultry as an advantage. 32.8% in Afyonkarahisar and 48.4% in Uşak consider goose breeding as an advantageous livestock activity because it meets the meat needs of the family and creates additional income. "What do you think are the biggest problems and difficulties you face in goose breeding?" for the question 52.8% of the producers in Afyonkarahisar, 77.3% in Uşak stated high feed costs, 17.6% and 14.7% low egg yield, 5.6% and 2 0.7 of them drew attention to the difficulties experienced in the supply of breeding animals. Similarly, "What do you think is necessary for the development of goose breeding in our province, region and country, what are the deficiencies, what are your demands against the problems you experience?" for the question 49.6% of the breeders in Afyonkarahisar and 44.0% in Uşak drew attention to the advertisement, promotion, and marketing of the products obtained from the geese. While the rate of those who want to goose breeding with high-yielding breeds is 16.0% in Afyonkarahisar, 32.0% in Uşak, 26.4% of the breeders in Afyonkarahisar and 20.0% in Uşak have slaughterhouses, feather my way stated that a cold storage is needed. Taşkın et al., (2017) reported that 50% of breeders stated that geese are easy to sell and resistant to diseases as an advantage. Researchers stated, that 40% of breeders; that they attach importance to goose breeding at the point of

meeting meat consumption and that they also state that the geese are compatible with the pasture as an advantage. It was stated that 50% of the breeders considered high feed prices and low egg production of geese as problems among the difficulties and difficulties they faced. Researchers reported that 20% of the producers declared that geese damage their farmland. As a result of this study, it was seen that goose breeders expect support, especially in terms of high feed costs and breeding animal supply.

**Table 8.** Slaughter time, slaughter age, live and carcass weight, feather plucking method and feather usage situation, place of sale, type and price of goose

| Slaughter time | Family (n) | SIF (%) | Family (n) | SIF (%) |
|---|---|---|---|---|
| | Afyonkarahisar | | Uşak | |
| October-November | 21 | 16.8 | 26 | 34.7 |
| December- January | 65 | 52.0 | 36 | 48.6 |
| February-March | 36 | 28.8 | 11 | 14.7 |
| Other months | 3 | 2.4 | 2 | 2.7 |
| Slaughter age (month) | | | | |
| 6-9 | 12 | 9.6 | 12 | 16.0 |
| 10-12 | 40 | 32.0 | 44 | 58.7 |
| 13-15 | 53 | 42.4 | 14 | 18.7 |
| 16-18 | 17 | 13.6 | 5 | 6.7 |
| ≥19 | 3 | 2.4 | 0 | 0.0 |
| Live weight (kg) | | | | |
| Do not know | 40 | 32.0 | 10 | 7.9 |
| 2-3 | 2 | 1.6 | 3 | 4.0 |
| 4-5 | 38 | 30.4 | 42 | 56.0 |
| 6-7 | 38 | 30.4 | 16 | 21.3 |
| ≥7 | 7 | 5.6 | 4 | 5.3 |
| Carcass weight (kg) | | | | |
| Do not know | 47 | 37.6 | 13 | 17.3 |
| 2-2.5 | 1 | 0.8 | 1 | 1.3 |
| 3-4 | 25 | 20.0 | 41 | 54.7 |
| 4.5-5 | 39 | 31.2 | 11 | 14.7 |
| ≥5 | 13 | 10.4 | 9 | 12.0 |
| Feather plucking method | | | | |
| Dry plucking | 12 | 9.6 | 14 | 18.7 |
| Wet plucking | 91 | 72.8 | 49 | 65.3 |
| Dry or wet plucking | 22 | 17.6 | 12 | 16.0 |
| Feather usage situation | | | | |
| Throwing | 101 | 80.8 | 65 | 86.7 |
| Pillow/quilt making | 20 | 16.0 | 7 | 9.3 |
| Selling to trader | 4 | 3.2 | 3 | 4.0 |
| Place of sale | | | | |
| No sale | 7 | 5.9 | 3 | 4.0 |
| Neighbor / friends in the village | 47 | 39.8 | 48 | 64.0 |
| Local animal markets | 59 | 50.0 | 17 | 22.7 |
| Web / social media | 5 | 4.2 | 7 | 9.3 |
| Sale type | | | | |
| No sale | 7 | 5.9 | 3 | 4.0 |
| Live | 72 | 61.0 | 51 | 68.0 |
| Carcass | 37 | 31.4 | 16 | 21.3 |
| Customer Request (Live/carcass/piece) | 2 | 1.7 | 5 | 6.7 |
| Sale price (TL) | | | | |
| 200-300 | 7 | 5.9 | 7 | 9.3 |
| 301-400 | 17 | 14.4 | 23 | 30.7 |
| 401-450 | 30 | 25.4 | 24 | 32.0 |
| 451-500 | 47 | 39.8 | 15 | 20.0 |
| >500 | 17 | 14.4 | 6 | 8.0 |

n= number of families surveyed, SIF= share in investigated family

## 4. Conclusion

Among these provinces, Kütahya and Afyonkarahisar, which were 42,000 and 35,000 in 2019, decreased to 33,000 and 21,000 at the end of 2022, and from 23,000 in 2020 to 12,000 at the end of 2022 in Uşak. This study has also shown that high feed costs are the most important problem for the sustainability of animal husbandry in Türkiye. In addition, "Goose Products, Collection and Sales Units, etc." within the scope of Municipal and Agricultural Organizations for the supply of breeding animals and the sale of goose products. should be created. These units can provide support to growers in the marketing of their products. Considering the goose production potential of Kütahya; The scope of the goose incentives stated by the Ministry of Agriculture and Forestry in "Supporting Economic Investments Based on Agriculture within the Scope of Rural Development Supports 2022-2023 Application Period, Communiqué No: 2022/24" is quite limited. In the relevant communiqué, it is stated that "applications for new facilities in 81 provinces, completion of partially made investments, capacity increase and technology renewal and/or modernization" will be taken into consideration only for turkey and goose breeding. In the continuation of the Communiqué, there is the statement "...No grant support is given for breeding eggs and/or egg production in goose breeding" (Anonymous, 2023). However, our breeders reported that they had the most problems with the supply of breeding eggs and breeding animals. Expanding the scope of the goose incentive will provide an opportunity to prevent losses in our goose stock and to increase our goose presence again in the future.

**Author Contributions**

The percentage of the author contributions is presented below. The author reviewed and approved the final version of the manuscript.

| | Y.A. |
|---|---|
| C | 100 |
| D | 100 |
| S | 100 |
| DCP | 100 |
| DAI | 100 |
| L | 100 |
| W | 100 |
| CR | 100 |
| SR | 100 |
| PM | 100 |
| FA | 100 |

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

**Conflict of Interest**

The author declared that there is no conflict of interest.

**Ethical Consideration**

This study was conducted within the scope of the decision of Uşak University Research and Publication Ethics Committee (protocol code: 2023/03-11 and date: 27 April 2023).

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# EVALUATION OF PLANT RESIDUES: SAMSUN PROVINCE

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**Abstract:** This study was carried out to determine the biomass potential, energy value, bio-composting material and composting possibilities produced from plant and animal residues in Samsun. Samsun is a province of Türkiye in the Black Sea region. Samsun province has 17 districts. Residue amounts of agricultural products grown in Samsun were calculated using the Turkish Statistical Institute (TUIK) 2021 and year product production data, Türkiye Biomass Energy Potential Atlas (BEPA) 2021 biomass data. The total amount of agricultural waste is approximately 877.812 tons wheat 254.154 tons, paddy 132,891 tons, maize 53.861 tons and oat 47.797 tons in cereals in fruits, hazelnut is 66.363 tons and peach is 125.065 tons. Total heating value was found as 5.439.003 GJ. Hazelnut was the highest contributor to this value with 27% as fruit for cereals, it was maize with 17.14%. The energy equivalents of the biomass amounts are respectively; 37.34% hazelnut, 24% paddy, 10% wheat, 17.4% maize and 11.26% other plants were found.

**Keywords:** Biomass, Sustainably energy, Agricultural residues, Bio-composite

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## 1. Introduction

With the increase in the human population, the demand for energy sources is increasing. The main energy sources can be listed as oil, natural gas and coal. As it is known, natural energy sources cause global climate change and consequently an increase in greenhouse gas emissions. In addition, it is known that natural energy resources are starting to decrease day by day. In summary, the use of natural energy sources has increased climate change and CO2 emissions in the world, and economic solutions have begun to be sought for the energy need, which is the lifeblood of the country's economies. In order to get rid of these negative situations, the importance of renewable energy sources has increased day by day (Pirlogea and Cicea, 2012; Shilev et al., 2007).

Türkiye is a developing country with rich biomass potential. Due to the scarcity of natural energy resources, the tendency to biomass energy, which is also among alternative energy sources, is increasing day by day (Baran et al., 2017; Lüle, 2019).

In this study, a research was carried out on the conversion of plant and animal residues to energy, bio-compost and bio-composite material possibilities in Samsun, which is located in the Black Sea Region of Türkiye.

## 2. Material and Methods

Samsun province in Türkiye has located between 41° 20' 1" E longitudes and covering 9,352 km² areas. Samsun Province is a province of Türkiye on the Middle Black Sea coast. Samsun province is divied into 17 districts, four of which (Ilkadım, Canik, Atakum, Tekkeköy, Asarcık, Kavak and Yakakent) are inculded in the municipality of Samsun city (Center). Other districts include Alacam, Ayvacik, Bafra, Carsamba, Havza, Ladik, Ondokuzmayis, Salipazari, Terme and Vezirkopru.

### 2.1. Energy Equivalents of Agricultural Residue

The amounts of residues from the crops cultivated in Samsun province were calculated using production data of crops with Turkish Statistical Institute for the 2021 seasonal years. The annual gross potential of agricultural residues was determined by using residues to product ratio (RPR). The available of the agricultural residues in Samsun province was calculated based on the equation 1 (Karaca et al., 2017).

$$(AAR)_i = (AAP)_i \times (RPR)_i \times (A)_i \quad (1)$$

Where (AAR)i is the available amount of agricultural residues of i'th crop in tons, (AAP)i the amount of agricultural product in tons or number of tree or planting area for pruning residues, (RPR)i residue-to product ratio of the i'th crop and (A)i the availability of residues.

The energy potential of residues for each district was calculated by multiplication of the heating values of a

selection of agricultural residues which was taken heating value per each residue (Table 1) with the available residue amount (equation 2).

$$(THV)_i = (AAR)_i \times (LHV)_i \quad (2)$$

Where; (THV)i the total heating value of agricultural residues of i'th crop in GJ, (AAR)i is the available amount of agricultural residues of i'th crop in tons and (LHV)i lower heating value of air dry residues of i'th crop in $MJkg^{-1}$.

**Table 1.** The ratio of product to residue, availability, heating value and ash of selected agricultural residues (Karaca et al, 2017)

| Field Crops | Residues | Ratio of Product to Residuce (RPR) | Availability (A) (%) | Heating Value (LHV) | Ash (%) | Referans |
|---|---|---|---|---|---|---|
| Wheat | Straw | 0.8 | 15 | 17.9 | 4.91 | (Mijailovic et al, 2014) |
| Barley | Straw | 0.9 | 15 | 17.5 | 2.71 | (Gümüs and Bayır, 2020) |
| Rye | Straw | 1.5 | 15 | 17.5 | 5.7 | (Wang et al, 2014) |
| Oats | Straw | 1.5 | 15 | 17.4 | 3.25 | (Gümüs and Bayır, 2020) |
| Maize | Stalks | 1 | 60 | 18.5 | 6.49 | (Mijailovic et al, 2014) |
| | Cob | 0.64 | 60 | 18.4 | 3.83 | (Mijailovic et al, 2014) |
| Paddy(rice) | Straw | 0.7 | 60 | 16.7 | 14.65 | (El-Sayed, 2006) |
| | Husks | 0.27 | 80 | 13 | 22.15 | (El-Sayed, 2006) |
| Tobacco | Stalks | 1100* | 60 | 16.1 | 8.6 | (Wu et al, 2019) |
| Sunflower | Stalks | 0.6 | 60 | 14.2 | 4.06 | (Demirbas, 2002) |
| Soybeans | Straw | 0.75 | 60 | 19.4 | 5.86 | (Liu et al, 2015) |
| Cannabis | Stems and Leaves | 2.50 | - | 26.7 | 14.36 | (Nakkliang et al, 2022) |
| Fruit Crops | | | | | | |
| Hazelnut | Shell | 0.3 | 80 | 19.3 | 1.36 | (Demirbas, 2002) |
| | Husks | 0.3 | 80 | 16 | 1.16 | (Demirbas, 2002) |
| | Pruning | 1500* | 80 | 18.8 | - | |
| Walnut | Pruning | 13.00** | 50 | 19 | - | |
| Almonds | Pruning | 7.40** | 80 | 18.4 | - | |
| Peach | Pruning | 8.00** | 80 | 18 | - | |
| Kiwi | Pruning | 8.00** | 80 | 18 | - | |
| Vineyard | Pruning | 6.00* | 80 | 18 | - | |

*Per planting area ($kg.ha^{-1}$), **Per number of trees ($kg.tree^{-1}$)

### 2.2. Evaluation of Agricultural Residues as Bio-Compost

Compost is defined as the conversion of different organic materials into biodegradable, stabilized and mineralized humus under suitable conditions (Uygun, 2012). It is widely used to change the elemental composition during composting and the C:N ratio is one of the main factors affecting this process. Because carbon and nitrogen elements are two essential nutrients required for the growth of microorganisms involved in the composting process (Ravindran et al., 2014). It has been determined that the C:N ratio in compost mixtures is generally between 20-50 (Wu et al., 2014).

### 2.3. Possibilities of Agricultural Residues to Produce Bio-Composite Materials

Today, in green chemistry, the use of sustainable and biodegradable biopolymers has increased due to the environmental risks posed by conventional petroleum-based polymers. Biopolymers can be produced from renewable resources, including agriculture, microbial resources and biomass. In addition, toxic or dangerous substances are not released during the degradation of biopolymers. Therefore, there are many studies focusing on the replacement of petroleum-based polymers with biopolymers.

Boonmee et al., in their study in 2016, found that a bio-composite material made from PLA (polylactic acid) can degrade 90% when it is under the ground for 90 days. Considering the physical properties of PLA, its important advantages include its strong sealing properties, low temperature adhesion, heat sealing on paper or cardboard, stability, transparency, thermoplastic and easy processing.

## 3. Results and Discussion

### 3.1 Energy Equivalent Results of Agricultural Residues

The energy equivalents of agricultural residues in Samsun and the usable amounts of agricultural residues are given in Table 2. According to the table, the amount of

plant residues that can be used in Samsun province and its districts are respectively; 35.3% hazelnut, 26.92% paddy, 9.72% wheat, 16.35% maize, 4.68% sunflower and 7.03% other plants. The energy equivalents of the residue amounts are respectively; 37.34% hazelnut, 24% paddy, 10% wheat, 17.4% maize and 11.26% other plants.

The amount of vegetable residue belonging to Samsun province and its districts is given in Table 3. Looking at Table 3, the highest amount of annual plants is seen in Bafra, Vezirköprü and Havza, respectively. The highest amount of residues from fruit trees is from Çarşamba, Terme and Salipazarı towns, respectively.

**Table 2.** The amount of agricultural product, available residues and Total heating value of residues in Samsun Province

| Field Crops | Amount of Agricultural Product in tons or Planting Area (AAP) (tons) or (ha) | Residues | Available Residues (AAR) (tons) | Percentage Distribution (AAR) (%) | Total heating Value (THV) (GJ) | Percentage Distribution (THV) (%) |
|---|---|---|---|---|---|---|
| Wheat | 254.154 | Straw | 30.498,50 | 9.72 | 545.923.15 | 10.04 |
| Barley | 16.955 | Straw | 2.289 | 0.73 | 40.057.5 | 0.74 |
| Rye | 651 | Straw | 146,4 | 0.05 | 2.562 | 0.05 |
| Oats | 47.797 | Straw | 10.754 | 3.43 | 187.119.6 | 3.44 |
| Maize | 53.861 | Stalks | 28.678 | 9.14 | 530.543 | 9.75 |
| | | Cob | 22.621,60 | 7.21 | 416.237.44 | 7.65 |
| Paddy | 132.891 | Straw | 55.814 | 17.78 | 932.093.8 | 17.14 |
| | | Husk | 28.704,50 | 9.14 | 373.158.5 | 6.86 |
| Tobacco | 6.897 | Stalks | 4.552 | 1.45 | 73.287.2 | 1.35 |
| Sunflower | 40.806 | Stalks | 14.690 | 4.68 | 208.598 | 3.84 |
| Soybeans | 7.714 | Straw | 3.471 | 1.11 | 67.337.4 | 1.24 |
| Fruits Crops | | | | | | |
| Hazelnut | 66.363 | Shell | 15.927 | 5.07 | 30.7391.1 | 5.65 |
| | | Husks | 15.927 | 5.07 | 254.832 | 4.69 |
| | 116.714,3* | Pruning | 78.972 | 25.16 | 1.484.673.6 | 27.30 |
| Walnut | 2.903 | Pruning | 18,9 | 0.01 | 359.1 | 0.01 |
| Peach | 125.065 | Pruning | 791,6 | 0.25 | 14.248.8 | 0.26 |
| Kiwi | 5.041 | Pruning | 32,3 | 0.01 | 581.4 | 0.01 |
| Total | 877.812,30 | - | 313.887,80 | 100 | 5.439.003,59 | 100 |

*Per planting area ($kg.ha^{-1}$)

**Table 3.** The amount of agricultural residues in district of Samsun

| District | Crops Residues (tons) | Fruit Crops Residues (tons) | Total Residues (tons) |
|---|---|---|---|
| Alacam | 39.608 | 350 | 389.608 |
| Ayvacik | 3.196 | 13.431 | 16.627 |
| Bafra | 219.618 | 3.33 | 222.948 |
| Carsamba | 30.243 | 69.212 | 99.455 |
| Center | 66.781 | 20.334 | 87.115 |
| Havza | 91.246 | 32 | 123.246 |
| Ladik | 34.338 | 77 | 111.338 |
| Ondokuz Mayıs | 12.668 | 4.135 | 16.803 |
| Salipazari | 129 | 21.161 | 150.161 |
| Terme | 29.332 | 41.254 | 70.586 |
| Vezirkopru | 109.826 | 395 | 504.826 |
| Total | 765.856 | 1.026.857 | 1.792.713 |

### 3.2. Composting

In general, it is possible to make a higher quality compost by reducing the C:N ratio of organic materials for composting (Tripetchkul, et al., 2012; Balasubramani and Mnkeni, 2016). In Table.4, the C:N ratios of vegetable and animal residues in Samsun province and its districts are given. The agricultural residues with the lowest C:N ratio were oats (straw), chicken (manure), tobacco (stalks)

and sheep (manure), respectively. Hazelnut husk agricultural residue combined with animal manure could be a promising source for composting since hazelnut production is very common in Samsun region and Türkiye is the largest producer of hazelnut on all over the world.

**3.3. Bio-composite Material**

PLA, a renewable polymer that can be produced from plant sources of corn, potato, molasses, tapioca, sugarcane and rice, is biodegradable and biocompatible (Gupta et al., 2007). Cellulose nanocrystals are one of the most attractive natural fillers for developing all-green nanocomposites due to their high specific surface area, high aspect ratio, high modulus, biocompatibility, non-toxicity and low density (Mariano et al., 2014).

According to the results of this research, the vegetable residue amount of wheat, corn and rice plants to be produced from PLA for the potential of plant residue bio-composite materials in Samsun province is 517 tons in total.

It is seen that PLA packaging is used in products such as beverage glasses, fresh pasta, bread and salad bags, thermoform containers for bakery products, agricultural covers and boxes (Cha and Chinnan, 2004). In addition, PLA materials are preferred because they do not steam in bread and bakery products (Ayhan, 2012). In addition, Almenar et al., (2008) stated that the shelf life of strawberries packed with packaging material produced from PLA extends.

In the light of the information received from the Cannabis Research Institute in Samsun Ondokuz Mayıs University, 250 tons of cannabis stem products were obtained for 2020. Approximately 60 tons of hemp fiber was obtained from the hemp stem produced. In addition, 1250 kg of cannabis seeds were produced. The oil content and caloric values of the produced seed are respectively; It is 35% and 3400 cal (Acar and Dönmez, 2019). Concerning the agricultural product pattern of Samsun region the products like rice, maize, hemp and hazelnut are compromising sources of PLA and good potentials for bio-composites.

**Tablo 4.** C:N ratios of agricultural residues in Samsun region

| Field Crops | Residues | Ratetion of C:N | Referans |
|---|---|---|---|
| Rye | Straw | 114.12 | (Gümüs and Bayır, 2020) |
| Oat | Straw | 12.3 | (Solowiej et al, 2017) |
| Maize | Stalks | 67.13 | (Gümüs and Bayır, 2020) |
| | Cob | 34.97 | (Gümüs and Bayır, 2020) |
| Paddy (rice) | Straw | 113.95 | (Gümüs and Bayır, 2020) |
| | Husks | 73.15 | (Liou and Wu, 2009) |
| Tobacco | Stalks | 19.7 | (Kopcic et al, 2014) |
| Sunflower | Stalks | 23.25 | (Gümüs and Bayır, 2020) |
| Soybeans | Straw | | |
| Fruit Crops | | | |
| Hazelnut | Shell | 32.25 | (Demirbas, 2002) |
| | Husks | 39.23 | (Demirbas, 2002) |
| Animal | | | |
| Chicken | Manure | 14.41 | (Ravindran and Mnkeni, 2016) |
| Cattle | Manure | 19.72 | (Suthar, 2008) |
| Sheep | Manure | 17.07 | (Tabrika et al, 2019) |
| Goat | Manure | 17.97 | (Zhang et al, 2013) |

## 4. Conclusion

Since Türkiye is a foreign-dependent country in terms of energy, it is important to search for alternative energy sources. In addition, bio-composite materials and compost produced from plant residues are gaining more importance day by day in terms of environmental friendliness and sustainability. The amounts of plant and animal residue belonging to Samsun province and its districts are given in tables.

Among the main products grown in Samsun, products such as hazelnut, paddy and wheat have high heat value and the amount of product is higher than other products, it is important in terms of biomass use.

Total biomass, renewable energy, bio-composite materials and compost production in Samsun province have great potential. However, this potential needs to be adequately evaluated. According to the results of the study, it was concluded that the high potential of plant residue in Samsun is important in terms of bioenergy, bio-composite materials and composting.

**Author Contributions**

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

| | C.G. | G.A.K.G. | B.D. |
|---|---|---|---|
| C | 52 | 25 | 50 |
| D | 60 | 20 | 20 |
| S | | 100 | |
| DCP | | | 100 |
| DAI | 50 | 30 | 20 |
| L | 50 | 25 | 25 |
| W | | 50 | 50 |
| CR | | 50 | 50 |
| SR | 10 | 60 | 30 |
| PM | 20 | 50 | 30 |
| FA | 20 | 50 | 30 |

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

**Conflict of Interest**

The authors declared that there is no conflict of interest.

**Ethical Consideration**

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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# BIBLIOMETRIC ANALYSIS FOR USE OF TIME SERIES IN ANIMAL SCIENCE

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**Abstract:** In this study, bibliometric analysis was applied to time series, which has been widely used in animal science studies in recent years. In the method part of the study, a bibliometric analysis was carried out for time series commonly used in animal science studies. In the study is to determine the trends in recent years in the field of animal science, by examining 3895 studies with the term "time series" in the title of the article published within the scope of SCI-Expanded between 1980 and 2023, within the scope of bibliometric analysis. Statistical evaluations were calculated using the R software belonging to the "bibliometrix" package. All data were generated bibliographically from the WoS system in plain text format. Time series has been one of the most popular research areas due to its application in many different fields such as cell biology, plant sciences, zoology, animal science, etc. There are many authors' works in the field of time series. According to the analysis, a total of 3202 studies, such as articles, journals, books, etc. by 14154 authors, were published on time series in animal science. As a result of the analysis, in the 14154 authors, only 247 studies has been single authored documents of afromentioned topic. Time series in animal science examined within the scope of author's collaboration that there were 0.275 authors per document. This study aims to conduct bibliometric analysis to determine the importance of time series in the field of agriculture, the number of publications by year, annual publication increase, and distribution by country and number of articles by keywords. The analysis results will be an important contribution to both readers and researchers.

**Keywords:** Animal sciences, Time series, Bibliometric

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## 1. Introduction

The main purpose of statistics is to examine variables from different aspects and interpret the results of data obtained from observations. The arrangement or ordering of the data obtained from the observations, taking into account some features, is referred to as a series or series. If the data obtained from these observations express the changes or movements of a variable over time, these data are called time series or time series. In other words, time series is also expressed as a sequential observation series (Çanga et al., 2021; Güneş et al., 2022; Tırınk, 2022).

Time series data are numerical data in which variables are ordered sequentially from one period to another. The most practical and easiest way to analyze the structure of the event and create future predictions by using data on variables in periods obtained over a certain period of time is to analyze it with time series. Time series data are also expressed in subgroups as economic, biological, physical and time control type data (Karaokur et al., 2019; Sözeyatarlar et al., 2021; Cui et al., 2023).

Numerical analyzes and statistical analysis of scientific studies can be defined as Bibliometrics. Bibliometric methods apply a quantitative approach to the description, evaluation, and interpretation of previously published research. Bibliometric analysis is one of the analysis methods used by researchers to interpret and evaluate research fields, countries, citation rates of publications or the number of journals. In bibliometric methods, researchers first discover the literature and show the researcher's work by presenting the most effective studies (Freire and Nicol, 2019; Donthu et al., 2021). In bibliometric analysis, it forms the number of articles in a certain time period and also shows how much the study influenced the studies done after it. The purpose of bibliometric methods is to obtain the findings of researchers and the collective bibliographic data produced by other researchers working in this field and to express the results through citation or writing. In addition, the bibliometric method is a research field that is gaining increasing attention in the scientific community and is determined by the rapid development of computers and the internet. The bibliometric method is a basic approach used to analyze research and is based on public library and information science (Persson et al., 2009; Merigó and Yang, 2017; Derviş, 2019; Han et al., 2020).

In this study, bibliometric analysis was applied to time series, which has been widely used in animal science studies in recent years (Mansioux and Carrot, 2012; Hotamışlı et al., 2014). In the method part of the study, a

bibliometric analysis was carried out for time series commonly used in animal science studies. All statistical evaluations were made using R software with the "bibliometrix" package (R Core Team, 2020). The data was bibliographically generated from the WoS system in plain text format. Then, the bibliographic data was converted into a data frame using the "convert2pdf" function with the "bibliometrix" package. Bibliometric analysis was performed by the biblioAnalysis function (Önder and Tırınk, 2022; Yavuz and Şahin, 2020). In this context, this article aims to conduct bibliometric, collaboration and co-citation analysis to determine the importance of Time series in animal science over the years.

## 2. Materials and Methods

Bibliometric analysis method was applied from the beginning of data collection to the interpretation of the analysis results. By applying the WoS database, a search was made for studies on Time series in the field of agriculture. During the data collection process, Web of Science (WoS) was searched with the keywords "animal sciences" and "agriculture". Information from 3895 agricultural field studies out of a total of 10072 studies conducted with time series between 1980 and 2023 was used as material (Olfaz et al., 2019; Öztürk and Kurutkan, 2020).

In the study, bibliometric analysis was applied to time series that have been widely used in agricultural studies in recent years. As a result of the analysis, statistical evaluations were calculated using the R software belonging to the "bibliometrix" package (R Core Team, 2020). All data were generated bibliographically from the WoS system in plain text format. The obtained data was then converted into data using the "convert2pdf" format with the "bibliometrix" package. Bibliometric analysis was implemented by the biblioAnalytics function. This study aims to conduct bibliometric analysis to determine the importance of time series in the field of agriculture, the number of publications by year, annual publication increase, distribution by country and number of articles by keywords (Aria and Cuccurulla, 2017; Yeksan and Akbaba, 2019; Sözeyatarlar et al., 2021).

## 3. Results and Discussion

There are many authors' works in the field of time series. According to the analysis, a total of 3202 studies, such as articles, journals, books, etc. by 14154 authors, were published on time series in animal science. In the 14154 authors, only 247 studies has been single authored documents of afromentioned topic. Time series in animal science examined within the scope of author's collaboration that there were 0.275 authors per document. The graphic of the number of publications in terms of yearly scientific output is given in Figure 1. According to Figure 1, while the number of time series studies in animal science was 3 in 1980 to 230 in 2022. Thus, how much this subject has been used over the years can be seen.

General information on bibliographic data on time series in animal science is given in Table 1. According to Table 1, journals, books, etc. a total of 3895 studies have been published in some sources between 1980 and 2023.

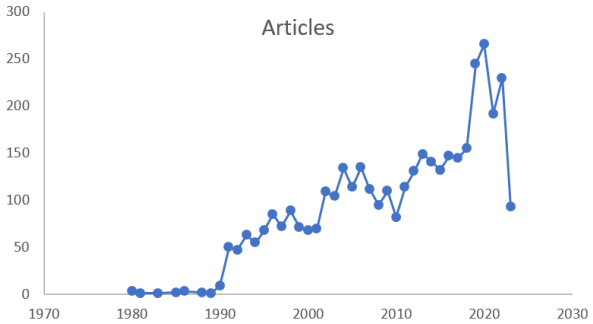


**Figure 1.** Annual scientific production on time series in animal science.

**Table1.** The primary information of the data

| Information | Number |
|---|---|
| Timespan | 1980:2023 |
| Documents | 3895 |
| Sources (Journals, Books, etc) | 364 |
| Average years from publication | 8.31 |
| Average citations per document | 14.9 |
| Average citations per year per document | 1.148 |
| Authors of single-authored documents | 247 |
| Documents per Author | 0.275 |
| Co-Authors per Documents | 4.54 |
| International co-authorships ( %) | 15.94 |

A total of 21930 studies were utilized about the time series. However, 3895 studies were used about the time series in the animal science. Document types of 1293 studies related to time series in animal science are given in Table 2. According to Table 2, the most common form of publication related to time series is the article. Also in Table 2, is given book chapters, earlaccess studies, proceeding papers and reviews about time series in animal science.

**Table 2.** Document types for time series

| Document Types | Number |
|---|---|
| Article | 3202 |
| Book chapter | 26 |
| Early access | 23 |
| Proceeding paper (Article) | 133 |
| Correction | 3 |
| Editorial material | 7 |
| Meeting abstract | 11 |
| Review | 98 |
| Proceeding paper | 382 |

Table 3 provides information about which journals the published articles are published in about the time series

selection in animal science. In Table 3, the top 7 journals of the list were shared. According to Table 3, the researchers published 316 articles in the Javma-Journal of the American Veterinary Medical Association as the first chosen journal. The second journal was Journal of veterinary Medicine Series a-Physiology with the number of 210 articles. The third journal was Veterinary Surgery with number of 192 articles.

**Table 3.** The most published articles in journals

| Sources | Number of Articles |
|---|---|
| Javma-Journal of the American Veterinary Medical Association | 316 |
| Journal of veterinary Medicine Series a-Physiology Pathology Clinical Medicine | 210 |
| Veterinary Surgery | 192 |
| Scientific Papers- Series D-Animal Science | 180 |
| Journal of Veterinary Medicine Series B-Infectious Diseases and Veterinary Public Health | 148 |
| Journal of Dairy Science | 123 |
| Animals | 100 |

The countries that publish the most in the field of time series in animal science and the number of articles by country are given in Table 4. According to Table 4, the country with the most articles is the USA with 1028 articles. The country with the most articles after USA is United Kingdom with 227 articles.

**Table 4.** The corresponding author's countries and number of articles

| Countries | Number of Articles |
|---|---|
| USA | 1028 |
| United Kingdom | 227 |
| Germany | 202 |
| Australia | 173 |
| Romania | 160 |
| China | 153 |
| Canada | 129 |
| Indonesia | 127 |
| Italy | 112 |
| Spain | 90 |

The countries with the most citations in the field of time series in animal science and their number of citations are given in Table 5. According to Table 4, the country with the most citations is the USA with 21632 total citations.

Figure 2 shows the total number of citations per year for studies on time series in animal science in the years 1980-2022.

The keywords most preferred by authors in publications are given in Table 6. According to Table 6, time series expression was used as the 10 most preferred keywords. In addition, expressions such as horse, dog, canine, time series, cattle, sheep, epidemiology, animal welfare, dairy cow and pig have been used extensively.

**Table 5.** Total citations per country

| Countries | Total Citations |
|---|---|
| USA | 21632 |
| United Kingdom | 4107 |
| Germany | 2974 |
| Canada | 2525 |
| Australia | 2229 |
| Italy | 1844 |
| Spain | 1622 |
| France | 1490 |
| Denmark | 1467 |
| China | 1410 |

**Table 6.** The most chosen keywords for time series

| Keywords | Total |
|---|---|
| Horse | 114 |
| Dog | 84 |
| Canine | 70 |
| Time series | 59 |
| Cattle | 54 |
| Sheep | 52 |
| Epidemiology | 36 |
| Animal welfare | 35 |
| Dairy cow | 28 |
| Pig | 27 |

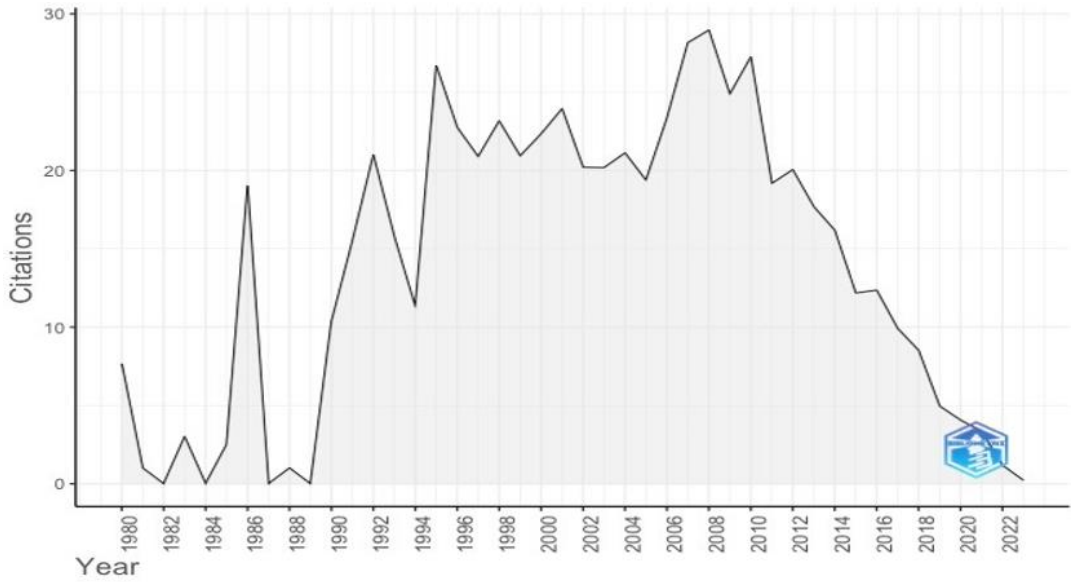


**Figure 2.** Avarage total cititaions per year for time series.

Table 7 shows the most leading authors for the time series researches. According to Table 7, the most leading author was Mayhew PD, Culp WTN and Kass PH, with 29, 25 and 23 articles about time series, respectively.

Figure 3 shows the authors' publications over time. According to Figure 3, studies in the field of time series were mostly carried out in the years 2012-2022.

Table 8 provides information on the most cited articles on time series. As a result of the bibliometric analysis made in this context, it was determined that the most citations were made to the article written by Marai IFM, published Small Ruminant Res in the journal in 2007 and article receives 29 citations in total. In addition total citation numbers of other journals are also given in the Table 8.

Figure 4 shows the common study network of studies on time series. Figure 5 shows the conceptual structure of the keywords used in studies in the field of time series. In the Figure, three clusters were formed according to the keywords used by the authors.

**Table 7.** The most productive authors for time series

| Authors | Total |
|---|---|
| Mayhew PD | 29 |
| Culp WTN | 25 |
| Kass PH | 23 |
| Brown DC | 19 |
| Monnet E | 19 |
| Withrow SJ | 19 |
| Singh A | 18 |
| Selmic LE | 17 |
| Ward MP | 17 |
| Berent AC | 16 |

**Table 8.** Top manuscripts per citations for time series

| Paper | Total Citation |
|---|---|
| Marai IFM, Small Ruminant Res | 29 |
| Jacobson RH, Rev SCI Tech OİE | 25 |
| Sorensen DA, Genet Sel Evol | 23 |
| Lopez-Gatius F, Theriogenology | 19 |
| Lind TC, J Dairy SCI | 19 |

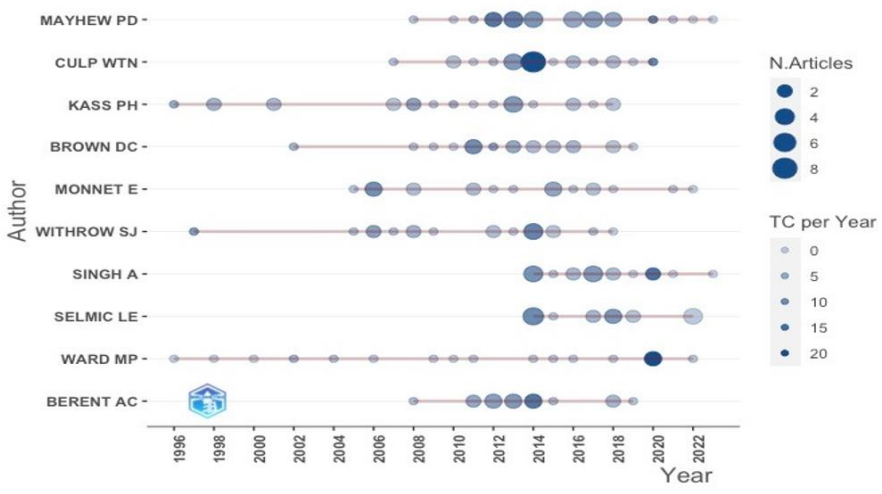

**Figure 3.** Authours publications over year.

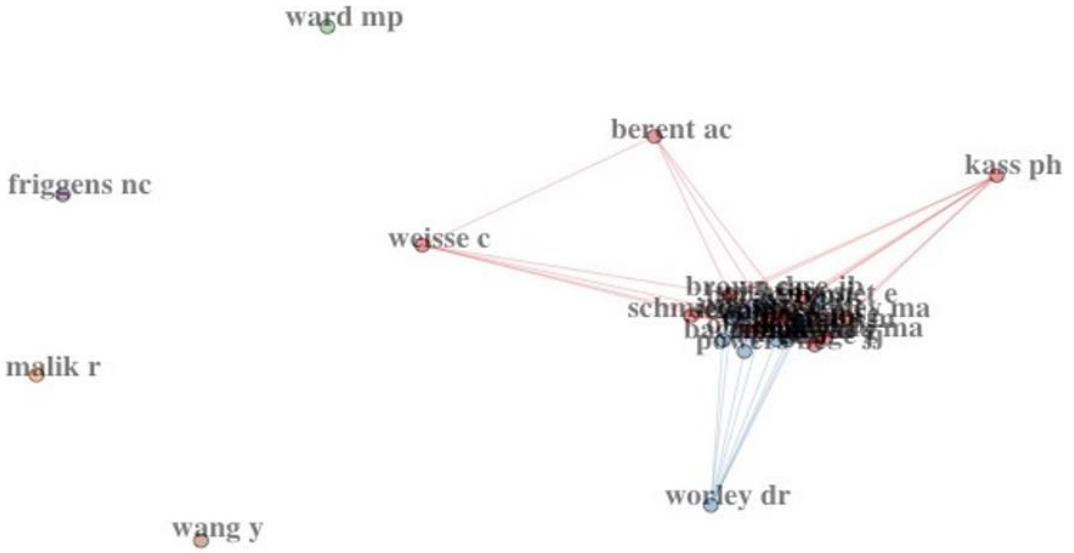


**Figure 4.** Collaboration for time series.

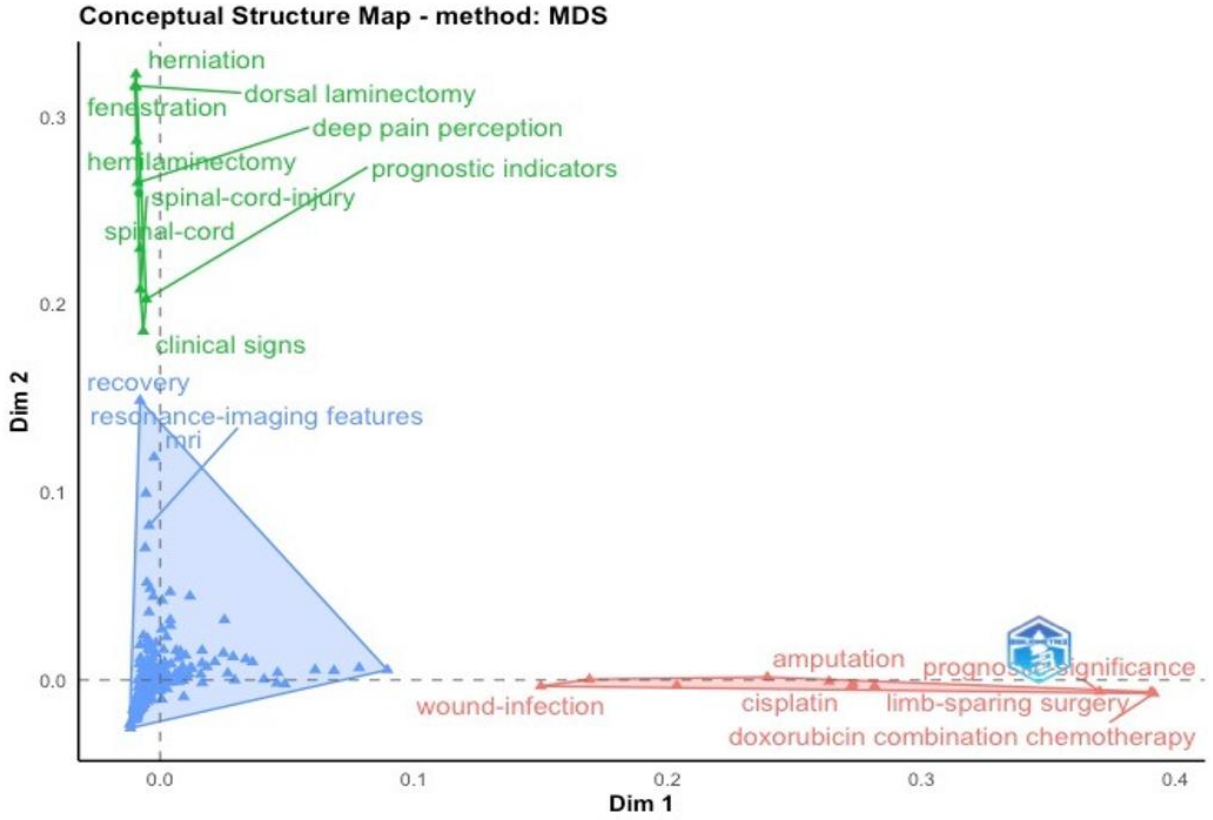


**Figure 5.** The conceptual structure for time series.

## 4. Conclusion

In this research, publications in the field of time series in animal science were examined on the basis of document, source, author and country with bibliometric analysis management and prepared for readers and researchers to reach them in the shortest way. Only Web of Science (WOS) database was used in the study. Data from other databases were not taken into account. What has not been achieved? All statistical evaluations were made using R software with the "bibliometrix" package. The depth of knowledge of the concept of time series, which always maintains its currency and importance, increases every year. With this study, the rich literature of time series management is presented to the reader by classifying, visualizing and interpreting in a way that will guide research.

Time series has been one of the most popular research areas due to its application in many different fields such as cell biology, plant sciences, zoology, animal science, etc. Especially in animal science, it is seen that there are

many studies on subjects such as meat quality, milk yield, gene expressions and reproduction, etc. Considering the distribution of 364 studies published between 1983 and 2023 on time series applications in animal science, it is seen that the most studies were done in 2020. In this context, the issue has not lost its importance and is a current issue.

In this context, as a result of the bibliometric analysis of time series in animal science, Journal of The American Veterinary Medical Association has the status of the journal with the most publications on this subject. Also, when the number of citations was examined, it was determined that the most effective author was Marai IFM. USA and United Kingdom stand out as the countries with the highest broadcasting rate.

In line with this information, it will be an important contribution that time series studies with bibliometric analysis are still up-to-date and that the studies to be done will increase their contribution to animal science. Additionally, this study facilitates the analysis of the literature by providing a holistic perspective on time series management. Researchers who will study or study on time series management will benefit from the results and have general information about the time series method.

**Author Contributions**

The percentage of the author contributions is presented below. The author reviewed and approved the final version of the manuscript.

| | E.Y. |
|---|---|
| C | 100 |
| D | 100 |
| S | 100 |
| DCP | 100 |
| DAI | 100 |
| L | 100 |
| W | 100 |
| CR | 100 |
| SR | 100 |
| PM | 100 |
| FA | 100 |

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

**Conflict of Interest**

The author declared that there is no conflict of interest.

**Ethical Consideration**

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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Research Article
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# THE EFFECTS OF ORGANIC FERTILIZATION ON THE QUALITY OF EGGPLANT SEEDLINGS

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**Abstract:** This study investigated the effects of chemical fertilizer (20-20-20) and two different organic fertilizers (compost and wood ash) applications on eggplant seedling quality. Peat was used as the growing medium. Some seedling quality parameters such as stem diameter, seedling height, number of leaves, leaf chlorophyll content, leaf area, root length and total seedling dry weight were investigated. In general, although applying chemical fertilizer is the best value in the seedling quality parameters, it was determined that statistically ($P<0.05$), similar values were obtained in applying compost. As a result, it was determined that compost fertilizer applications could compete with compound fertilizers. Compost application, which stands out regarding seedling dry weight, leaf chlorophyll content and leaf area values, has been determined to provide height control in seedlings compared to chemical fertilizer application.

**Keywords:** Eggplant, Seedling quality, Compost, Wood ash

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## 1. Introduction

Türkiye ranks 4th in world vegetable production with 31.7 million tons of vegetable production. Eggplant production, which constitutes approximately 3% of this vegetable production, is 832,938 tons in an area of 167 thousand/da (TSI, 2021). Plants used in eggplant production can be planted directly on the field by sowing seeds or planting seedlings. However, seedling cultivation has many advantages (earliness, high yield, high resistance to root diseases, homogeneous growth, energy, seed, land and time savings) compared to direct seed sowing (Özer and Kandemir, 2016; Yılmaz et al., 2018; Demir et al., 2020; Tüzel et al., 2021). The advantageous use of ready-made seedlings has increased the use of seedlings in greenhouse cultivation to 100% (Demir et al., 2020). In recent years, in parallel with the increase in vegetable production, the production of vegetable seedlings has also increased rapidly. In the ready-made seedling sector in Türkiye, only 588 million seedlings of 9 vegetable types are produced in companies registered with the Seedling Producers Sub-Association in Antalya. Eggplant seedling production constitutes 4% (23 million) of the total seedling production (Anonymous, 2023).

Although the media used to grow the seedlings differed, peat was the most preferred medium. It is used by mixing in specific proportions with materials such as peat, perlite or vermiculite. It is preferred due to its many advantages (free from diseases and high water holding capacity) (Tüzel et al., 2021). However, although peat gives very successful results in germination and emergence, fertilization is needed, especially when the seedlings reach the 2-leaf stage. For this reason, those who want to grow organic seedlings, especially those who want to grow seedlings, carry out alternative growing environments or organic fertilizers (Yılmaz et al., 2018; Tüzel et al., 2021). Since seedling production, the growing period is short and plant roots develop in a very small medium volume, it is necessary to pay attention to fertilizer applications to prevent nutrient deprivation of the plant. Nitrogen is the most important factor affecting seedling development, and giving nitrogen in the form that plants can take significantly increases tomato seedlings' fresh and dry weights (Tüzel et al., 2021).

Additional fertilizer has become an essential input item for seedling growers. For this, preparing preparations to be used as different organic fertilizers may potentially reduce the input cost and increase the seedling quality. Therefore, this study aimed to investigate the effects of different organic fertilizers that can be used as an alternative to commercial fertilizers on the quality of tomato seedlings.

## 2. Material and Methods

The research was carried out in the glass greenhouse of Ondokuz Mayıs University, Faculty of Agriculture, Department of Horticulture, between April 15 and May 30, 2023. The study used seeds of eggplant (*Solanum melongena* L. cv. Aydın siyahı) cultivar as plant material. The seeds were sown in 216 mesh EPS viols with outer dimensions of 695 x 470 x 75 mm and inner dimensions of 31 x 31 x 65 mm (mesh volume). Viols were filled with peat (100%). The seeds were kept in the germination cabinet at 27 °C until germination and emergence. Then, the viols were placed on the seedling benches in the greenhouse, the temperature of which was adjusted to 23-24 °C during the day and 19-21 °C at night. Fertilization was applied twice when the seedlings reached their first true leaf stage and the third leaves started forming until all leaves and growing media were wet.

The research used chemical fertilizer (20-20-20) and two different organic fertilizers (compost water and wood ash) to improve seedling quality. In the preparation of compost water, the stem and leaf wastes of tomato plants were composted using the heap method (Inckel et al, 2005). Holes with a diameter of 2 cm (10 cm spacing) were drilled in the plastic (770L) container used to implement this method. Tomato pruning residues (5 cm in diameter) were divided into pieces, forming a 25 cm heap. On top of this pile, 10 cm high burnt animal manure was applied. Garden soil with a height of 2 cm was added over the animal manure. This process was continued until the container was full. Finally, the heap was moistened and put on hold. The composting process continued for 60 days. During this period, the batch was mixed weekly.

Waste oak tree wood ash (*Quercus* sp.) used in lye production was taken from the bakery (Yazıcıoğlu brothers' bread oven) that produces bread in the Samsun region. Compost and ash analyses (pH, E.C., nitrogen, phosphorus and potassium) were determined according to Kaçar and İnal (2008) (Table 1).

**Table 1.** Some chemical properties of compost and wood ash fertilizer

| Values | Compost | Wood ash |
|---|---|---|
| pH | 7.73 | 12.0 |
| EC (dS $m^{-1}$) | 3.58 | 14.04 |
| Nitrogen (%) | 0.40 | 0.13 |
| Phosphorus (%) | 0.84 | 0.79 |
| Potassium (%) | 0.44 | 6.76 |

EC= conductivity

The compound fertilizer used in the study was prepared at 1000 ppm. The conductivity (EC) values of the solution obtained from the compound fertilizer were measured as 1.3 ds/m. Strainers were prepared by adjusting the EC values of ash and compost water according to the value of the compound fertilizer (1.3 ds/m). The strains obtained were used in fertilization two times as the first true leaf period and the 2-3 leaf period. While fertilizing, infiltrates were applied until all the leaves of the seedlings and the seedling growing medium were wet.

In order to determine the quality of the seedlings, when the seedlings come to the planting stage (four-five true leaf periods), the following measurements will be made in 3 replications and ten seedlings in each replication, in total 30 eggplant seedlings;

*Leaf chlorophyll content:* Chlorophyll content (CCI) in the leaves will be determined in the leaves of the seedlings between 09:00 and 11:00 in the morning using a chlorophyll meter (CCM-200, Opti-Sciences, U.S.A.).

*Leaf area:* All sheets were fixed on A3 paper and photocopied. Leaf areas were measured using a planimeter (Placom Digital Planimeter, SOKKISHA Planimeter Inc., Model KP-90) on the photocopier.

*Seedling height:* In measurement plants, the parts of the seedlings from the root collar to the growth point will be measured with a ruler.

*Stem diameter:* It will be measured with the help of a caliper 1 cm above the root collar in measurement plants.

*Number of leaves:* It will be determined by counting the total number of leaves in the measurement plants.

*Root length:* In measurement plants, the parts of the seedlings from the root collar to the tip of the longest root will be measured with a ruler.

*Root, stem and leaf fresh and dry weights:* For measurement, the roots will be washed and separated so that there is no root loss during the removal process of the seedlings. Then, the roots, stems and leaves will be divided into parts and their wet weights will be weighed. Leaves, roots and stems separated from the plant will be placed separately in small paper bags in an oven at 80 $^{o}$C. The drying process will be carried out for at least 48 hours. Whether the drying process has been completed by applying the weight change method on the samples that have not completed their drying within this period will be decided. When it is understood that the samples are completely dry, the dry weights of the leaves, roots and stems will be weighed with a scale sensitive to 0.01 g.

### 2.1. Statistical Analysis

The research will be set up using the 3-replication Random Plots trial design. IBM SPSS version 20.0 statistical analysis program will be used to analyze the variance of the result obtained from the research and determine the differences between the means (Tukey test).

## 3. Results and Discussion

Different organic fertilizer applications' significant ($P<0.05$) effects on plant height, stem diameter, leaf number, leaf chlorophyll content, leaf area and total seedling dry weight were determined in eggplant seedling cultivation. When the seedling quality parameters were examined, it was determined that the highest plant height, stem diameter, number of leaves,

leaf chlorophyll content, leaf area and relative growth rate values were obtained from chemical fertilizer application. It was determined that the highest total seedling dry values were obtained from wood ash application. In addition, in compost application, similar values were obtained for the number of leaves, leaf chlorophyll content and leaf area values with chemical fertilizers (Table 2). Seedling height (3-19 cm) and stem diameter (3-4 mm) were determined in eggplant seedling cultivation. In the study examining the effect of different paclobutrazol applications for seedling height counter, 7-12 cm spacing of eggplant seedlings was evaluated as ideal (Geboloğlu et al., 2015).

**Table 2.** The effects of different organic fertilizer (compost and wood ash) and chemical fertilizer (20-20-20) applications on stem diameter, plant height, leaf number, leaf chlorophyll content, leaf area, root length and total seedling dry weight

| | Stem diameter (mm) | Plant height (cm) | Leaf number (piece) | Chlorophyll content (CCI) | Leaf area ($cm^{2)}$ | Root length (cm) | Total dry weight (g) |
|---|---|---|---|---|---|---|---|
| Control | 1.6 c | 4.68 d | 2.0 b | 8.4 b | 8.8 b | 10.6 | 0.073 b |
| Compost | 2.3 b | 7.96 b | 3.0 a | 13.8 a | 28.8 a | 11.9 | 0.227 a |
| Wood ash | 1.8 c | 6.30 c | 2.0 b | 9.2 b | 10.7 b | 11.0 | 0.320a* |
| Chemical | 2.8 a* | 9.25 a* | 3.3 a* | 16.5 a* | 35.1 a* | 10.9 | 0.277 a |

*P<0.05

In our study, seedling height values varied between 4.68-9.25 cm considering the average size of the eggplant seedlings, the similar values of chemical fertilizer and compost applications offered an opportunity to give an idea on many issues. The seedling height values were determined to be within the desired ranges in chemical fertilizer and compound fertilizer applications. However, compost application came to the fore regarding seedling height control. Mainly when the 4-5 leaf periods of eggplant seedlings are considered, it has been observed that chemical fertilizer applications may exceed the desired values. It has been a scientific fact that the nitrogen content of chemical fertilizers is decisive here (Table 1-2). In studies conducted similar to our study, it is reported that the highest seedling length in eggplant is reached in chemical fertilizer applications (Fadıllıoğlu, 2022). In a different study, it was reported that the seedling height values in eggplant varied between 13 and 21 cm and the highest values were obtained in the control application. In contrast, 400 ppm tebuconazole application provided significant seedling height control (Öztürk and Dursun, 2020). In our study, a significant amount of height control was achieved with the application of wood ash without any chemical application.

In the study, where one of the important indicators of seedling quality was the number of leaves, it was reported that the number of leaves in eggplant seedlings varied between 3.4 and 4.8. The study determined that the number of leaves was a determinant in height control and paclobutrazol application provided height control by reducing the number of leaves (Geboloğlu et al., 2015). In our study, we can characterize the number of leaves as less than the control application as a nutritional problem. However, it is thought that the antagonistic effect due to the high potassium level can be mentioned in the wood ash (Table 1). Considering that compost application is similar to chemical fertilizer in terms of other quality criteria, it has been determined that it increases seedling quality and provides height control (Table 2). Stem diameter, plant height and dry matter content of seedlings play a decisive role in seedling quality and height control (Uçan and Uğur, 2021). When the seedling root lengths in eggplant are examined, it has been reported that it varies between 9-11 cm (Öztürk and Dursun, 2020). Although similar values were obtained in our study, no statistical difference was found.

The fact that the production period is short in seedling cultivation and the growing environments are limited in nutrients has made fertilization necessary. Nitrogen is one of the most important factors affecting seedling growth. For nitrogen to be quickly taken up by plants, it must be decomposed by microorganisms. Therefore, it is reported that the decrease in mineral N content due to mineralization in organic fertilization limits growth (Tüzel et al., 2021). In our study, it is thought that results stemming from a similar problem emerged. However, it has been determined that especially the compost application is competitive. When the leaf chlorophyll content, total seedling dry weight and relative growth rate values were examined, the chemical bore application came to the fore. In contrast, the compost application showed similarity with the chemical fertilizer application. However, it was determined that wood ash application came to the fore in total seedling dry weight values and this value also affected the relative growth rate (Table 2).

## 4. Conclusion

As a result of the study, it was determined that compost application could compete with compound fertilizer in eggplant seedling cultivation. It has been determined that using compost as fertilizer comes to the forefront regarding seedling quality values and especially seedling

height control compared to chemical fertilizer application.

Fertilizer use is an essential input in seedling cultivation. Compost application is one of the materials that can be prepared quickly and cheaply as a homemade solution. In addition to all these advantages, the use of compost increases the dry weight of the plant, the root development is balanced, and in addition to all these, it provides limited height control. It has revealed the potential to reduce seedling production costs significantly.

**Author Contributions**

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

| | K.M | A.Ç.T | A.A | H.Ö |
|---|---|---|---|---|
| C | 15 | 15 | 30 | 40 |
| D | 20 | 20 | | 60 |
| S | | | | 100 |
| DCP | 30 | 30 | 20 | 20 |
| DAI | 10 | 30 | 10 | 50 |
| L | 20 | 30 | 20 | 30 |
| W | 20 | 30 | 20 | 30 |
| CR | 20 | 30 | 20 | 30 |
| SR | 20 | 30 | 20 | 30 |
| PM | 20 | 30 | 20 | 30 |
| FA | 20 | 30 | 20 | 30 |

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

**Conflict of Interest**

The authors declared that there is no conflict of interest.

**Ethical Consideration**

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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# COMPARISON OF TEN DIFFERENT MATHEMATICAL MODELS USED IN IN-VITRO GAS PRODUCTION TECHNIQUE

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**Abstract:** In this study, the usability of models commonly used in in vitro gas production techniques in different feed sources was comparatively investigated. For this purpose, Richard, Logistic, Orskov, Verhulst, Janoschek, Weibull, Bridges, Mitscherling, Monomolecular and Von Bertalanffy models, which are widely used in the literature, were used. In comparing these models, criteria such as mean square error (MSE), coefficient of determination($R^2$), corrected coefficient of determination ($\bar{R}^2$), accuracy factor (AF), bias factor (BF), Akaike information criterion (AIC) and Bayesian information criterion (BIC) were used. As a result of the research, according to these criteria, the best model in Arbutus andrachne plant was determined as Richard, and the worst model was determined as Janoscheck and Weibull model. For Arbutus unedo, *Ceratonia siliqua* and *Laurus nobilis* L. plants, the best models were determined as Orskov, Mitscherling, Monomolecular and Von Bertalanffy models, and the worst models were Logistic and Verhulst models.

**Keywords:** In-vitro, Ruminant, Model comparison

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## 1. Introduction

Different methods such as in-vivo, in-vitro and in-situ are used to determine the feed value of feeds used in ruminant animal feeding. Although the most reliable results are obtained from in-vivo studies, they are not preferred because they are difficult to study, costly and require large amounts of feed. For these reasons, the in-vitro method based on the measurement of fermentation residues (gas) is preferred. In this method, gas measurements are made at certain intervals after the start of fermentation (3, 6, 12, 24, 48, 72, 96 and 120 hours). The relationship between rumen fermentation and gas production has been known for a long time. It has been reported that the applications of fermentative gas measurement technique in the rumen date back to 1939 and that this technique is the measurement of microbial activity (Getachew et al., 1998, Canbolat et al., 2005).

By using the amount of gas produced, the performance of animals, feed consumption, microbial protein digestion, digestibility levels of feeds, metabolic energy and net energy values of feeds, determination of protein and dry matter degradability in the rumen, in vitro degradation rate and amount of feeds can be determined. Due to advances in computers and software, many new equations have been developed in modeling gas production curves. It is extremely important to choose the most statistically accurate and meaningful model or models in terms of animal nutrition among these equations. Values of gas measurements show a sigmoidal distribution and it is extremely difficult to model this distribution with linear models. For this reason, it became necessary to use non-linear models, which are more complex than linear models. After the models are created, it is extremely important to compare the models statistically and choose the most appropriate model. In comparing models, criteria such as error mean squares, coefficient of determination, corrected coefficient of determination, accuracy factor, deviation factor, Akaike information criterion and Bayesian information criterion are used.

In this study, 10 different models used in the literature were applied on the gas production values of 4 different feed sources. At the same time, it is aimed to create an important reference source in the relevant field by obtaining model comparison criteria used in the literature.

## 2. Materials and Methods

### 2.1. Materials

In this study, gas production values obtained from *Arbutus andrachne*, *Arbutus unedo*, *Ceratonia siliqua* and *Laurus nobilis* L. plants were used. Gas values of these plants were obtained in the laboratories of KSÜ, feed and animal nutrition department. For this purpose, the amounts of gas produced from these four different feed samples were measured at different time periods (at 3, 6, 12, 24, 48, 72 and 96 hours) using the in-vitro gas production technique.

### 2.2. Methods

#### 2.2.1. Equations used in modeling

In modeling the gas values produced from four different feed sources, Richard, logistic, Orskov, Verhulst, Janoschek, Weibull, Bridges, Mitscherling, Monomolecular and Von Bertalanffy models, which are widely used in the literature, were used (Brody, 1945; Richards, 1959; Schofield et al., 1994; Groot et al., 1996; Orskov and Mcdonald, 1979). SAS statistical package program was used to estimate the parameters of the models and obtain the estimated gas production curves (SAS, 1999). The models used and their explanations are given in Table 1, and the in vitro gas production parameters of the models are given in Table 2 (Lopez et al., 1999; Kamalak et al., 2004; Canbolat et al., 2007; Üçkardeş and Efe, 2014).

**Table 1.** Mathematical models used in in-vitro gas production technique

| Models | Equations |
|---|---|
| Richard | $Y = a(1+be(-ct))^{d}$ |
| Logistic | $Y = a / (1 + e^{b-ct})$ |
| Orskov | $Y = a + b (1 - e^{ct})$ |
| Verhulst | $Y = a / (1 - be^{ct})$ |
| Janoscheck | $Y = a - (a - b)e^{-ct^{d}}$ |
| Weibull | $Y = a - be^{-ct^{d}}$ |
| Bridges | $Y = a + b (1 - e^{-(ct^{d})})$ |
| Mitscherling | $Y = a(1 - be^{-ct})$ |
| Monomolecular | $Y = a - be^{-ct}$ |
| Von Bertalanffy | $Y = a - (a - b)e^{-ct}$ |

**Table 2.** The models used in the study and parameter expressions

| Models | A | B | C | TG | SP |
|---|---|---|---|---|---|
| Richard | $a(1+b)^{d}$ | $a- a(1+b)^{d}$ | c | a | d , b |
| Logistics | a/(1+e(b)) | a-a/(1+e(b)) | c | a | b |
| Orskov | a | b | c | a+b | - |
| Verhulst | a/(1-b) | a- a/(1-b) | c | a | b |
| Janoscheck | b | a-b | c | a | d |
| Weibull | a-b | b | c | a | d |
| Bridges | a | b | c | a+b | d |
| Mitscherling | a(1-b) | a- a(1-b) | c | a | - |
| Monomolecular | a-b | b | c | a | - |
| Von Bertalanffy | b | a-b | c | a | - |

A= amount of gas produced from easily fragmented part, B= amount of gas produced from the slowly degraded part, C= gas production rate, TG= total gas, SP= shape parameter.

#### 2.2.2. Model Comparison Criteria

In modeling studies, it is not enough to obtain models with the appropriate equations for the existing data set (Özkan and Sahin, 2006; Sahin et al., 2011; Bayazıt et al., 2022). It is also necessary to evaluate how statistically sufficient the created models are in describing the data set. For this purpose, in the studies of modeling gas production curves, as in all disciplines, in the statistical comparison of the models obtained, the mean squares of error, coefficient of determination, corrected coefficient of determination, accuracy factor, bias factor, Durbin-Watson autocorrelation value, Akaike information criterion and Bayesian information criterion is used (Korkmaz et al., 2011; Cankaya et al., 2014; Tahtalı et al., 2020; Gök et al., 2021). Equations of these comparison criteria are given in Table 3.

**Table 3.** Model comparison criteria

| Criterion | Equality |
|---|---|
| Error Mean Squares | $EMS = ESS/EDF$ |
| Coefficient of Determination | $R^2 = 1 - (ESS/TSS)$ |
| Adjusted Coefficient of Determination | $\bar{R}^2 = 1 - (1 - R^2)(n - 1/(n - p - 1))$ |
| Accuracy Factor | $AF = 10^{\sum_{i=1}^{n}\lvert\log(\hat{Y}_i/Y_i)\rvert/n}$ |
| Bias Factor | $BF = 10^{\sum_{i=1}^{n}\log(\hat{Y}_i/Y_i)/n}$ |
| Durbin-Watson Value | $DW = \frac{\sum_{i=2}^{n}(e_1 - e_2)^2}{\sum_{i=1}^{n} e_1^2}$ |
| Akaike Knowledge Criteria | $AIC = nxln\left(\frac{ESS}{n}\right) + 2k$ |
| Bayesian Information Criterion | $BIC = nxln\left(\frac{ESS}{n}\right) + kln(n)$ |

ESS= error sum of squares, EDF= error degrees of freedom, TSS= total sum of squares, n= sample size, p= Number of independent variable, $\hat{Y}_i$= estimated value, $Y_i$= observation value, $e_i$= the term residual, k= Number of parameters.

## 3. Results and Discussion

3. Results and Discussion

Parameter estimates for ten different models for four different feed sources are given in Table 4, Table 5, Table 6 and Table 7. Additionally, for four different feed sources, in Table 8, Table 9, Table 10 and Table 11, mean square error, coefficient of determination, corrected coefficient of determination, bias and accuracy factors, Durbin Watson, Akaike information criterion and Bayesian information criterion values of 10 different models are given.

**Table 4.** Parameter Estimates for *Arbutus andrachne*

| Models | Parameters | | | |
|---|---|---|---|---|
| | a | b | c | d |
| Richard | 56,87 | -0,006 | 0,002 | 0,17 |
| Logistic | 41,91 | 0,23 | 0,09 | - |
| Orskov | 16,8 | 25,69 | 0,05 | - |
| Verhulst | 41,91 | -1,26 | -0,09 | - |
| Janoscheck | 295,6 | -754,7 | 1,33 | 0,01 |
| Weibull | 152,5 | 665,6 | 1,58 | 0,02 |
| Bridges | -535,1 | 619,2 | -2,17 | 0,04 |
| Mitscherling | 42,49 | 0,6 | 0,05 | - |
| Monomolecular | 42,49 | 25,69 | 0,05 | - |
| Von Bertalanffy | 42,49 | 16,8 | 0,05 | - |

**Table 5.** Parameter Estimates for *Arbutus unedo*

| Models | Parameters | | | |
|---|---|---|---|---|
| | a | b | c | d |
| Richard | 41,3 | 0,05 | 0,04 | 0,4 |
| Logistic | 41,25 | 0,23 | 0,08 | - |
| Orskov | 16,29 | 25,34 | 0,06 | - |
| Verhulst | 41,25 | -1,26 | -0,08 | - |
| Janoscheck | 42,34 | 12,25 | 0,13 | 0,76 |
| Weibull | 42,34 | 30,08 | 0,13 | 0,76 |
| Bridges | 12,258 | 30,08 | -0,13 | 0,76 |
| Mitscherling | 41,64 | 0,6 | 0,06 | - |
| Monomolecular | 41,64 | 25,34 | 0,06 | - |
| Von Bertalanffy | 41,64 | 16,29 | 0,06 | - |

**Table 6.** Parameter Estimates for *Ceratonia siliqua*

| Models | Parameters | | | |
|---|---|---|---|---|
| | a | b | c | d |
| Richard | 32,14 | 0,32 | 0,7 | 0,98 |
| Logistic | 41,95 | 0,67 | 0,124 | - |
| Orskov | 10,48 | 31,83 | 0,07 | - |
| Verhulst | 41,95 | -1,96 | -0,12 | - |
| Janoscheck | 42,33 | 10,44 | 0,07 | 0,99 |
| Weibull | 42,33 | 31,88 | 0,07 | 0,99 |
| Bridges | 10,44 | 31,88 | -0,07 | 0,99 |
| Mitscherling | 42,33 | 0,75 | 0,07 | - |
| Monomolecular | 42,32 | 31,83 | 0,07 | - |
| Von Bertalanffy | 42,32 | 10,48 | 0,07 | - |

**Table 7.** Parameter Estimates for *Laurus nobilis* L.

| Models | Parameters | | | |
|---|---|---|---|---|
| | a | b | c | d |
| Richard | 144,4 | -0,0001 | 0,0001 | 0,25 |
| Logistic | 43,01 | 0,47 | 0,05 | - |
| Orskov | 14,47 | 30,21 | 0,03 | - |
| Verhulst | 43,01 | -1,6 | -0,05 | - |
| Janoscheck | 176,5 | -19,8 | 0,15 | 0,19 |
| Weibull | 147 | 161 | 0,169 | 0,2 |
| Bridges | -26,67 | 168,8 | -0,24 | 0,16 |
| Mitscherling | 44,69 | 0,67 | 0,031 | - |
| Monomolecular | 44,69 | 30,21 | 0,031 | - |
| Von Bertalanffy | 44,69 | 14,47 | 0,031 | - |

When Table 4 and Table 7 are examined, it is seen that the "a" parameter values are equal in the Logistic and Verhulst models in the *Arbutus andrachne* and *Laurus nobilis* L. plants. A similar situation is also valid in the Mitscherling, Monomolecular and Von Bertalanffy models. When Table 5 and Table 6 are examined, the "a" parameter was found to be equal in Logistic and Verhulst, Janoscheck and Weibull, Mitscherling, Monomolecular and Von Bertalanffy models for *Arbutus unedo* and *Ceratonia siliqua* plants. In the Verhulst model, "b" and "c" parameters were obtained as negative in all feed sources. The same applies to the “c” parameter of the Bridges model.

When Table 8 is examined, it can be seen that for the *Arbutus andrachne* plant, all model comparison criteria of the Orskov, Mitscherling, Monomolecular and Von Bertalanffy models are equal, except for the bias factor. A similar situation is valid for logistic and Verhulst models. Considering the goodness of fit criteria, the best model in *Arbutus andrachne* is the Richard model. It can be said that Orskov, Mitscherling, Monomolecular and Von Bertalanffy models are in second place. The worst results were obtained from Janoscheck and Weibull models. The positions of the curves according to the point distribution given in Figure 1 support the results obtained. When the values in Table 9 for the *Arbutus unedo* plant are examined, it is seen that the best models are the Orskov, Mitscherling, Monomolecular and Von Bertalanffy models. Considering the mean squares of error, Akaike information criterion and Bayesian information criterion, the worst results were obtained in the Logistic and Verhulst models. It can be said that there is a negative autocorrelation problem in the Rizhard, Janoscheck, Weibull and Bridges models (Durbin-Watson negative autocorrelation limit value = 3.525). High coefficient of determination values were obtained in all models. The positions of the obtained curves according to the point distribution given in Figure 2 support this situation.

**Table 8.** Comparison criteria for *Arbutus andrachne*

| Models | EMS | ?² | $\bar{R}^2$ | AF | BF | DW | AIC | BIC |
|---|---|---|---|---|---|---|---|---|
| Richard | 3,10 | 0,999 | 0,999 | 1,023 | 1,002 | 2,676 | 34,209 | 101,389 |
| Logistic | 5,98 | 0,997 | 0,997 | 1,048 | 1,002 | 2,206 | 73,440 | 122,129 |
| Orskov | 4,69 | 0,961 | 0,954 | 1,039 | 1,002 | 2,361 | 58,898 | 108,103 |
| Verhulst | 5,98 | 0,997 | 0,997 | 1,048 | 1,003 | 2,206 | 73,440 | 122,129 |
| Janoscheck | 20,6 | 0,872 | 0,847 | 1,085 | 1,003 | 0,950 | 182,587 | 244,502 |
| Weibull | 8,38 | 0,948 | 0,938 | 1,053 | 1,002 | 1,489 | 78,917 | 144,510 |
| Bridges | 4,59 | 0,972 | 0,966 | 1,037 | 1,008 | 2,194 | 46,863 | 113,594 |
| Mitscherling | 4,69 | 0,961 | 0,954 | 1,039 | 1,015 | 2,361 | 58,898 | 108,103 |
| Monomolecular | 4,69 | 0,961 | 0,954 | 1,039 | 1,004 | 2,361 | 58,898 | 108,103 |
| Von Bertalanffy | 4,69 | 0,961 | 0,954 | 1,039 | 1,001 | 2,361 | 58,898 | 108,103 |

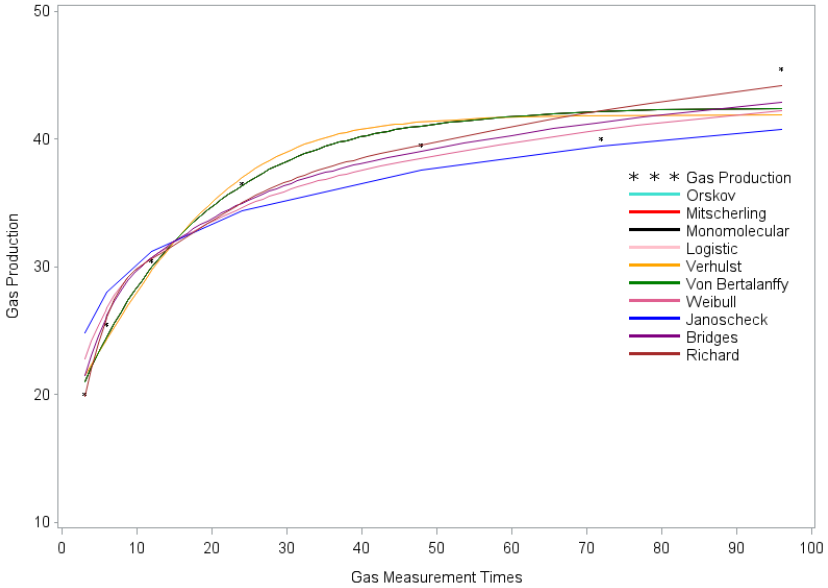


**Figure 1.** Curves were obtained from 10 different models for gas production values of *Arbutus andrachne*.

**Table 9.** Comparison criteria for *Arbutus unedo*

| Models | EMS | ?² | $\bar{R}^2$ | AF | BF | DW | AIC | BIC |
|---|---|---|---|---|---|---|---|---|
| Richard | 0,26 | 0,999 | 0,999 | 1,008 | 1,000 | 3,52 | 10,204 | 78,23 |
| Logistic | 1,02 | 0,999 | 0,999 | 1,024 | 1,001 | 2,11 | 23051,2 | 22284,6 |
| Orskov | 0,39 | 0,997 | 0,996 | 1,013 | 1,000 | 2,64 | 1287,9 | 1293,5 |
| Verhulst | 1,02 | 0,999 | 0,999 | 1,024 | 1,001 | 2,11 | 23051,2 | 22284,6 |
| Janoscheck | 0,27 | 0,998 | 0,998 | 1,008 | 1,000 | 3,45 | 1289,9 | 1312,6 |
| Weibull | 0,27 | 0,998 | 0,998 | 1,008 | 1,000 | 3,45 | 1289,9 | 1312,6 |
| Bridges | 0,27 | 0,998 | 0,998 | 1,008 | 1,000 | 3,45 | 1546,4 | 1559,9 |
| Mitscherling | 0,39 | 0,997 | 0,996 | 1,013 | 1,000 | 2,64 | 1287,9 | 1293,5 |
| Monomolecular | 0,39 | 0,997 | 0,996 | 1,013 | 1,000 | 2,64 | 1287,9 | 1293,5 |
| Von Bertalanffy | 0,39 | 0,997 | 0,996 | 1,013 | 1,000 | 2,64 | 1287,9 | 1293,5 |

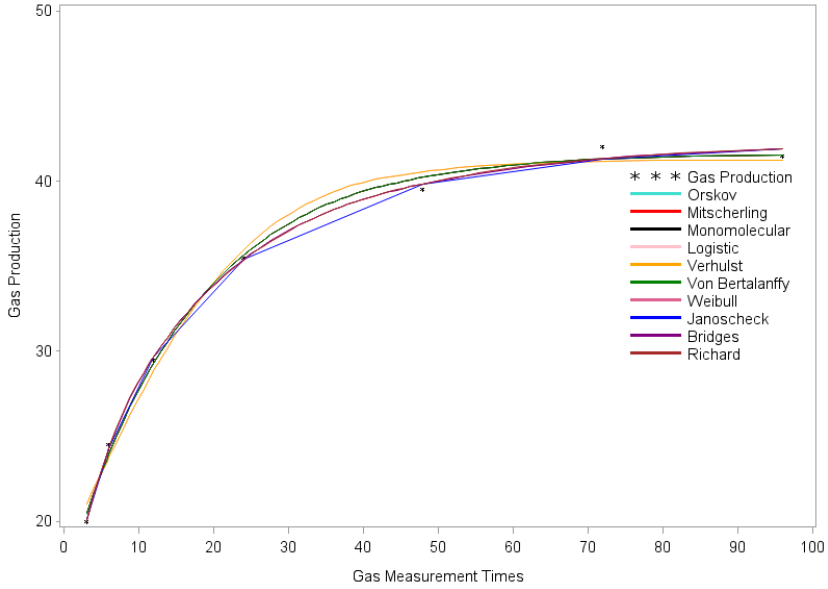


**Figure 2.** Curves were obtained from 10 different models for gas production values of *Arbutus unedo*.

When the values given in Table 10 are examined for the *Laurus nobilis* plant, it can be said that the best results belong to the Orskov, Monomolecular and Richard models. Logistic and Verhulst models have the worst results due to the very high Akaike information criterion and Bayesian information criterion values, and the Janoscheck model has the worst results due to the very high mean squares of error value. It can be said that there is a positive autocorrelation problem in the Mitscherling and Von Bertalanffy models (Durbin-Watson positive autocorrelation limit value = 0.475). The positions of the curves according to the point distribution given in Figure 3 support the results obtained. When the results in Table 11 for the *Ceratonia siliqua* plant are examined, it is seen that the best results are obtained from the Orskov, Mitscherling, Monomolecular and Von Bertalanffy models. It can be said that the Logistic and Verhulst models have the worst results due to their high mean squares of error, Akaike information criterion and Bayesian information criterion values. The positions of the curves according to the point distribution given in Figure 4 support the results obtained.

As a result, in terms of model fit criteria, it was concluded that the best model for Arbutus andrachne was the Richard model, and the worst models were the Janoscheck and Weibull model. In Arbutus unedo, Ceratonia siliqua and Laurus nobilis L. plants, the best models were determined as Orskov, Mitscherling, Monomolecular and Von Bertalanffy models, and the worst models were Logistic and Verhulst models. These results are parallel to the results obtained by Üçkardeş and Efe (2014).

Gas production curves were obtained after making coefficient estimates of Richard, Logistic, Orskov, Verhulst, Janoschek, Weibull, Bridges, Mitscherling, Monomolecular and Von Bertalanffy models for four different feed sources are shown in Figure 1, Figure 2, Figure 3 and Figure 4.

**Table 10.** Comparison criteria for *Laurus nobilis* L.

| Models | EMS | $R^2$ | $\bar{R}^2$ | AF | BF | DW | AIC | BIC |
|---|---|---|---|---|---|---|---|---|
| Richard | 14,4 | 0,994 | 0,993 | 1,042 | 1,003 | 1,96 | 130,1 | 193,8 |
| Logistic | 6,08 | 0,997 | 0,996 | 1,042 | 1,003 | 1,96 | 20297,9 | 19628,9 |
| Orskov | 3,25 | 0,981 | 0,977 | 1,042 | 1,003 | 1,96 | 1946,3 | 1928,6 |
| Verhulst | 6,08 | 0,997 | 0,996 | 1,063 | 1,007 | 1,85 | 20297,9 | 19628,9 |
| Janoscheck | 11,5 | 0,950 | 0,940 | 1,063 | 1,007 | 1,85 | 1948,3 | 1947,6 |
| Weibull | 4,96 | 0,978 | 0,974 | 1,042 | 1,003 | 1,96 | 1948,3 | 1947,6 |
| Bridges | 4,69 | 0,980 | 0,975 | 1,034 | 0,980 | 1,23 | 1948,3 | 1947,6 |
| Mitscherling | 3,25 | 0,981 | 0,977 | 1,077 | 0,928 | 0,59 | 1946,3 | 1928,6 |
| Monomolecular | 3,25 | 0,981 | 0,977 | 1,032 | 0,984 | 1,36 | 1946,3 | 1928,6 |
| Von Bertalanffy | 3,25 | 0,981 | 0,977 | 1,081 | 0,925 | 0,57 | 1946,3 | 1928,6 |

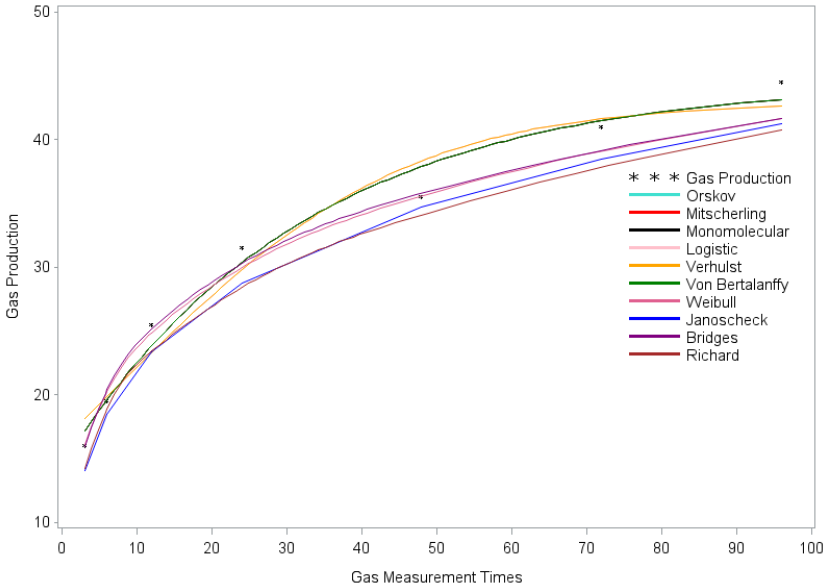


**Figure 3.** Curves were obtained from 10 different models for gas production values of *Laurus nobilis* L.

**Table 11.** Comparison criteria for *Ceratonia siliqua*

| Models | EMS | ?² | $\bar{R}^2$ | AF | BF | DW | AIC | BIC |
|---|---|---|---|---|---|---|---|---|
| Richard | 0,06 | 0,999 | 0,999 | 1,005 | 1,000 | 3,29 | 8,57 | 76,6 |
| Logistic | 0,56 | 0,999 | 0,999 | 1,019 | 1,002 | 2,06 | 23578,2 | 22792,9 |
| Orskov | 0,05 | 0,999 | 0,999 | 1,005 | 1,000 | 3,28 | 1815,0 | 1801,89 |
| Verhulst | 0,56 | 0,999 | 0,999 | 1,019 | 1,002 | 2,06 | 23578,2 | 22792,9 |
| Janoscheck | 0,06 | 0,999 | 0,999 | 1,005 | 1,000 | 3,29 | 1817,0 | 1820,92 |
| Weibull | 0,06 | 0,999 | 0,999 | 1,005 | 1,000 | 3,29 | 1817,0 | 1820,92 |
| Bridges | 0,06 | 0,999 | 0,999 | 1,005 | 1,000 | 3,29 | 1817,0 | 1820,92 |
| Mitscherling | 0,05 | 0,999 | 0,999 | 1,005 | 1,000 | 3,28 | 1815,0 | 1801,89 |
| Monomolecular | 0,05 | 0,999 | 0,999 | 1,005 | 1,000 | 3,28 | 1815,0 | 1801,89 |
| Von Bertalanffy | 0,05 | 0,999 | 0,999 | 1,005 | 1,000 | 3,28 | 1815,0 | 1801,89 |

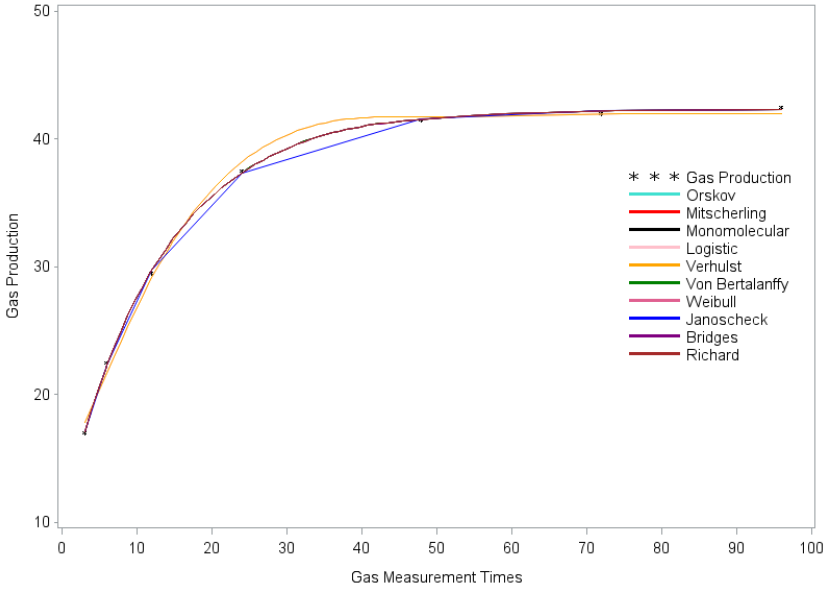


**Figure 4.** Curves were obtained from 10 different models for gas production values of *Ceratonia siliqua*.

As can be seen here, although gas production curves show a certain sigmoidal distribution, they may differ slightly in different feed sources and studies. For this reason, it is extremely important to include as many equations as possible in modeling studies to obtain reliable curves and parameters. On the other hand, it is of particular importance that parameters such as the amount of gas produced from the easily degraded part, the amount of gas produced from the slowly decomposed part, gas production rate and total gas production are easily interpretable and meaningful values in terms of animal nutrition. Ignoring residual values in model selection will lead to erroneous determinations and erroneous interpretations. It would be more statistically accurate to consider one or more of the model comparison criteria such as Durbin Watson, deviation factor, accuracy factor, Akaikle information criterion and Bayesian information criterion, which take into account the error terms of the models, together with other criteria.

## 4. Conclusion

As a result, it was determined that the models used could give different results in different feed sources, in other words, the models showed different reactions. For this reason, the use of more than one model in gas production curves is extremely important in choosing the right model and naturally in making correct interpretations and determinations. In addition, in this study, it was determined that fit criteria such as Durbin-Watson, Bias factor, accuracy factor, Akaikle information criterion and Bayesian information criterion based on error terms are extremely effective in model selection. Considering all the criteria, it was concluded that statistically models other than Logistic and Verhulst models can be easily used in modeling in vitro gas production curves.

**Author Contributions**

The percentage of the author contributions is presented below. The author reviewed and approved the final version of the manuscript.

| | M.Ş. |
|---|---|
| C | 100 |
| D | 100 |
| S | 100 |
| DCP | 100 |
| DAI | 100 |
| L | 100 |
| W | 100 |
| CR | 100 |
| SR | 100 |
| PM | 100 |

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management.

**Conflict of Interest**

The author declared that there is no conflict of interest.

**Ethical Consideration**

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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Review
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# PRODUCTION SYSTEMS AND PHENOTYPIC VARIABILITY OF THE GUINEA FOWL (*Numida meleagris*) IN SUB SAHARA AFRICA

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**Abstract:** The diversity of natural biological resources is at the heart of concerns on food security and climate change. Originating in Africa, guinea fowl constitutes an alternative source of income and protein of animal origin that can be easily mobilized, mainly for rural populations. How-ever, relatively little information is available on its production system, while its genetic diversity remains an enigma in most Sub Sahara African countries. This study therefore aimed to review the production systems and phenotypic variability of the guinea fowl (*Numida meleagris*) in Sub Sahara Africa. It revealed that this species is distributed almost all over the Africa; it is more frequent in many African countries where some studies showed that this species is extensively family farm with a variability of morphometric characters that is a function of sex and environment as well as the production system. This variability seems to indicate its adaptation to environmental conditions. The present review also revealed the need to extend studies to all the agro-ecological zones of Sub Sahara Africa in order to undertake global actions for a sustainable exploitation of the guinea fowl. This would necessarily include variability studies for its genetic improvement, preservation and conservation.

**Keywords:** Production system, Phenotype, Guinea fowl, Sub Sahara Africa

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## 1. Introduction

Biodiversity refers to the biological diversity of living organisms (fauna, flora, microorganisms) at different levels of ecological organization. It is expressed in terms of specific, genetic and ecosystem diversity and includes its composition, structure, and functions. One of the challenges is to maintain these properties, the evolutionary capacities of living organisms and their ability to adapt to global changes. Agriculture depends on biodiversity, on the proper functioning of ecosystems through the biological diversity necessary for domestication, soil fertility or pollination (Couvet, 2015). Consequently, the future of biodiversity and agriculture are linked. For this to, it is necessary to understand their interactions and therefore their present and future dynamics, especially since the current needs of an ever-growing human population are linked to the proper control of current resources of all kinds with a view to their sustainable exploitation. Therefore, the characterization of local species and their production systems was identified as the first field of global interest for the sustainable management of animal genetic resources (FAO, 2008). Indeed, the optimal use of animal genetic resources depends on a good knowledge of their genetic basis, especially in view of the constantly changing environment (FAO, 2008; Groeneveld et al., 2010). The absence of coherent management strategies for domesticated species has resulted, among other things, in the weakening of performance, uncontrolled crossbreeding and the loss of their diversity (Chepnda, 2012). In general, the state of biodiversity loss of animal resources in the world is alarming and is relatively undocumented on the African continent in general and in Sub Sahara Africa in particular (FAO, 2015a).

A lot of attention has been paid to industrial livestock farming which has become very unsustainable and fragile

in the face of global changes; yet non-conventional species are better adapted and resistant to weather as well as to diseases in various environments. They are sources of animal protein and income mainly for rural and peri-urban populations (McGowan et al., 2012). Among these species, the guinea fowl represents 3% of the world's poultry population (FAO, 2008). In addition to its socio-economic and environmental interest (Annor et al., 2013; Dongmo et al., 2016; Djonwe, 2017; Massawa et al., 2020), the genetic diversity of guinea fowl remains puzzling. Hence, this study aims at reviewing the production systems and phenotypic variability of the guinea fowl species in order to undertake actions for its sustainable exploitation as well as its genetic improvement and preservation.

## 2. Origin and Domestication of Guinea Fowl

*Numida meleagris* is the wild ancestor of the West African subspecies *Galeata* which gave rise to the domestic guinea fowl. The Latin name 'Meleagris' comes from Greek mythology where the legend of the Argonauts reveals that when the Greek hero Meleager lost his sisters, they cried themselves to death, so the goddess Artemis transformed them into birds called 'guinea fowls' to save them from the flames of hell. Guinea fowl are therefore Meleager's sisters, and Aristotle described them as Meleagris in the fourth century BC (Le Coz-douin, 1992; Savadogo, 2013).

The guinea fowl originated in Africa (Hasting, 1984; Moreiki and Seabo, 2012). Because of its origin in North Africa and Guinea, it was called the 'Numidian fowl' and the 'Guinea fowl' respectively by the Romans; hence the English name 'guinea fowl' by Le coz-douin (1992). Because of the colorful appearance of its plumage, the Portuguese called it "pintata", then "pintada" by the Spanish and finally "pintade" by the French. According to the Robert dictionary, this term first appeared in the French language in 1643 (Nagalo, 1984). In his “Systema Naturae", the 18th century Swedish naturalist Charle Linnaeus describes the common guinea fowl as *Numida meleagris* (Le coz-douin, 1992), thus associating its legendary origin with its geographical origin.

The guinea fowl is widely distributed in Africa where it lives either in small groups or in large flocks (Cauchard, 1971 cited by Nagalo, 1984) and where it is widely reared in farms (Nwagu and Alawa, 1995). Meleagriculture, which has long been practiced by the Peuhl in a purely traditional way, is nowadays widespread in most parts of the world (Boko, 2004). However, history reveals that the guinea fowl was first domesticated in ancient Greece and by the Romans (Salichon, 1983; Ikani and Dafwang, 2004; Moreki and Seabo, 2012). Genetic selection from African strains, characterised by better zootechnical performance, has led to the industrial production of guinea fowl in Europe, North America and Australia (Bonds, 1997; Boko et al., 2012).

## 3. Geographic Distribution

In addition to its undoubted African origin (Hastings, 1984), the guinea fowl is also reared extensively in temperate zones where it represents a significant market (Gnassimgbe, 1983). It was introduced to the Americas around 1508 by the Genoese together with the first black slaves from Guinea led by Spanish settlers (Nagalo, 1984). The expansion of the guinea fowl occurred in two periods as presented first by Cauchard (1971), then by Gnassimgbé (1983) and Nagalo (1984):

- Ancient times during which guinea fowl migrated from West Africa to North Africa, specifically to Egypt and progressively to Ancient Greece and then to the Mediterra-nean coast;
- Modern times during which the expansion of the guinea fowl was made from the Af-rican cradle to Portugal, then to France, Siberia, the Antilles and Java. France is now the largest producer of guinea fowl (Champagne and Leveque, 2007; Agreste, 2011; Champagne and Segret, 2013).

In Africa, almost all guinea fowl species are still found either in the wild, sometimes semi-domesticated and/or domesticated, and very few in captivity, depending on the region. This population seems to be poorly characterized due to a general lack of information. In Came-roon, guinea fowl farming is highly concentrated in the northern, Far North, North and Ada-mawa regions respectively; but also in the Central and Littoral regions, mainly at poultry mar-kets, while in West Cameroon, the species present seems to be dominated by imported strains (Ngandeu and Ngatchou, 2006; DREPIAEN, 2011; MINEPAT, 2014) (Figure 1).

## 4. Production Systems

In Sub Sahara Africa, guinea fowl farming is practiced in a free roaming system and is not very productive in general, but there are a few modern farms that mainly use improved strain from imports (Sayila, 2009; Boko et al., 2012; Annor et al., 2013; Dongmo et al., 2016; Massawa et al., 2020). The daily monitoring of flocks of up to 15 guinea fowls is generally carried out by women and children, but decisions are made by men (Laurenson, 2002; Dahouda et al., 2007; Bouba, 2017; Dao, 2018). However, as there are no prohibitions, meleagriculture is practiced by all social classes. The principal production goal of guinea fowl is for sales (90%) and own consumption of eggs and meat; however, this species is also used for gifts and prestige. Its breeding constitutes a form of saving and relatively easy to carry out (Ikani and Dafwang, 2004; Djovonou, 2010)

This type of farming is similar to that of local chickens, with the difference that guinea fowl require more space, that is about 5 adults guinea fowl per square meter instead of about 10 in the case of chicken (CTA, 1990; Meutchieye and Djiotsa, 2015).

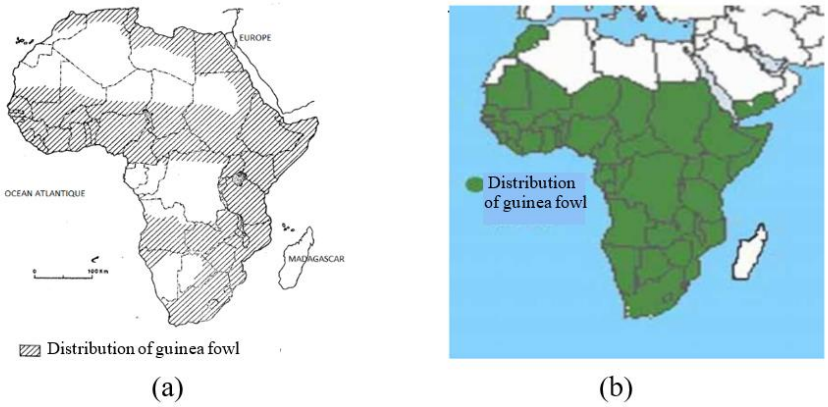


**Figure 1.** Distribution of guinea fowl in Africa (a)= ancient times; (b)= modern times (Gnassimgbé, 1983).

The birds roam throughout the day and find shelters around family compounds at the end of the day for the night. No importance is generally attached to rearing equipment such as feeders, troughs, and other (Saina et al., 2005; Sanfo et al., 2007$_a$; Moreiki, 2009; Moreiki and Seabo, 2012).

Guinea fowl's diet consists of greens, kitchen scraps and seeds. Guinea fowl has an omnivorous tendency. Very few producers formulate complete feeds for guinea fowl. However, there are a few pasture-based farms and sometimes distribution of maize seed, oilcake, meal and millet as supplements (Bastianelli et al., 2002; Sanfo et al., 2009; Dahouda, 2009; Savadogo, 2013; Dongmo et al., 2016; Massawa et al., 2020).

The guinea fowl can fall victim to almost all health and technical constraints encountered in village chicken farming. Although it is less susceptible to Newcastle disease, it remains an important reservoir. The guinea fowl is particularly susceptible to internal parasites (Nagalo, 1984; Susan, 1992; Sidibe, 2001; Bastianelli et al., 2002; Massawa et al., 2020).

Sex ratio is generally not respected; however, a ratio of one male to four females is sometimes observed. A female guinea fowl lays between 80-100 eggs of 43g on average per year. Laying starts from the 8th month and the brooding period is 28 days. Because of the guinea fowl's poor brooding ability, its eggs are usually given to hens or ducks for brooding (SAILD, 2001; Sanfo, 2005; Sanou, 2005; Sanfo et al., 2007$_b$; Sanfo et al., 2008; Dongmo et al., 2016; Massawa et al., 2020). Meleagriculture is still primitive, and its practice is essentially traditional in Sub-Sahara Africa.

Faced with the multifactorial constraints that reduce guinea fowl productivity and despite empirical research, breeders develop endogenous know-how, including the cutting of feathers to prevent guinea fowl from flying away, the provision of supplementary feed, generally consisting of a few handfuls of cereals for habituation and the placing of eggs under hens and ducks for brooding (Biagini, 2006; Dongmo et al., 2016).

Although the commercial circuit of Meleagriculture products is not well defined in Cameroon, the average market price of an adult guinea fowl (12 weeks old) of about 2.5 kg live weight is around 2.5 euros (SAILD, 2001; Dongmo et al., 2016). This price is generally higher for females than males because of weight dimorphism in favor of females. Guinea fowl appears to be less expensive in rainy season than in dry season. However, the average price of the products of mixed farming remains lower than those of the local hen (Fotsa et Poné, 2001).

## 5. Morphometric Characteristics

Preliminary studies of the phenotypic biodiversity of guinea fowl in Sub Sahara Africa reveal variability in morphological (Figure 2) and biometric (Table 1) characteristics.

In addition, the same source inventoried nineteen colors whose photos could not be represented. These include in particular: blue coral, Buff White, Chamois, Coral White, Dundotte, Grey, Lakenpur, Light gray, Lilac, opal white, Pearl, porcelain white, Silverwing, Splattered, Spotted, white-breasted pearl, White-breasted purple, White of Dondotte, Wild type.

With few exceptions, most of the work carried out in Africa on the variability of the qualitative traits of the local guinea fowl populations listed almost the same colors as recorded by AU-IBAR (2015). The morphological characteristics observed in guinea fowl are variable, which is mostly due to environmental and/or genetic effects as well as the production system. Plumage and tarsi coloration change with age (AU-IBAR, 2015), while mumps color differs according to light and certain periods of excitement (Boussini, 1995; Agbolosu et al., 2015); the color of the skin is linked to the breeding system but mainly the diet (Agbolosu et al., 2015; Panyako et al., 2016). Sex discriminating variables are crest shape, live weight and barbels development as well as calls. Males have more developed barbels directed towards the front of the bill while females have less developed barbels directed towards the back of the bill. Similarly, a male has more developed crests than female. Some producers differentiate sex based on egg shape: eggs with pointed tips are males while round tips represent females (Annor et al., 2013; Issoufou, 2016, Bouba, 2017, Djonwe, 2017; Meutchieye et al., 2018;

Mwandwe, 2019; Gondebne, 2019). However, the studies of visible polymorphism in guinea fowl in many countries of Africa are limited to a few localities, although guinea fowl seems to be present in almost all agro-ecological zones of the Sub Sahara Africa.

Table 1 summarizes some of the measurable variables that allow for better discrimination of guinea fowl in Sub Sahara Africa.

Table 1 shows a diversity of values for the quantitative variables considered. From the same authors, it was revealed that there were intra and inter-meleagre population differences ac-cording to zone and sex. This suggests that these variables are the most discriminating of guinea fowl populations in Sub Sahara Africa. However, the influence of farming systems and socio-economic characteristics on all guinea fowl phenotypic variables remains.

Preliminary analysis of phylogenetic relationships and the structure of common guinea fowl populations in the Sudano-Sahelian zone of Cameroon based on 13 body measurements made it possible to distinguish 03 genetic types categorized into 2 subgroups according to genetic distances and inter and intra population variation (Dongmo et al., 2020).

## 6. Genetic Identification of Guinea Fowl

### 6.1. Karyotype

In the Galliformes order, the chromosome number is quite conservative and ranges from 76 to 84 (Takagi and Sasaki, 1974; Stock and Bunch, 1982; Belterman and de Boer, 1984) and that of the guinea fowl is estimated to be between 74 and 78 chromosomes (Piccinni and Stella, 1970; Takahashi and Hirai, 1974). Research by Shibusawa *et al* (2002) indicates that the guinea fowl genetic material consists of 38 pairs of autosomes and one pair of homogamous gonosomes in the male (ZZ) and heterogamous gonosomes in the female (ZW) in the ZZ/ZW system, i.e., 2n = 78. Indeed, almost all birds, some reptiles, butterflies, fish and amphibians are grouped in this system, whereas mammals are in the XX/XY system (Manjeli et al., 2009). As in the hen, karyotyping of the guinea fowl can be done following either of the protocols described by Eldrige (1985); Austic and Nesheim (1990); Popescu *et al*. (1998). However, compared to chicken, the karyotype shows differences in telocentric chromosome 3, chromo-metacentric chromosome 5, acrocentric chromosomes 6 and 7, and submetacentric chromosome Z (Shibusawa et al., 2002). Goswami and Harpreet (1996) showed that the hen and guinea fowl are genetically very close and the crossing of these two species results in a hybrid ('Numigal') with a karyotype of 70 chromosomes (2n = 70).

### 6.2. Genetic formulas

The genetic formula proposed by Colona quoted by Dams (1996) with corresponding feather colors are shown in Table 2.

Thus, the color Isabelle (Table 2) is determined by a gonosomal gene, i.e., linked to sex. The male guinea fowl carrying this gene is therefore homogamous recessive (is is) or dominant (Is Is), whereas the female guinea fowl with two different sex chromosomes, one of which is identical to the male's, is heterogamous (Is Is). Apart from the Isabelle gene, the other genes are dependent on an autosomal recessive gene, i.e., not linked to sex.

According to the reference classification (version 5.1, 2015) of the International Ornithological Congress (phylogenetic order), there are 04 genera of guinea fowl divided into 06 species (Table 3).

**Table 1.** Average values of some bodies measurements of local guinea fowl in Sub Sahara Africa

| Sources | Variables | | | | | | |
|---|---|---|---|---|---|---|---|
| | PV | PT | LA | EA | LCo | LP | LT |
| Fajemilehin (2010) | 967.12 | 30.19 | 23.02 | - | 41.75 | 13.74 | 8.94 |
| Ogah (2013) | 1420.00 | 35.37 | 19.34 | - | - | 11.87 | 7.73 |
| Payako *et al.* (2015) | 1446.60 | - | 25.30 | - | 44.04 | - | 9.68 |
| Issoufou (2016) | 1064.00 | 25.82 | - | 42.77 | 40.44 | 12.88 | 6.41 |
| Dongmo *et al.* (2018) | 1210.00 | 32.36 | - | 36.56 | 43.40 | 11.99 | 6.64 |
| Massawa (2018) | 2110.00 | 31.62 | 22.83 | 45.65 | 37.93 | 8.97 | 6.14 |

*PV expressed in grams and other variables in centimeters; PV= live weight, PT= thoracic circumference, LA= wing length, EA= wingspan, LCo= body length, LP= leg length, LT= tarsal length.*

**Table 2.** Genetic formula with different colors of the corresponding guinea fowl plumage (Dams, 1996)

| Guinea fowl with pearl | | Guinea fowl with no pearl | |
|---|---|---|---|
| Pure gray | PP LL CC Is- (Is Is for males) | Violet | pp LL CC Is- (Is Is for males) |
| Lilac | PP ll CC Is- (Is Is for males) | Azure | pp ll CC Is- (Is Is for males) |
| Isabelle | PP LL CC is- (is is for males) | Rachel | pp LL CC is- (is is for males) |
| Chamois (white) | PP LL cc Is- (Is Is for males) | Fulvette | pp LL cc Is- (Is Is for males) |


**Figure 2.** Color diversity of guinea fowl in Sub Sahara Africa (AU-IBAR, 2015)

**Table 3.** Genus, species, and description of the guinea fowl in the taxonomic order (Annor et al., 2013)

| Genus | Species | Description |
|---|---|---|
| Agelaste | *Agelaste meleagrides* | White-breasted guinea fowl |
| | *Agelaste niger* | Black-breasted guinea fowl |
| Numida | *Numida meleagris* | Helmeted guinea fowl |
| Guttera | *Guttera plumifera* | Black-breasted guinea fowl |
| | *Guttera pucherani* | Crested guinea fowl |
| Acryllium | *Acryllium vulturinum* | Vulturine guinea fowl |

## 7. Conclusion

The guinea fowl is an alternative protein resource, a source of income and animal protein for the rural population essentially, and its breeding is not subject to any prohibition. In Sub Sahara Africa, this breed is practiced in an extensive family system, with a variability of morphometric traits. Under the influence of gender and environment as well as the production system, this variability reflects its adaptation to environmental conditions. However, the information currently available is insufficient to make decisions related to the genetic improvement of guinea fowl in sub-saharan Africa as well as their conservation. In addition, its exploitation dominated by direct harvesting from the wild contributes to a genetic imbalance with the consequence of the disappearance of the species. The present review reveals the need to undertake global actions for the sustainable exploitation of the guinea fowl which would necessarily include a study of its variability for genetic improvement and conservation. It would also be necessary to consider genotypic research in order to determine candidate genes linked to the adaptation traits of guinea fowl as well as to meat and egg production for future exploitation.

**Author Contributions**

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

| | D.D.F. | F.M. | M.J. | D.K.F. | M.K.J.P. |
|---|---|---|---|---|---|
| C | 30 | 20 | 20 | 10 | 20 |
| D | 100 | | | | |
| S | | 40 | | 20 | 40 |
| DCP | 60 | | 40 | | |
| DAI | 50 | 10 | 20 | 10 | 10 |
| L | 60 | 10 | 10 | 10 | 10 |
| W | 60 | 10 | 10 | 10 | 10 |
| CR | 20 | 20 | 20 | 20 | 20 |
| SR | 45 | 15 | 10 | 15 | 15 |
| PM | 20 | 20 | 20 | 20 | 20 |

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management.

**Conflict of Interest**

The authors declared that there is no conflict of interest.

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# PRODUCTION, NUTRITIONAL QUALITY AND MICROBIAL SAFETY OF SELECTED NIGERIAN DRIED MEAT PRODUCTS: A REVIEW

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**Abstract:** Production and consumption of dried meat products are increasing considerably across all nations because they are nutritious, low in fat, easily accessible, and convenient for customers to eat. Over the years, its roles have become vital in human diet as they are consumed to combat protein malnutrition and boost food security of undernourished people in underdeveloped and developing nations. Initially, dried meat products are made to satisfy consumer expectations for sensory and nutritional attributes as well as to reduce meat wastage and increase the meat shelf life during prolonged transportation and storage. Recently, the discovery of contamination that is above the minimal threshold advised for meat safety has made the safety of dried meat products the focus of microbiological evaluation. It is well recognized that eating meat products with poisoning microorganisms could put customers at risk for health problems. As a result, it is critical to refocus the research to determine the viability of dried meat products for eating after production by evaluating the production processes, nutritional quality, and microbial safety. Therefore, this review aimed to highlight the production procedures, nutritional quality and microbial safety of dried meat products and their suitability for consumption after production.

**Keywords:** Dried meat products, Mycotoxin, Microbial safety, Kilishi meat, Tinko meat

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## 1. Introduction

Traditional dried meat is one of the most important meat products that reflect the culture, heritage and identity of people across the world (Karabasil et al., 2018) because they are usually produced using ingredients and procedures from ancient times. Frequently, traditional meats are prepared to satisfy the expectations of consumers (regarding sensory, nutritional aspects and safety), reduce meat wastage and combat protein malnutrition (protein deficiency) and boost food security in the nation (Laranjo et al., 2017). They are usually produced from high quality and carefully selected raw meat and produced and consumed as rich source of protein and other essential nutrients such as minerals, vitamins and amino acids.

Dried meats are products made from the muscle or carcass of animal which have been subjected to dehydration by thermal treatment in order to enhance taste and extend shelf life during transportation and storage (Mediani et al., 2022). Additionally, it refers to meat products whose moisture content has been dehydrated to a level below 25% with expression of water activity ranging between 0.00 and 0.60 (Mishra et al., 2017). The low moisture content of dried meat guarantees longer shelf life because of the low water activity which inhibits microbial growth (Eke et al., 2013).

The most common dried meat products in Nigeria include kilishi, tinko, catfish etc. In other countries, different varieties of dried meat products such as biltong (South Africa) pastirma (Turkey), bundner fleisch (Switzerland), beef jerky (USA), rougan and shafu (PR China) etc. have been developed (Mediani et al., 2022; Sivaranjani et al., 2022). Dried meat and meat products may play a major role in providing protein-rich food to under nourished people in underdeveloped and developing nations. These products are of much interest since they do not require refrigeration during marketing as well as storage. Until recently, the consumption of dried meat products has been regarded as being safe because of the heat treatments that are usually involved during their processing and production (Ribah et al., 2020), which enabled preservation for a longer period of time without any sign of deterioration and off-flavor. However, the discovery of contaminants such as mycotoxins in dried meat products has created a public health concern around its consumption in recent times. Since there is an increase in demand and consumption of meat across the world, it is needed to highlight the production process, nutritional and safety quality of dried meat and factors that influence their production and consumption.

## 2. History and Consumption of Dried Meat Products

For thousands of years, countries all over the world have used different traditional processing methods such as sun drying and smoking to preserve and extend the shelf-life of meat and meat products. The idea of drying meat began with the necessity to reduce the moisture content and weight of meat as well as maintain nutritional content for longer periods as a source of protein for merchants and military officers traveling long distances. There is evidence that production of dried meat began in ancient times in Asia, North America, Africa, and Europe, while in South America, the drying of meat was reported to begin in year 1500's. However, statistics on the production and consumption of dried meat are scarce in Nigeria. It is known that dried meat products hold a large share in local meat production and consumption in Nigeria and across the world. In Nigeria, the consumption of dried meats (e.g kilishi meat) is common in the northern part of Nigeria, partly because of the abundance of cattle and camels for production and also due to intensity of sunlight (Akerele et al., 2010). In recent times, however, the consumption of dried meat product has gone beyond the borders of northern part of the country and become delicacies that cut across different ethnic groups, religion, social and economic class.

## 3. Types of Dried Meat in Nigeria

In Nigeria, several dried meat products have been developed over time with Banda or kundi or tinko (boiled and sundried meat), Balangu (smoked chunks of meat), Dambu-nama (spiced, cooked, pounded, shredded and dried meat) and Kilishi (sliced, coated and sundried meat) being the most prominent products majorly prepared in the Northern parts of the country (Muhammad et al., 2010; Ajiboye et al., 2011; Adeyeye et al., 2016). Others include Ndariko, Jiorge etc (Ajiboye et al., 2011) which are prepared from meats of donkeys, asses, horses, camel, buffalo and wild-life (Ajiboye et al., 2011). The production of the different types of meats is further discussed in the next sections.

## 4. Production of Kilishi Meat

Kilishi is a popular, traditionally processed ready-to-eat meat product usually produced in the northern part of Nigeria. The processed meat is originally made from fresh carcass of cattle but has now been extended to other ruminant and non-ruminant animals such as sheep, goat, pig and camel (Ayorinde, 2015). The production of kilishi involves four essential stages of technology that include meat preparation, infusion of ingredients/spices, application of heat and storage/packaging (Figure 1).

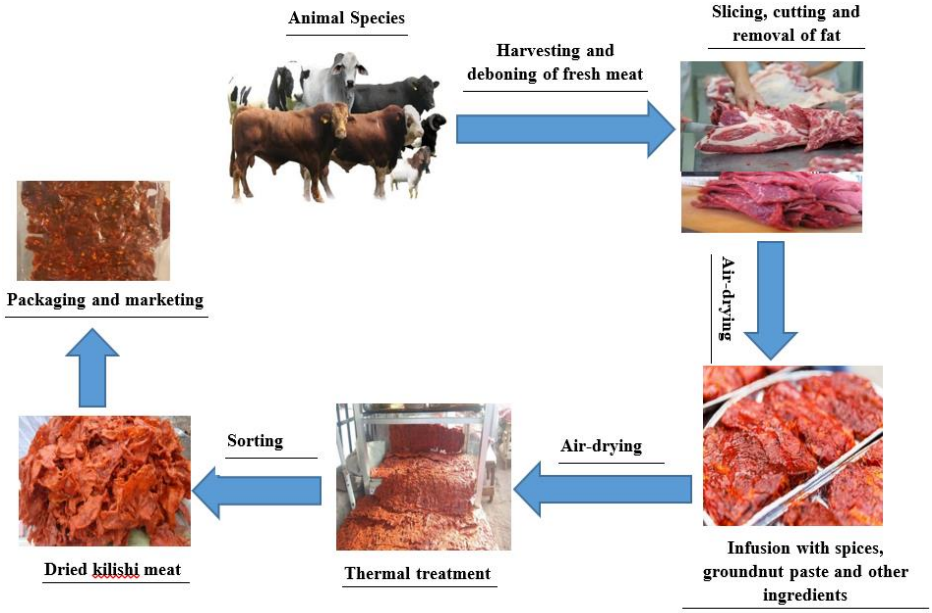


**Figure 1.** The stages in production process of Kilishi meat.

During meat preparation, it is important to select a fresh muscle especially the Longissimus or Semimembranosus muscles of the carcass, and thereafter clean, debone and trim of fat and excess connective tissues in order to obtain the lean meat. Thereafter, the lean meat is cut into small portions and each portion is sliced into a sheet of one meter or less to allow easy drying. The next stage is the preparation of ingredients for infusion. The key ingredient in the processing of Kilishi is the groundnut paste. The groundnut paste is normally obtained from dehulled roasted groundnut seeds after milling, kneading and extraction of oil (Ogunsola and Omojola, 2008). The groundnut pastes together with other ingredients such as spices (onion, pepper, ginger, thyme, cloves etc) salt and honey are mixed thoroughly with water to form a slurry. The meat sample will be marinated/soaked in the infusion slurry for an hour. After that the sample is removed and carefully spread out under the sun for drying for about 6 hours and then roasted in the oven to heat-seal the infused ingredients (Ogunsola and Omojola,

2008). Finally, the finished products can be cooled at room temperature, packed and heat sealed in high density polyethylene bags for preservation and storage (Iheagwara and Okonkwo, 2016).

## 4.1. Nutritional Quality of Kilishi Meat

Different meats have been shown to have potentially important nutritional differences. The proximate composition of the kilishi meat made from different species of livestock is presented in Table 1. Irrespective of species, it is evident that kilishi meat is characterized with high protein content which ranges between 57.00 and 71.00%, moderate fat content between 6.40 and 13.33 and ash content between 2.55 and 8.87% as well as low moisture/water content between 9.75 and 26.33%. However, Kilishi made from camel meat contained higher moisture (15.92-28.33) and protein (58.55-70.45) while those made from beef have the lowest moisture (9.75-14.10) and protein (57.02 – 64.40) contents. The variation in the levels of the various nutrients in kilishi meat within species could be as a result of differences in the carcass composition, age at slaughter, sex, technique of analysis and amount of groundnut cake paste used. In comparison to other meat products, kilishi meat contained higher protein content than raw beef, mutton, chevron, pork and camel meat which ranges from 16-23%. This is because the moisture content of kilishi meat has been significantly dehydrated by drying thereby improving and concentrating the percentage protein content and other nutrients in the meat. Reports have shown that cooking or heat processing usually decreases moisture content and concentrates other nutrients in meat products (Iheagwara and Okonkwo, 2016). In addition, the type and amount of slurry agents used may also influence the protein content of kilishi meat (Idowu et al., 2010; Iyiola et al., 2021). Previous research has shown that the addition of groundnut cake paste tend to increase or contribute to higher protein content of kilishi meat when compared to other meat products (Idowu et al., 2010).

**Table 1.** Proximate composition of Kilishi meat

| Parameters (g/100g) | Beef | Camel | Chevron | Pork | Mutton |
|---|---|---|---|---|---|
| Moisture | 9.75[a]- 14.10[b] | 15.92[b]-28.33[h] | 17.17[h] -26.27[h] | 9.92[d]-26.33[h] | 9.33[e] |
| Crude Protein | 48.1[f]- 64.40[g] | 58.55[h]-70.45[h] | 57.91[h]- 69.18[h] | 59.41[d]- 66.63[h] | 38.68[e] |
| Crude Fibre | 0.40[g] | - | 2.83[e] | - | 2.83[e] |
| Crude Fat | 6.21[j] - 18.10[a] | 6.40[b] | 17.00[e] | 13.33[d] | 22.00[e] |
| Ash | 7.10[a] - 8.87 | 5.12[h] - 5.79[h] | 2.55[h]-7.88[h] | 6.96[d] -7.55[h] | 16.00[e] |
| Carbohydrate | 2.30[g] - 8.05 | - | 28.78[e] | - | 23.82[e] |

e=Adeyi et al., 2015, a=Idowu et al., 2010, b= Ayorinde and Muhammad, 2017, d= Ogunsola and Omojola, 2008, f= Olusola et al., 2017, g= Adeyeye et al., 2020a h= Fakolade and Fatola, 2021, j=Iyiola et al 2021.

## 4.2. Mineral, Fatty Acids, Amino Acids and Vitamins Composition of Kilishi Meat

Mineral generally refers to the elements other than C, H, O, and N that are present in foods (Falowo, 2021). Minerals can occur in relatively low concentrations in foods but play key functional roles in both living systems and foods (Falowo, 2021). They can be classified as either major or trace elements. The major elements include calcium, phosphorus, magnesium, sodium, potassium, and chloride, while the trace elements are iron, iodine, zinc, selenium, chromium, copper, fluorine, lead, and tin. The mineral composition of kilishi meat is presented in Table 2. Like other meat products, the kilishi meats made from beef, camel, chevron and pork contain the relative amount of mineral elements. Kilishi beef possesses higher mineral elements with about 29.6mg/100g calcium, 81.6mg/100g magnesium, 320mg/100g sodium, 781mg/100g phosphorus, 985mg/100g potassium, 18.3mg/100g zinc, 8.62mg/100g iron, 0.05mg/100g selenium and 0.32mg/100g copper compared to kilishi made from camel, chevron and pork (Table 2). The substantial variation in the mineral composition of kilishi meats may be due to differences in species, nutrition and processing methods.

Previous research conducted on the fatty acid of Kilishi beef meat has showed that it contains higher unsaturated fatty acid than the saturated fatty acids (Table 2). The values of unsaturated fatty acids reported for kilishi are higher than those reported for fresh beef while the saturated fatty acids are less than those reported in fresh beef (Adeyeye et al., 2020b). There is a report that kilishi beef meat are very rich in unsaturated fatty acid compared to fresh meat. Unsaturated fatty acids are essential in the human diet for maintaining the impermeability barrier of the skin and are involved in cholesterol transport and metabolism (Adeyeye et al., 2020b). Also, the kilishi made for beef was reported to possess higher linoleic fatty acid than those made from camel meat (Ayorinde, 2015).

Previous research conducted on amino acids revealed that beef kilish is rich in essential and non-essential amino acid. On estimate, beef kilishi contains 38.2g/100g essential amino acid and 58.1g/100g non-essential amino acids. In addition, previous research conducted on vitamin content of kilish meat showed that they contained an appreciable quantity of vitamins (Table 2). The chevron and goat kilishi showed higher values of Vitamin D and Vitamin B12 than the beef kilishi. However, the quantity of vitamins reported in kilishi meat are relatively lower than those found in fresh meat. This decrease or reduction might be due to the

volatilization of the vitamin during thermal treatment. Prolonged thermal treatment has been shown to destroy fat-soluble vitamins and water-soluble vitamins, thereby making them volatile (Fakolade and Fatola, 2021).

**Table 2.** Mineral, fatty acids, amino acids and vitamins composition of kilishi meat

| Parameters | Beef | Camel | Chevron | Pork |
|---|---|---|---|---|
| Minerals (mg/100g) | | | | |
| Ca | 29.6[g] | 11.84[h]-12.39[h] | 11.92[h]-12.59[h] | 11.76[h]-12.19[h] |
| Mg | 81.6[g] | 15.85[j]-16.51[h] | 15.99[h]-16.59[h] | 14.73[h]-15.97[h] |
| Na | 320 [g] | | | |
| P | 781[g] | | | |
| K | 985[g] | 235[h]-251[h] | 238[h]-258.67[h] | 243[h]-246.00[h] |
| Fe | 8.62[g] | | | |
| Zn | 18.3[g] | | | |
| Se | 0.05[g] | | | |
| Cu | 0.32[g] | | | |
| Fatty Acid (%) | | | | |
| Saturated fatty acid | 33.4[j] | | | |
| Mono unsaturated fatty acid | 41.2[j] | | | |
| Poly unsaturated fatty acid | 25.4[j] | | | |
| Total unsaturated Fatty acid | 66.6[j] | | | |
| Amino Acid (g/100g protein) | | | | |
| Total essential amino acid | 38.2[k] | | | |
| Total non-essential acid | 52.1[k] | | | |
| Total amino acid | 90.3[k] | | | |
| Vitamins (mg/100g) | | | | |
| Vit A | 1.94[g] | | | |
| Vit. B12 | 0.0013[g] | 0.14[h] | 0.11[h] | 0.08[h] |
| Vit. B2 | 0.29[g] | 0.005[h] | 0.006[h] | 0.001[h] |
| Vit. D | 0.001[g] | 0.79[h] | 0.88[h] | 0.73[h] |
| Vit C | 6.53[g] | | | |
| Vit E | 1.38[g] | | | |

h= Fakolade and Fatola, 2021 g= Adeyeye et al., 2020a, j = Adeyeye et al., 2020b k= Adeyeye et al 2020c

## 5. Tinko Meat and Its Production Processes

Tinko is a Yoruba name that refers to traditional meat products made by sun-drying (Figure 2). In other tribes like Igbo and Hausa, the sun-dried meat product is called banda and Kundi, respectively (Adeyeye et al., 2016). Like kilishi meat, tinko meat is usually prepared from carcass of cattle and other transport animal such as donkeys, asses, horses, camel, buffalo as well as wild-life (Ajiboye et al., 2011). Nevertheless, Oladejo and Adebayo-Tayo, 2011 and Adeyeye et al (2016) in their study reported that tinko meat is made mostly from rejected cattle and discarded transport animals. Tinko meat is, however, widely consumed in Nigeria and other Africa countries. This is due to its readily availability, affordability and a prolonged shelf life (up 6–12 months under ambient temperature) after production (Adeyeye et al., 2016). Besides, tinko meat is consumed as a good source of animal protein to combat protein malnutrition in human diet (Oladejo and Adebayo-Tayo, 2011;

Adeyeye et al., 2016). The production of tinko meat involves five stages. This include collection of meat sample (animal carcass), cutting of the meat samples in cube shapes or small pieces, cooking of the meat sample for 15-30mins, drying and smoking for about 18-30hrs and lastly cooling, storage and packaging.

### 5.1. Nutritional Composition of Tinko Meat

The Nutritional composition of tinko meat is presented on Table 2. Meat is known to be rich in protein, lipid, carbohydrate, mineral and other nutrients. Precisely, it contains about 7.0 -12.6% moisture, 44.0 – 65.0% protein, 0.5 – 1.4% crude fibre, 1.2 – 24.5% ash and 10.0-14.0% carbohydrate contents (Oladejo and Adebayo-Tayo, 2011, Adeyeye et al., 2016, Table 2). In comparison, the range of protein, moisture, ash, crude fibre and carbohydrate content in tinko meat is similar to those of Kilish beef meat but higher than fresh meat. This indicates that processing methods do not have negative effects on the nutritional content of the meat especially if they are made from the same species of animal. Like kilishi meat and other meat products, tinko meat contains a relative amount of sodium (0.35-1.84%), calcium (1.24-6.76%), phosphorous (0.8-3.45%), magnesium (0.12-0.43%), potassium (0.09-0.53%), copper (1.5 -8.6 %), manganese (2.6-9.1%), zinc (120-449%) and iron (132-443%) among other nutrients (Oladejo and Adebayo-Tayo, 2011; Adeyeye et al., 2016, Table 3 and 4). However, there is little empirical research

on the amino acid, fatty acids and vitamins profiles as well as biosafety status of tinko meats. Further research should be conducted in order to provide more useful information about the complete nutritional content of tinko meat and its promise to meet the demands of consumers and the meat industry.

**Figure 2.** Image of Tinko meat.

**Table 3.** Proximate composition of Tinko meat

| Parameters | Moisture (%) | Protein (%) | Fat (%) | Crude fiber (%) | Ash (%) | Carbohydrate (%) |
|---|---|---|---|---|---|---|
| | 7.48[a] - 12.63[b] | 44.21[a] - 64.5[b] | 3.17[a] - 12.27[b] | 0.55[a] -1.37[b] | 1.27[b] - 24.30 | 10.00[a]-14.00[a] |

a=Oladejo and Adebayo-Tayo, 2011, b= Adeyeye et al., 2016.

**Table 4.** Mineral composition of Tinko meat

| Minerals | Na | Ca | P | Mg | K | Cu | Mn | Zn | Fe |
|---|---|---|---|---|---|---|---|---|---|
| | 0.35-1.84[a] | 1.24-6.76[a] | 0.8- 3.45[a] | 0.12-0.43[a] | 0.09-0.53[a] | 1.5 –8.6[a] | 2.6-9.1[a] | 120- 449[a] | 132- 443[a] |

a=Oladejo and Adebayo-Tayo, 2011, b= Adeyeye et al., 2016.

## 6. Dambu-Nama and Balangu Meat Product

Dambu-nama is another nutritious dried meat product commonly produced in northern part of Nigeria. It is usually made from fresh meat obtained from carcasses of cattle, goat, sheep or camel meat (Figure 3). The meat is mostly preferred by consumers because of its soft and tender taste compared to the hard, non-soft kilishi product (Eke et al., 2012). Dambu-nama meat is reported to contain about 5.50 -7.60% moisture, 39.19-46.51% protein, 4.90-5.76% ash, 0.015- 0.72% crude fibre, 15.65-24.94% crude fat and 22.64- 26.54% carbohydrate (Eke et al., 2012). Reports on mineral concentration revealed that Dambu-nama meat contains about 1242.50-764.20 mg/kg sodium, 1112.20 - 1384.00 mg/kg potassium, 565.10 - 764.20 mg/kg potassium, 43.10-50.10 mg/kg calcium, 221.60-286.40 mg/kg magnesium and 128.73-181.73 mg/kg manganese (Eke et al., 2013). The production of Dambu-nama meat is said to involve the collection of fresh meat, trimming and removing of fat and connective tissues, cutting of the meat samples into nearly equal size, curing and mixing of the meat samples with seasonings and spices, cooking of the meat samples, pounding of the cooked meat into shredded structure, frying or oven drying of the meat to doneness and finally, cooling and packaging/marketing (Koleosho, 2013; Eke et al., 2013b). On the other hand, balangu refers to meat that has been grilled over wood or coal fire without the addition of seasoning in order to retain its natural flavor (Ribah et al., 2020). Like other traditional meat products, balangu is mainly produced in Northern part of Nigeria and other part of Africa. Its preparation includes collection of fresh boneless meat or offal/viscera from goat, sheep, camel and cattle, cutting of the meat sample into chucks, and grilling or roasting of the meat samples on a wire mesh over a smokeless or coal fire (Gambo et al., 2012; Ribah et al., 2020). Nutritionally, balangu meat products contain 62.70% moisture, 20.26% protein, 32.51% fat and 2.10% ash (Muhammad et al., 2010). Reports on mineral concentration revealed that balangu meat products contained 3.44 mg/g sodium, 8.38 mg/g potassium, 565.10 - 764.20 mg/kg phosphorus, 2.08mg/g calcium, 4.82mg/g magnesium, 1.30mg/g iron and 9.29mg/g zinc (Muhammad et al., 2010).

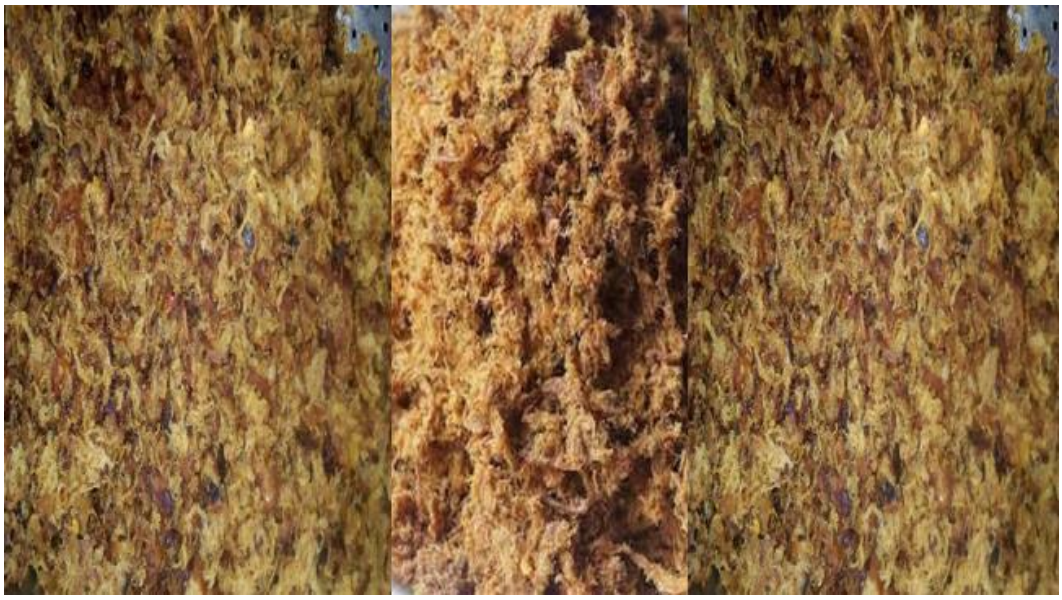

**Figure 3.** Image of Dambu-nama dried meat products.

## 7. Microbiology Safety of Dried Meat Products

Consumer awareness of the quality and safety of what they consume has increased in recent times. Microbial quality and safety of meat products are very important in ensuring consumer health and food security after production. Several research articles have emerged showing that the quality of meat and meat products could be compromised by spoilage organisms (bacteria, fungi, yeast and molds) especially during processing, handling, storage and marketing (Kovac et al., 2020, Anjorin et al., 2022). However, the production, handling and storage of Kilishi meat under a condition free of microbial contamination has been seen as a process usually difficult to achieve in Nigeria and Africa. This is because dry meat products, for instance kilishi meats are rich in nutrients which serve as a good culture medium for many micro-organisms (bacteria, yeasts and molds) to grow. The degree of spoilage is usually influenced by the concentration and availability of nutrients, presence of oxygen, storage temperature, pH at storage, initial microbial load at the beginning of production and the environment where the product is being processed etc. (Adeyeye et al 2020c; Iheagwara and Okonkwo, 2016). Different spoilage organisms such as Salmonella, E. coli, Staphylococcus and coliform bacteria (not microorganisms, mycotoxins are toxic compounds produced by certain types of fungi) that cause rapid deterioration and even poisoning have been identified and isolated from meat and meat products. These spoilage organisms could be introduced into meat by butchers and workmen, or through water and air in the dressing, cooling and cutting rooms or tables and even from the environment (Olusola et al., 2017). The consumption of these meat products, when contaminated with poisoning microorganisms, expose consumers to health threats and affects global trade. Nonetheless, the emerging results on evaluation of microbial safety of kilishi meat (using nutrient agar and isolation of organisms on a medium methods) has shown that the level of microbial counts in the meat products are below the recommended range of 5.4 - 8.0 log10, which is acceptable level of microbial load of ready-to-eat food products (Iyiola et al., 2021; Olusola et al., 2017). Recently, the use of analytical techniques such as High-Performance Liquid Chromatography (HPLC) method to detect the microbial load on food products showed that the kilish product may be unsafe for consumption especially when contaminated with fungi such as mycotoxins (Anjorin et al., 2022). Based on this, researchers are now advocating that the level of mycotoxins among other contaminants should be evaluated in food products because of their potential to cause cancer and suppress the immune system as well as decrease reproductive capacity and can cause allergies in consumers (EC 2006; Iqbal et al., 2014; Anjorin et al., 2022).

### 7.1. Occurrence, Detection and Prevention of Mycotoxins in Kilishi Meat

Mycotoxins are toxic secondary metabolites produced by various toxigenic species of fungi/mold that grow on meat products during storage. They can cause cancer and exert mutagenic effect when ingested by consumers of meat products (Montanha et al., 2018). There is evidence that about five billion people are constantly exposed to mycotoxins across the world (Khodaei et al., 2021, Pandey et al., 2023), thereby making it a public health concern around the world. On meat products, mycotoxins are produced by several species of fungi of the genus such as Aspergillus, Fusarium, Penicillium, Alternaria and Claviceps (Montanha et al., 2018). Among these species of fungi in meat products, Aflatoxins B1 (AFB1), B2 (AFB2), G1 (AFG1), and G2 (AFG2), Fumonisin B1 (FB1) , Ochratoxin A (OTA) and Ochratoxin A (OTB) (Anjorin et

al., 2022) are reportedly the most prominent. The level of AFB1 and total aflatoxins that have been recommended as permissible in food by European Union is estimated at 2µg/kg and 4µg/kg, respectively (EC 2010; Mahato et al., 2019; Dada et al., 2020). In Nigeria, Anjorin et al. (2022) in their study discovered that the consumption of kilishi meat from the central part of Nigeria could be unsafe because of the prevalence of Aflatoxin B1 and B2, OTA and OTB contamination levels recorded in their samples which were above the maximum limit reported by European Union (EU). Similarly, Oladejo et al (2011) in their study observed the high level of contamination of aflatoxins B1, B2, fumonisin (FB1 and FB2) and ochratoxin (OTA) in tinko meat sampled from the western part of Nigeria, which exceeded the maximum limit permitted in most countries by EU. In another study in South West of Nigeria, Dada et al. (2020) found that the concentration of aflatoxin in dried beef samples sold in the market greatly exceeded the EU (4 µg/kg) permissible level in food. Therefore, the occurrence of mycotoxins in meat products showed that consumers are at risk of contracting severe health problems when they are consumed. Hence, this call for an urgent need for concerned regulatory bodies to impose necessary measures to safeguard the health of consumers. This also requires that standard methods for identification should be put in place in order to reduce the inflow of contaminated products into the market to minimize the deleterious effects caused by their consumption.

To control the incidence of mycotoxins and establish microbial safety in meat products, emphasis should be placed on the prevention and determination of contaminants especially, toxigenic fungi growth (Núñez et al., 2015). There is evidence that fungus growth can be efficiently controlled in food products by using chemical preservatives (such as salting), modified atmosphere packaging, drying and ripening methods, gamma irradiation application etc. Household-practiced physical methods such as cooking and baking could be used to control the growth of fungi on meat products.

## 8. Conclusion and Future Prospects

The production and consumption of dried meat are increasing geometrically across Nigeria nowadays although there are few studies indicating the presence of microbial contamination which may hamper or compromise their production in the future. Moreover, findings from this study have revealed that dried meat products are essentially rich in nutrient and could be consumed when properly processed and handled. There is a need to consistently examine the microbiological properties of meat products especially with modern day techniques and equipment's in order to ensure the development of safe food products for consumption. To do this, efforts should be geared towards reducing the source of contamination during production chain especially during processing, drying, storage and point of sale. The inability to know the exact species of animal used for production of tinko meat during marketing is still a challenge in Nigeria. The government and all stakeholders should enact a policy that will ensure that dried meat products such as tinko are properly labelled to indicate meat type and species of animal used during production before marketing to consumers. More studies should be conducted in different parts of the country using modern technology such as High-Performance Liquid Chromatography (HPLC) in order to ascertain the microbial safety of dried meat products for post-production consumption**.**

**Author Contributions**

The percentage of the author contributions is presented below. The author reviewed and approved the final version of the manuscript.

| | A.B.F. |
|---|---|
| C | 100 |
| D | 100 |
| S | 100 |
| DCP | 100 |
| DAI | 100 |
| L | 100 |
| W | 100 |
| CR | 100 |
| SR | 100 |

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision

**Conflict of Interest**

The author declared that there is no conflict of interest.

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# COMPARISON OF THE NUTRITIONAL VALUE OF COW'S MILK AND PLANT-BASED MILKS

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**Abstract:** Malnutrition and micronutrient deficiencies are observed among people in many developing countries. The high cost of cow's milk and poverty make it difficult for people to access nutritious food. For this reason, low-cost foods that can be an alternative to cow's milk are important. In addition, the fact that cow's milk causes lactose intolerance, high cholesterol, constipation and bloating in some individuals has led people to other alternatives. Apart from these, alternatives for vegan individuals have begun to be considered. All these reasons have increased the demand for alternative milk of plant origin worldwide. Plant-based milks have been the subject of research with different names in the literature. For example: vegetable milk, non-dairy milk, imitation milk, dairy substitute. This review is focused on comparing nutrient composition of cow's milk and plant-based milk alternatives.

**Keywords:** Cow's milk, Plant-based milk, Vegetable milk, Non-dairy milk

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## 1. Introduction

Cow's milk is a complete food that contains all its essential components, such as fat and carbohydrates, as well as being a good source of protein (Padma et al., 2022). Cow's milk, which has high nutritional value, is used as a staple food in many diets. Cow's milk has a wide range of uses. Although milk is consumed as a beverage, it is also added to various beverages like coffee, smoothie. In addition, many dairy products such as ice cream, yogurt, cheese and butter are produced from cow's milk (Bocker and Silva, 2021). But, due to problems such as lactose intolerance and milk allergy caused by cow's milk consumption, the demand for alternative milk of plant-based has increased worldwide (Vanga and Raghavan, 2018). Milk alternatives are water extracts of plants (Tangyu et al., 2019). Although there are many varieties of plant-based milk, the most common are rice milk, soy milk and coconut milk (Rasika et al., 2021). Known for its lactose-free, animal protein-free and cholesterol-free properties, plant-based milks are known as an important food for individuals with sensitivity to the specified properties (Bernat et al., 2014). There are also some disadvantages of plant-based milks. Among these disadvantages are that they are nutritionally unbalanced and their taste profiles are difficult to accept. Fermentation is recommended to produce more valuable and delicious products (Tangyu et al., 2019). Fermented plant-based milk ice cream can be a good alternative that can be used as a new functional food (Aboulfazli et al., 2016). Existence of soy milk in ice creams was reported as a significant improvement in probiotic tolerance against gastrointestinal conditions (Aboulfazli and Baba, 2015). In general, plant-based milk alternatives have lower protein content, calcium availability, and higher GI values, than cow's milk (Chalupa-Krebzdak et al., 2018). However, these milks are rich in phenolic compounds, unsaturated fatty acids and bioactive compounds (Aydar et al., 2020). There are many methods for producing plant-based milk substitutes. Because they have many common steps, one flowchart is prepared for general plant-based milk substitute production in this study (Figure 1) (Makinen et al., 2016). However, there is very little research in the literature to understand the nutritional effects of consuming these plant-based milk drinks, which are popularly promoted as healthy, in the short and long term (Vanga and Raghavan, 2018).

Nutritional comparison of cow's milk and some plant-based milks will be made by using the literature. Although there are many plant-based milks, only rice milk, soy milk, coconut milk, almond milk, tigernut milk, peanut milk and cashew nut milk were the subject of this study. The compared milk types are shown in Figure 2.

### 1.1 Animal-Based Milk

#### 1.1.1. Cow's milk

Milk is an important food. The four components that predominate in the content of milk are water, fat, protein and lactose. Minerals, enzymes, vitamins and dissolved gases are minor components of milk (Guetouache et al., 2014).

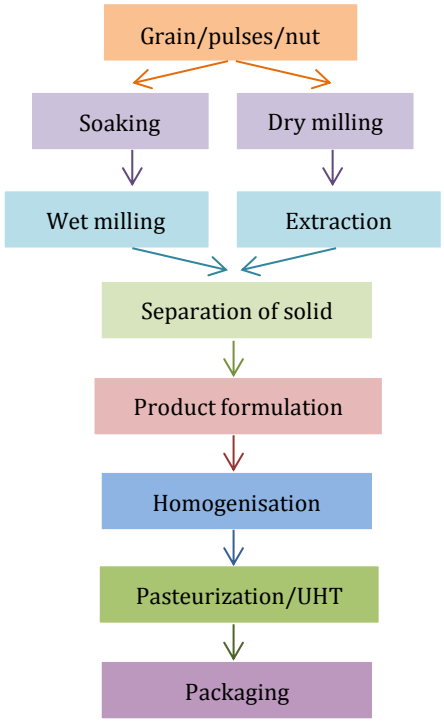


**Figure 1.** The general manufacturing process of vegetable milk alternatives (Makinen et al., 2016).

### 1.2 Plant-based Milk Types

There are many types of plant-based milk. The most common plant-based milks are soy and rice milk. In this review, data in the literature on soy, rice, coconut, almond, tiger nut, peanut and cashew nut milk varieties will be examined. A brief literature information about these milk types is written below.

#### 1.2.1. Rice milk

Rice milk is not an adequate source of protein (Mori et al., 2015). However, it has been the subject of many different studies. Rice milk is a milk alternative beverage that can be used in kefir production (Sulistyaningtyas et al., 2019). In addition, lactic acid bacteria contribute to rice milk fermentation and these bacteria produce products such as yoghurt and cheese. It is thought that rice yogurt can be used as a supplementary to colon anticancer therapy (Fawzi et al., 2022).

#### 1.2.2. Soy milk

Soy milk is a traditional beverage popular in Asia (Ng and Loh, 2018). Soy milk, a plant-based beverage, is a rich source of nutrients. But soy milk contains several harmful compounds, including allergens, anti-nutritional factors, and biogenic amines (Mollakhalili-Meybodi et al., 2022). Soy milk substitute in cake production has increased the overall nutritional composition of the products, and besides milk it is a good source of protein (Erfanian and Rasti, 2019).

#### 1.2.3. Coconut milk

Coconut milk is an oil-in-water emulsion extracted from coconut (Chiewchan et al., 2006). Coconut milk is a dairy alternative source rich in various nutrients. Low-fat coconut milk is an alternative to cow's milk in the production of kefir-based beverages (Abadl et al., 2022). Coconut milk contains negligible levels of cholesterol. This situation makes coconut milk suitable for a group of populations suffering from lactose intolerance and heart disease (Tulashie et al., 2022).

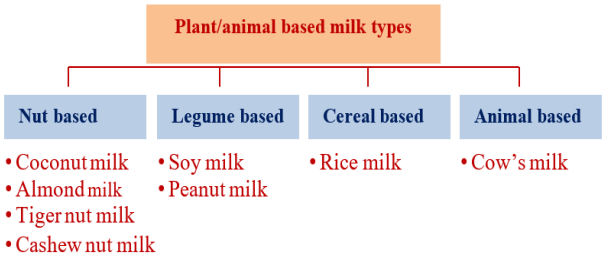


**Figure 2.** Milk types.

#### 1.2.4. Almond milk

Almond milk is a nutrient-dense milk that is lower in calories than cow's milk. This milk is an important beverage for gastrointestinal and cardiovascular health (Alozie Yetunde and Udofia, 2015). Probiotic yoghurts produced by adding almond milk to dairy products compensate the expectations of consumers who demand food products with high nutritional value (Yılmaz-Ersan and Topcuoglu, 2022).

#### 1.2.5. Tiger nut milk

Tiger nut milk is a widely produced and consumed beverage also called "kunun aya" in Nigeria (Opeyemi and Obuneme, 2020). Tiger nut milk is a nutrient-rich beverage. Tiger nut milk is a perishable beverage. Therefore, extending the shelf life of commercialized tiger nut milk is an important topic (Codina-Torrella et al., 2018; Costa Neto et al., 2019). It has been determined that microencapsulation application increases the shelf life of tiger nut milk (Costa Neto et al., 2019). In addition, in many studies, tiger nut milk is also referred to as "chufa milk".

#### 1.2.6. Peanut milk

The use of peanut milk will provide an alternative to animal milk and will also help to overcome malnutrition (Yadav et al., 2010). Peanut milk has higher fat, protein content and calorific value than cow's milk (Gamlı and Atasoy, 2018). In addition, in many studies, peanut milk is also referred to as "groundnut milk, bambara groundnut milk".

#### 1.2.7. Cashew nut milk

Cashew nut milk is promoted in rural communities where the availability and cost of animal milk poses great challenges to people. Cashew nut milk can be preferred as a milk substitute due to it's reduce the cost of diary milk and its high nutritional content (Tamuno and Monday, 2019).

## 2. Comparison of Plant-Based Milks and Cow's Milk

In this review article protein, fat, carbohydrate, sugar contents and energy values of cow's milk and plant-based milk will be compared (Figure 3).

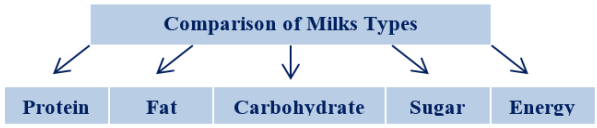


**Figure 3.** Comparison of plant-based milks and cow's milk.

### 2.1 Content of Protein

Proteins are one of the essential nutrient for healthy life, growth and cell reparation. However, with the increasing world population, protein sources are decreasing day by day and this situation causes an increase in the demand for new alternative protein sources. Plants, which are low cost compared to animal protein sources and preferred by special consumer groups such as vegan and vegetarian, are a good alternative protein source (Çetiner and Ersus Bilek, 2018). Encouraging the use of protein-rich foods can reduce the problem of malnutrition (in terms of protein and energy) (Oyeyinka et al., 2019).

In general, plant-based milk alternatives have lower protein content than cow's milk (Chalupa-Krebzdak et al., 2018).

Table 1 show the protein contents of milk samples. Soy milk and peanut milk have similar protein content as cow's milk. Compared to cow's milk, rice milk, almond milk, tigernut milk, cashew nut milk contains less protein.

### 2.2 Content of Fat

Fat content is one of the important parameters in determining food quality in many food products (Guthausen et al., 2004). Fats from plant-based sources have shown positive alterations in gut microbiota biodiversity studies (Muralidharan et al., 2019). Table 2 show the fat contents of milk samples. With the literature review, it was concluded that the fat content of coconut milk is higher than other plant-based milks and cow's milk. Rice milk, soy milk, tigernut milk and cashew nut milk have lower fat content than cow's milk.

**Table 1.** Protein content of milk types

| Reference | Protein (%) | Reference | Protein (%) |
|---|---|---|---|
| Cow's Milk | | Almond Milk | |
| (Jemaa et al., 2021) | 3.32 | (Vanga and Raghavan, 2018) | 1 |
| (Asres et al., 2022) | 3.40 | (Maghsoudlou et al., 2016) | 1.06 |
| (Abou-Dobara et al., 2016) | 3.65 | (Kundu et al., 2018) | 1.3 |
| Rice Milk | | Tigernut Milk | |
| (Vanga and Raghavan, 2018) | 1 | (Wakil et al., 2014) | 1.66 |
| (Silva et al., 2023) | 1.48 | (Abdulfatai et al., 2013) | 2.24 |
| (Abou-Dobara et al., 2016) | 1.62 | (Neto et al., 2017) | 2.6 |
| Soy Milk | | Peanut Milk | |
| (Makinen et al., 2014) | 2.95 | (Isanga and Zhang, 2009) | 3.71 |
| (Kundu et al., 2018) | 3.17 | (Jain et al., 2013) | 3.8 |
| (Abou-Dobara et al., 2016) | 3.54 | (Abou-Dobara et al., 2016) | 3.91 |
| Coconut Milk | | Cashew nut Milk | |
| (Tulashie et al., 2022) | 2.22 | (Cardello et al., 2022) | 0.4 |
| (Ayah et al., 2022) | 2.30 | (USDA, 2019) | 0.42 |
| (Szparaga et al., 2019) | 3.23 | (Drewnowski, 2022) | 0.87 |

**Table 2.** Fat content of milk types

| Reference | Fat (%) | Reference | Fat (%) |
|---|---|---|---|
| Cow's Milk | | Almond Milk | |
| (Ceballos et al., 2009) | 3.42 | (Maria and Victoria, 2018) | 1.6 |
| (Isanga and Zhang, 2009) | 3.54 | (Angelino et al., 2020) | 2.3 |
| (Abou-Dobara et al., 2016) | 3.6 | (Vanga and Raghavan, 2018) | 2.5 |
| Rice Milk | | Tigernut Milk | |
| (Drewnowski, 2022) | 1.21 | (Abdulfatai et al., 2013) | 1.23 |
| (Lalić et al., 2014) | 2.4 | (Amponsah et al., 2017) | 1.81 |
| (Vanga and Raghavan, 2018) | 2.5 | (Muhammad et al., 2019) | 2.84 |
| Soy Milk | | Peanut Milk | |
| (George and Awopetu, 2017) | 1.83 | (Bucker et al., 1979) | 4.4 |
| (Angelino et al., 2020) | 2 | (Abou-Dobara et al., 2016) | 4.5 |
| (Kundu et al., 2018) | 2.35 | (Elsamani, 2016) | 5.0 |
| Coconut Milk | | Cashew nut Milk | |
| (Azlin-Hashim et al., 2019) | 11.02 | (Cardello et al., 2022) | 1.4 |
| (Tulashie et al., 2022) | 14.12 | (Sumner and Burbridge, 2020) | 2 |
| (Masia et al., 2020) | 17.67 | (Drewnowski, 2022) | 2.26 |

**2.3 Content of Carbohydrate**

Carbohydrates are important in foods as a major source of energy (Jebb, 2015; Campos et al., 2022). Carbohydrates form a significant component of a healthy and balanced diet. Carbohydrates, which provide 50-70% of energy intake, are divided into three main groups in human nutrition. These are sugars, starch and non-starch polysaccharides (Lunn and Buttriss, 2007). Table 3 show the carbohydrates contents of milk samples. With the literature review, it was concluded that the carbohydrate content of rice milk and tigernut milk is higher than other plant-based milks and cow's milk. Other plant-based milks have similar carbohydrate content as cow's milk.

**2.4 Content of Total Sugar**

Total sugars are described as the total of all free monosaccharides and disaccharides (such as glucose, fructose, lactose, and sucrose) (BeMiller, 2010). Table 4 show the sugar contents of milk samples. With the literature review, it was concluded that the sugar content of rice milk is higher than other plant-based milks and cow's milk. Soy milk, coconut milk, almond milk, peanut milk and cashew nut milk have lower sugar content than cow's milk.

**2.5 Energy Value**

The risk of obesity and cardiometabolic disease increases with calories from any food (Stanhope et al., 2018). The dietary energy of cow's milk varies based on the fat content of the milk. Most of the energy in milk alternatives consists of carbohydrates and sugars. These alternatives drinks relatively raise the glycemic index (Chalupa-Krebzdak et al., 2018). Table 5 show the energy value of milk samples. With the literature review, it was concluded that the energy value of coconut milk and peanut milk is higher than other plant-based milks and cow's milk. Almond milk and cashew nut milk have lower fat content than other plant-based milks and cow's milk. Rice milk and soy milk have similar energy value as cow's milk.

**Table 3.** Carbohydrate content of milk types

| Reference | Carbohydrate (%) | Reference | Carbohydrate (%) |
|---|---|---|---|
| Cow's Milk | | Almond Milk | |
| (Asres et al., 2022) | 4.32 | (Devnani et al., 2020) | 2.3 |
| (Gamlı and Atasoy, 2018) | 4.61 | (Ceylan and Özer, 2020) | 2.44 |
| (Mohamed et al., 2019) | 4.96 | (Maria and Victoria, 2018) | 2.71 |
| Rice Milk | | Tigernut Milk | |
| (Atwaa et al., 2019) | 10.27 | (Costa Neto et al., 2019) | 7.61 |
| (Silva et al., 2023) | 11.33 | (Wakil et al., 2014) | 8.34 |
| (Angelino et al., 2020) | 12 | (Abdulfatai et al., 2013) | 10.73 |
| Soy Milk | | Peanut Milk | |
| (USDA, 2021b) | 3 | (Pahane et al., 2017) | 4.2 |
| (Al and Oladimeji, 2008) | 3.49 | (Gamlı and Atasoy, 2018) | 4.24 |
| (Vanga and Raghavan, 2018) | 4 | (Singh et al., 2018) | 4.7 |
| Coconut Milk | | Cashew nut Milk | |
| (Clegg et al., 2021) | 3.70 | (Craig and Brothers, 2021) | 3 |
| (Mepba et al., 2006) | 3.84 | (Oyeyinka et al., 2019) | 5.17 |
| (USDA, 1984) | 5.54 | (Tamuno and Monday, 2019) | 5.95 |

**Table 4.** Sugar content of milk types

| Reference | Sugar (%) | Reference | Sugar (%) |
|---|---|---|---|
| Cow's Milk | | Almond Milk | |
| (Coyle et al., 2019) | 4.4 | (Sumner and Burbridge, 2020) | 2.4 |
| (Sumner and Burbridge, 2020) | 4.7 | (Drewnowski, 2022) | 2.58 |
| (Cardello et al., 2022) | 4.8 | (Angelino et al., 2020) | 3.0 |
| Rice Milk | | Tigernut Milk | |
| (Drewnowski, 2022) | 5.05 | (Neto et al., 2017) | 3.70 |
| (Cardello et al., 2022) | 5.8 | (Okyere and Odamtten, 2014) | 6.00 |
| (Angelino et al., 2020) | 6.2 | (Costa Neto et al., 2019) | 6.20 |
| Soy Milk | | Peanut Milk | |
| (Awasthi and Singh, 2020) | 2.2 | (Naliapara and Cholera, 2017) | 0.08 |
| (Angelino et al., 2020) | 2.6 | (Elgazouly et al., 2018) | 0.41 |
| (Vanga and Raghavan, 2018) | 3 | (Hardy and Jideani, 2018) | 0.5 |
| Coconut Milk | | Cashewnut Milk | |
| (Sumner and Burbridge, 2020) | 1.9 | (Craig and Brothers, 2021) | 0 |
| (Drewnowski, 2022) | 2.12 | (Drewnowski, 2022) | 1.88 |
| (Clegg et al., 2021) | 2.28 | (Sumner and Burbridge, 2020) | 2 |

**Table 5.** Energy value of milk types

| Reference | Energy value (kcal/100 g) | Reference | Energy value (kcal/100 g) |
|---|---|---|---|
| Cow's Milk | | Almond Milk | |
| (Gamlı and Atasoy, 2018) | 58.27 | (USDA, 2021a) | 19 |
| (Şahan and Say, 2001) | 62.13 | (Vanga and Raghavan, 2018) | 35 |
| (Bhat et al., 2022) | 65.72 | (Angelino et al., 2020) | 38 |
| Rice Milk | | Tigernut Milk | |
| (Cardello et al., 2022) | 50.87 | (Abdulfatai et al., 2013) | 62.97 |
| (Silva et al., 2023) | 52.03 | (Ntukidem et al., 2019) | 69.41 |
| (Drewnowski, 2022) | 53 | (Aly et al., 2022) | 74 |
| Soy Milk | | Peanut Milk | |
| (Awasthi and Singh, 2020) | 50 | (Singh et al., 2018) | 72 |
| (Alozie Yetunde and Udofia, 2015) | 57.36 | (Isanga and Zhang, 2009) | 86.32 |
| (Mepba et al., 2006) | 62.65 | (Gamlı and Atasoy, 2018) | 90.52 |
| Coconut Milk | | Cashew nut Milk | |
| (Awasthi and Singh, 2020) | 70 | (Cardello et al., 2022) | 17.43 |
| (Mauro et al., 2022) | 77.48 | (Oyeyinka et al., 2019) | 20.25 |
| (Drewnowski, 2022) | 95 | (Drewnowski, 2022) | 36 |

## 3. Conclusion

Cow's milk is a good source of fat, protein and micronutrients. But plant-based milks also have a rich protein content similar to cow's milk and are good non-dairy alternatives. For these reasons, there has recently been an interest in milk alternatives derived from plant-based sources. With this study, we wanted to compare the nutritional contents of some plant-based milks as well as knowing that cow's milk is a valuable food. The following conclusions were reached with the literature review.

- Soy milk and peanut milk have similar protein content as cow's milk.
- Coconut milk has a higher fat content than other plant-based milks and cow's milk.
- Rice milk and tigernut milk have higher carbohydrate content than other plant-based milks and cow's milk.
- Peanut milk and cashew nut milk have lower sugar content than other plant-based milks and cow's milk.
- Coconut and peanut milk have higher energy value than other plant-based milks and cow's milk.

There is no doubt that cow's milk is beneficial for the health of individuals who are not allergic. However, plant-based milk is recommended for individuals who do not consume cow's milk due to various reasons such as lactose intolerance and vegan diet. Although the nutritional content of each plant-based milk is not the same, these alternatives are thought to be beneficial for human health.

**Author Contributions**

The percentage of the author contributions is presented below. The author reviewed and approved the final version of the manuscript.

| | R.T.M. |
|---|---|
| C | 100 |
| D | 100 |
| S | 100 |
| DCP | 100 |
| DAI | 100 |
| L | 100 |
| W | 100 |
| CR | 100 |
| SR | 100 |

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision.

**Conflict of Interest**

The author declare that there is no conflict of interest.

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# SUSTAINABLE MANAGEMENT OF SUCKER PROBLEM IN HAZELNUT CULTIVATION

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**Abstract:** Suckers that develop rapidly in hazelnut bottoms compete with the main branches, resulting in yield and quality losses as well as a risk for pest and diseases. Hazelnut suckers are controlled by mechanical, physical and chemical methods. A majority of mechanical methods are impractical in large production areas and physical and chemical methods come to the forefront. Among the physical methods, flaming is an effective method preferred for this purpose. As a result of studies carried out in different countries on the effect of herbicides on suckers, 2.4-D, glufosinate ammonium, paraquat, carfentrazone-ethyl, and saflufenacil have been recommended. For this purpose, 2.4-D, diquat and glyphosate are applied in Türkiye. Aside from herbicides, some nitrogen fertilizers and plant growth regulators were also effective. In Türkiye, hazelnut growers prefer mechanical and chemical applications for controlling suckers, which they consider as a problem. Within the scope of this study, in order to determine effective, economical, and practical methods and to develop control strategies for Türkiye, a literature review was carried out on the methods for controlling hazelnut suckers in Türkiye and in other countries. In the light of compiled information, current methods and their alternatives have been evaluated. As a result, it is concluded that scientific research is needed and region-specific management strategies should be developed by integrating cultural, physical, mechanical and chemical methods which are effective, economical, and practical for the sustainable management of suckers in hazelnut orchards in Türkiye.

**Keywords:** Hazelnut, Sucker, Alternative methods, Chemical control, Integrated management

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## 1. Introduction

Hazelnut (*Corylus avellana* L.) is the second most produced hard-shelled fruit and a widely consumed dried fruit worldwide (Anıl et al., 2018). Turkish hazelnut, grown in the temperate regions of Türkiye, is a preferred product in the international market due to its quality, taste, and variety. The high volume of hazelnut exports indicates the global demand and successful presence of Turkish hazelnut in a competitive market. The total hazelnut cultivation area worldwide reached 1 million hectares in 2020. Türkiye ranked first with a production area of 734 thousand hectares, followed by Italy with 80 thousand hectares, Azerbaijan with 45 thousand hectares, and Chile and Iran both having 24 thousand hectares (FAO, 2022). According to the Black Sea Exporters Association (KIB), the revenue from hazelnut exports in Türkiye was approximately 1.75 billion dollars in 2022, with Germany being the top importing country with an export value of around 490 million dollars (Anonymous, 2022).

Hazelnut is a tree species that grows in shrub form and requires maintenance. Numerous methods must be done in order to produce high-yielding and high-quality products, including irrigation, fertilization, disease, pest, and weed control and management of hazelnut suckers. During the growth season, suckers emerge from adventitious buds at the base of the trunk and develop rapidly each year (Figure 1). Suckers inhibit growth by sharing the nutrients and water of the tree, as well as preventing air circulation and sunbathing in hazelnut hearths. Sucker restricts the growth of hazelnut main branches, leading to a significant decrease in yield, and they serve as a source of inoculum for many plant diseases such as powdery mildew, and create habitats for harmful insects. (Okay et al., 1986; Mehlenbacher and Smith, 1992; Tous et al., 1994; Beyhan and Pınar, 1996; Figen et al., 2021).

Neglecting the regular removal of suckers can have adverse effects on the yield of hazelnut trees and increase the risk of diseases (Karadeniz et al., 2009). It is highly crucial to struggle hazelnut suckers to cultivate high-quality hazelnuts and increase their yield and it is

determined that hazelnut suckers removal twice a year resulted in 42.5% more yield than hazelnut plants that were not treated at all. (Figen et al., 2021). In hazelnut cultivation, it is recommended to control suckers twice a year. These control practices ensure that the suckers in hazelnut orchards are kept under regular control (Okay et al., 1986; Beyhan et al., 1999; Karadeniz et al., 2009; Serdar et al., 2017).

## 2. Management of Controlling Suckers in Hazelnut Cultivation

Suckers are suppressed by some cultural, mechanical, physical, and chemical methods in different countries. (Figure 2). In Turkish hazelnut orchards, they are controlled through mechanical and chemical methods.


**Figure 1.** Hazelnut groves with sucker control (a) and without sucker control (b).


**Figure 2.** Tools and machines used in the control of the suckers, a. Hazelnut knife (Anonymous 2023a), b. Sucker mower, c. Back-mounted flamethrower (Anonymous 2023b), d. Tractor-pulled flamethrower (Anonymous 2023c), e. Steam machine (Anonymous 2023d).

**2.1. Cultural Control**

Materials in Türkiye, sucker control in hazelnut orchards is typically applied twice a year, usually in July before the harvest and in the fall (October or November). However, some growers may perform this process once a year or once every two years (Kaya, 1986; Kurnaz and Serdar, 1993). When all maintenance operations in hazelnut orchards are calculated in terms of time, the control of bottom shoots, which has a time period of 42%, causes a high labor force, and due to the high labor force, it is applied less than necessary by some growers. It is essential that all hazelnut cultivation practices, including sucker control, are carried out at the right time and frequency.

Hazelnuts are mainly grown in the bottom area (ocak in Turkish) system in Türkiye (Tekgüler, 2021). This system is a multi-stemmed planting system (İslam, 2018) and usually, six or eight saplings planted are around a circle 1–1.2 m in diameter in a bottom area (Beyhan, 2007). The planting method of hazelnut saplings, the number of sapling in each bottom area, and proper pruning of branches are crucial factors that can influence the formation or development of suckers. After planting saplings in the bottom areas, regular inspections should be conducted, and suckers emerging especially in the middle of the bottom areas should be promptly removed to prevent their development. Pruning performed at the right time and using the correct techniques not only positively affects hazelnut yield but also helps to hinder the growth of suckers (Beyhan et al., 1999).

The preference for hazelnut varieties without suckers (*Corylus colurna* L.) has become an important method to facilitate sucker control in hazelnut orchards. The presence of hazelnut trees without suckers in orchards has been found to have both positive and negative effects on hazelnut production (Rovira, 2021). The first trials of *C. colurna* rootstocks, which are suckerless, were conducted between 1940 and 1970 in Oregon, with *C. avellana* rootstocks (Lagerstedt, 1975). The absence of suckers in *C. colurna* trees positively affects hazelnut quality and yield by preventing the diversion of water and minerals from the hazelnut roots to the suckers (Bijelic et al., 2021). This leads to improved quality and higher yield of hazelnuts, enabling better access to underground nutrient sources due to the deep-rooting potential of hazelnut trees. The compatibility of *C. colurna* with *C. avellana* varieties prevents the deterioration of hazelnut trees, making it a positive factor (Mehlenbacher, 1991). Moreover, hazelnut trees without suckers can also exhibit specific characteristics in terms of shell color and texture when compared to *C. avellana* species. However, there are some drawbacks to hazelnut production without suckers. The necessity for hazelnut seeds to be stored for more than two years and the need for at least two years for grafting seedlings extend the orchard establishment process. Additionally, the formation of a taproot with a few lateral roots and the weakness of the root system make hazelnut trees without suckers more susceptible to root diseases and pests. As a result, hazelnut varieties without suckers has significant importance as an alternative solution to sucker control. However, considering both the positive and negative effects is crucial for planning and management when using this method (Bijelic et al., 2021; Pacchiarelli et al., 2022).

**2.2. Mechanical Control**

In Türkiye, mechanical control of suckers is generally applied manually using some cutting tools such as hazelnut knives or blades, and this practice is commonly mentioned as "cleaning" among the hazelnut growers. Sucker control is one of the most labor-intensive maintenance tasks in hazelnut orchards (İlkyaz, 1986). Moreover, hazelnut orchards' hilly and rugged lands, it may not always be possible to achieve sufficient cleaning. In sloping orchards, finding skilled workers who can clean suckers at the right time and using the correct technique has become a significant challenge (İlkyaz, 1986). Manual sucker control poses the risk of damaging hazelnut branches and roots when performed by inexperienced individuals. Additionally, this process can be time-consuming, physically demanding, and ergonomically challenging, potentially affecting the health of the workers (Kopuzoğlu and Şen, 1991).

To overcome this trouble, some implements and machines (motorised scythe and hazelnut cutter) for using mechanical control of suckers have been developed. Mechanical equipment reduces labor while ensuring a faster and more effective cleaning process. These implements are designed to cut, remove, or clean suckers from the ground. Mechanical cleaning methods not only reduce the workload of workers but also minimize the risk of damaging hazelnut trees (Beyhan et al., 1996).

As a result, keeping up with technological advancements and utilizing mechanization can enable more efficient and cost-effective sucker control. By reducing labor costs, the use of mechanical sucker control implements can encourage growers to be more proactive in managing suckers. The use of these implements for sucker control cause lower labor costs and increase the efficiency. This method helps achieve more effective and ergonomic sucker control in hazelnut orchards. Investing in technological developments and mechanization can provide significant advantages to hazelnut growers in this regard. In Türkiye, some implements have been developed for this purpose and are attached to motorized trimmers to cut the suckers. These implements should be evaluated primarily in terms of effectiveness, and if necessary, effective, practical, and economic implements and machines should be developed and widely used in hazelnut production areas.

**2.3 Physical Control**

In other countries, physical methods applied for controlling suckers include flame and steam applications. As an alternative approach to control suckers, the use of these methods is also recommended in hazelnut orchards

in Türkiye. Additionally, hot water has been recently used for weed control in different areas, and it could also be used for controlling suckers in hazelnut orchards (Tomasone et al., 2010; Tekgüler, 2021). These physical control methods can provide effective and environmentally friendly alternatives for managing suckers. Their utilization helps reduce the use of pesticides and minimizes environmental impacts while ensuring efficient control of suckers. Research and trials are important to evaluate the effectiveness of hot water treatment on suckers and better understand its applicability in hazelnut orchards (Tomasone et al., 2010; Tekgüler, 2021). The implementation of these alternative methods contributes to sustainable and eco-friendly hazelnut cultivation practices, which can be beneficial for hazelnut growers in Türkiye and around the world.

**2.3.1. Flaming**

Flaming is a preferred method due to its low cost, ergonomic, and low fuel consumption for sucker control. In Italy, flame applications of 30 and 60 seconds were performed on hazelnut suckers, and the 30-second application was found to be more suitable in terms of sucker management and time efficiency. Applying an average of 6 seconds of flaming to each sucker resulted in moderate to good results (Tomasone et al., 2010). May was recommended for flaming due to the being suckers in the early stage of that period. The most suitable period for this application is early morning or before sunset (Tomasone et al., 2010). Various durations and pressures of flame treatments were applied for controlling suckers in Türkiye. These applications included 30, 60, 90, 120, 150, and 180 seconds of treatment with 1, 2, 3, and 4 bar pressure levels. The most effective application was accomplished with a period of 150 seconds and a pressure of 3 bars which reduce LPG consumption drastically and control the suckers 90% (Tekgüler, 2021). This study represents a valuable research on the combinations of duration and pressure levels to reduce LPG consumption and minimize environmental impact. The results indicate that a 150-second treatment with 3 bars of pressure can be recommended to increase the efficiency of LPG usage and to reduce suckers substantially. Such studies provide important insights into developing strategies for the more sustainable and efficient use of energy resources. Tractor-pulled flame machines are ideal for bigger hazelnut orchards, whereas backpack-mounted flame machines are suitable for smaller orchards. Manual flame method was found to be more effective compared to tractor-pulled flame application for controlling suckers. The narrow spacing between hazelnut trees in the orchards led to adverse effects on the main hazelnut branches and reduced the effectiveness of flame treatment on suckers in the tractor-pulled application. Flame application is advantageous as it also controls weeds in the orchard, in addition to sucker control (Tekgüler, 2021).

**2.3.2. Steaming**

Steaming is one of the physical control methods used for both weed management and sucker control in hazelnut orchards. A small steam machine was used for steam application in Italy. Steam was applied to hazelnut suckers for a duration of 30-60 seconds to ensure complete contact with the suckers, and the temperature of the steam outlet was measured to reach 300 °Cduring the application. Special equipment or careful monitoring of the steam machine's pipe is required due to the high temperature it reaches during the application (Tomasone et al., 2008). Therefore, steaming needs to be carried out at a slow pace. Compared to flaming, steaming may require more expensive equipment, more fuel, more water, and a longer duration, resulting in more time consumption (Tomasone et al., 2008). Although successful results have been achieved in controlling suckers through the steam application, it was reported that the application needs to be conducted swiftly due to the rapid dispersal of steam into the air (Tomasone et al., 2008).

**2.3.3. Hot water application**

This application is a cost-effective weed control strategy that is less expensive than the other two physical control methods. Water at 98 °C was sprayed to some weed species (*Plantago major* L., *Amaranthus blitoides* (L.) S. Watson., *Chenopodium botrys* L., *Heliotropium europaeum* L. and *Cynodon dactylon* (L.) Pers.) at three different time periods (morning, noon, and late afternoon) and at different development stages. The best time period for hot water application was discovered to vary based on the weed species and was most effective during the early development stages. The most effective period for application is in the morning or evening hours (Koç, 2019). When examining the effect of hot water on hazelnut suckers, it is hypothesized that this method. It is predicted that this method could yield favorable outcomes for controlling hazelnut suckers.

**2.4. Chemical Control**

Some organic and inorganic chemicals are also used to suppress hazelnut suckers. Herbicides or other chemical substances can be used to prevent the development of suckers or to completely eradicate them by drying in hazelnut orchards. The use of herbicides for controlling suckers is the most widely used method because of quick implementation and lower cost compared with other methods. Herbicides were first applied in Italy and Oregon (USA) in 1960 for controlling suckers (Serdar and Akyüz, 2017). The effectiveness of herbicides against hazelnut suckers varies depending on the growth stage of the suckers and the number of herbicide applications. Additionally, the droplet size created by herbicide pulverizer also affects the herbicide effectiveness (Creech et al., 2015). However, the chemical substances used for the control of suckers should be applied with caution due to their potential to cause environmental pollution, reduction in soil microbial activity, and phytotoxic effects on agricultural products (Dolci et al., 2000).

**2.4.1. Herbicides**

The efficacy of some effective substances for controlling

suckers have been studied by many researchers during the early development period of hazelnut. Chemicals containing amitrole, bromacil, cacodylic acid, chlorthiamid, cypromid, dicamba, dichlobenil, dinoseb, diquat, glyphosate, paraquat, picloram, 2,4-dichlorophenoxy-acetic acid (2.4-D), and 2,4,5-trichlorophenoxy-acetic acid (2,4,5-T) were reported to be used against suckers (Reich and Lagerstedt, 1971; Peterson et al., 2016; Pacchiarelli et al., 2022). These substances investigated for their effects on suckers belong to the groups of Auxin, PPO, PSI, and CS in terms of their mode of action (WSSA, 2023) (Table 1).

2,4-D, glufosinate ammonium, paraquat, carfentrazone-ethyl, and saflufenacil were found to be effective against to suckers in Italy (De Souza and Moratti, 2020). Throughout the growing season, multiple applications were recommended for the control of hazelnut suckers (Serdar and Akyüz, 2017; De Souza and Moratti, 2020).

1-naphthaleneacetic acid (NAA) is known as a plant growth regulator that does not have volatile properties. When used above certain doses, it can act as a herbicidal substance that stimulates the production of abscisic acid and hydrogen peroxide, leading to inhibition of plant growth, tissue necrosis, and eventually plant death, or it can be mixed with herbicides. According to recent studies, NAA sprayed on the plant has no negative effect on growth and yield (Dolci et al., 2000; Dolci et al., 2004). In addition, a more effective result was obtained by applying a mixture of NAA and herbicide against suckers (Pacchiarelli et al., 2022).

**2.4.2. Nitrogen Fertilizers**

Nitrogen is a fundamental macro-nutrient essential for hazelnut, both in the early growth stages of plants and in mature plants. Proper calibration of nitrogen provided through fertilization is crucial to ensure appropriate plant growth and yield.

**Table 1.** Site actions and chemical group of the herbicides used for suckers control (WSSA, 2023)

| Herbicide | WSSA/ HRAC Code | Site of Action | Chemical Group |
|---|---|---|---|
| 2,4,5-tricloro-fenoksi-acetic acid (2,4,5-T) | 4 | Auxin Mimics | Phenoxy-carboxylates |
| 2,4 diclorofenoksi-acetic acid (2.4-D) | 4 | Auxin Mimics | Phenoxy-carboxylates |
| Amitrole | 34 | Inhibition of Lycopene Cyclase (LC) | Triazole |
| Bromacil | 5 | Inhbition of Photosynthesis at PSll - Serine 264 Binders (PS II) | Uracils |
| Cacodylic Acid | 0 | Unknown | Other |
| Carfentrazone-ethyl | 14 | Inhibition of Protoporphyrinogen Oxidase (PPO) | N-Phenyl-triazolinones |
| Chlorthiamid | 29 | Inhibition of Cellulose Synthesis (CS) | Nitriles |
| Cypromid | 0 | Unknown | Anilide |
| Dicamba | 4 | Auxin Mimics | Benzoates |
| Dichlobenil | 29 | Inhibition of Cellulose Synthesis (CS) | Nitriles |
| Dinoseb | 24 | Uncouplers | Dinitrophenols |
| Diquat | 22 | PS I Electron Diversion (PS I) | Pyridiniums |
| Glufosinate ammonium | 10 | Inhibition of Glutamine Synthetase (GS) | Phosphinic acids |
| Glyphosate | 9 | Inhibition of Enolpyruvyl Shikimate Phosphate Synthase (EPSP) | Glycine |
| Paraquat | 22 | PS I Electron Diversion (PS I) | Pyridiniums |
| Picloram | 4 | Auxin Mimics | Pyridine-carboxylates |
| Saflufenacil | 14 | Inhibition of Protoporphyrinogen Oxidase (PPO) | N-Phenyl-imides |

However, excessive nitrogen application can have negative effects on the plant, leading to vegetative abnormalities. The effect of different nitrogen fertilizers (21% ammonium sulfate and 26% calcium ammonium nitrate) at different dosages (0%, 10%, 15%, and 20%) on hazelnut suckers was investigated in Samsun, Türkiye. The 10% solution of 21% ammonium sulfate was found to be effective at a level comparable to herbicides (Serdar et al., 2022). On the other hand, the impact of nitrogen solution fertilizers used in hazelnut orchards on hazelnut yield, shell hardness or softness, and soil properties is not fully understood. However, the general observation is that the use of nitrogen fertilizers in hazelnut orchards provides positive contributions. Nevertheless, while it has been confirmed that nitrogen solutions applied to suckers cause the withering of the suckers, whether they promote the emergence of new suckers is yet to be determined (Serdar et al., 2022).

**2.4.3. Inorganic ingredients (Rock Salt)**

The use of rock salt at a concentration of 10% is a common practice among hazelnut growers for controlling suckers. However, scientific studies specifically investigating the effects of rock salt on hazelnut suckers have not been reported, yet. When rock salt is applied at a 10% concentration to wet the suckers, it is estimated that approximately 100 grams of salt are applied per bottom area. It is also assumed that a small dose of salt will not have a negative effect on hazelnut trees. To draw a definitive conclusion on this matter, further scientific research is needed to examine the effects of rock salt at different concentrations on the soil, suckers, hazelnut trees, and the quality and yield of hazelnuts. Conducting such research would provide valuable insights into the potential benefits or risks associated with using rock salt for controlling suckers in hazelnut cultivation.

## 3. Conclusion and Recommendations

Studies conducted worldwide have revealed that various methods are being explored to combat the problem of sucker growth in hazelnut orchards. When comparing their effectiveness and costs, mechanical and chemical control methods have been found to yield the best results. However, it is noted that chemical control should be applied more cautiously due to potential adverse effects. Regarding the application of substances such as nitrogen-based fertilizers and inorganic salts, no conclusive scientific evidence has been obtained on their effects on hazelnut plants, yields, and soil. Therefore, comprehensive studies regarding these practices are needed. Research conducted on sucker growth management in hazelnut orchards highlights the need for careful consideration of the chosen control methods. While mechanical and chemical approaches have proven effective, the potential adverse effects associated with chemical control require diligent application. The long-term effects of substances like nitrogen-based fertilizers and inorganic salts on hazelnut plants, yields, and soil remain inconclusive, underscoring the necessity for extensive research in this regard.

The study on the effects of flaming and steaming on sucker growth has determined that flame application is more effective and economical. All alternative methods should be subjected to necessary scientific research. In order to achieve sustainable hazelnut production and effective sucker growth management, practical and cost-effective methods should be integrated and implemented by growers according to their production areas. Increasing yield and quality in Türkiye, the main producer of hazelnuts in the world, can be achieved by determining and widely implementing successful production and management strategies.

**Author Contributions**

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

| | M.S.B. | Z.F.A. |
|---|---|---|
| C | 50 | 50 |
| D | 40 | 60 |
| S | 20 | 80 |
| DCP | 70 | 30 |
| DAI | 60 | 40 |
| L | 70 | 30 |
| W | 70 | 30 |
| CR | 30 | 70 |
| SR | 70 | 30 |

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision.

**Conflict of Interest**

The authors declared that there is no conflict of interest.

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