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RESEARCHPAPER

Determination of General Structure of Enterprises Producing Eggs in Free Range System in Ordu Province

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Abstract

In this research, the general structure of the enterprises that produce eggs in the free range system in Ordu province was determined from face-to-face surveys with 54 producers.

It has been determined that 68.5% of the enterprises producing eggs in the free range system are located in the field under hazelnut, and 42.6% prefer to use concrete materials in the construction of the poultry house. It has been stated that 50% of the enterprises provide ventilation in the hen house with fans, 44.4% use nipple type drinkers and 42.6% prefer thick sawdust as litter material. In addition, 61.1% of enterprises do not use workers. The egg yield was more than 81% in 26.9% of the enterprises. Moreover, the average feed consumption per chicken in 42.5% of the enterprises was between 100-120g. According to the statements, 72.2% of the producers were not members of the "Egg Producers Association" and 81.5% of the producers pointed out that they did not meet the expectations of the “Egg Producers Association”. At the same time, 70.4% of the producers told that the Covid-19 epidemic affected the egg sales prices, and 50% of the producers affected by the epidemic stated that they were negatively affected.

Introduction

Most consumers prefer eggs produced through alternative production systems that apply food safety regulations since animal rights started to be considered important in egg production systems (Anderson, 2009). There are various alternative production systems available such as free range, organic, enriched cage, aviary and deep-litter systems (Şekeroğlu et al. 2010; Türker and Alkan, 2018). There has been a resurgence of interest in free-range poultry farming in recent years in developed countries, as a result of welfare concerns associated with farming of poultry under intensive conditions. For the “best positive welfare outcome”, hens should be free from hunger, thirst, discomfort, pain, injury, disease, fear and distress and able to express normal behaviors (Brambell, 1965).

In free-range system laying hens breeding, where animal rights and welfare are more considered, animals are raised in conditions suitable for their nature as much as possible, the movements of hens are not restricted, their skeletal systems are better developed and the wishes and sensitivities of consumers who seek different tastes are taken into account. In this kind of breeding system, the use of cages is minimized and hen house is a breeding system in which hens can move freely in certain areas during the day and benefit from the green grass in these areas. Therefore, with this system, hens benefit from both the grass in the open area and sunlight at the maximum level. Since the stress level is lower in hens that actively use the open space, plucking and cannibalism events are also less common. The stocking density should not exceed 7 hens/m2 of available floor space in the free range system (Thear, 1997).

Again, under free range system conditions, the hens show high vigor, a firm and strong feather

coverage and wattles (Bogdanov, 1997). Birds show typical signs of calmness and comfort, such as dust and solar bathing, stretching wings and beak cleaning and preening of their feather (Bogdanov, 1997). In addition to these, beak trimming should not be necessary for hens in this system (Sorensen, 1994; Yenilmez ve Uruk, 2016; Durmuş ve Alkan, 2015; İpek ve Sözcü, 2015).

Ordu province is located between 40-41 North parallels and 37-38 East meridians. It is geographically rugged and almost all of the agricultural lands consist of hazelnut gardens. While hazelnut producers generate income from hazelnuts at certain times of the year, these gardens remain empty during the rest of the year. Developing and implementing alternative production activities in addition to hazelnuts in the Ordu province is very important in preventing migration from the village to the city. In this regard, producers may provide additional income by making free system egg chickens under hazelnut gardens. With this production system, approximately 10% savings can be achieved in the feed consumption of the hens grazing in the hazelnut gardens and the weeds in the gardens are cleaned by the hens without requiring additional labor costs (Anonymous, 2014; Derebaşı and Alkan, 2018). It is stated by historical sources that commercial egg production and export in Ordu has been taking place since 1900s (Köse and Durmuş, 2014). However, the egg sector in the area could not develop rapidly due to the ruggedness of the region, the high humidity and the lack of knowledge of the producers about the poultry sector (Anonymous, 2014).

The best method for the management of the grazing area in free range laying is the rotational grazing system. In this way, the grasses in the rotated areas grow sufficiently and grass with high nutrient content is obtained. In the rotational grazing system, the basic principle is to divide the grazing area into 4 equal parts and rotating it and moving to the other grazing area when there is no grass in one grazing area. In free range system laying hens; there exists two parts as a walking area and a closed poultry area (floor system or perch type) (Durmuş and Alkan, 2015; İpek and Sözcü, 2015; Sözcü and Yılmaz, 2014). A planning should be made in such a way that at least 4 m^2 of green area per hen in the walking area will be provided. In the henhouse, closed area should be planned so that there will be 5-6 chickens per m^2. By increasing the area allocated per hen in the hen house, the problems to be experienced due to ammonia, temperature and humidity increase in the hen house can be reduced (Durmuş and Alkan, 2015).

In this research, we aimed to determine the general structure of the enterprises that produce eggs in the free range system in Ordu province.

Material and Method

In this study, face-to-face interviews were used as data collection method and questionnaire forms prepared to measure research variables were used as data collection tool. After the questionnaire was explained to the owners of the enterprises to which the questionnaires were to be applied, it was ensured that the participants answered the questions in a healthy way. In this study, 9 of the questions in the questionnaires are about demographic characteristics, 29 of them are about poultry enterprises, 11 of them are about health and nutrition of hens, 11 of them are about sales and marketing of eggs, 10 of them are egg producers' association, 3 of them are about Covid-19 epidemic, and a total of 75 questions were used in the survey.

Sample of the Study

The sample of the study consisted of 54 enterprises that produce eggs in the free range system in Ordu province.

Statistical Analysis

First of all, frequency values (n and %) were calculated by frequency analysis of the answers given by the participants to all the questions in the survey. SPSS (2008) statistical package program was used in the calculations.

Findings and Discussion

Demographic Characteristics of Producers

The numerical (n) and percentage (%) frequency values of the individual characteristics of egg producers in free-range system and socio-economic characteristics of families are given in Table 1. It was determined that 53.7% of the producers were between the ages of 31-50, while 37% were older than 51 years. It has been determined that 55.55% of the producers are farmers, 16.16% are self-employed, 11.11% are civil servants and 16.66% are retired. It was also determined that none of the producers were veterinarians or agricultural engineers. It was determined that 35.18% of the producers participating in the research were primary/secondary school graduates, 46.29% were high school, 16.66% were university graduates and 1.85% were illiterate. It was determined that 62.96 % of the producers had more than 4 households, 35.18% had 4-6 and 1.85% had more than 7 households. When the ownership status of the enterprises is examined, it has been determined that 81.5% of the enterprises belong to the producers themselves, 11.1% to the rent and 7.4% to both the rent and the producers. At the same time, 33.3% of the producers had Social Security Organization for Artisans and the Self-Employed, 39.8% Social security agency and 1.9% Green Card social security, while 25.9% did not have any social security. While 25.9% of the producers attended the course or training related poultry, 74.1% did not. Of those who did not attended a course or training on poultry, 70% stated that they wanted to attended, 17.5% did not want to participated, and 12.5% stated that they did not have any idea about this issue. While establishing their enterprises, 35.18% of the producers used credit from

the bank, 1.85% from the Agriculture and Credit Cooperative, on the other hand, 62.96% did not use any credit. Çimrin et al., (2019) in their study on laying hens in the province of Hatay, found that approximately 40% of the producers were between the ages of 36-50, 35% were over the age of 51, and Cönk (2006) found that 42.6% of the producers were between 36- 50 years old. He reported that among 50 years of age, 50% were 51 years or older. Again, 55.6% of the producers stated that they are engaged in farming as a profession and 63% of them stated that the number of households is less than 4 people. It was determined that 1.9% of the producers included in the research were illiterate, 35.2% were primary school-secondary school graduates, 46.3% were high school graduates and 16.7% were university graduates. In the study conducted by Sarıca et al. (2020) average age of the producers was found to be 53. It was also determined that 50.7% of the enterprise owners were primary school graduates, 33.6% were middle school graduates, 13.6% were high school graduates, and 5.8% were university graduates. Moreover, cattle breeding is practiced in 96.1% of the poultry enterprises, sheep breeding is practiced in 35.9% of the poultry enterprises and, in only 1.1% of the enterprises, there is no other production other than poultry breeding. It was determined that 98.9% of the enterprises were not vaccinated at all and only 1.1% of the enterprises were vaccinated against Newcastle disease. In a study conducted by Aydın and Çelen (2011) in Gaziantep, Diyarbakır, Şanlıurfa, Batman, Adıyaman, Kilis and Mardin provinces, it was found that all poultry enterprise owners in Batman province were primary school graduates, whereas all poultry enterprise owners in Gaziantep province were university graduates. Köse and Durmuş (2014) reported that 31.5% of the producers were primary school graduates, 58% were secondary and high school graduates, and 10.5% were university graduates in their study conducted in poultry enterprises in Ordu province. When these data are compared, it is understood that over the years, university graduates have participated in free system egg poultry with a higher rate compared to previous years in Ordu province. While 74.1% of the producers stated that they did not attend any training or course related to poultry, 70% of them that they wanted to attend. It was determined that 38.9% of the producers included in the research were dependent on the social security institutions in terms of social security. While 81.5% of the producers stated that they owned the enterprises, 63% stated that they did not use any agricultural credit. In the study conducted by Çimrin et al. (2019) in Hatay province, it was determined that 1.9% of the producers used bank loans while establishing their enterprises, while 79.6% used equity.

Table 1. Demographic Characteristics of Producers

No	Demographic Characteristics	Options	Frequency	
			n	%
1	Participant's age	less than 30 years old	5	9.3
		31-40 years	15	27.8
		41-50 years	14	25.9
		over 51 years old	20	37.0
2	Occupation of the participant	Farmer	30	55.5
		Self-employment	9	16.6
		Veterinarian or agricultural engineer	0	0
		Officer/Worker	6	11.1
		Retired	9	16.6
3	Participant's educational status	illiterate	1	1.8
		Primary -Middle School	19	35.1
		High school	25	46.2
		University	9	16.6
4	Number of households of the participant	less than 4	34	62.9
		4 to 6	19	35.1
		more than 7	1	1.8
5	Participant's enterprise property	Own	44	81.5
		Rent	6	11.1
		Own-Rent	4	7.4
		State land	0	0
6	Social security of the participant	None	14	25.9
		Social Security Organization for Artisans and the Self-Employed	18	33.3
		Social security agency	21	38.9
		Green Card social security	1	1.9
			14	25.9

7	Participant's participation in poultry training or course	I joined		
		I did not participate	40	74.1
8	If you haven't joined, would you like to join?	Yes	28	70.0
		No	7	17.5
		I have no idea	5	12.5
9	The participant's use of agricultural credit	Bank	19	35.1
		Agriculture and Credit Cooperative	1	1.8
		Chamber of Agriculture	0	0
		I did not use	34	62.9

3.2. Main Findings for Enterprises

The main findings (n and %) regarding the general characteristics of the enterprises are given in Table 2.

In the study, 68.5% of the producers stated that their enterprise is under hazelnuts, 24.1% in open land and 7.4% in other ways. At the same time, it was determined that 42.6% of the producers preferred to use concrete in the construction of poultry houses, 25.9% of them used sandwich panels and 31.5% of them used other building materials. Keeping the temperature and relative humidity at optimum levels in the henhouses, removing the harmful gases released by the animals and animal welfare are directly related to the ventilation quality of the house (Akkaya ve İşgüzar, 2006). Again, nearly half of the producers stated that they provide ventilation in the poultry houses by using fans, 27.8% using windows and 22.2% using chimneys and windows. Also, 57.4% of the producers included in the research stated that they use individual nesting-box, 38.9% of them use group nesting-box, and 3.7% do not use nest boxes. Nest boxes should be above ground level to avoid floor-laid eggs; a common problem for free-range hens. Loose material in the nest boxing is preferred by hens. Thear (1997) suggested that straw is better than hay in nest boxes. The Australian Code of Practice recommends 7 birds/nest box (SCARM, 1995).

Again, 44.4% of the producers stated that they used nipple type drinkers, 42.6% of them used round type drinkers and 13% of them used trough type drinkers or other types. As substrate material, it was determined that 42.6% of the producers used thick sawdust, 14.8% rice husk, 14.8% fine sawdust and 27.8% other substrate materials. It was determined that while 44.4% of the enterprises applied 16 hours of lighting to the hens, 37% of them were illuminated as much as daylight, 13% of them 12 hours and 5.6% of them 24 hours of lighting. Also, 79.6% of the producers stated that hens can find green grass in the open area throughout the year. At the same time, 51.9% of the producers participating in the survey stated that the number of hens in their enterprise is 250 and below, 35.2% of them are between 250-750 and 13% of them are 750 and above. The ideal free-range egg layer should have adequate body weight at the start of lay and a good hen-housed egg production (Thear, 1997). More importantly these birds should reproduce and survive under very harsh environmental conditions (Huque, 1999). Modern strains can be successfully raised in a free-range condition with a slightly reduced rate of lay during summer (Glatz and Ru, 2002). Local breeds are inseparable from the rural scenario due to their adaptability under harsh environmental conditions. In a study conducted by Demircan et al. (2010) in Afyonkarahisar province, it was determined that 75% of the hybrid genotypes used in poultry enterprises were Lohmann, Nick Chicks, Bovans White, while 25% were Hy line, Brown Nick. Çimrin et al. (2019) found that ATAK'S genotype hens are more preferred because they are more resistant to adverse environmental conditions in Hatay province and are more suitable for free range egg production system.

Lohman Brown genotype hens are used in 63% of the enterprises, Atak-S genotype in 25.9%, Tinted genotype in 9.3% and Nick Brown genotype in 1.9%. In this study, it was determined that Lohman Brown genotype (63%) was used in free-range system in Ordu province. Similarly, in the study conducted by Köse and Durmuş (2014) in Ordu province, Lohman Brown and Hy-Line Brown hens were used in most of the poultry enterprises, and in the study conducted by Çimrin et al., (2019) in the province of Hatay, it was reported that hen breeds such as Atak-S were used in 52.18% of the enterprises and Lohman Brown and Nick Brown were used in 47.82. Again, 57.4% of the producers stated that they bought their hens at the age of 16-18 weeks, 22.2% under the age of 12 weeks, 11.1% over the age of 18 weeks and 9.3% at the age of 12-14 weeks. At the same time, it was determined that 51.9% of the producers used hens 71 weeks and over, 44.4% between 51-70 weeks of age and 3.7% less than 50 weeks in production. Again, 87% of the producers reported that they purchased hens by their own means, 11.1% through the Egg Producers Association and 1.9% through the Chamber of Agriculture. Half of the producers participating in the survey stated that they wanted to rais the chicks they used, and half of them did not want to. While 27.8% of the producers reported 6 or more hens per m^2 in the walking area of their enterprise, 27.8% of them stated that 4 hens, 27.8% of them 3 hens and 16.7% 5 of hens were raised. Australia Free Range Egg Producers Association

(FREPA, 1998) recommends maximum stocking density of 750 birds/hectare. The UK Soil Association requires that the stocking rates should not exceed 250 hens/acre (625/hectare) (Thear, 1997). Again, 37% of the producers reported that there were 6 or more hens per m² indoors, while 29.6% of producers stated that there were 4 hens, 18.5% of them 5 hens, and 14.8% of them 3 hens. At the same time, 72.2% of producers stated that the mortality rate in their enterprises was less than 5%, 13% of them between 6-10%, 9.3% of them more than 15% and 5.6% of them between 11-15%. In the study conducted by Tuğluk and Yalçın (2004) in Nevşehir Kozaklı, it was stated that the average mortality rate in laying hen enterprises was 5.9%. It was determined that 96.3% of the producers regularly make egg yield calculations in their enterprises. It was determined that the egg yield was between 61-80% in 46.2% of the enterprises for which egg yield calculations were made, more than 81% in 26.9% of enterprises and less than 60% in 26.9% of enterprises. Similarly, in the study conducted by Tuğluk and Yalçın (2004) in Nevşehir Kozaklı, it was stated that the average egg yield in laying hen enterprises is between 70-80%. Again, 61.1% of the producers stated that there are no employees in their enterprises, and 35.2% of producers stated that they employed 1 (18.5%) or 2 (16.7%) people. In another study conducted by Çimrin et al. (2019) in Hatay province, it was determined that more than half of the enterprises met their labor needs as permanent labor force from outside the enterprise.

In the study conducted by Köse and Durmuş (2014) in poultry enterprises in Ordu province, it was determined that 82.4% of the enterprises employed 1 person and 17.6% of them employed 2. Since the egg poultry enterprises in Ordu are not very large, either one worker or none is generally needed. This situation is considered to be due to the fact that the producers try to keep their enterprises sustainable by reducing their labor costs against increasing costs. Also, 59.3% of the producers reported that they have a feed storage in their enterprises. Again, 63% of the producers stated that they made poultry as an additional source of livelihood, while 16.7% of producers reported that they adopted egg poultry as their main source of livelihood. Also, 57.4% of the producers reported that they have been hen breeding for more than 4 years, 18.5% of producers for 1 year, 18.5% of producers for 3 years and 5.6% of producers for 2 years. At the same time, it has been determined that 75.9% of the producers are positive about continuing poultry, 44.4% are considering expanding their enterprises, whereas 16.7% are considering to leave egg poultry. Contrary to the results of this study, Cönk (2006) reported that in the study conducted in Afyonkarahisar, approximately 67% of poultry breeders would not continue production.

Again, while 64.8% of the breeders reported that they did not breed any other animal species other than hens, it was determined that 63.2% of those who raised other animals in addition to poultry breeding were engaged in cattle breeding, 10.5% in sheep-goat breeding and 10.5% in beekeeping. Also, 72.2% of the breeders stated that their enterprises were adequately controlled. Also, it was determined that 55% of the breeders asked for feed support, 18.5% cash aid, 14.8% pullet support and 11.1% training-course support from public institutions and organizations.

Table 2. Main Findings for Free Range Egg production System Enterprises

No	Questions	Options	Frequency	
			n	%
1	What is your reason for doing egg production?	For basic livelihood	9	16.6
		For additional livelihood	34	62.9
		Because it's profitable	5	9.2
		Cause I have nothing else to do	6	11.1
2	How many years have you been egg production?	1 years	10	18.5
		2 years	3	5.6
		3 years	10	18.5
		4 years and more	31	57.4
3	Do you raise other animals other than hens?	Yes	19	35.2
		No	35	64.8
4	If your answer is yes, which animal are you raising?	Cattle	12	63.2
		Sheep-Goat	2	10.5
		Bee	2	10.5
		Other	3	15.8
		Yes	24	44.4
		No	21	38.9

5	Are you considering expanding your enterprise?	I'm thinking of quitting	9	16.7
		I have no idea	0	0
6	What is the land structure where your enterprise is located?	Under hazelnut	37	68.5
		Open land	13	24.1
		Other	4	7.4
7	What material did you use in the construction of the hen house?	Concrete	23	42.6
		Sandwich panel	14	25.9
		Other	17	31.5
8	What type of nesting-box do you use in the hen house?	Individual	31	57.4
		Group	21	38.9
		I don't use	2	3.7
9	How do you provide ventilation in your hen house?	Fan	27	50.0
		Window	15	27.8
		Chimney	0	0
		Chimney + Window	12	22.2
10	How do you give water to hens in your hen house?	Trough type drinker	1	1.9
		Nipple drinker	24	44.4
		Hanging type round drinker	23	42.6
		Other	6	11.1
11	How much lighting time do you apply to hens during the laying period?	as daylight	20	37.0
		12 hours	7	13.0
		16 hours	24	44.4
		24 hours	3	5.6
12	Can hens find green grass in the grazing area all year?	Yes	43	79.6
		No	11	20.4
13	How many people work in your enterprise?	None	33	61.1
		1	10	18.5
		2	9	16.7
		3 and above	2	3.7
14	How many hens do you have in your hen house?	Less than 250	28	51.9
		Between 250-500	9	16.7
		Between 500-750	10	18.5
		More than 750	7	13.0
15	What do you use as litter material in your hen house?	Thick sawdust	23	42.6
		Fine sawdust	8	14.8
		Rice glume	8	14.8
		Other	15	27.8
16	Which hen breeds do you have in your hen house?	Tinted	5	9.2
		Lohman Brown	34	62.9
		Nick Brown	1	1.8
		Atak-S	14	25.9
		Hy-Line Brown	0	0
17	How many weeks old do you buy hens to start production?	less than 12 weeks	12	22.2
		Between 12-14 weeks	5	9.3
		Between 16-18 weeks	31	57.4
		Over 18 weeks	6	11.1
18	Would you consider raising the chicks you use in production yourself?	Yes	27	50.0
		No	27	50.0
19	Do you calculate the egg yield at regular intervals in your hen house?	Yes	52	96.3
		No	2	3.7
20	What is your average egg yield in your enterprise?	Less than 60%	14	26.9
		Between 61-70%	11	21.2
		Between 71-80%	13	25.0
		More than 81%	14	26.9
		Less than 5%	39	72.2

21	What is the % mortality from various causes in your hen house?	Between 6-10%	7	12.9
		Between 11-15%	3	5.5
		More than 15%	5	9.2
22	How many weeks old do you use hens in production?	Less than 50 weeks	2	3.7
		Between 51-60 weeks	12	22.2
		Between 61-70 weeks	12	22.2
		More than 71 weeks	28	51.8
23	How do you supply your hens?	At my own facility	47	87.0
		Egg Producers Association	6	11.1
		Chamber of Agriculture	1	1.9
24	Do you think to continue egg production?	Yes	41	75.9
		No	13	24.1
25	How many hens per square meters (m²) in the grazing area in your enterprise?	3	15	27.7
		4	15	27.7
		5	9	16.6
		6 and more	15	27.7
26	How many hens per square meters (m²) indoor area in your enterprise?	3	8	14.8
		4	16	29.6
		5	10	18.5
		6 and more	20	37.0
27	Is there an egg and feed storage unit in your enterprise?	Yes	32	59.3
		No	22	40.7
28	Is your enterprise adequately controlled?	Yes	39	72.2
		No	15	27.8
29	What are your expectations from public institutions and organizations?	Feed support	30	55.6
		Training/course support	6	11.1
		Pullet support	8	14.8
		Cash support	10	18.5

Main Findings on Health and Nutritional Status of Hens

The findings (n and %) regarding the health and nutritional status of hens used in free range system laying hens are given in Table 3.

In the current study, 51.9% of the producers stated that they received help from self-employed veterinarians and 88.9% stated that they applied routine disinfection to prevent possible diseases that may occur in the hen houses. In addition, it was determined that 57.4% of the producers had the quality control of the water given to the hens at regular intervals. Also, 83.3% of the producers stated that they buy the feed from any feed factory, 9.3% from the Egg Producers Association and 7.4% stated that they make their own feed. It has been determined that 50% of the producers who produce their feed needs by their own means prefer this way because it is cheaper than the feed they buy. At the same time, 44% of the producers, who meet their feed needs by purchasing, stated that they prefer to buy feed from a factory or Egg Producers' Association because they do not have sufficient technical knowledge. It has also been determined that 88.9% of the producers have sufficient information about the content of the feed they use. Again, 87% of the producers stated that they fed the hens continuously and 70.4% of the breeders stated that they did not feed their hens other than the recommended feed. Moreover, it was determined that the feed consumption was calculated in all hen enterprises and the feed consumption per hen in 42.59% of the enterprises varied between 100-230 grams. In the study conducted by Çimrin et al. (2019) in Hatay province, it was determined that 86.95% of the enterprises purchased feed.

Table 3. Findings on Health and Nutritional Status of Hens

No	Questions	Options	Frequency	
			n	%
1	Is disinfection applied in your hen houses?	Yes	48	88.9
		No	6	11.1

2	How do you fight diseases in your hen house?	From the Provincial/District Directorate of Agriculture and Forestry	12	22.2
		From self-employed veterinarians	28	51.9
		By my own facility	14	25.9
3	Do you continually feed the hens?	Yes	47	87.0
		No	7	13.0
4	Where do you buy the feed?	I'm doing it myself	4	7.4
		From any feed factory	45	83.3
		From the Egg Producers Association	5	9.3
5	Do you give your hens any other feed other than the recommended feed?	Yes	16	29.6
		No	38	70.4
6	Do you calculate the feed consumption of hens?	Yes	54	100.0
		No	0	0
7	Do you have any information about the content of the feed you use?	Yes	48	88.9
		No	6	11.1
8	How many grams is your average daily feed consumption per hens?	less than 100 g	3	5.5
		Between 100-120 g	23	42.5
		Between 21-130 g	21	38.8
		Between 131-141 g	5	9.2
		More than 140 g	2	3.7
9	If you meet your feed needs by producing it with your own facility, what is the most important reason for this?	Because I produce cheaper than I buy from factory	2	50.0
		To earn additional income by selling feed to other producers	1	25.0
		Because I produce better quality feed than feed factories	0	0
		To produce healthier feed	1	25.0
10	If you supply your feed needs from the factory, what is the most important reason for this?	Because it's cheaper	7	14.0
		Because I don't have enough area to store raw materials	6	12.0
		Because I don't have enough technical knowledge	22	44.0
		Because I think it's healthier	15	30.0
11	Is the quality control of the water you give to the hens carried out at regular intervals?	Yes	31	57.4
		No	23	42.6

Main Findings on the Sales and Marketing of Eggs

The findings (n and %) regarding the sale and marketing of the eggs by the producers are given in Table 4.

In our research, 92.6% of the producers stated that the eggs obtained from hens raised in the free range system were better than other eggs production systems. Again, while 70.4% of the producers stated that the eggs produced in this system were healthier, 16.7% the producers stated that they were more nutritious. Consumers have the perception that free-range eggs are healthy and wholesome foods, low in calories and saturated fats, high in protein and vitamins. Many consumers are prepared to pay an increased price for such a product because of the higher cost of production associated with the greater land area required, increased labor output per bird, higher feed consumption and poor economies of scale in grading, packaging and distribution as compared to the cage industry (Miao et al. 2005).

When marketing eggs, 46.3% of the producers stated that they preferred open viols, 20.4% closed cardboard viols, 18.5% gelatin-coated viols and 14.8% plastic viols. While 88.9% of the producers market their eggs themselves, 7.4% stated that they give the eggs wholesale to the Egg Producers Association. According to this result, producers market a very important part of the eggs they obtain with their own facility, and they do not prefer institutions such as the

Egg Producers' Association and Cooperative. It is thought that this situation is due to the decrease in the trust of the producers in these institutions due to the negativities that occur from time to time in the mentioned institutions. In the study conducted by Çimrin et al. (2019) in Hatay province, it was determined that 94.4% of the enterprises marketed the eggs themselves. In the study conducted by Köse and Durmuş (2014) in Ordu, it was determined that only 23% of the producers marketed eggs by their own facility, whereas 77% of them marketed them through Cooperative. At the same time, 75.9% of the producers stated that they are waiting egg prices to increase when there are decreases in egg prices in the short terms and they continue to sell eggs, 18.5% of the producers stated that they sold the hens at low prices and stopped egg production, and 5.6% stated that they reduced the feed they gave to the hens.

In the study, 40.7% of the producers stated that in order to increase egg sales, television and radio programs that encourage egg consumption should be made and 27.8% stated that information studies should be carried out in which the benefits of eggs for health. Again, 3.7% of the producers stated that they thought that promotional studies should be carried out on the process ability of eggs with different products, 13% of them thought that the benefits of eggs should be better explained in schools and 14.8% of them thought that negative and false news about eggs should be prevented. At the same time, 42.6% of the producers stated that they sold their hens to a slaughterhouse at the end of the production period, 46.3% of them sold their hens to a wholesale any company and 11.1% of them sold through the Egg Producers' Association. Also, 72.2% of the producers stated that they used the manure they obtained from their hens on their own land. In the study, 38.9% of the producers stated that free range egg production system would be better in the future, while 38.9% stated that it would be worse. In a study conducted by Benli and Durmuş (2015) in Ordu province, it was reported that advertising and promotional activities should be given importance in order to better explain the nutritional value of eggs.

Table 4. Findings Regarding the Sales and Marketing of Eggs

No	Questions	Options	Frequency	
			n	%
1	Do you think eggs from hens raised in the free-range system are better?	Yes	50	92.6
		No	0	0
		I have no idea	4	7.4
2	In your opinion, why should the consumer prefer eggs obtained from hens raised in the free-range system?	Because it is more nutritious	9	16.6
		Because it's healthier	38	70.3
		Because of the importance of animal welfare	4	7.4
		I have no idea	3	5.5
3	In your opinion, what will be the future status of free-range egg production system?	It will be better	21	38.9
		it will be worse	21	38.9
		No change	10	18.5
		I have no idea	2	3.7
4	How do you pack the eggs?	on open viol	25	46.3
		Viol covered with gelatin	10	18.5
		In closed cardboard viol	11	20.4
		On the sparkling viol	0	0
		On plastic viol	8	14.8
5	How do you market your eggs?	I'm selling myself	48	88.9
		I give to the Wholesale Egg Producers Association	4	7.4
		I market myself + I give to the Egg Producers Association	2	3.7
6	What do you do with your hens at the end of the production period?	I sell to any slaughterhouse	23	42.6
		I'm selling to a wholesale any company	25	46.3
		I sell through the Egg Producers Association	6	11.1
7	How do you evaluate the manure of your hens?	I do not evaluate	7	13.0
		I'm selling	8	14.8
		I use it on my own land	39	72.2
8		I reduce the feed I give to hens	3	5.6

	What measures do you take in the periods when egg prices fall sharply in the short term, and you must sell at a loss?	I wait egg prices to rise and continue to sell eggs without cutting the feed.	41	75.9
		Hoping that egg sales will increase in the short term; I keep eggs in cold storage	0	0
		I sell hens at a loss and stop production	10	18.5
9	In your opinion, what should be done to increase egg sales?	Television and radio advertisements should be made to encourage egg consumption	22	40.7
		Information studies should be conducted to explain the benefits of eggs for health	15	27.8
		Introductory studies should be carried out on the processability of eggs with different products	2	3.7
		The benefits of eggs should be better explained in schools, and students should be encouraged to consume eggs	7	13.0
		Negative and false news about eggs should be prevented	8	14.8

Main Findings on the Egg Producers Association

The findings (n and %) regarding the Egg Producers' Association of Producers are given in Table 5. As can be seen from the table, 81.5% of the producers stated that the Egg Producers Association did not meet their expectations, and 72.2% of the producers were not members of the "Egg Producers Association". At the same time, 43.6% of the producers stated that they are not members of the Egg Producers Association because they can better market the eggs they produce with their own facility. Also, 80% of the producers who are members of the Egg Producers Association stated that they received their payments in the form of money, feed and viol, 13.3% in the form of money only, and 6.7% in the form of feed or viol. Again, 80% of the producers who are members of the Egg Producers Association stated that they received their payments on time and of those who could not get it on time, 66.7% stated that they received payments with a delay of 5 months, and 33.3% of them 3 months late. Tuğluk and Yalçın (2004), in their research on egg poultry enterprises, stated that the participants do not have long-term receivables related to egg sales and they expect payments in a short-term period of 15-20 days. While 66.7% of the producers stated that a sufficient number of meetings were held at the Egg Producers' Association, 60% of producers stated that a meeting should be held at least once a month. Again, 73.3% of the producers who are members of the Egg Producers Association stated that they are not being informed about the meeting date and the decisions taken in the Egg Producers Association.

Table 5. Main Findings Regarding the Egg Producers Association

No	Questions	Options	Frequency	
			n	%
1	Are you a member of the Egg Producers Association?	Yes	15	27.8
		No	39	72.2
2	If you are not a member of the Egg Producers Association, what are the reasons?	The egg purchase price determined by the Egg Producers Association does not cover the costs	13	33.3
		The payment term determined by the Egg Producers Association is too long	1	2.6
		The Egg Producers Association is monopolized by certain producers, and I am not satisfied with this situation	4	10.3

		I think the Egg Producers Association is not well managed	2	5.1
		Not all egg producers produce the same quality eggs	2	5.1
		I can market better with my own facility	17	43.6
3	Does the Egg Producers Association meet your expectations?	Yes	10	18.5
		No	44	81.5
4	How do you receive your payments from the Egg Producers Association?	Money	2	13.3
		Food/Viol	1	6.7
		Money / Food /Viol	12	80.0
5	Do you receive your payments on time after the egg sale?	Yes	12	80.0
		No	3	20.0
6	How many months are you delaying your payments?	1 month	0	0
		3 months	1	33.3
		5 months	2	66.7
		12 months and above	0	0
7	Do you think there are enough meetings held at the Egg Producers Association?	Yes	10	66.7
		No	5	33.3
8	If your answer is no, how many times a month should the meeting be held?	1	3	60.0
		2	2	40.0
		3	0	0
9	Are you aware of the meeting dates held or to be held and the decisions taken?	Yes	11	73.3
		No	4	26.7

Main Findings on the Covid-19 Pandemic

The findings (n and %) regarding the impact of the producers from the Covid-19 pandemic are given in Table 6. 70.4% of the producers stated that the Covid-19 pandemic affected egg sales prices, and 50% of the producers affected by the epidemic stated that they were negatively affected. At the same time, 81.6% of the producers stated that they expected the effect of the epidemic to last longer than 12 months.

Table 6. Main Findings on the Impact of the Covid-19 Epidemic

No	Questions	Options	Frequency	
			n	%
1	Has the Covid-19 epidemic affected your egg sales prices?	Yes	38	70.4
		No	16	29.6
2	If your answer is yes, how was it affected?	Positive	18	47.4
		Negative	19	50.0
		I have no idea	1	2.6
3	How long do you think the impact of the COVID-19 epidemic on your enterprise will last?	3 months	3	7.8
		6 months	2	5.2
		9 months	2	5.2
		12 months and above	31	81.5

Conclusion and Recommendations

In order for people to lead a healthy life, they need to consume an adequate amount of animal-derived nutrients. One of the foods of animal origin that should be consumed is eggs. The success of laying hen enterprises varies depending on the rearing system, the breed, care-feeding, yield level, marketing status, health and climatic conditions. One of the egg production systems is free range egg production system. Free range laying hen system is a system

where animal rights and welfare are observed more, animals are raised in conditions suitable for their nature as much as possible, the movements of hens are not restricted, their skeletal systems are better developed and the use of cages and hen houses are minimized. At the same time, free range laying hen system is a breeding system in which the wishes and sensitivities of consumers seeking different tastes are taken into account, hens can move freely in certain areas during the day and benefit from green grass in these areas. According to this study, 55.6% of the producers that produce eggs in the free range laying hen system stated that feed support is very important for the production to be sustainable because approximately 70% of the costs of laying hen enterprises are feed costs. For this reason, producers should be supported as much as possible in the production of feed raw materials leading to reduction in the costs of the enterprises. In addition, with the contracted production model, the production of products such as soy and corn can be increased. In the study, 72.2% of the producers stated that they were not members of the "Egg Producers' Association" and 81.5% of producers stated that they did not meet the expectations of the Egg Producers' Association. Selling the eggs produced by the enterprises from a single source ensures continuity and uniformity in marketing. This is possible if the producers are able to work in harmony with the union or cooperative where they are located. In order to achieve this, the necessary trust must be established between these institutions and the producers. These institutions should pay their members for the eggs on time without delay. Most of the producers stated that they want to attend the training/course on hen breeding. For this reason, the necessary training should be given to the producers by the relevant institutions. In order for the producers to be less affected by the supply-demand imbalance that occurs from time to time, warehouses with should be established to store the eggs produced. Since eggs are processable, egg processing technologies should be implemented and expanded in order to make the produced eggs more suitable for export. Thus, by converting eggs into liquid yolk, liquid white, frozen and dry egg powder forms, the transportation of eggs is facilitated and more income can be obtained from the unit product. Innovative technological steps should be taken in Ordu, these studies should be supported and investors should be encouraged. Chicks, feed, medicine, technical information and marketing support should be given to enterprises through unions and cooperatives. Again, consumers should be sufficiently conscious about free range egg production system, which is one of the alternative egg production systems.

Author Contributions:

First Author: Collection and analysis of data

Second Author: Supervision of data collection and analysis, Writing - review and editing of the manuscript

Conflict of Interest:

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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RESEARCH PAPER

The Effects of Different Plant Extract Supplementation into Drinking Water Egg Laying Performance and Egg Quality in Second Production Cycle

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Abstract

This study aimed to investigate the effects of different plant extract supplementation into drinking water egg laying performance and egg quality parameters in second production cycle under commercial conditions. A total of 16657 Nick Brown laying hens in a second production cycle, kept in free-range system, used in the experiment between 75-83 weeks of age. The experimental groups were consisted of three treatment: CONT - control group (no supplementation), PEXT1 - combination of artichoke (Cynara scolymus) and turmeric (Curcuma longa) supplementation, PEXT2 – combination of cinnamon (Cinnamomum venum) + echinacea (Echinacea purpurea) + turmeric (Curcuma longa) supplementation as liquid form into drinking water. The supplementation was performed twice on weekly basis with an amount of 1 l / ton drinking water. The mean value of hen-housed and hen-day egg production, and egg weight were interestingly increased in the control group, than the PEXT1 and PEXT2 groups. However, a more efficient of FCR was found for the PEXT2 group. The breaking strength was found to be the highest in the eggs of PEXT1, whereas a higher value for shell thickness was observed in PEXT1 and PEXT2 groups. Egg yolk had a darker tone in the PEXT1 and PEXT2 groups. According to the results of the present study, it could be concluded that using a combination of cinnamon, echinacea and turmeric could improve feed efficiency. Furthermore, supplementation of plant extracts enhanced the egg quality, namely shell strength and yolk color, during the second cycle of egg production.

During last decade, the world production of egg has shown a great increment by 24.4%, reached to 1,627 trillion eggs produced globally in 2022 (FAO, 2022), and it is expected to increase further because of the rapid growth of world population and high demand for animal originated protein sources. Accordingly, the total of global demand for food shows an increment from 35% to 56% between 2010 and 2050 years (Van Dijk et al., 2021). To meet this huge demand for egg and broiler meat, some serious problems have been observed under today's commercial conditions which are performed using high yield hybrid strains which have higher performance yield, with optimum nutritional practices and management conditions. When regarding of these factors, some critical problems, for example, a higher incidence of disease, chronical stress, behavioral and welfare problems, could be largely observed, and cause reduction in performance, death and economical losses (Dilawar et al., 2021).

Due to the banning of antibiotics usage as growth promotors (O'Neill, 2016), some alternatives have been searching for possible stimulating effects for enhancing of growth and performance, modulating of immunity and health of birds. One of these alternatives of feed additives is plant extracts which have different bioactive compounds having possibility to substitute instead of antibiotics, due to their biological and aromatic properties (Gholami-Ahangaran et al., 2022). These plant extracts include the plant secondary metabolites and their derivate, and defined as phytobiotics that could be added into feed or drinking water as feed additives. It is highlighted that more than 8000 phytochemicals for example phenols, flavonoids,

tannins, saponins, essential oils, have been extracted from fruits, vegetables, legumes, whole grains, nuts and herbs (Yadav et al., 2016). Besides, plant extracts have advantages in respect to reduced or zero toxicity to animals, and naturally available (Gholami-Ahangaran et al., 2022).

It is well known that the phytobiotics have much kind of desirable effects including anti-oxidative, anti-fungal, anti-carcinogenic, anti-inflammatory, and antimicrobial effects (Bahmani et al., 2014; Gholami-Ahangaran et al., 2020). According to the these properties, phytobiotics, using as individual compounds or mixed preparations, could provide improvements in the performance parameters, efficient utilization of nutrients, stimulating of immunity, antioxidant and antibacterial properties in poultry nutrition, under normal or stressful conditions (Hashemi and Davoodi, 2012; Gholami-Ahangaran et al., 2021; Kikusato, 2021).

Commercial management standards could cause stress related to high yield of modern hybrids, nutritional aspects, environmental conditions, metabolic disorders in poultry production (Farghly et al., 2019). One of the management tools that could be acted as stressor is forced molting which promote stress, inducing birds to stop eggs laying (Roland and Brake, 1982). It is an easy application and could be obtained desired results for egg production during second cycle. However, it seriously creates fasting and aggression, therefore it has to be take account of animal welfare (Landers et al., 2005, 2008).

Recently, a huge interest has been exhibited to plant extract as feed additive in poultry nutrition (Garcia et al., 2019; Gholami-Ahangaran et al., 2022; Ürüşan, 2023). In this respect, the objective of this study was to investigate the possible effects of two different plant extract supplementation (first combination: artichoke (*Cynara scolymus*) and turmeric (*Curcuma longa*), second combination: cinnamon (*Cinnamomum venum*), echinacea (*Echinacea purpurea*) and turmeric (*Curcuma longa*) into drinking water on egg laying performance and egg quality parameters in second production cycle (between 75-83 weeks of age).

MATERIALS AND METHODS

In this study, a total of 16657 Nick Brown genotype and forced-molted laying hens in a second production cycle from a commercial laying facility were used in the experiment. This study was performed after completing of molting process according to the farm procedures, and between 75 and 83 weeks of age. Three free-range houses with similar dimensions and conditions were randomly selected and used as different plant extract supplementation groups (n: 5519 hens/experimental group).

The experimental groups were consisted of two different plant extract supplementation (commercial product) as liquid form into drinking water: CONT - control group (no supplementation), PEXT1 - combination of artichoke (*Cynara scolymus*) + turmeric (*Curcuma longa*) supplementation (Natual, Techna Smart Feed and Good Health); PEXT2 – combination of cinnamon (*Cinnamomum venum*) + echinacea (*Echinacea purpurea*) + turmeric (*Curcuma longa*) supplementation (Groupe Techna). The supplementation was performed twice on weekly basis with an amount of 1 l / ton drinking water. The concentration of plant extraction in each combination (PEXT1 and PEXT2) was 0.95 kg and 1 kg per liter product, respectively. A standard commercial layer diet for post-molting period with a content of 16% CP and 2800 ME kcal/kg was formulated according to the National Research Council (NRC, 1994) to meet nutritional specifications. The content of the diet was given in Table 1. During the experimental period, feed and water were offered ad-libitum. All hens were kept under a constant lighting schedule (16 h light and 8 h dark periods daily).

Table 1. Composition and calculated analyses of the basal diet

Ingredients	%
Corn	60.2
Soybean meal, 48%	15.6
Sunflower meal, 33.5%	5.0
Soybean oil	2
DDGS (Golden)	4.9
Black cumin meal	5.0
Limestone	4.2
Di-calcium phosphate	1.6
Lysine sulphate	0.42
Sodium chloride	0.24
Sodium sulphate	0.19
Betaine	0.13
DL-methionine	0.02
Premix*	0.50
Calculated analysis	
Metabolizable energy (kcal/kg^{-1})	2810.35
Crude protein (%)	15.85
Calcium (%)	4.35
Available phosphorus (%)	0.40

* Vitamin premix provided per kg of diet: Vitamin A: 2.40 mg; Vitamin D3: 75.00 µg; Vitamin E: 5.00 mg; Vitamin K3: 2.20 mg; Vitamin B1: 1.50 mg; Vitamin B2: 4.00 mg; Vitamin B3: 8.00 mg; Vitamin B5: 35.00 mg; Vitamin B6: 2.50 mg; Vitamin B9: 0.50 mg; Vitamin B12: 10.00 µg; Vitamin H2: 0.15 mg; Choline: 468.70 mg; Mn: 80.00 mg; Fe: 75.00 mg; Zn: 64.00 mg; Cu: 6.00 mg; Se: 0.30 mg.

Performance Parameters

The eggs in each house were collected manually at 10.00 a.m. each day, and the number of eggs laid was recorded daily. The abnormal eggs (broken eggs, shell-less, or soft shells) were excluded when counting of daily egg lay. The egg production percentage was calculated as the hen-day egg production (HDEP) and egg mass were calculated by multiplying the average egg weight with HDEP. The percentage of broken eggs

was calculated by dividing the number of broken and soft-shell eggs by the total number of eggs laid.
Daily feed intake and egg weight were recorded on weekly basis. Egg mass and feed conversion rate were calculated as ratio between egg production and egg weight, and ratio between feed intake and egg mass.
To determine the body weight, randomly selected hens (approximately 10% from each experimental group) were individually weighed at 75 and 83 weeks of age.

Egg Quality Parameters

Internal and external egg quality parameters were measured at 83rd weeks of age by randomly selected 12 eggs per each treatment group. The quality parameters measurements were performed 24 h after oviposition. After weighing the eggs with ±0.01 g precision, the length and width of the eggs were measured by using a digital caliper with 0.01 mm precision (Mitutoyo, 300 mm, Neuss, Germany). The measured values were used to calculate the egg shape index with a formula of (egg width/egg length) ×100 (Reddy et al., 1979). Eggshell breaking strength (kg/cm^2) was determined by using an eggshell force reader machine (Egg Force Reader, Orka Food Technology, Israel). The eggs were broken to obtain the albumen and yolk, and then the yolk weight was measured with ±0.01 g precision. These yolk samples were also used for further yolk color measurements. The egg yolk color (n = 12 eggs per experimental group) was measured visually assessing with Roche egg yolk color fan (Roche Ltd., Switzerland). Pigmentation of yolk was determined by scoring ranging from the lightest pigmentation (score 1) to the darkest pigmentation (score 15). The egg shells were carefully washed and dried 24 h in a drying oven at 105°C (Nuve FN-500, Ankara, Turkey) and then weighed with a precision of 0.01 g. Albumen weight was calculated by subtracting yolk and shell weights from the egg weight.
Eggshell thickness was measured using a special caliper with a precision of ± 0.01 mm, and it was given as the average thickness of the upper, middle, and lower end of the shell. Egg yolk diameter, albumen length, albumen width (mm) were measured with digital caliper with a precision of ± 0.01 mm (Mitutoyo, 300 mm, Neuss, Germany). The albumen and yolk height (mm) were measured using a tripod micrometer. Egg yolk index, albumen index, and Haugh unit were calculated using the formulas given by Funk (1948), Heiman and Carver (1936), and Haugh (1937), respectively:

Yolk index = (Yolk height / Yolk diameter) × 100
Albumen index = (Albumen height / (Albumen length + Albumen width)/2)) × 100
Haugh unit = 100 ×log (Albumen height + 7.57 - 1.7 × EW$^{0.37}$)

To determine the dry matter of albumen and yolk, the sampled eggs were used for the analysis. The dry matter analysis was performed according to the method (method number 934.01) given by AOAC (2006).

Statistical Analysis

Data obtained in the current study was subjected to statistical analysis using the general linear model (GLM) procedure in a randomized complete block design (Minitab 17). The following statistical model was used to determine the effects of the treatment:
$Y_{ij} = \mu + \alpha_{ij} + e_{ij}$
where μ = general mean, e_{ij} = random error, α_i = effect of dietary treatments, and Y_{ij} = response variable.
For performance parameters measured repeatedly in time (between 75 and 83 weeks of age) from the same chickens, a statistical model for repeated measurements in time was used. For egg quality measurements, an egg was the experimental unit (n: 15 eggs/experimental group). The means were presented with the standard error of the mean (SEM). The differences among treatments were analyzed by Duncan's multiple range tests. Differences were considered significant at P≤0.05.

RESULT AND DISCUSSION

The current study clearly indicated that the responses of forcing-molted laying hens to plant extracts supplementations could be challenging under commercial conditions with high capacity of laying hens in facilities. Both of egg production and egg quality parameters have been independently affected in all treatment groups.
The effects of different plant extract supplementation into drinking water on performance parameters during second production cycle of laying period was presented in Table 2. The highest level of hen-housed and hen-day egg production was obtained in the control group between 75 and 83 weeks of age (81.7 and 82.5% respectively) (P<0.05). The percentage of dirty eggs was found to be the highest in the PEXT2 (24.3%) and the lowest in the control (18.4%) group (P=0.007). Similarly, Esenbuga and Ekinci (2023) found a decline in egg production and feed intake in laying hens fed diets supplemented with anise, black cumin seed, and thyme extracts when compared to the control group. On the other hand, Awad et al. (2021) reported that egg laying performance showed an increment with dietary *Echinacea purpurea* powder supplementation (2.5–7.5 g/kg) by 35.4%–40.2% compared to the control group in ducks.
This contradiction among the results could be related to some issues, for example animal species, presence of stressful conditions, animal health status, egg production level of birds before molting process period, or the plant species used in combination (Jahanian et al., 2017). Another important issue to obtain remarkable effects is the supplementation amount of the plant extract into diet, single usage or combine usage of plant extracts, and usage types (supplementation into diet or water) of the extracts

(Bozkurt et al., 2012; Abadjieva et al., 2020). Similar to our findings, Roth-Maier et al. (2005) and Böhmer et al. (2009) found any significant differences in performance of laying hens by supplementation of *Echinacea purpurea* extract. However, Abadjieva et al. (2020) indicated any significant differences for hen-day egg production (control group 71.5%, supplementation group 73.3%) when the diet supplemented with an amount of 3g of dried and milled artichoke per kg feed. On the other hand, Suwarta and Suryani (2019) investigated the possible effects of cinnamon and turmeric powder mixture on egg production performance and egg quality in quails. According to this study, turmeric and cinnamon powder supplementation with different amounts (10 g turmeric + 10 g cinnamon, 20 g turmeric + 20 g cinnamon, 40 g turmeric + 40 g cinnamon / kg of feed) improved egg production of quails compared to the control group. This improvement was explained as stimulating effects of turmeric and cinnamon on intestinal absorption, relevant to increment in intestinal length, villus depth and width (Şimşek et al. 2015). In another study performed by Park et al. (2012), the egg production showed a significant increment when Lohmann Brown laying hens fed with supplementation of 0.10, 0.25, and 0.5% turmeric powder between 60 and 67-week-old age.

Table 2. The effects of different plant extract supplementation into drinking water on performance parameters during post-molted laying period

Performance parameters*	Experimental groups			*P values*
	Control	PEXT1	PEXT2	
Hen-housed egg production (%)	81.7 ± 7.5^{a}	73.5 ± 4.9^{b}	78.2 ± 4.5^{ab}	0.023
Hen-day egg production (%)	82.5 ± 8.0^{a}	74.0 ± 4.9^{b}	79.0 ± 4.4^{ab}	0.021
Percentage of dirty eggs (%)	18.4 ± 7.7^{ab}	13.2 ± 6.8^{b}	24.3 ± 6.1^{a}	0.007
Percentage of cracked eggs (%)	0.29 ± 0.18	0.36 ± 0.16	0.47 ± 0.67	0.634
Percentage of soft eggs (%)	0.002 ± 0.005	0.002 ± 0.004	0.01 ± 0.03	0.439
Egg weight (g)	63.9 ± 1.4^{a}	61.8 ± 1.6^{b}	61.8 ± 1.5^{b}	0.009
Egg mass (g)	52.2 ± 4.4^{a}	45.5 ± 3.6^{b}	48.3 ± 3.2^{ab}	0.004
Feed consumption (g/hen)	136.5 ± 12.4^{a}	125.3 ± 11.5^{ab}	112.3 ± 11.1^{b}	0.001
FCR	2.63 ± 0.29^{ab}	2.76 ± 0.22^{a}	2.34 ± 0.31^{b}	0.010
Body weight at 75 wks of age	1625.5 ± 128.1	1645.9 ± 120.8	1602.9 ± 143.7	0.787
Body weight at 83 wks of age	1632.3 ± 102.8^{b}	1771.7 ± 137.1^{ab}	1786.8 ± 144.4^{a}	0.034

* For performance parameters measured repeatedly between 75 and 83 weeks of age, the statistical model for repeated measurements in time was used.

As seen in Table 2, the mean value of egg weight and egg mass were found to be higher in the control group (63.9 g and 52.2 g), than the PEXT1 (61.8 g and 45.5 g) and PEXT2 (61.8 g and 48.3 g) groups (P<0.001). The laying hens in the control group consumed more feed than the other groups during the experimental period (136.5 g vs. 125.3 and 112.3 g, P=0.001), whereas a higher feed efficiency was found for the PEXT2 group (2.34, P=0.010).

Contrary to current findings Radwan et al. (2008) found a significant increment in egg weight by 0.5% level of turmeric powder supplementation. In another study, the turmeric powder supplementation with different levels (0.1, 2 and 4%) caused a reduction in feed intake in Hisex Brown laying hens between 80 and 92 weeks of age (Rahardja et al., 2016). Suwarta and Suryani (2019) indicated that both of turmeric and cinnamon supplementation and the supplementation amount into feed affected the egg weight and also feed efficiency in quails. The eggs obtained from quails fed with 10 g turmeric + 10 g cinnamon per kg of feed was found to be the heaviest among the treatment groups, also the best feed efficiency was also observed in the same group. Previous studies indicated that the supplementation of turmeric powder with different amount into feed caused a decline in feed consumption in laying hens during late period of laying (Riasi et al., 2012; Rahardja et al., 2016). On the other hand, the supplementation of *Echinacea purpurea* powder with different amount (2.5, 5. 7.5 and 10 g per kg of diet) had any significant effect on both egg weight and egg mass, but provided a significant improvement in feed efficiency in laying hens fed with 5 and 7.5 g per kg of diet between 44 and 49 weeks of age. Ürüşan (2023) investigated the possible effects of 5, 10 and 15 g/kg supplementation of artichoke leaf powder on productive performance of laying hens at 70 weeks of age. It was found that any significant effect for egg weight, but a higher egg production (89.4%) and the best value of feed efficiency (1.59) were observed in 10 g / kg artichoke leaf powder group.

The body weight of the hens was similar among the experimental groups at 75 weeks of age, whereas a higher body weight was observed in the PEXT2 group (1786.8 g) than the control and PEXT1 groups (1632.3 g and 1771.7 g, P=0.034). Observed differences among the experimental groups could be attributed to the possible positive effects of turmeric as increasing of excretion of digestive enzymes, pancreatic lipase enzyme, and stimulating villus size in intestine, subsequently enhancing nutrient absorption (Rajput et al., 2013).

The effects of different plant extract supplementation into drinking water on egg content during post-molted laying period was given on Table 3. At 83 weeks of age, the egg weight was found to be the highest with a value of 68.7 g in the PEXT1 group (P<0.001). The yolk ratio was the highest in the control group, and the lowest in the PEXT1 and PEXT2 groups (P<0.001). The highest albumen ratio was observed in the PEXT1 group, whereas the eggshell ratio was found to be the highest in the PEXT2 group (P<0.001). Ürüşan (2023) reported any significantly differences for egg weight when the diet was supplemented with 5, 10 and 15 g/kg of artichoke leaf powder, but the highest numerically value of egg weight (63.45 g) was observed in 15 g/kg of artichoke leaf powder supplementation group. Conversely to the findings, some reports indicated any differences for egg weight with artichoke supplementation (Abadjieva et al., 2020; Wen et al., 2021). Suwarta and Suyani (2019) found a higher weight of yolk, albumen and eggshell when quail feed supplemented with different amounts of cinnamon and turmeric (10 g, 20 g and 40 g) compared to the control group.

Table 3. The effects of different plant extract supplementation into drinking water on egg content during post-molted laying period

Egg content	Experimental groups			*P values*
	Control	PEXT1	PEXT2	
Egg weight (g)	63.9 ± 2.63[b]	68.7 ± 3.06[a]	65.6 ± 2.25[b]	<0.001
Yolk ratio (%)	26.8 ± 0.80[a]	23.3 ± 1.55[b]	24.3 ± 1.47[b]	<0.001
Albumen ratio (%)	63.4 ± 0.91[c]	66.8 ± 1.77[a]	65.2 ± 1.72[b]	<0.001
Eggshell ratio (%)	9.8 ± 0.38[b]	9.9 ± 0.84[b]	10.5 ± 0.44[a]	0.004

n: 12 eggs/treatment groups

The effects of different plant extract supplementation into drinking water on egg quality parameters during post-molted laying period was given on Table 4. The breaking strength was found to be the highest in the eggs of PEXT1 group, whereas a higher value for shell thickness was observed in PEXT1 and PEXT2 groups (P<0.05). Egg yolk had a darker tone in the PEXT1 and PEXT2 groups (12.6 and 12.4 vs. 10.8 in the control; P<0.001). On the other hand, yolk index was the highest in the eggs obtained from PEXT1 group (P<0.05). The dry matter content was found to be the highest in the egg yolk obtained from PEXT2 and in the albumen obtained from PEXT1 groups (P<0.05).

Table 4. The effects of different plant extract supplementation into drinking water on egg quality during post-molted laying period

Egg quality parameters	Experimental groups			*P values*
	Control	PEXT1	PEXT2	
Shape index (%)	77.7 ± 3.15	77.8 ± 1.74	78.4 ± 3.60	0.792
Breaking strength (g/cm^2)	1.810 ± 0.649[b]	2.475 ± 0.758[a]	2.386 ± 0.760[ab]	0.032
Shell thickness (mm)	0.388 ± 0.04[b]	0.430 ± 0.03[a]	0.434 ± 0.02[a]	<0.001
Yolk colour	10.8 ± 0.78[b]	12.6 ± 0.51[a]	12.4 ± 0.51[a]	<0.001
Yolk index (%)	46.3 ± 3.48[ab]	48.3 ± 2.70[a]	45.5 ± 0.65[b]	0.012
Albumen index (%)	8.4 ± 1.45	8.4 ± 1.65	7.7 ± 0.64	0.197
Haugh unit	88.2 ± 3.85	87.8 ± 4.11	85.8 ± 3.13	0.322
Yolk dry matter (%)	36.8 ± 5.97[b]	42.8 ± 7.67[ab]	44.3 ± 4.83[a]	0.040
Albumen dry matter (%)	22.0 ± 7.59[ab]	23.8 ± 7.36[a]	17.2 ± 2.63[b]	0.017

n: 12 eggs/treatment groups

Egg shape index and breaking strength are important egg quality criteria's and has importance for transporting and storing of eggs. The current findings clearly showed that the supplementation of various plant extracts positively affected the egg quality parameters. When the shell thickness showed an increment, eggshell breaking strength increased in the current study. Previous reports were similar the findings related eggshell breaking strength with supplementation of artichoke (Klementavičiūtė et al. 2018; Torki et al. 2018). This could be explained by rich content of inulin and oligofructose in artichoke which potentially provide a better mineral absorption, especially for calcium (Ürüşan, 2023). Many previous studies highlighted that aromatic plant additives have a positive effect on yolk color (Jahanian et al., 2017; Garcia et al., 2019; Suwarta and Suryani, 2019; Ürüşan, 2023). The darker of yolk color could be arisen due to carotenoid and phenolic components of the used plants in the experiment. Suwarta and Suryani (2019) reported a darker yolk color when quail feed supplemented with different amounts of cinnamon and turmeric (10 g, 20 g and 40 g) compared to the control group. The increment in yolk index in PEXT1 could be attributed to the anti-oxidative properties of artichoke and turmeric. These compounds such as

caffeic acid, cynarin, luteolin, apigenin in artichoke (Abbasi and Samadi, 2014) and curcumin, tetrahydrocurcuminoids, demethoxycurcumin, and bisdemethoxycutcumin in turmeric (Osawa et al., 1995; Wuthi-udomler et al., 2000) have higher antioxidant capacity that provide protection against ageing and vascular diseases. Therefore, it could be hypothesized that this potential could also provide a protective effect for yolk membrane against oxidation and also damages (Jin et al., 2019; Gholami-Ahangaran et al., 2022). Similar to current results, Jahanian et al. (2017) reported any significant differences for albumen index and Haugh unit with dietary supplementation of *Echinacea purpurea* powder.

CONCLUSION

This study was performed under commercial conditions; therefore the response of birds could be differed according to the various factors. Current findings clearly showed that artichoke and turmeric supplementation (PEXT1) could have a potential effect for enhancement of feed efficiency and eggshell strength during second cycle of laying period. However, it must be highlighted that the selection of plant extracts should be decided according to the health status, laying period and egg performance capacity of flock, and also regarding to cost- benefit in egg production.

Ethical Statement: Not applicable
Your Ethical Statement: The ethical approval is not required for this study. This is a study field performed by standard free-range process. Any animal was suffered during this study. Only performance parameters, egg content and egg quality parameters were measured without giving any suffer to chickens.

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RESEARCH PAPER

POULTRY STUDIES

Different Concentrations of High Fructose Corn Syrup in Broiler Diet Cause Different Effects on Selected Hematological Parameters

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Abstract

This study was aimed to explore how the consumption of high fructose corn syrup (HFCS) affects blood parameters of broilers. Total 120 chickens were divided into three groups including 4 subgroups in each with free access to food and water for 42 days. The control group received no additional treatment, while the second and third groups were fed diets containing 5% or 10% HFCS, respectively. On the last day, broilers were euthanized, and 5 mL blood samples were collected for hematological analyses. Results indicated no significant differences in red blood cell (RBC) or hemoglobin (HGB) levels between the control and 5% or 10% HFCS-treated groups. However, 10% HFCS treatment significantly increased packed cell volume (PCV) and mean corpuscular volume (MCV), while 5%-HFCS increased mean corpuscular hemoglobin (MCH) and decreased mean corpuscular hemoglobin concentration (MCHC) compared to the control group. Total leukocyte counts (TLC) and monocyte values remained unaffected by HFCS treatments. Notably, 5% HFCS treatment increased basophil, heterophil, and heterophil/lymphocyte ratio while decreasing lymphocyte. Conversely, 10% HFCS treatment reduced eosinophil and heterophil, increasing lymphocyte counts. In conclusion, our study suggests that dietary fructose intake can modify certain hematological parameters, potentially serving as early indicators of future systemic or metabolic issues.

Introduction

Cells necessitate a continuous influx of energy to maintain the essential biological processes that sustain their vitality. This energy is sourced from the chemical bond energy present in food molecules, which consequently act as cells' energy fuel (Alberts 2002). Carbohydrates serve as a vital energy source, play a role in regulating blood glucose and insulin metabolism, contribute to cholesterol and triglyceride metabolism, and are involved in fermentation. The process of carbohydrate breakdown into monosaccharides is initiated in the digestive tract (Goñí et al., 2007; Şen and Başalan 2017). Monosaccharides are also called carbohydrate sweeteners and highly desired due to their sweetening properties, as they elevate the flavor and overall enjoyment of a wide range of foods (Parker et al., 2010). Fructose monosaccharide is one of the three primary monosaccharides, along with glucose and galactose, and is the naturally sweetest among them (Skoog and Bharucha 2004). It is found in nearly all processed foods, fruits, vegetables, grains, and cereals. In addition, fructose is extensively utilized as a sweetener alternative to glucose or sucrose in the food industry and has become a significant component of contemporary diets (Okon et al., 2019). Following the development of high fructose corn syrup (HFCS) as a cheaper alternative to natural sugars, the use of HFCS in foods and beverages industry has gradually increased and reached approximately 40 percent of additive sweeteners in US (Parker et al., 2010). Therefore, fructose is employed in the production of desserts, condiments, and carbohydrate beverages. This situation has led to serve fructose as a big energy source for people of all ages worldwide (Fedewa and Rao 2014). However, as reviewed by (Okon et al., 2019), the excessive consumption of refined carbohyd-

rates in food and beverages elevates the risk of conditions such as dyslipidemia, obesity, insulin resistance, and heart disease. Furthermore, it is well known that the measurement of hematological parameters or indices has proven to be a valuable method for diagnosing diseases and evaluating the health of both animals and humans. This is because blood frequently provides specific indications of ongoing physiological processes in the body, assisting in the diagnosis and assessment of health status (Theml et al., 2004; Kabakci 2022; Kabakci and Kara 2023).

Recently, there has been a growing utilization of fruit-derived by-products in poultry farming. However, the immediate impact of fructose on the metabolism of young chickens remains unclear, as these by-products likely contain fructose. This mean that it is imperative to conduct research to assess the fructose consumption among poultry (Goñí et al., 2007; Ebrahimi et al., 2013; Akhlaghi et al., 2014). Therefore, this study was conducted to evaluate the effects of dietary different concentrations of high fructose corn syrup (HFCS) consumption on selected hematological parameters in broilers. We also assessed the heterophil to lymphocyte ratio (H/L) as stress and well-being parameter in poultry which is still remains justified (Scanes 2016).

Material and Methods

The experimental design of this study was approved by Kirikkale University Local Ethical Committee by the number of 2020-06/06. Following a power analysis, the experiment was carried out with one hundred twenty 0-day-old Ross 308 broiler chickens and lasted 42 days. Broiler chickens were divided into 3 groups with 4 subgroups, each 10 chickens were housed in an area of 1 m^2 on the ground as described in Ross Manuel User (Aviagen 2014). The groups consisted of as follows; group I fed with an HFCS-free diet (control), group II fed with diet containing 5% HFCS (low) and group III fed with diet containing 10% HFCS (high). The nutritional needs of the broiler chickens were determined according to (NRC 1994). The diets were prepared as isocaloric and isonitrogenic. Diets and water are recommended as *ad libitum* in compliance with the Ross broiler breeder's handbook (Aviagen 2014). The HFCS purchased from Sunar Mısır (Sunar Mısır Entegre Sanayi ve Ticaret A.Ş. Seyhan/Adana) and contained 42% fructose. The broiler chickens were housed in a heated environment with electric heaters and illuminated with fluorescent lights for 24 hours. Lighting periods were performed as previously described by Ouchi et al. (2023). The environmental temperature was initially set at 35 ºC and then gradually reduced as recommended in the Ross broiler breeder's handbook (Aviagen 2014).
At the end of the experimental days, animals were killed by cervical dislocation and blood samples (5mL) were collected bleeding jugular vein into test tubes with anticoagulant (K_3EDTA) for hematological analysis. Total leukocyte counts (TLC) and red blood cells (RBC) were counted using hemocytometer methods with Turk solution and Hayem solution (Tamzil et al., 2016), respectively. Hematocrit (PCV) and hemoglobin (HGB) concentration were determined by microhematocrit methods and cyanmethemoglobin methods (Campbell and Ellis 2007), respectively. Then, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) as red blood cell indexes were calculated from the values of RBC, PCV, and HGB. Percentage of leukocyte subtypes were determined by manually counting total 100 cells: Basophil, Eosinophil, Heterophil, Lymphocyte, and Monocyte from the blood smear under a light microscope. Afterwards heterophil/lymphocyte (H/L) rate was calculated for each sample (Gross 1983).
Descriptive and statistical analysis of the data were performed by SPSS 20.0 with one-way ANOVA following the Normality test. The significancy of difference between the groups were evaluated by post-hoc Tukey test. $P<0.05$ was considered as statistically significant.

Results

Effects of %5 or 10% levels of dietary HFCS intake on selected hematological parameters in broiler were presented in Table 1. Although the application of 5% dietary HFCS intake decreased the number of erythrocyte and high dietary HFCS intake increased the number of erythrocytes, these were not statistically different from control group ($P>0.05$). However, RBC was significantly higher in 10%-HFCS group than that of 5%-HFCS group (*P=0.019*). HGB did not affected by any concentrations of HFCS (*P=0.970*). HFCS treatment significantly ($P<0.008$) elevated the PCV and MCV values of broilers in high dose groups rather than low dose group compared to the control group. The MCH from the erythrocyte indexes differentiated by HFCS application, which was increased by low dose while decreased by high dose, but they were individually similar with control group in terms of statistical evaluation ($P=0.149$). On the other hand, 10%-HFCS treatment remarkably reduced MCH and MCHC values compared to 5%-HFCS treatment ($P=0.001$) and non-treated control groups ($P=0.040$) in broilers, respectively.
Although HFCS application did not change total leukocyte counts (TLC) of broiler ($P=0.896$), it affected leukocyte subtypes of broiler chickens in various forms depending on the dose (Table 2). Monocyte was not affected from the 5% or 10% HFCS treatment ($P=0.593$). However, percentage of basophil significantly increased in the animals treated 5%-HFCS ($P=0.016$) while it was similar to control in the animals treated 10%-HFCS ($P=0.951$). High level dietary HFCS intake decreased the eosinophil values of broilers compared to both control and the animals applied 5% dietary HFCS ($P=0.035$). Effects of 5% or 10% HFCS

Table 1. The alterations of selected hematological parameters of broilers by 5% or 10% dietary intake of high fructose corn syrup.

	Control (n=12)			5% HFCS (n=12)			10% HFCS (n=12)			*P* value
	Mean	±	SE	Mean	±	SE	Mean	±	SE	
RBC (10^6/µL)	1.84	±	0.13^{ab}	1.57	±	0.11^{a}	2.28	±	0.24^{b}	*P*<0.05
HGB (g/dL)	10.03	±	0.46	10.18	±	0.31	10.03	±	0.66	*P*>0.05
PCV (%)	28.92	±	0.90^{a}	30.33	±	0.69^{ab}	33.33	±	1.15^{b}	*P*<0.01
MCV (fL)	23.13	±	1.26^{a}	23.64	±	0.97^{a}	28.52	±	1.47^{b}	*P*<0.01
MCH (pg)	57.05	±	3.82^{ab}	67.53	±	4.15^{b}	46.52	±	3.57^{a}	*P*<0.01
MCHC (g/dL)	34.76	±	1.25^{b}	33.68	±	1.22^{ab}	29.98	±	1.48^{a}	*P*<0.05

^{a,b}: Different letters in the same row represents statistical significancy as *P*<0.05 or *P*<0.01.

Table 2. The alterations in leukocyte subtypes of broiler fed with different concentrations of high fructose corn syrup.

	Control (n=12)			5% HFCS (n=12)			10% HFCS (n=12)			*P* value
	Mean	±	SE	Mean	±	SE	Mean	±	SE	
TLC (10^3/µL)	2.89	±	0.27	2.81	±	0.13	2.72	±	0.34	*P*>0.05
Basophil (%)	0.67	±	0.26^{a}	3.08	±	0.89^{b}	0.92	±	0.40^{a}	*P*<0.05
Eosinophil (%)	1.83	±	0.60^{b}	1.75	±	0.55^{b}	0.08	±	0.08^{a}	*P*<0.05
Heterophil (%)	21.75	±	1.74^{b}	35.58	±	2.23^{c}	11.75	±	1.78^{a}	*P*<0.001
Lymphocyte (%)	75.50	±	1.69^{b}	58.75	±	3.32^{a}	86.50	±	1.89^{c}	*P*<0.001
Monocyte (%)	0.25	±	0.18	0.83	±	0.66	0.75	±	0.30	*P*>0.05
H/L	0.29	±	0.03^{a}	0.65	±	0.07^{b}	0.15	±	0.03^{a}	*P*<0.001

TLC: Total leukocyte counts, H/L: Heterophil / Lymphocyte. ^{a,b,c}: Different letters in the same row represent statistical significancy as *P*<0.05 or *P*<0.001.

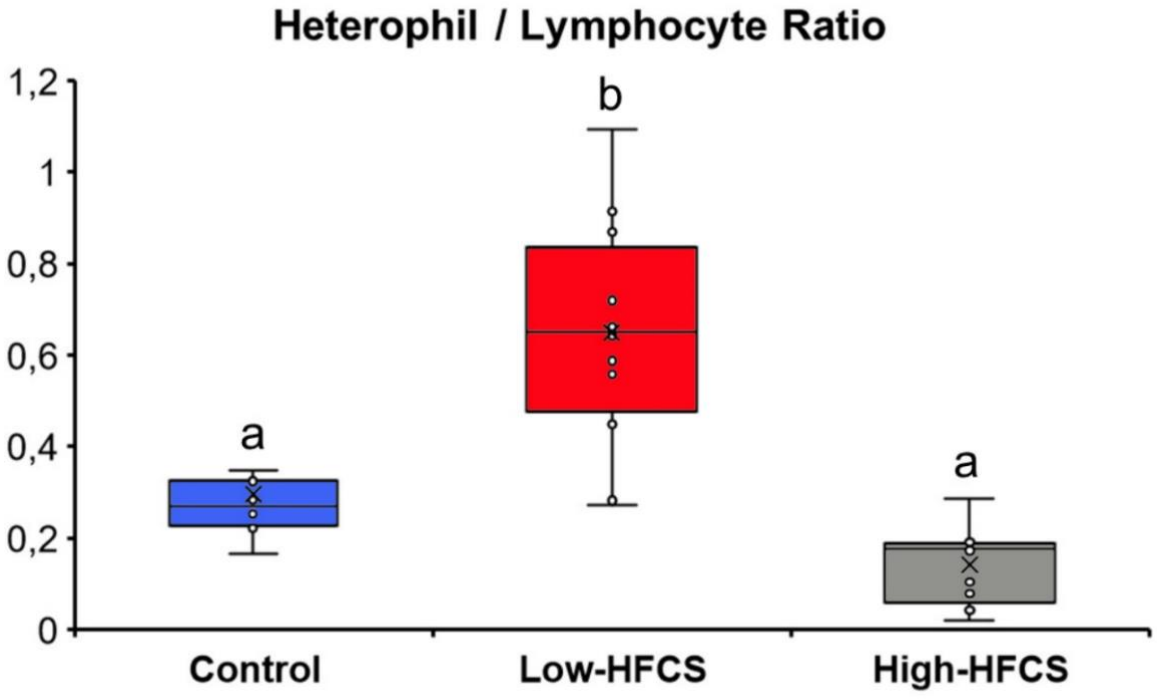

Figure 1. Effects of different concentrations (5% or 10%) of high fructose corn syrup treatments on heterophil/lymphocyte ratio in broiler chickens. ^{a,b}: Different letters on the boxplots represent statistical significancy as *P*<0.001.

treatment on heterophil and lymphocytes were the opposite of each other; the 5%-HFCS significantly increased heterophil and decreased lymphocyte while the 10%-HFCS decreased heterophil and increased lymphocyte values, respectively (*P*=0.000). Heterophil/lymphocyte (H/L) ratio did not change by high dietary HFCS intake but significantly increased by 5% dietary HFCS intake in broiler as seen in Figure 1 (*P*=0.000).

Discussion

Following the first development of HFCS at 1960s, usage of it in various foods and beverages has gradually increased over the years worldwide. Even the impact of HFCS on the health is debated intensely, excessive consumption of HFCS is reported to cause rising the incidence of disorders such as obesity, diabetes, cardiovascular diseases, and metabolic syndromes (Parker et al., 2010). Recently, fruit-derived by-products which contain fructose have been progressively utilized in poultry farming, however, the impact of fructose on the broiler chickens' metabolism is still unclear. The findings of our study conducted for this purpose firstly showed that different concentrations of HFCS intake by diet affected certain hematological parameters including heterophil and lymphocyte ratio in broiler chickens.

In the present study, rather than 5%-HFCS, 10%-HFCS treatment significantly elevated PCV and MCV, and increased RBC as well even it was insignificant compared to control group. Previous studies proved that MCV reduced in rats after oral administration of high fat/high fructose diet (Nurliyani et al., 2018), while it increased by fructose application in obese rats (Bakalov et al., 2021). In both reports it was found as a common result that RBC was not affected by fructose application. Fructose intake by HFCS may lead to hyperinsulinemia (Galderisi et al., 2019), which is associated with proliferation of erythrocytes and increasing of the RBC indices (Stonestreet et al., 1989). Stimulated red blood cell proliferation is most probably responsible for the increased MCV which can be resulted from reticulocytosis (Bakalov et al., 2021). This is also resulted in increased PCV as seen in our findings.

On the other hand, neither 5% nor 10%-HFCS affected HGB values of broiler chickens in the present study. In addition, the observed differences in MCH values by 5% or 10% HFCS treatment was not statistically significant. Similar results were also reported in rats fed with high fat-high fructose diet (Bakalov et al., 2021). However, MCHC values of broilers treated 10%-HFCS in our study significantly decreased compared to control groups' values. Although there are few different reports which were revealed any changes (Bakalov et al., 2021) or increments (Stonestreet et al., 1989) in MCHC values of rats followed by high fructose intake, the increased MCV and PCV values are most probably responsible for the reduced MCHC without non alterations in HGB of broiler chickens in the present study.

According to our findings, any concentrations of HFCS used in this study did not significantly change total leukocyte counts and monocyte percentage. However, other leukocyte subtypes and H/L ratio remarkably differed from the those of control group by 5% or 10% HFCS treatment. Findings of previous studies conducted in rats (Mihafu et al., 2020) and goats (Nurliyani et al., 2018), in which TLC and some of leukocyte subtypes did not change by high fat high fructose diet, are consistent with our results. Conversely, (Abd Elmonem and Ali 2011), reported that TLC and granulocytes decreased by consumption of fructose rich soft drink in balb/c mice. Nurliyani et al., (2018) also showed that HFHF decreased lymphocyte while increased neutrophil in goats as seen in our study following 5%-HFCS treatment. These alterations observed in percentage of heterophils, and lymphocytes may be resulted from HFCS which was shown as stimulator of stress and inflammation (Rippe and Angelopoulos 2013).

Furthermore, we found that unlike 10%-HFCS, 5%-HFCS elevated H/L ratio in broiler chickens. The H/L ratio is a common parameter that has been used for years to assess the stress status of chickens. It represents physiological changes that influence the long-term environmental conditions around the body. Because the number of lymphocytes and heterophils of broiler chickens decreased and increased, respectively, in response to stressor, the H/L ratio elevates as a stress indicator (Gross 1983). In this study, the source of stress leading to an increase in the H/L ratio may be acute allergic inflammation induced by 5%-HFCS, as the basophil percentage was also significantly increased in this group. Consistent with our results, (Jung et al., 2018) et all reported that fructose can cause anaphylactic reaction by stimulating basophil activation in human drinking fructose-rich beverages. Another study found an association between frequent consumption of free-fructose drinks and allergy in children and adolescents (Yu et al., 2018).

On the other hand, 10%-HFCS treatment did not affect basophils and H/L ratio but significantly increased lymphocytes. The reason of this increment observed in the percentage of lymphocyte in our study may be intestinal damage caused by HFCS. Moughaizel et al., (2022) et all showed that high fructose consumption via HFCS sweetened drinks led to disruption of intestinal barrier and increased gut permeability. In case of intestinal barrier damage, undigested foods leak into the bloodstream. As a result of this, lymphocytes located in the small intestine wall as an agent of immune system elevates to induce systemic inflammatory processes (Stewart et al., 2017; Arnone et al., 2022).

Conclusion

In conclusion, this study demonstrated for the first time that HFCS treatment can affect hematological parameters of broiler chickens. According to our results, low (5%) HFCS treatment increased basophil

and H/L ratio (consistent with the changes observed in heterophil and lymphocytes) while high (10%) HFCS treatment increased PCV, MCV, and lymphocytes. Thus, we suggest that low-dose HFCS consumption may lead to allergic reactions, while high-dose HFCS consumption may cause disruption of the intestinal wall, but this needs to be comprehensively elucidated by microscopic investigation in further studies.

Ethical Statement
The scientific design of this study was approved by the Local Ethical Committee of Kırıkkale University with the decision date and number of 2020-06/06.

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Author Contributions
First Author (TA): Writing -review and editing, Data Curation, Resources; Second Author (GŞ): Conceptualization, Writing -review and editing, Data Curation, Investigation, Supervision, Project Administration, Methodology, Visualization and Writing -original draft; Third Author (RK): Conceptualization, Supervision, Funding Acquisition, Resources, Writing -review and editing, Formal Analysis.

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REVIEW PAPER

Uses of Nanotechnological Feed Additives and Nanofeeds in Poultry Feeding

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Abstract

In poultry, which has an important place in meeting the need for healthy animal protein, the production of ration substance compositions with alternative sources is one of the popular topics of the last period. Nanoparticles produced by nanotechnology of various minerals added to poultry diets are increasingly being used as feed additives that can reduce feed costs, reduce the risk of environmental waste, replace antibiotics and do not pose residue risk in animal products, both in terms of supporting growth and development and strengthening the immune system. The aim of this study was to bring together the studies on the effects of using nanoparticles as feed additives on growth, development performance and immune system in poultry.

Introduction

In order to meet the need for sufficient and healthy animal protein for the rapidly growing human population, it is estimated that 200 million tons of meat production is required annually (Ghasemzadeh, 2012). In view of the rapid production and cost of meat production, poultry meat production comes to the forefront, which leads to an increase in the number of studies on alternative poultry meat production, especially chicken meat. In order to ensure the continuity of production, issues such as reducing production costs, sustainability of animal health and product health are being discussed. In recent years, the development of alternative feeds has made it possible to increase the feed utilization rate, protect animal health and obtain clean products. Intensive efforts are also being made to develop raw materials that do not compete with humans and to produce relatively inexpensive, high-quality products with a lower environmental impact in animal nutrition.

Infection with microbial agents adversely affects growth, development, and yield characteristics and is one of the major threats to the poultry industry, causing economic losses. Infectious diseases, generally controlled by vaccination and the use of antibiotics, are associated with the emergence of drug resistance in susceptible microbial populations (Doyle et al., 2006). Some countries have restricted the use of antibiotics as growth promoters as well as chemoprophylactics against poultry diseases because the residual concentrations of these drugs in poultry products may pose a risk to consumer health (Ohimain & Ofongo, 2012). Researchers are therefore looking for consumer-friendly and residue-free alternatives to develop a profitable poultry business.

Feed additives can only be placed on the market if they have been scientifically evaluated and approved as having no adverse effects on human or animal health or the environment, and if they can increase growth and/or egg production efficiency, prevent diseases, and improve feed utilization. Five categories of feed additives are defined: zootechnical (enzymes, probiotics, prebiotics, some phytogenics), nutritional (vitamins and amino acids), technological (organic acids, antioxidants, pellet binders, etc.), sensory (flavourings) and coccidiostats. However, the use and development of enzymes, phytogenics and probiotics in poultry nutrition has gained momentum (Pirgozliev et al., 2019).

In light of these increases, modern technologies such as nanotechnology will be used in agriculture and food to improve health, performance, productivity, efficiency and unit production. Nanomaterials may play an important role in facilitating the productivity gains needed to transform agriculture and, in the context of this review, the poultry industry.

Over the past decade, there has been a significant increase in interest in investigating the potential use and efficacy of nanomaterials in animal production. The main applications of these nanomaterials in animal nutrition as supplements, drugs and probiotics are being rigorously investigated. The major application of nanotechnology in poultry nutrition is mainly in the form of nanominerals due to their increased bioavailability and reduced antagonistic behavior in the intestine (Gopi et al., 2017). Nano-Zn supplementation improved growth performance in broilers (Mohammadi et al., 2015a). Similarly, the addition of silver (nano-Ag) and selenium (nano-Se) nanoparticles to broiler diets has been shown to reduce oxidative stress due to their strong antioxidant activity (Ahmadi & Kurdestani, 2010a; Aparna & Karunakaran, 2016). Furthermore, copper nanoparticles (nano-Cu) not only improved growth performance but also enhanced immune responses in poultry (Wang et al., 2011). It has also been reported that the supplementation of nano-Fe improved the growth performance and hatchability of poultry (Saki & Abbasinezhad, 2014; Sizova et al., 2015).

In this study, studies on growth and development and immunological effects of nanoparticles as feed additives in poultry diets were evaluated.

THE EFFECTS OF NANOPARTICULES ON GROWTH AND DEVELOPMENT CHARACTERISTICS

The high surface area to volume ratio makes gold nanoparticles an attractive option for use in poultry. Their surface can be coated with hundreds of molecules. Supplementation of chicken embryos in-ovo with golden nanoparticles, taurine or taurine conjugates has been shown to improve pectoral muscle organization through the activation of molecular mechanisms such as the expression of nuclear antigens of proliferative cells to enhance growth performance (Zielinska et al., 2009).

Copper is an essential trace mineral involved in many physiological and biochemical processes, such as angiogenesis, vasculogenesis, hemoglobin synthesis, and redox processes (Mroczek-Sosnowska et al., 2015). Nanocopper stimulated vascular development at the molecular and systemic level, prolonging the predominance of hyperplasia over hypertrophy until the end of embryogenesis and improving performance in broilers and layers (Leeson, 2009; Karimi et al., 2011). Muscle growth during embryogenesis depends on vascular development stimulated by copper, and the number of muscle fibers is mainly determined during the prenatal period. This stimulation of cell proliferation by copper can have a significant effect on the subsequent growth of chickens (Mroczek-Sosnowska et al., 2016). Sawosz et al. (2018) reported that it was possible to reduce the level of copper supplementation in chicken feed and its excretion into the environment using nanocopper. Another study reported that in-ovo injection of nanocopper on different days during incubation significantly altered oxygen consumption and heat production, indicating changes in metabolic rate. This may be related to reduced fat oxidation and suppression of organ development (Anwar et al., 2019).

Zinc is an essential trace element for growth, wound healing, immune function, fertility, metabolism and ROS scavengers in animals (Feng et al., 2010; Liu et al., 2011). The bioavailability of organic zinc is higher than that of inorganic zinc, but its use in animal nutrition is limited due to its higher cost (Anwar et al., 2019). However, high levels of zinc in the diet can lead to excessive excretion, which can be a source of contamination (Broom et al., 2003). High levels of zinc supplementation can also have an effect on the balance of other elements in the body and on the stability of vitamins and other nutrients. In addition, long-term exposure may increase the risk of residues (Sundaresan et al., 2008). The effects of interactions between different fatty acids, zinc sources and levels on carcass and meat quality characteristics of broiler chicks showed that using palm oil containing nano-zinc oxide at 80 mg/kg in the diet improved carcass characteristics and meat quality of broilers reared under summer heat conditions (Selim et al., 2014). An adequate dietary concentration of nanocinoxide was shown to improve growth performance and antioxidant capacity in broilers at a dose of 20 mg/kg (Zhao et al., 2014). The addition of nanocinoxide to the dry diet improved the carcass yield and increased the relative weight of the digestive and lymphoid organs of broilers in the initial period as compared to the wet diet (Mohammadi et al., 2015b; Esfahani et al., 2015).

Essential minerals for optimal poultry growth and performance are calcium and phosphorus. Reducing calcium and phosphorus in poultry diets can result in bloody meat and broken bones during carcass processing (Chen & Moran Jr., 1995). Supplementing minerals in nano form increases their bioavailability and utilization efficiency. By reducing the amount of mineral supplementation, nano-sized calcium-phosphorus supplementation can improve broiler

growth performance and reduce feed costs. Since phosphorus is an expensive mineral source, its requirement as dicalcium phosphate can be replaced with the nano form of Ca-P at levels as low as 50%. Compared to the control, birds fed 50% and 60% Ca-P-NP had significantly higher BW gains. Birds fed 50% Ca-P NPs substitution had the best FCR, which was significantly different from the control group (Vijayakumar & Balakrishnan, 2014).

Adding Se-NPs to chicken feed has significant effects on broiler growth (Cai, 2012). The addition of Se-NPs at a dose of 0.3 mg/kg to the feed increased the growth rate of broiler chickens, as reported by Senthil Kumaran et al. (2015). Furthermore, nano vitamin D3 affects the quality of femur and production performance of chickens (Yang et al., 2014). Supplementation of poultry with nano minerals and vitamins reduces stress and improves productivity and meat quality. Therefore, Se-NPs can be used as potential anti-stress factors in poultry to increase production.

EFFECTS OF NANOPARTICLES ON THE IMMUNE SYSTEM

Ag NPs showed antimicrobial activity against *Escherichia coli* and *S. aureus* with cell wall disruption at minimum inhibitory concentrations of 100 and 50 ppm (Cho et al., 2005). NPs attach to pathogens and remove them from chicken's body. Elkloub et al. (2015) found that broilers fed with Ag-NPs at a dose of 4 ppm per kilogram of feed demonstrated a reduction in the number of harmful bacteria (*E. coli*), while the population of beneficial lactobacilli remained unaltered. Due to these antimicrobial activities, these Ag-NP were reported to improve growth performance, body weight (BW), feed intake (FI) and feed conversion ratio (FCR) at 900 ppm (Ahmadi, 2009).

From an immunological perspective, it is accepted that phagocytosis of Ag-NPs stimulates inflammatory signalling by accumulating reactive oxygen species (ROS) in macrophage cells, followed by secretion of activated macrophage cell-derived tumor necrotic factor alpha (TNF-α). As TNF-α levels increase, it causes cell membrane damage and apoptosis. Incorrect recognition of Ag-NPs as foreign particles by immune cells can lead to a multi-level immune response and ultimately to toxicity in the host (Park et al., 2010). Conversely, when Ag-NPs are spontaneously recognized or in the absence of immune recognition, their ability to stimulate an immune response may be the determinant of Ag-NPs' fate in the host. In vivo studies have shown that NPs can promote inflammation (Nygaard et al., 2009). The immune defense and T helper 1 (Th1)/T helper 2 (Th2) cell balance may be affected by the inflammatory response induced by NPs. Grodzik and Sawosz (2006) evaluated the effect of Ag-NPs at 10 ppm on fetal bursa Fabricius and growth, showing decreased size and number of follicles and no significant effect on growth of chickens. Ahmadi and Kurdestany (2010b) investigated the changes in the relative weight of the bursa after application of 20, 40 and 60 ppm concentrations of Ag-NPs. The results reported decreased follicle size and number. This may be due to the antimicrobial properties of Ag-NPs affecting the microbial populations in the gut. Ag-NPs transport available oxygen and certainly reduce the growth of anaerobic microorganisms. This in turn has negative effects on the growth of the bursa Fabricius.

Matsumura et al. (2003) suggest that the same effect of Ag-zeolite may be due to Ag+ uptake in bacterial cells when they come in contact with Ag-zeolite, which inhibits cellular functions and damages the cell. On the other hand, it could be explained by the formation of reactive oxygen molecules that inhibit the cellular respiration. It is logical that for the growth and development of the bursa in healthy broilers, the presence of microorganisms in the gastrointestinal tract is necessary. Ag NPs do not appear to affect immunoglobulin M (IgM) and immunoglobulin G (IgG) levels (Pineda et al., 2012). Furthermore, Ag-NPs together with amino acids (cysteine and threonine) were shown to enhance innate and adaptive immunity in chickens during embryonic development (Bhanja et al., 2015; Saki et al., 2017). Another study reported antiviral activity of Ag NP solution against infectious bursal disease virus in embryonic chicken eggs (Pangestika & Ernawati, 2017).

Au-NPs are attractive for use in poultry because their surface can be coated with hundreds of molecules due to their high surface area-to-volume ratio. In-ovo supplementation of chicken embryos with Au-NPs, taurine, or taurine-conjugated Au-NPs improved pectoral muscle organization by activating molecular mechanisms such as nuclear antigen expression of proliferating cells to enhance growth performance (Zielinska et al., 2010). Improvements in growth performance can be maximized by controlling infectious diseases. However, early detection of disease is essential. Therefore, an effective on-farm disease control program for early detection of diseases is essential for profitable poultry production. Nanotechnology provides a platform for rapid and early detection of various poultry diseases. Au-NPs were used in diagnostics to detect avian influenza H5 hemagglutinin-derived peptides with a detection limit of 2.2 pg/mL (Jarocka et al., 2014). Au NPs have been used to coat an immobilien polyvinylidene difluoride membrane with some of the immunodominant sequences of the non-structural 1 protein of the avian influenza virus for the detection of this virus in the serum of infected birds (Emami et al., 2012).

Chitosans are thought to possess a variety of biological effects, including immunomodulatory and antimicrobial activity (Jan et al., 2012; Alishahi 2014). It is used as effective adjuvant for the delivery of biological substances such as drugs and vaccines. Compared to chitosan-free vaccines, chitosan-adjuvanted vaccines increased antibody titres against influenza. Wang et al (2011) reported that the addition of Cu-loaded chitosan NPs at a dose of 100 mg/kg can improve growth performance, immunity, protein synthesis and caecal microbiota in broilers. In another

study, avian influenza (H9N2) vaccine-loaded chitosan-NPs induced protective antibody titres after single vaccination and required low antigen dose (Khalili et al., 2015). Chitosan NPs loaded with Newcastle disease virus (La Sota) showed stronger cellular, humoral and mucosal immune responses when administered by the intranasal route (Dai et al., 2015).

METABOLISM and POSSIBLE SIDE EFFECTS of NANOFEEDS

Nanoparticles can enter the gut by several routes, including direct ingestion from feed or water and therapeutic nanodrug delivery. Inhalation of nanoparticles can also enter the GI after clearance from the respiratory tracts (Surai et al., 2017). Due to gastrointestinal barriers, bioavailability is generally reduced with oral administration. Intestinal mucosa and liver are associated with incomplete absorption. Nanoparticles, however, have shown 100% bioavailability when administered by intravenous injection due to direct entry into the systemic blood circulation (Geraet et al., 2014). Nanoparticles diffuse through intestinal mucus to reach intestinal cells and blood more rapidly (Surai et al., 2017). Little is known regarding gastrointestinal uptake of nanoparticles. Studies have shown that, depending on size, nanoparticles either pass through the gastrointestinal tract without absorption and are rapidly eliminated from the body (Geraet et al., 2014), or that nanoparticles cross the intestinal mucosa and enter the bloodstream, from where they are transferred to other organs. The physicochemical properties of these particles (e.g., charge, size, and solubility) have a significant impact on their fragmentation, absorption, distribution, and excretion (Choi & Choy, 2014). Recent studies have proposed a possible mechanism for the conversion of nano-Se to selenite in monogastric animals, suggesting that the gut microbiota can convert nano-Se to selenite, Se-phosphate and/or H_2Se, ultimately leading to the synthesis of selenoprotein (Thulasi et al., 2013; Surai et al., 2017).

Nano minerals tend to be rapidly distributed from the circulation into tissues. Primarily, the highly perfused reticuloendothelial system (RES), including organs such as the spleen and liver, are target tissues for nano minerals. Nanoparticles have even been observed in protective membranes, although at lower levels (Geraet et al., 2014). The tissue distribution patterns of ZnO nanoparticles are highly dependent on the animal, the exposure route and the physicochemical properties of ZnO nanoparticles. Studies have shown that kidney and liver are common target tissues for nano-ZnO, regardless of exposure routes, physicochemical properties, and animals tested. There is also evidence that the clearance of ZnO nanomaterials may be largely dependent on faecal excretion (Choi & Choy, 2014).

Toxic effects have been reported in addition to the benefits of nanoparticles. Toxicity of conventional sources of Zn in food and feed has been reported. Zn toxicities are mediated by oxidative stress, lipid peroxidation, cell membrane damage and oxidative DNA damage (Lin et al., 2009). The toxic effects of nanoparticles are generally size dependent, and nano-sized Zn has been shown to be more toxic than micro-sized Zn at the same dose (Chen et al., 2007). In a similar way, copper nanoparticles cause systemic toxic effects with morphological and functional changes in the liver, the spleen and the kidneys. In summary, the in vivo toxicity of Cu nanoparticles as compared to their ionic form is most likely related to their higher solubility and biodistribution in physiological media (Wang et al., 2014). Nano-Cu toxicity caused decreased growth parameters and increased malonaldehyde concentration (indicator of cellular oxidation), total SOD activity, total GHS-Px concentration and Na(+)/K(+)-ATPase activity. However, adverse effects were observed in the brain (Suttle, 2010). The literature recommends further studies on the toxic effects of nanoparticles.

CONCLUSION

In conclusion, it is seen that nanoparticles used as feed additives in poultry provide advantages such as increasing growth and development performance, strengthening the immune system and reducing feed costs. The use of these minerals produced by nanotechnology is an important development in terms of reducing the risk of environmental waste and providing natural alternatives to antibiotics. Considering the economic perspective that this method brings to poultry farming, the economic advantages of using nanoparticles as feed additives can play an important role. The reduction in feed costs, coupled with improved growth and development performance, could positively impact farmers' profit margins. Furthermore, factors such as reduced use of antibiotics, reduced risk of residues in animal products and reduced risk of environmental waste can increase sustainability and consumer confidence in the sector. However, factors such as the cost, production processes and commercial viability of this technology need to be evaluated in more detail. In conclusion, the economic advantages of using nanoparticles to farmers require a comprehensive economic analysis in terms of long-term profitability and sustainability in the sector. These studies can be considered as an important step to increase productivity and sustainability in poultry farming. However, research in this area needs to be further deepened and long-term impacts need to be evaluated more comprehensively.

Author Contributions

First Author: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Visualization and Writing -original draft, Funding Acquisition, Project Administration, Resources, Writing -review and editing.

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