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From The Editor;

Dear Readers and Authors,

As “International Journal of Science Letters (IJSL)”, we are pleased and honored to present the first issue of 2020. IJSL, is an international double peer-reviewed open access academic journal published on the basis of research- development and code of practice.

The aims of this journal are to contribute in theoretical and practical applications in relevant researchers of Life Sciences, Biology, Biotechnology, Bioengineering, Agricultural Sciences, Food Biotechnology and Genetics institutions and organizations in Turkey, and to publish solution based papers depending on the principle of impartiality and scientific ethics principles, focusing on innovative and added value work, discussing the current and future.

With these thoughts, we are especially thankful to academicians honoring with the articles, valuable scientists involved in editorial boards and reviewers for their contributions to the evaluation processes with through their opinions/ideas/contributions/criticisms in this issue of "International Journal of Life Sciences and Biotechnology".

27.02.2020

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Research Article

The effects of concentration based on the absorbance form the Ultraviolet–Visible (UV-VIS) spectroscopy analysis

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Abstract

This experiment utilizes the material characterization technique known as UV-Vis spectroscopy. The aim of this research is to study the UV-Vis absorption spectroscopy readout in order to investigate the effect of concentration on absorbance for gold solution and to determine the value of band gap for powder sample (TiO_2). The types of TiO_2 specimens can be characterized on a solid form of powder, thin film and/or in a liquid form. The preparation of each state of specimen are varies. In this current research, Liquid samples of Au nanoparticles dispersed in different concentrations of ethanol were characterized using UV-Vis technique. The absorbance versus concentration plot of the Au-ethanol samples were found to obey Beer's law. When examining the solid state in a form of TiO_2 powder, the sample need to be packed before running the experiment. To calculate the band gap of each matter from the reflectance data of UV-Vis, the graph of energy (eV) versus is $\left[\ln\left(\frac{R_{max}-R_{min}}{R-R_{min}}\right)\right]^2$ plotted. The J-curve is identified quickly, extrapolating the tangent to intersect the x-axis to obtain the band gap value.

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Keywords

Material characterization, Spectroscopy, Sample concentration vs absorbance

1. Introduction

Ultraviolet visible (UV-Vis) spectroscopy have been widely used to provide characteristic information for about any type of materials. For example, UV-Vis spectroscopy can be used to observe organic or inorganic, solid or liquid groups such as organic molecule and functional group (Aimanant and Ziemann, 2013; Ranney and Ziemann, 2016), reflectance measurements for coatings, paints, textiles (Bisulca et al., 2008), biochemical analysis (Schmid, 2001), dissolution kinetics (Jargalan et al., 2015), band gap measurements (Dharma

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et al., 2009) etc. These informations are provided by the UV-Vis depending on the extent of absorbance or transmittance of a different wavelengths beam light and the different response of samples (Thomas and Causse, 2017).

1.1. Electromagnetic Spectrum

Spectroscopic processes rely on the fact that electromagnetic radiation interacts with atoms and molecules in discrete ways to produce characteristic absorption or emission profiles. The property of electromagnetic radiation (EMR) that determines the range of colour perceived is wavelength. The part of the electromagnetic spectrum that the eye can detect is known as the visible region. EMR may be simply represented as a sine wave. These visible wavelengths cover a range from approximately 400 to 800nm (Thomas and Causse, 2017).

Basically, when measuring the optical density using spectrophotometers, visible light corresponds to a particular wavelength or color, is absorbed and disappears. The remaining light, lacking this color, shows the remaining mixture of colors as non-white light. Figure 1 illustrates the approximate complementary relationship between the wavelengths of light absorbed and the wavelengths transmitted. For example, in a blue substance, there would be a strong absorbance of the complementary (opposite it in the color wheel) color of light, orange (Sommer, 2012).

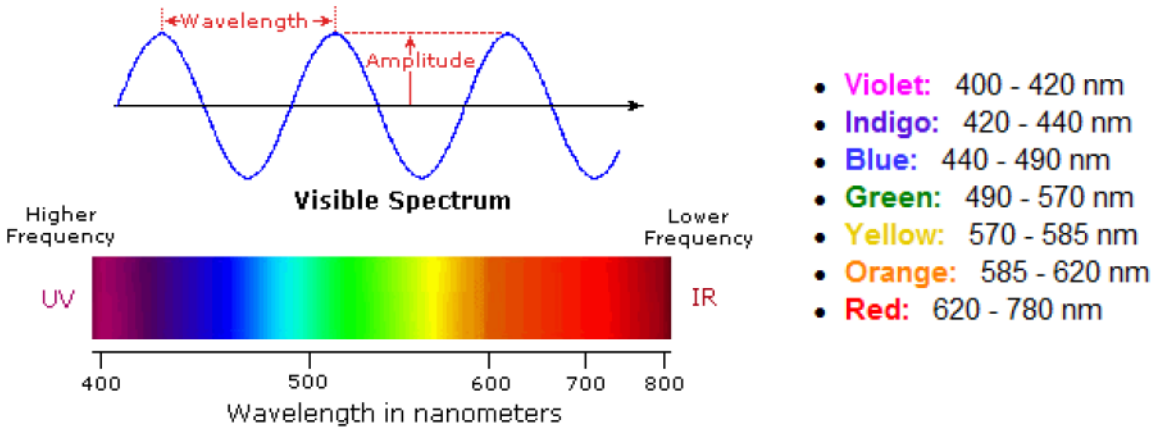

Figure 1. Sine wave representation of electromagnetic radiation and electromagnetic spectrum

Wavelength is the distance between adjacent peaks or troughs (Figure 2). The wavelength, λ, of EMR can be expressed as a function of its frequency, ν, and the speed of light, c, by the following simple equation:

$$\nu = \frac{c}{\lambda} \tag{1}$$

EMR behaves as a particle and as a wave (the dual nature of light), and the wavelength of such a particle, a photon, is related to energy by the equation:

$$E = \frac{hc}{\lambda} 10^{9} \tag{2}$$

Where h is the Planck's constant (6.63 x 10-34Js), c is the speed of light in vacuum (2.998 x 108 ms-1), E is the energy of the photon and is the wavelength in nm.

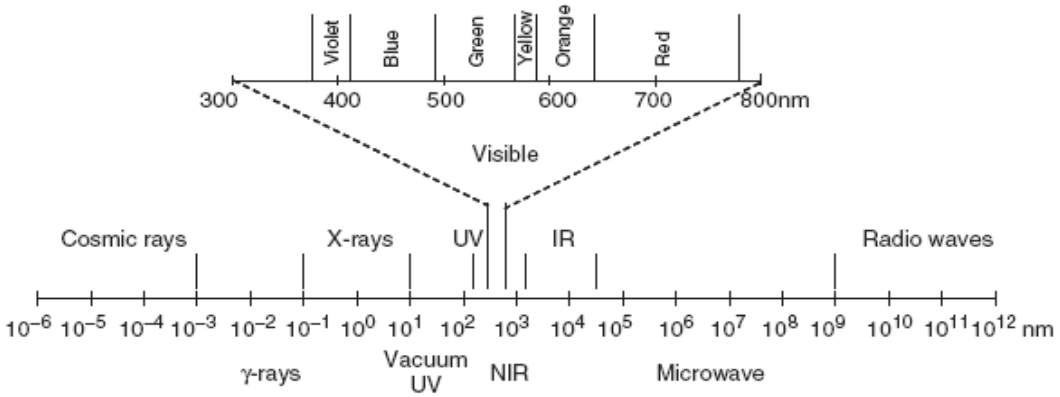

Figure 2. The electromagnetic spectrum (Thomas and Causse, 2017)

1.2. Electronic Transition

When an atom or molecule absorbs energy, electrons are promoted from their ground state to an excited state. In a molecule, the atoms can rotate and vibrate with respect to each other. These vibrations and rotations also have discrete energy levels, which can be considered as being packed on top of each electronic level. The absorption of UV or visible radiation corresponds to the excitation of outer electrons. In this analysis on the transitions involving π, σ and n electrons will be considered (Kalantar-zadeh, 2008).

Table 1. Electronic energy levels and transtitions (Kalantar-zadeh, 2008)

Transition	Region of electronic spectra	Example
$\sigma \rightarrow \sigma^*$	Vacuum ultraviolet	CH_4 at 125 nm
$\pi \rightarrow \pi^*$	Far-ultraviolet, sometimes near-ultraviolet	Acetone at 190 nm; methylamine at 213 nm
$n \rightarrow \sigma^*$	Ultraviolet	Saturated aldehydes at 180 nm
$n \rightarrow \pi^*$	Near-ultraviolet and visible	Acetone at 277 nm; nitroso-t-butane at 665 nm

1.3. Beer's Law

When a beam of electromagnetic radiation is passed through an absorbing substance, the intensity of the incident radiation (Io) will be greater than that of the emergent radiation (I). The absorption of radiant energy by matter can be described quantitatively through the general principle known as Beer's law. Beer's law states that the amount of radiation absorbed (absorbance, A) or transmitted by a solution or medium is directly proportional to the concentration of the absorbing substance present, c (moles per liter) and the path length of radiation through the sample, b (centimeters). Hence, plot of absorbance versus concentration should give a straight line passing through the origin with slope equal to Єb (Gary and O'Reilly, 1986).

$A = - \log \left(\frac{I}{Io} \right) = Єbc$, where $Є = k/2.303$

The constant ϵ is called the molar absorptivity and is independent of the concentration and path length (Kalantar-zadeh, 2008). Beer's law equation can be used to determine the concentration of an organic substance by identifying its maximum absorbance in the UV-Vis absorption spectrum, provided that the path length and molar absorptivity are known (Kalantar-zadeh, 2008).

1.4. Mechanism of Double Beam UV-VIS Spectroscopy

The function of this instrument is relatively straightforward. A beam of light from a visible and/or UV light source (red) is separated into its component wavelengths by a prism or diffraction grating. Each monochromatic (single wavelength) beam in turn is split into two equal intensity beams by a half-mirrored device. One beam, the sample beam (magenta),

passes through a small transparent container (cuvette) containing a solution of the compound being studied in a transparent solvent. The other beam, the reference (blue), passes through an identical cuvette containing only the solvent. The intensities of these light beams are then measured by electronic detectors and compared. The intensity of the reference beam, which should have suffered little or no light absorption, is defined as Io. The intensity of the sample beam is defined as I. Over a short period of time, the spectrometer automatically scans all the component wavelengths in the manner described. The ultraviolet (UV) region scanned is normally from 200 to 400 nm, and the visible portion is from 400 to 800 nm (Pavia et al., 2008).

Different methods have been widely used to determine concertation of a substance in solution. Such as, acid-base titration to find the concentration of the hydrogen ion (determining the pH of solution). There are other properties of a solution can be changed with concentration e.g. density, conductivity and colure. Beer's law relates colour intensity and concentration. Comparing to pH titration, The use of colour-based method can be much faster especially when there are samples with different concentrations of the same substances. (Fujishima et al., 2000; Nakata and Fujishima, 2012).

Au nanoparticles and TiO_2 have always been one of the most important topics in the research of photocatalysts due to its special activity and stability (Bickley et al., 1991). According to Zeng et al. (2019) and Padikkaparambil, Narayanan, Yaakob, Viswanathan, and Tasirin (2013) it has always been difficult to obtain nano-TiO_2 with a small particle size, high dispersion and high photocatalytic activity. In this study, the spectroscopy (UV-vis), was used to obtain pure TiO_2 with a small particle size, good dispersibility. Owing to its relatively high intrinsic band gap, Ultraviolet (UV) irradiation is required for TiO_2 photoactivity.

2. Materials and Methods

2.1. Sample preparation

UV-Vis can be used to characterize organic and inorganic samples in the form of gaseous, liquid or solid. Each different type of materials mentioned needs different sample preparation techniques. In this experiment, two types of samples were analyzed. The first type is Gold (Au) nanoparticles obtained from Sigma Aldrich (St. Louis, USA). Au was suspended in

ethanol solution (Sigma-Aldrich, USA) with four different concentrations (2 ppm, 5 ppm, 10 ppm, and 20 ppm) respectively, which may be differentiated by the intensity of purple color. After the sample is properly poured into cuvette, it is ready to be sent for characterizing using UV-Vis instrument Lambda 35 UV–vis spectrophotometer (Perkin-Elmer Inst., Norwalk, CT). The effect of concentration on absorbance was investigated. Absorption edge is expected to be observed at particular wavelength. For this sample, the liquid used in the reference sample has to be the same with that of which the Au nanoparticles diluted with. Examples of solution available are water, alcohol, acetone, chloroform, and toluene. In this experiment, the solution used is ethanol thus the reference sample is ethanol. The second type sample is the powder sample of TiO_2 (Sigma-Aldrich, USA). For the powder sample, the sample needed to be compacted into the sample holder before it can be analyzed and no sample reference is needed.

2.2. Instrumental Principle

In this experiment, is used to carry out the samples characterization. The light from the source, after passing through the monochromator (prism), is split into two separate beams; one for the sample and the other for the reference (Kalantar-zadeh, 2008). The absorption signal produced by the reference cell is automatically subtracted from the absorption signal produced by the sample cell, giving a net signal that corresponds to the absorption of the sample solution (Pavia et al., 2008). As a result, a plot of UV-Vis spectra (absorbance or reflectance versus wavelength) will be generated in the monitor of computer.

2.3. Data Interpretation

From the raw data for the Au nanoparticles, the absorbance peak of each concentration is plotted against the respective concentration of the solution. The data for TiO_2 is initially generated in the form of wavelength against reflectance. The data for wavelength is converted into energy using the equation and the reflectance is calculated by $\left[\ln\left(\frac{R_{max}-R_{min}}{R-R_{min}}\right)\right]^2$.

3. Results and Discussion

3.1. The Effect of Sample Concentration on the Absorbance

UV-Vis analysis is able to provide valuable information on the size, structure and aggregation properties of gold nanoparticles. The absorbance is an important parameter that can be used to calculate the nanoparticle concentration or estimate the nanoparticle size.

Figure 3 shows the results for wavelength against absorption (absorption curves) for Au nanoparticles suspended in ethanol solution with concentrations 2 ppm, 5 ppm, 10 ppm, and 20 ppm; from the wavelength of 438.8 nm to 600 nm. The peak wavelengths of each curve are labeled in the Figure 3.

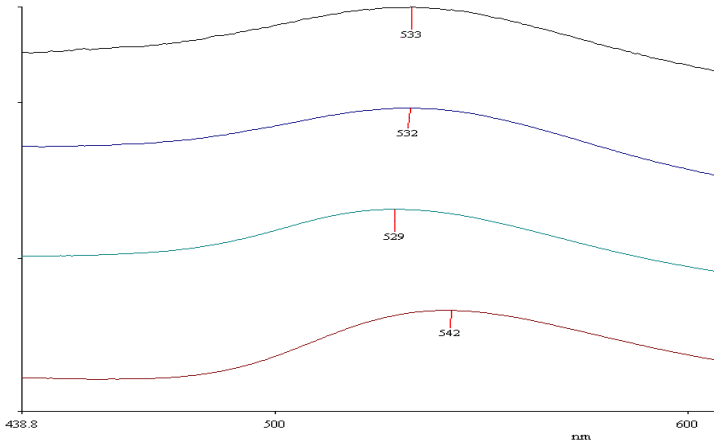

Figure 3. UV absorption spectra of Au nanoparticles dispersed in ethanol with different concentration from wavelength of 438.8 nm to 710.2 nm

According to Beer's law, the absorbance should be propositional to the concentration. However, the gold solution with 20 ppm shows the highest absorptance value (Figure 4). This concentration values is also the highest. The lowest value of the gold solution with 2 ppm is also has the lowest concentration. This means that the effect of the concertation on the absorbance obey the Beer's law.

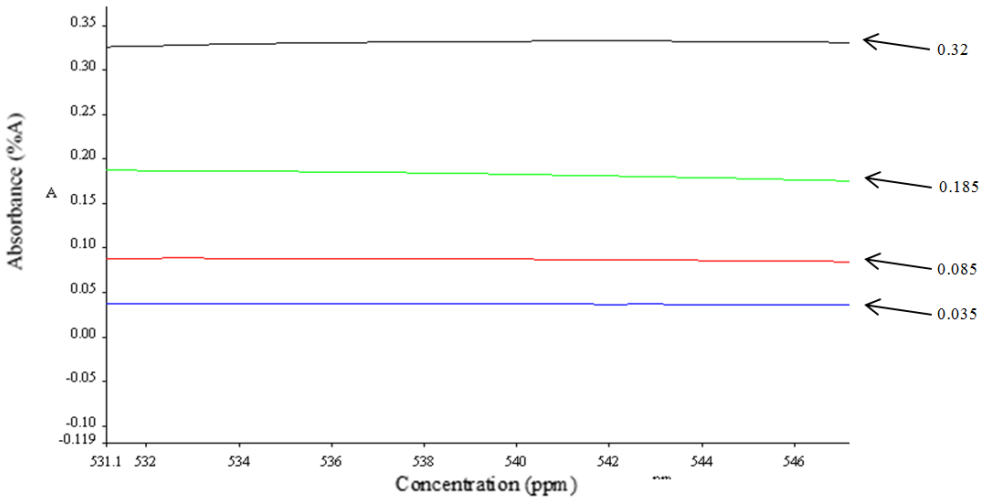

Figure 4. Graph of absorbance versus concentration from 531.1 nm to 546 nm

Figure 5 shows the plot of concentration versus absorbance for the Au ethanol solution. The plot is a straight line, thus satisfying Beer's law. This plot can be used to predict the concentration of an unknown Au ethanol solution, with the absorbance results obtained from the UV-Vis analysis (Liu et al., 2007).

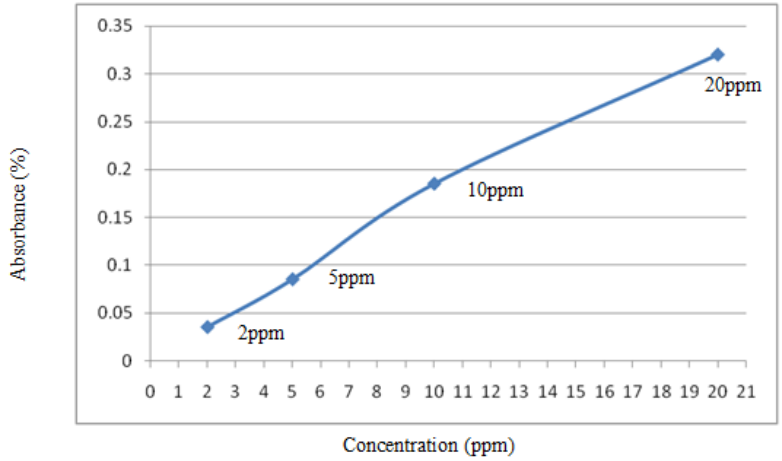

Figure 5. Graph of concentration versus absorbance obeying Beer's law

3.2. Band gap measurement of powder sample: TiO_2

UV-Vis is an important material characterization technique to determine band gap of a material. TiO_2 is semiconducting material used in optoelectronic devices (Singla et al., 2009). The band gap of the semiconducting material TiO_2 is determined from the data obtained from

UV-Vis analysis. The data obtained is tabulated in terms of energy (eV) and [ln (Rmax-Rmin)/(R-Rmin)]^2.

Figure 6 shows the plot of energy (eV) against [ln (Rmax-Rmin)/(R-Rmin)]^2 for TiO_2 in the full eV range and as well as the J-curve region. The J-curve is used to determine the band gap values by extrapolating the tangent of the J-curve.

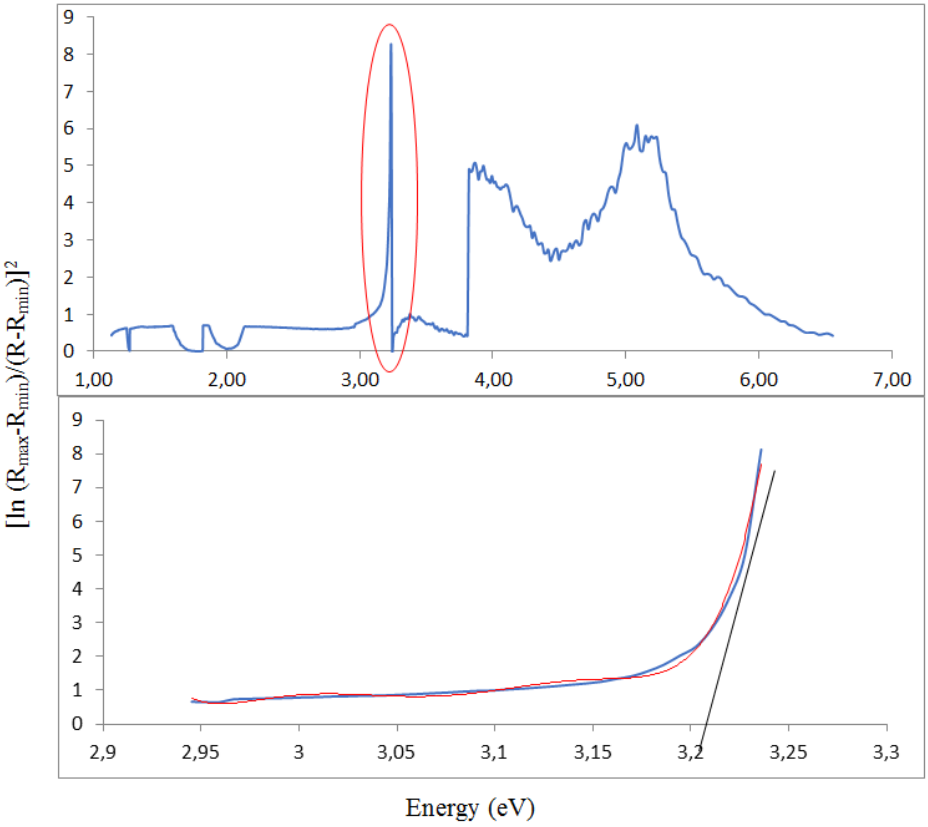

Figure 6. (a) Graph of [ln(Rmax-Rmin)/(R-Rmin)]^2 versus energy (eV) for TiO_2; (b) J-curve identified from the circled area

From the J-curve of TiO_2 the band gap value of the respective sample is determined (Table 2). The theoretical value for the band gap is compared to the one which was determined from this experiment.

Table 2. Experimental and theoretical band gap values of TiO_2 (Singla et al., 2009)

Sample	Experimental band gap value (eV)	Theoretical band gap value (eV)
TiO_2	3.20	3.18

There is a slight difference between the theoretically and experimentally determined band gap values. This difference is most probably due to the misalignment of mirrors as monochromator in the instrument or errors during the sample preparation process.

As such, precautionary measure is to be taken before starting the experiment. The alignment of instrument has to be checked before characterization being carried out. Powder sample must be compacted sufficiently in order to give precise analysis and results.

4. Conclusion

UV-Vis spectroscopy is a technique which utilizes the light absorption principle to characterize materials. This technique is useful in predicting the effects of concentration based on the absorbance form the UV-Vis analysis. As such, it is a very useful for evaluating the direct band gap of materials. Different sample types would require different preparation techniques. In this experiment, liquid samples and powder sample were used. In this experiment, gold solutions with 4 different concentrations are analyzed using UV-Vis technique. In accordance to Beer's law, the concentrations are directly proportional to the absorbance. The band gap values for TiO_2 powder is determined experimentally to be 3.20eV.

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Review

Sperm storage and artificial insemination in honey bees (*Apis mellifera* L.)

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Abstract

To obtain the expected yield from a honeybee colony, queen bee is required to have high egg capacity, the colony is also expected to be resistant to diseases, to have a low tendency for swarming, to be resistant to looting, and to be able to adapt to the climatic conditions of the region. In this context, it is important to protect the pure bee races and to improve them by conducting breeding research. To maintain the high yield aspect of honeybee colony, the queen bee needs to be replaced at most every 2 years. Queen bee becomes adult in as short as 16 days after hatching, and mating is realized in the air outside the colony, which makes it difficult to preserve the genetic line of the queen bee. At this point, artificial insemination and protection of gene resources become important. Honey bee (Apis mellifera L.) sperm can be stored for short and long periods. However, due to the delicate structure and biology of honey bee sperm, the high success rate in mammals could not be achieved in storing bee sperm. Due to the genetic damage exposed during the freezing of honey bee sperm, long-term storage difficulties are experienced. In addition, the concentration, motility and viability of spermatozoa decrease due to the short-term storage and storage conditions. In the breeding studies in the same region, after a period of time, gene resources decrease, and accordingly, the risk of inbreeding occurs. Instead of replacing the bee colonies that are at risk of inbreeding, a new different genome addition to the colony whose current yield characteristics are known can be made through the sperm storage of other colonies. Thanks to the long-term storage of sperm, long-period genetic studies can be carried out as in mammals, which is important for improving yield characteristics genetically. In addition, the long-term storage of honeybee sperm is a hope for the protection of regional races that are in danger of extinction due to unconsciousness and improper breeding policies.

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Sperm

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1. Introduction

In artificial insemination, the great accomplishments that can be repeated has been achieved (Cobey, 2007). Recent studies in the field of artificial insemination, especially in bees, and the benefits which artificial insemination provides are attracting great interest. In the past, artificial insemination in bee was difficult for a breeder, but it is now easily available, and there are many interested breeders. However, it is necessary to have suitable colonies to take advantage of honey bees (*Apis mellifera* L.) to increase their yield, to make genetic progress and to do breeding studies with the help of artificial insemination. To prevent inbreeding and diseases caused by the queen bee, it is necessary to renew the queen by obtaining the proper one from the colonies of the desired races and desired characteristics. It's a serious problem to just depend on our environment in so sensitive issues. Thus, the storage of honey bee sperm is the key to solve such problems. Since the 1970s, many different methods have been developed for freezing and thawing honey bee sperm, and studies are still being conducted (Hopkins and Herr, 2010).

2. Colony Members of Honey Bees

Honey bees are social insects that live together. A honey bee colony has 3 types of individuals: worker bee, drone, and queen (Genç and Dodoloğlu, 2002).

2.1. Work Bee

The worker bee and queen are female. They are born from fertilized eggs. Their chromosomes are diploid. The colony's greatest labor force is worker bees. They have many tasks in the colony such as hive cleaning, wax production, royal jelly production, care and feeding of the offspring and queen, hive guard, providing nectar, pollen, propolis, and ripening of honey (Genç and Dodoloğlu, 2002).

2.2. Queen

The queen determines the future of the colony. It lays eggs for the continuity of the colony and manages the colony with its pheromones. Other colony individuals are fed royal jelly for the first three days after hatching, and then honey and pollen, while the queen is fed royal

jelly until the end of its life. A young queen can lay an average of as many as 1500-2000 eggs per day. It lays two kinds of eggs, fertilized and unfertilized. The young queen lays fertilized eggs on the worker bee honeycomb, while lays unfertilized eggs on the cells of the drones that are larger males. During mating, the queen transfers the inherited characteristics that it receives from the drone to the colony by combining them with their own hereditary characteristics. Environmental interactions with inherited traits observed in worker bees are reflected as the value of the colony. This is why it is vital for a beekeeper to breed quality bees. Female reproductive organs are a pair of ovaries, a pair of lateral oviducts, a large oviduct formed by the combination of the lateral oviducts, spermatheca where sperm is stored, spermatheca canal, and vagina (Genç and Dodoloğlu, 2002).

2.3. Drones

They have 16 chromosomes, i.e. they are haploid. They develop from unfertilized eggs. Their only mission in the colony is to mate (Winston, 1987). They are of great genetic importance because they are haploids (Laidlaw, 1979). The drone has a pair of testicles, a pair of vas deferens, a pair of mucus gland, a pair of ductus ejaculatorii as sexual organs (Den boer et al., 2009). Endophallus is also the outward part of the reproductive organs (Koeniger, 1990).

3. Breeding of the Queen

Queen breeding is divided into two as the natural ways of breeding and controlled queen breeding. Breeding by natural ways occurs in the form of loss of the queen, swarming, and renewal of the queen. Although there are many methods in controlled beekeeping, the most commonly used method is the Doolittle method (Vatansever, 2004). The queen loss or Doolittle method is used for breeding quality queens for artificial insemination purposes.

3.1. Queen Breeding with Queen Loss

Frames with larvae less than 3 days old are placed in the hive without the queen. According to population size, the colony is divided into several hives. In this way, each colony produces a queen. It is an easy method and costs less. But the queen may be low-quality (Vatansever, 2004).

3.2. Doolittle Method

It is the transfer of 1-day-old young larvae into artificially prepared special queen-cell molding tools. The queen-cell molding tools are attached to the molds placed on an artificially prepared frame. The queen of a strong hive is taken 3 days before the transfer and the colony is left without a queen. Before the transfer, all open-off frames in the colony where the queen has been taken away are removed. Meanwhile, present royal jelly in the cells made by the bees to transform larvae into the queen is taken. The taken royal jelly is placed at sufficient amounts in the artificial queen-cell molding tool prepared for transfer, thus increasing the chance of success of the transfers. A suitable frame with 1-day-old larvae is taken from the breeding colony for transfer. Young larvae are carefully transferred into artificial queen-cell molding tools. Vaccination must be performed on the larvae's royal jelly. The frame of the hive in which transfer is done is given to the pre-prepared queenless starter hive, i.e. the starter colony (Vatansever, 2004).

The starter colony is fed with abundant pollen cakes and syrup. After the transfer frame stays 24-36 hours here, they are taken to the finisher colony. The finisher colony is the strong colony with the queen and 2-3 honeypot. The queen stays downstairs in the brood chamber and is not allowed to go out to the honey chamber. It is placed between the vaccination frames that are about to be left and the frames where young larvae are in. Queens that will emerge 11 days after vaccination are taken to the incubator or transferred to pre-prepared queenless mating core hives (Vatansever, 2004).

4. Maturation of the Drone

Honey bee development takes place in 4 stages. These are eggs, larvae, pupae and adults (Vatansever, 2004). The drone undergoes sexual maturity within eight to ten days after leaving the pupae. During this time, the reproductive system and the maturation process of the sperm develop (Colonello-Frattini and Hartfelder, 2009). This stage is managed by the bee's endocrine system. Juvenile hormone is effective in the transform of insects from larva to pupae and adults. It also encourages flying. Corpora allata, the gland that secretes the juvenile hormone, is activated during the maturation period (Giray and Robinson, 1996; Tozetto et al., 1997).

5. Mating of the Drone

In honey bees, mating occurs in the air. When the drone takes a mating position, the endophallus is pushed back and go out as a result of the pressure generated through the contraction of the abdominal muscles by hemolymph (Woyke and Ruttner, 1958). The cornua bend downwards (Woyke, 1955). When the endophallus partially rotates within the mating organ of the queen, the drone is paralyzed (Woyke, 2011). When the drone is separated from the queen, a part of the endophallus remains in the needle compartment of the queen. This piece is called the mating sign. The mating sign is a chitin layer of the endophallus containing mucus (Woyke, 2011). With the increase of mucus, the sperm is transferred to the queen. As the drone leaves the queen, the mating sign is also pushed into the needle of the queen (Woyke, 2008). Mucus is slightly alkaline and becomes viscous during maturation of the drone (Moors et al., 2005). Protein synthesis takes place in the lumen of the mucus gland. These proteins are essential for sexual maturity. When the endophallus is torn, the mucus in the part of the queen and the mating sign solidify, forming a mating stopper. Thanks to the stopper created by mucus, other drones are quickly prevented from mating (Koeniger and Koeniger, 2000). There is bulbus gland secretion between the mucus secretion and sperm (Colonello and Hartfelder, 2003). When the endophallus turns outward, it is torn, and the drone is separated from the queen by transmitting the sperm, secretion of the bulbus gland, cornu gland and mucus mating sign into the queen (Collins et al., 2006).

The sperm produced in the testicle is transmitted by the contraction of the testicular vesicle, while a large amount of seminal secretion is taken from the accessory gland. The accessory gland also produces the mucus that makes up the mating stopper (Den Boer et al., 2009).

6. Sperm Collecting from Drone

Drones flying before giving sperm, thus warming up, provide an advantage during sperm collecting (Collins, 2003). Wegener and Bienefeld (2012) used the method described by Harbo (1979). The genital organ, which is stimulated by the pressure applied to thorax and abdomen of the drone which reaches the desired maturity to give the sperm, turns outwards and appears. Sperm is seen in smaller amounts on the mucus layer. Sperm is collected with the help of a special syringe developed for this purpose (Harbo, 1974). The syringe contains

sterile saline solution. A sufficient amount of antibiotics is added to the saline solution and necessary precautions against contamination are taken. In addition, syringes and their parts are treated with 70% alcohol and then cleaned with pure water (Paillard et al., 2017). There are many instructions related to saline solution. For example, Paillard et al. (2017) used 1000 mL of pure water, 10 g of sodium chloride and 0.25% dihydrostreptomycin.

7. Sperm Examination in Honey Bees

7.1. Motility Test

In an Eppendorf tube, 0.5 mL of 0.9% NaCl and sperm are mixed. Subjective evaluation is made (Gontarz et al., 2016). According to Taylor et al. (2009), evaluations are made in the form of percentages (0, 20, 40, 60, 80, 95).

7.2. Concentration of Spermatozoon

To calculate the number of spermatozoon in the spermatheca, the queens are killed and dissected and their spermathecae are removed. One mL of dilution (Kiev solution, saline...), 4 mL of cold water are gently ground with the help of a mortar. Also, 8 squares of the hemocytometer are filled with approximately 10 µL of the taken sample, and spermatozoa are appropriately counted. The obtained value is multiplied by 50000 and the approximate result is estimated (Gül et al., 2017).

For arranging the density of spermatozoon, 1 µL of sperm is diluted with 1mL Kiev solution. The specimen added to the hemocytometer is examined by light microscopy at 400x magnification. After counting the spermatozoa in the four middle square, the overall spermatozoa are calculated by multiplying the obtained value and 10 and the amount of dilution (Cobey et al., 2013).

7.3. Morphological Examination

7.3.1. Water test

One μL of sperm is added to 250 μL of pure water at room temperature, and after waiting 5 minutes, a drop is taken on the lam on the phase-contrast microscope and examined at 400x magnification. It is evaluated in terms of morphological differences (Nur et al., 2012).

7.3.2. Staining of spermatozoon

In particular, it helps the acrosome, head, and nucleus to look better and the tail to be structurally evaluated. Two methods have been used in the studies for this purpose. Staining with $AgNO_3$ (silver nitrate) aids in the evaluation of chromatin proteins of acidic structure. Silver nitrate is an alkaline dye (Andraszek and Smalec, 2011). More sufficient results are obtained from it compared to the eosin and gentian violent complex. This acidic dye stains spermatozoa well and is used to distinguish morphological characteristics (Kontracki et al., 2005).

7.4. Examination of the Dead and Living Spermatozoa

Collins and Donoghue (1999) mixed 2-3 μL of sperm with 1 mL of Kiev solution (D-glucose, 0.3 g; potassium chloride, 0.41 g; sodium bicarbonate, 0.21 g; sodium citrate-2 hydrate, 2.43 g; in 100 mL pure water) as described by Moritz (1984), and after diluting it with 200 μL SYBR-14 and Propidium iodide, it is kept at room temperature for 15 minutes. It is examined under a microscope at 40x magnification. SYBR-14 adheres to nucleic acid and stains it green, while Propidium Iodide also distinguishes dead cells by staining them red.

For the purpose of dead-living cell examination of frozen sperm, Gül et al. (2017) added 1 μL of SYBR-14 and 1 μL of propidium iodide to 1 μL of sperm for every 50 μL of DMSO, i.e. 4 microliters of SYBR-14 and 4 microliters propidium iodide for every 200 μL of solution, and after incubating 30 ºC in 10 minutes, they examined the specimens by taking a sample of 5 μL.

8. Storing Honey Bee Sperm

After mating, spermatozoa of the honey bee can live for a long time in the spermatheca of the queen. The oxygenated spermatheca contains the protein, sugar, and antioxidants necessary for the survival of spermatozoa (Phiancharoen et al., 2004). A suitable buffer diluter should have around 8.5 pH and the necessary amino acids, sugar and antibiotics (Moritz, 1984).

8.1. Short-Term Storage of Honey Bee Sperm

Fresh sperm can be stored in a capillary glass tube. There should be some diluter on both sides of the tube. It can be stored by closing the two ends of the tube so that sperm and air are kept in the middle (Burley et al., 2008). If the sperm stored for the purpose of eliminating harmful effects for the queen is diluted, it is centrifuged in 1.5 mL eppendorf tube for 10 min at 1000 rpm, so the sperm is re-separated (Wegener et al., 2014). Cobey (2007) argued the negative effects of temperature on sperm when storage temperature was below 10 °C and above 32 °C, based on the study of Harbo and Williams (1987).

8.2. Long-Term Storage of Honey Bee Sperm

The sperm diluted with the proper diluter is closed with air on both sides of the pipette. It is then cooled to +4ºC in two hours to protect the sperm from cold shock. It is then cooled to -40ºC by decreasing 3ºC per minute with the help of a special cooler and then transported into -196ºC liquid nitrogen. When reused, it is dissolved by waiting 30 sec. at +37ºC (Paillard et al., 2017). In a study, the sperm cooled quickly to 0ºC was transported into liquid nitrogen (-196 ºC) (Hopkins and Herr, 2010).

According to Taylor et al. (2009), after diluting 10 mL of sperm by 1/5, it is cooled to a temperature of 5 ºC in 1-hour in the water bath. After waiting for equilibrium at +5 °C for 2 hours, the straws are filled with 0.25 cc of this dilution. The two sides of the straws are covered with air and some diluent or a substance such as polyvinyl alcohol or hot press. After freezing it in liquid nitrogen vapor at -110 ºC for 10 min, it is stored in -196ºC liquid nitrogen.

When honey bee sperm kept at -196ºC for 48 hours, the fertilized queen is seen to lay more drones than worker bee (Harbo, 1977). In light of this information, many different cryoprotectant agents are used. Gül et al. (2017) emphasized that the best result was obtained from the mixture of 60% sperm, 10% DMSO (dimethyl sulfoxide) and 30% saline solution described by Harbo (1979). Depending on the storing time of sperm, the number of worker bees decreased. Cobey (2007) stated that there was a decrease in the number of worker bees after the long-term storage of sperm described by Harbo (1979). The cause of this situation is the genetic damage of sperm due to freezing, as stated by Harbo (1981).

In order for honeybee sperm to be stored for a long time, appropriate storage techniques are needed (Cobey, 2007). Factors affecting the success of freezing methods include toxicity of cryoprotectants, freezing speed, temperature sensitivity and cold shock (Hopkins and Herr, 2010).

Whether cryoprotectants caused cell death has been investigated. DMSO is the least damaging agent among other cryoprotective. Also, 95% motility is observed at room temperature in 1-hour; and 35% living cells are observed in distillation with glycerol, but no motility is observed. In the mixture with DMSO and glycerol, it is observed that living spermatozoa are around 65% in 1-hour. When the water baths of spermatozoa at temperatures of 30, 35, 40 and 45ºC were used, there was no significant difference in the thawing of sperm up to 40ºC (Hopkins and Herr, 2010). The egg yolk, which is used successfully in mammals, is not preferred because it clogs the sexual canals of the queen. Frozen and thawed sperm are used after centrifugation, but spermatozoa are damaged during this process. Honey bee spermatozoa are very delicate and sensitive. Therefore, the centrifuge process is more successful in fresh sperm. Success is expressed as the percentage of worker bees in the eggs of inseminated queens (Wegenner et al., 2014).

Honey bee sperm can be stored at temperatures between +16 ºC and -196 ºC. In a study conducted for this purpose, the viability rates of sperm, which was stored at temperatures of 16 ºC and -196ºC and used by being centrifuged and not centrifuged at different time intervals, were examined. As a result, all spermatozoa stored for a long time at 16 ºC died. In frozen and thawed samples, the viability rate before centrifuge was 78±3%, while after centrifuge it was 75±3%. The centrifuge process reduces the viability of sperm.

In the study, according to the number of spermatozoa in the spermatheca, half of the 36 queens were inseminated after centrifugation and the other half were inseminated without centrifugation. Since all spermatozoa stored for a long time at 16 ºC died, the evaluation was conducted through the spermatozoa stored at -196 ºC. When the spermathecas of the queens were examined, alive spermatozoa were observed at a rate of 82%, and 13% of them were found to have empty spermatheca. Spermatozoon numbers were determined between about 5.000 and 1.500.000. Spermatozoa viability was found between 10% and 71%. In the same study, when the situation between temperature and sperm viability was examined, it was observed that the viability rate of the sperm stored at +16ºC in 90 days (77% ± 5) was higher than the viability rate of the sperm stored at -196ºC (61% ± 5). Sperm viability at +16 ºC (69±8%) and -196ºC (64±8%) approached each other at 180 days, but all of the spermatozoa stored at +16ºC for 330 days died out (Paillard et al, 2017).

In a study of different cryoprotective and dilution rates, 3 different cryoprotective (DMSO, DMA, glycerol) and 5 different dilution rates (1:1, 3:1, 6:1, 9:1, 12:1) were used. DMSO was found to be more successful than other agents. As the sperm dilution rate was increased, the sperm's survival rate also increased. When the amount of dilution increases, it becomes easier for cryoprotectant diluent to be diffused into the cell and wrapped spermatozoa better (Taylor et al., 2009).

Sodium, potassium, calcium, magnesium ions are found in bee sperm. These ions, also found in mammals, are essential for maintaining sperm motility and viability. Hence they are required to be present in the diluent (Moritz, 1984; Elits, 2005). HCO_3^- (bicarbonate), which is found in seminal fluids in mammalian animals and is essential for sperm functions, is important. HCO_3^- increases intracellular pH. It stimulates motility by causing an increase in Ca^{+2} and cAMP (cyclic adenosine monophosphate) between cells (Gagnon and Lamirande, 2006). It was determined that sperm motility was higher in diluents where bicarbonate and potassium were higher (Taylor et al., 2009).

Taylor et al. (2009) studied the effects of different diluents on sperm and compared between diluents with and without bicarbonate, and found that after freezing and thawing, the rate of viability in the sperm diluted with bicarbonate-containing diluents was higher. In the study, a bicarbonate-free diluent with a pH of about 9.7 that is the pH of the spermatheca of a queen was found to be more successful. This result supported the study of Camargo (1975).

Antioxidants can prevent damage to the cell due to oxidative stress by different mechanisms (Rahal et al., 2014). Catalase, a natural antioxidant, is important for the viability of spermatozoa (Collins et al., 2004).

9. Insemination of the Queen

After the sperm is collected, the queen is anaesthetised with carbon dioxide (CO_2). Queen is placed upside down in the tube. The amount of CO_2 that will come to the queen tube is adjusted. The main reasons for the CO_2 application are to prevent the queen from moving during insemination and to enable the queen to lay eggs in a short time after insemination. During insemination, after the queen is anaesthetised, it is fixed in the appropriate position to the insemination device. For passing the vaginal valve in the queen, fixation of the queen holder with the injector needle at an angle of around °45 provides an advantage. The vagina emerges with the help of a special pair of hooks. One of the hooks is the abdominal hook, and the other holds the needle. By lifting the hook holding the needle slightly upward, the position is created for artificial insemination. The injector end of the artificial insemination device is gently directed to the vaginal opening. The injector end of the insemination device is gently directed up and forward, and the vaginal valve is passed. Also, 8-12 µL of sperm that must be transplanted is given to the queen (Cobey et al., 2013). Cobey (2007) suggested that sperm is stored by flowing into the lateral oviduct canal and then passing into the spermatheca, which Koenigerin (1986) stated it before regarding the path of sperm in artificial insemination of honey bees.

Sperm can be used in artificial insemination in honey bees by storing them for long and short periods. Studies show that artificial insemination with short-term stored sperm gives more successful results. In addition, sperm taken from male honey bees for artificial insemination does not include some disease risks found in mammals (Colins, 2000).

Cobey (1998) found that naturally mated bees have a close life span to that of queens fertilized by artificial insemination. Wilde (1994) stated that naturally mated bees start to lay egg earlier than bees with artificial insemination. Cobey (2007) published a review article

comparing the queens with natural mating and artificial insemination, Harbo (1986) found more spermatozoa in naturally mating bees than those fertilized by artificial insemination.

In addition, the study found that the artificial insemination in queens was successful in terms of colony management and resistance to the difficulties in natural conditions. Artificial insemination in honeybees has provided a great advantage especially for bee breeding studies (Cobey et al., 2013).

10. Conclusion

Artificial insemination in honey bees has become an increasingly important area in the world. Beekeepers are aware of the advantages of artificial insemination in controlled beekeeping. In the studies related to the short and long storage of honeybee sperm, the desired results as sufficient as in mammals have not been achieved. But there has been great progress recently. These developments have been a hope for the development of Turkey's and the World's beekeeping. Thanks to the freezing and long-term storing of honeybee sperm, yield characteristics can be increased in controlled beekeeping in the near future, just as in some mammalian animal breedings. Longer-term scheduled genetic studies can be done. But these studies are expected to be conducted consciously to be useful. That is why, in this case, it is possible to protect regional races, while gene pollution is likely to spread more quickly.

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Research Article

Effects of grape (*Vitis vinifera* L.) seed oil and St John's wort (*Hypericum perforatum* L.) extract supplementation into laying hens diets on performance, egg quality, and some blood parameters

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Abstract

This study set out to determine the effects of dietary supplementation of grape seed oil and Hypericum perforatum L. extract to the laying hens (Lohmann White, 40 weeks of age) on performance, egg quality and some blood parameters. For this purpose, a total of 336 hens were randomly distributed to the control and other treatment groups, 12 replicates/group with 4 hens/replication. Birds were fed with basal diet only (control group) and the basal diet supplemented with different levels (100, 200, 300 mg/kg) of grape seed oil (GSO-1, GSO-2, GSO-3) and *Hypericum perforatum* L. extract (HPE-1, HPE-2, HPE-3). Dietary treatments had no significant effect on performance parameters. GSO-3 and HPE-1 supplemental groups presented with increased shell weight ($p<0.01$) while only GSO-1 group showed increased egg albumen index ($p<0.01$) and Haugh unit ($p<0.05$). Among blood parameters, except for serum HDL and total protein, the other blood parameters (serum triglyceride, total cholesterol, LDL, AST, Ca and P) were not affected by the treatments. In conclusion, results showed a partial improvement in some egg quality traits, however, further studies are needed to fully investigate the beneficial effects of these additives in laying hens reared under different stresses.

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1. Introduction

Various feed additives have been used in the poultry diets for different purposes especially for the improvement in feed efficiency, and quantity and quality of animal products. In animal feeding, with the ban on antibiotics as growth promoters due to bacterial resistance and residues in animal products (Brenes and Roura, 2010), a great number of efforts have been made searching for new additives that could be used as an alternative to the antibiotics (Alagawany et al., 2018). Various organic acids (Soltan, 2008; Swiatkiewicz et al., 2010), probiotics (Deng et al., 2012; Wijayanti et al., 2019), prebiotics (Sarangi et al., 2016; Abdel-Hafeez et al., 2017), synbiotics (Radu-Rusu et al., 2010; Sarangi et al., 2016) and herbal extracts (Murugesan et al., 2015; Ahsan et al., 2018) are some notable examples of such alternative feed additives. Recently, herbal extracts owing to their bioactive components have received a lot of attention (Wallace et al., 2010) and showed beneficial effects such as antimicrobial, antioxidant, anti-inflammatory, and antiparasitic (Cheng et al., 2014). In poultry nutrition, many studies have evaluated the use of herbs as additives, for example, black cumin (El-Bagir et al., 2006; Yalçın et al., 2009), thyme (Al-Kassie, 2009; Ghasemi et al., 2010), sage (Demir et al., 2008; Rasouli et al., 2019), rosemary (Yeşilbağ et al., 2011), olive leaf (Parsaei et al., 2014), *Hypericum perforatum* L. extract (Landy et al., 2012; Banisharif et al., 2016) and grape seed oil (Tekeli et al., 2014; Salman, 2019).

The fruit grape (*Vitis vinifera*) is widely cultivated in the world, and the oil obtained from its seeds is highly valuable in terms of unsaturated fatty acids (Salman, 2019) particularly oleic and linoleic acids compared to other oil seeds (Tekeli et al., 2014). Grape seed oil is reported to possess various phenolic compounds with powerful antioxidant effects (Salman, 2019), capable of increasing HDL cholesterol while lowering LDL cholesterol (Tekeli et al., 2014). In a study investigating the effects of various aromatic oils (grape seed oil, coriander oil, laurel leaf oil) and vitamin E in broiler diets on intestinal microbiology and oxidative stability, Okur (2010) observed lowest coliform while highest lactic acid bacteria counts in ileum of birds supplemented with grape seed oil (200 mg/kg). In another study with broilers (Erkan, 2013), no significant differences were observed between groups supplemented with grape seed oil (300 mg/kg), vitamin E and selenium in terms of body weight, body weight gain, feed consumption and feed conversion ratio. Similar results were obtained in terms of body weight gain, feed consumption, blood parameters (plasma glucose, cholesterol, triglyceride, VLDL, acid phosphatase) in broilers when fed diets supplemented with grape

seed oil (5, 10, 15 g/kg), however, 15g/kg supplementation of grape seed oil improved the feed efficiency (Tekeli et al., 2014). Salman (2019) also investigated the use of grape seed oil (1%, 2%) and black seed oil (1%, 2%) in broiler diets and found significant effects on some blood parameters (total protein, globulin, cholesterol, HDL, LDL) except albumin and VLDL. An experiment with laying hens showed no effects of grape seed oil supplementation (1%, 2%, 4%) on feed consumption, egg weight, egg production, feed conversion ratio, and body weight but grape seed oil at 2% level increased the egg albumen index and lowered blood plasma glucose and cholesterol levels (Ozgan, 2008).

The *Hypericum perforatum* L. plant, also known as St John's Wort, belonging to Hypericeae family (Etemad et al., 2011) contains many bioactive components (hypericin, hyperforin, flavonoids) with antioxidant, antimicrobial and antidepressant properties (Landy et al., 2012). Supplementation of dried Hypericum perforatum L. (5 and 10 g/kg) in broiler diets as a substitute for antibiotic growth promoter had no effects on serum total protein, albumin, LDL-cholesterol and triglyceride but it provided with the highest feed conversion ratio compared to other groups (Landy et al., 2012). Landy et al. (2012) concluded that *Hypericum perforatum* L. supplementation to broilers had no positive effects on growth performance. In another study with broilers, Hosseini et al (2015) also found no effect of supplemental *Hypericum perforatum* L. (aqueous extract, 150, 300, 450 mg/kg) on performance parameters except feed consumption. They also observed higher HDL and lower glucose levels at the end of 24 days with 150 mg/kg of *Hypericum perforatum* L. supplementation while the group having 300 mg/kg of the additive had higher AST and ALT enzyme activities, however, the differences in these enzyme activities were non-significant at the end of 42 days. Addition of different herbal extracts (*Matricaria chamomilla* L., *Melissa officinalis* L., and *Hypericum perforatum* L.) to the drinking water (2ml/L) of broilers resulted in reduced cholesterol and increased immunoglobulin level (Skomorucha and Sosnowka-Czajka, 2013). *Hypericum perforatum* addition also resulted in increased body weight. Similarly, Davoodi et al. (2014) also reported that Hypericum perforatum extract (150, 200 and 250 mg/L) added to the drinking water of broilers lowered plasma triglyceride, cholesterol, and LDL levels while increasing HDL levels. The present study was proposed to determine the effects of different doses (0, 100, 200 and 300 mg/kg) of grape seed oil and *Hypericum perforatum* L. extract supplementation into the diets of laying hens on performance, egg quality, and some blood parameters.

2. Materials and Methods

2.1. Animals and Experimental Design

Three hundred and thirty-six Lohmann white commercial laying hens 40 weeks of age were used in the present experiment. The birds were raised in the Poultry Research and Application Unit of the Agricultural Management at Atatürk University Agricultural Faculty. The experiment consisted of seven groups (one control and six treatment groups as GSO-1, GSO-2, GSO-3, HPE-1, HPE-2, and HPE-3) and birds were distributed to 3-layer cages with 12 replicates per group and four chickens per replicate. Before hens were placed in cages, they were weighed and the SPSS package software was used to test the homogeneity of the groups in terms of body weight (Table 1).

Table 1. Experimental design

Group	Feed Additive (mg/kg)	No of replicates	Hens per replicate	Total hens per treatment group
Control	0*	12	4	48
GSO-1	100	12	4	48
GSO-2	200	12	4	48
GSO-3	300	12	4	48
HPE-1	100	12	4	48
HPE-2	200	12	4	48
HPE-3	300	12	4	48

GSO: Grape seed oil; HPE: Hypericum perforatum L. extract
*Basal diet

2.2. Basal Diet Composition and Nutritive Value

In the present study, first-period laying hen feed was used as a basal diet and was obtained from a feed factory. A basal diet with no additive served as control while for treatment groups basal diet was supplemented with different doses (100, 200, 300 mg/kg) of grape seed oil (GSO) and *Hypericum perforatum* L. extract (HPE). Grape seed oil and *Hypericum perforatum* L. extract used in the trial were obtained from private companies. The nutrient composition of basal diet used in the experiment was determined by the methods described in AOAC (1990) (Table 2).

Table 2. Ingredients and chemical composition of the basal diet

Ingredients	%	Chemical composition	
Corn	52.73	Dry matter, %	88.2
Soybean meal (46% CP)	18.72	Crude protein, %	16.6
Sunflower seed meal (36% CP)	5.35	Crude fiber, %	4.18
Bonkalite	7.50	Crude fat, %	2.74
Fish meal	2.16	Crude ash, %	13.6
Vegetable oil	0.50	ME (kcal/kg)**	2653
Limestone	9.80		
Dicalcium phosphate (DCP)	2.04		
Salt	0.25		
DL-Methionine	0.40		
L-Lysine	0.25		
Vitamin+mineral premix*	0.30		

*****Vitamin-mineral content of each kilogram of premix: 15.000.000 IU Vitamin A, 1.500.000 IU Vitamin D_3, 30.000 mg Vitamin E, 5.000 mg K_3, 3.000 mg Vitamin B_1, 6.000 mg Vitamin B_2, 20.000 mg Nicotinamide, 8.000 mg Calcium D Pantothenate, 5.000 mg Vitamin B_6, 15 mg Vitamin B_{12}, 1.000 mg Folic acid, 80.000 mg Manganese, 60.000 mg Zinc, 30.000 mg Iron, 5.000 mg Copper, 2.000 mg Iodine, 150 mg Selenium.

******ME **(**Metabolizable energy) was calculated according to the equation of Carpenter and Clegg (1956).

2.3. Determination of Performance Parameters

To determine the change in body weight, the hens were weighed at the beginning and end of the experiment. Egg weight, egg production, feed consumption, feed conversion ratio and cracked egg ratio were determined every 15 days of the experiment (Kaya, 2009).

Feed conversion ratio (FCR) = kg feed intake/kg egg

2.4. Determination of Egg Quality Traits

Egg quality parameters such as shape index (%), shell strength (kg/cm^2), shell weight (g), shell thickness (mm), albumen index (%), yolk index (%), and Haugh units were determined every 14 days. Egg width and length, yolk diameter, albumen width and length were measured using a caliper; albumen and yolk heights were measured using a three-leg micrometer while shape index (%), albumen index (%), yolk index (%) and Haugh units were calculated using the following formulas (Sarica and Erensayin, 2009).

Shape index (%) = egg width (cm)/egg length (cm) x 100

Albumen index (%) = albumen height (mm)/avarage of albumen length (mm) and albumen width (mm) x 100

Yolk index (%) = yolk height (mm)/yolk diameter (mm) x 100

Haugh unit = 100 Log ($H+7.57-1.7 \times W^{0.37}$) [H = Albumen height (mm), W = Egg weight (g)]

Shell strength was measured as kg/cm^2 using a breaking strength measuring tool. Eggshells free from albumen residues and membranes were weighed using precision scales. Later on eggshells were sampled from 3 different points and the average shell thickness was determined using a micrometer.

2.5. Blood Sampling and Analyses

At the end of the experiment, a total of 35 blood samples (5 hens/group) were taken from vena cutanea ulnaris into the coagulation activator tubes to determine the levels of serum triglyceride, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), total protein, aspartate aminotransferase (AST), calcium (Ca) and phosphorus (P). The tubes were centrifuged for 5 minutes at 3000 *g* to remove the blood serum samples which were then stored at -80 °C until analyzed. Measurements of serum parameters were made using the autoanalyzer in the Laboratory of Biochemistry Department of Atatürk University Faculty of Medicine.

2.6. Statistical Analysis

All the data were analyzed by variance analysis with Duncan test for comparisons between groups using GLM (General Linear Model procedure). The effects of increased doses of grape seed oil and *Hypericum perforatum* L. extract were determined through polynomial analysis using SPSS 10.01 package software. The effects (significance) of the treatments were evaluated at $p<0.05$ level.

3. Results and Discussion

3.1. Effects of Grape Seed Oil and Hypericum perforatum L. *Extract Supplementation on Performance Parameters*

The results of different performance parameters (live weight, feed consumption, egg production, FCR, egg weight and cracked egg ratio) are presented in Table 3.

Table 3. Effects of grape seed oil and *Hypericum perforatum* L. extract supplementation on performance parameters

Group	Level (mg/kg)	Feed consumption (g)	Egg production (%)	FCR	Egg weight (g)	Cracked egg ratio (%)	Live weight (g)		
							Initial	Final	Gain
Control	0	127.71	87.35	2.29	65.34	0.65	1602.56	1649.76	47.20
GSO-1	100	133.55	86.81	2.45	63.99	1.09	1602.06	1654.29	52.23
GSO-2	200	132.35	87.44	2.38	64.42	0.49	1591.38	1615.88	24.51
GSO-3	300	128.90	86.52	2.32	65.40	0.28	1595.00	1655.19	30.19
HPE-1	100	132.15	85.54	2.38	67.06	0.55	1585.31	1637.23	51.98
HPE-2	200	128.71	86.91	2.40	65.53	0.26	1586.29	1623.42	37.13
HPE-3	300	130.37	84.57	2.46	65.56	1.21	1591.71	1671.20	79.49
Pooled SEM		2.88	2.66	0.08	0.94	0.30	7.19	21.27	21.30
P value		0.743	0.998	0.746	0.384	0.256	0.489	0.522	0.627
Polynomial Contrasts									
GSO	Linear	0.846	0.858	0.980	0.813	0.278	0.294	0.210	0.368
	Quadratic	0.094	0.933	0.153	0.049	0.361	0.770	0.904	0.986
	Cubic	0.694	0.792	0.440	0.633	0.365	0.439	0.309	0.449
HPE	Linear	0.729	0.607	0.195	0.861	0.246	0.331	0.612	0.416
	Quadratic	0.635	0.929	0.851	0.461	0.029	0.122	0.180	0.404
	Cubic	0.326	0.610	0.779	0.348	0.163	0.670	0.528	0.447

GSO: Grape seed oil, HPE: Hypericum perforatum L. extract, FCR: Feed conversion ratio (kg feed intake/kg egg)

The present study showed that grape seed oil and *Hypericum perforatum* L. extract supplementations to laying hen diets had no significant effect on final body weight, body weight change, feed consumption, egg production, FCR, egg weight and cracked egg ratio. These results were consistent with the previously published studies using grape seed oil at 300 mg/kg (Erkan, 2013) and at 1, 2 and 4% (Ozgan, 2008) in broilers and laying hens, respectively. They also reported no significant effect of grape seed oil addition on body weight, body weight gain, feed consumption, and feed conversion ratio. On the contrary, Tekeli et al. (2014) reported an improvement in feed conversion ratio in broilers fed diets supplemented with 15 g/kg grape seed oil. In another study with Japanese quails, Banisharif et al. (2016) reported an increase in feed consumption with *Hypericum perforatum* L. (0.2, 0.4 and 0.6%) supplementation. Although the present study showed no difference among groups for body weights, Skomorucha and Sosnowka-Czajka (2013) reported an increase in the body weight of broilers provided drinking water with added *Hypericum perforatum* L. extract.

3.2. Effects of Grape Seed Oil and Hypericum perforatum L. *Extract Supplementation on Egg Quality Traits*

Results obtained for the egg quality characteristics (shape index, shell strength, shell thickness, shell weight, yolk index, albumen index and Haugh unit) are presented in Table 4.

Table 4. Effects of grape seed oil and *Hypericum perforatum* L. extract supplementation on egg quality

Group	Level (mg/kg)	Shape index (%)	Shell strength (kg/cm^2)	Shell thickness (mm)	Shell weight (g)	Yolk index (%)	Albumen index (%)	Haugh unit
Control	0	76.62	2.79	0.46	8.21^{c}	39.78	9.39^{b}	84.49^{b}
GSO-1	100	76.54	3.02	0.45	8.35^{bc}	40.24	10.22^{a}	87.20^{a}
GSO-2	200	76.96	2.70	0.44	8.17^{c}	40.27	9.65^{ab}	85.19^{ab}
GSO-3	300	76.18	2.84	0.46	8.75^{a}	39.83	9.52^{b}	84.59^{ab}
HPE-1	100	75.59	2.58	0.45	8.73^{a}	40.12	9.27^{b}	83.86^{b}
HPE-2	200	76.30	2.66	0.47	8.64^{ab}	40.27	9.15^{b}	83.16^{b}
HPE-3	300	76.07	2.69	0.45	8.41^{abc}	40.18	9.11^{b}	83.39^{b}
Pooled SEM		0.35	0.14	0.01	0.12	0.34	0.21	0.88
P value		0.171	0.361	0.071	**0.002**	0.884	**0.007**	**0.036**
Polynomial Contrasts								
GSO	Linear	0.544	0.808	0.491	0.020	0.921	0.858	0.684
	Quadratic	0.284	0.735	0.019	0.105	0.210	0.048	0.046
	Cubic	0.253	0.109	0.609	0.078	0.991	0.092	0.153
HPE	Linear	0.553	0.736	0.827	0.301	0.336	0.245	0.269
	Quadratic	0.267	0.386	0.744	0.001	0.491	0.813	0.593
	Cubic	0.096	0.574	0.045	0.359	0.970	0.903	0.785

a, b, c: The averages with different superscripts in the same column differ significantly ($p<0.05$). **GSO:** Grape seed oil, **HPE:** *Hypericum perforatum* L. extract

The findings observed in the present study suggested no significant effects of supplemental grape seed oil and *Hypericum perforatum* L. extract on egg shape index, shell strength, shell thickness, and yolk index ($p> 0.05$). However, significant differences were observed between the groups in terms of shell weight, albumen index and Haugh unit. Eggshells from GSO-3 and HPE-1 groups were the heaviest compared to other groups ($p<0.01$) while only GSO-1 group presented with the highest values for the albumen index ($p<0.01$) and Haugh unit ($p <0.05$). Scientific data on the effects of grape seed oil and *Hypericum perforatum* L. extract on egg quality is scarce. The present finding in this study regarding albumin index was in agreement with Ozgan (2008) who also reported an increase in the egg albumin index when diets of laying hens were supplemented with grape seed oil (2 %). Ozgan (2008) attributed this improvement in the albumen index to the antioxidant properties of grape seed which might have preserved the β-ovomucin responsible for the gelatinousness of albumin resulting

in the increase in the albumin height. Also, for Haugh Units, there was a similarity between the results of the present study and that described by Kaya et al. (2014) who stated a significant effect on Haugh units when laying hens' diets were supplemented with grape seeds and grape seed extract. Egg shape index, shell strength and shell thickness remained unaffected in their experiment. In a study conducted by Kara et al. (2016), it was shown that grape pomace added to the laying hen diets had no effect on the egg albumen index and Haugh unit.

3.3. Effects of Grape Seed Oil and Hypericum perforatum L. *Extract Supplementation on Some Blood Parameters*

The average values of some blood serum parameters evaluated in this study are presented in Table 5. The effects of both additives (GSO and HPE) on serum triglyceride, cholesterol, LDL, AST, Ca and P levels were non-significant, whereas significant variations among groups were observed for the levels of serum HDL and total protein. Although no difference was observed among groups for HDL when compared to the control group, the GSO-2 and GSO-3 presented with numerically higher values. Compared to the control group a reduction in total protein ($p<0.05$) in HPE-2 was observed. In an experiment with broilers supplemented with dried *Hypericum perforatum* L. (5, 10 g/kg), Landy et al. (2012) also found no effects on serum triglyceride and LDL levels. On the contrary, Davoodi et al. (2014) with supplemental *Hypericum perforatum* L. (150, 200 and 250 mg/L) in broiler drinking water and Salman (2019) with supplemental grape seed oil (1 and 2%) in broiler diets showed lowering effects of these additives on LDL levels. Additionally, the 2% grape seed oil in diets of laying hens (Ozgan, 2008), 1 and 2% grape seed oil in broiler diets (Salman, 2019), 2 ml/l *Hypericum perforatum* L. in broiler drinking waters (Skomorucha and Sosnowka-Czajka, 2013) resulted in lower cholesterol levels.

Table 5. Effects of grape seed oil and *Hypericum perforatum* L. extract supplementation on some blood parameters

Group	Level (mg/kg)	TG	TC	HDL	LDL	TP	AST	Ca	P
Control	0	1399.20	152.60	37.00^{ab}	52.60	6.66^{a}	235.60	31.30	6.00
GSO-1	100	1045.00	122.68	26.68^{b}	47.64	6.00^{abc}	192.32	30.77	6.78
GSO-2	200	1281.40	180.00	45.20^{a}	40.00	6.14^{abc}	195.00	33.56	6.58
GSO-3	300	913.40	162.60	44.00^{a}	67.80	6.24^{ab}	175.40	24.00	4.50
HPE-1	100	1244.00	127.76	25.20^{b}	35.76	6.08^{abc}	206.00	27.88	5.33
HPE-2	200	1277.00	150.50	39.00^{ab}	43.26	5.53^{c}	202.00	30.76	6.23
HPE-3	300	1243.00	137.80	35.00^{ab}	43.80	5.92^{bc}	189.60	31.64	5.32
Pooled SEM		47.15	21.13	4.36	6.95	0.21	13.75	2.45	0.53
P value		0.090	0.507	**0.016**	0.063	**0.031**	0.125	0.174	0.056
Polynomial Contrasts									
GSO	Linear	0.045	0.288	0.064	0.334	0.332	0.030	0.060	0.078
	Quadratic	0.957	0.729	0.319	0.073	0.149	0.488	0.073	0.021
	Cubic	0.051	0.059	0.026	0.032	0.250	0.374	0.117	0.073
HPE	Linear	0.291	0.830	0.665	0.240	0.001	0.041	0.732	0.599
	Quadratic	0.507	0.788	0.339	0.023	0.004	0.555	0.403	0.810
	Cubic	0.532	0.416	0.026	0.061	0.171	0.601	0.469	0.131

a, b, c: The averages with different superscripts in the same column differ significantly ($p<0.05$). **GSO:** Grape seed oil, **HPE:** *Hypericum perforatum* L. extract, **TG:** Triglyceride (mg/dL), **TC:** Total cholesterol (mg/dL), **HDL:** High-density lipoprotein (mg/dL), **LDL:** Low-density lipoprotein (mg/dL), **TP:** Total protein (g/dL), **AST:**Aspartate aminotransferase (unit/L), **Ca:** Calcium (mg/dL), **P:** Phosphorus (mg/dL).

4. Conclusion

In conclusion, the present study showed that grape seed oil and *Hypericum perforatum* L. extract can be used in the diets of laying hens without any negative effects on birds' performance. The results also showed partial improvement in some egg quality parameters including egg shell weight, albumin index and Haugh units with grape seed oil and *Hypericum perforatum* L. extract supplementation. Further studies are needed to determine the effects of these supplements in laying hens reared under different stresses.

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Review

Agriculture history and policy

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Abstract

The ancient Fertile Crescent in particular, is commonly comprehended as the origin of agriculture. The location of Western Asia covers the area of Mesopotamia and the Levant, and is limited by Syrian Desert to the south and the Anatolian Plateau to the north. First of all, a good diagnosis is needed to treat the disease, it is necessary to make a good diagnosis and definition for our sectors such as agriculture which connects our past, current and future life. In the agricultural sector, like other sectors, if we ask for a timely diagnosis and treatment of issues; certainly, we don't have to demand more because of neglect. The basis of the agricultural sector also is a science. World population has been growing and natural resources such as land and water is already under stress. To make a nation healthy and to meet food demand along with nutrition security, agriculture would continue remain as the top most priority sector for any country. Agriculture provides food, fibre and raw materials to industry. It contributes significantly to Gross Domestic Product (GDP). Agriculture provides cleaner, better environment for people to lead healthy life on earth. Moreover, it provides employment to large section of the people. If a nation has to be strong, then it has to be self-reliant coupled with strong agricultural economy that supports ecological and nutritional security. The history of agriculture has showed an important value in human development. More than half of all human around the World once efforted in farming, and even today, few—if any—humans could survive without it.This paper depicts the agricultural history and policy reforms and structural changes in World from past to the current times.

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1. Introduction

The most important change in human history begins with the development of agriculture. Scholars from many disciplines, such as religiology, archaeology, historical linguistics, biology, anthropology and history, have investigated southwest Asia, South Asia, China, Japan, Southeast, Middle East, Asia and Pacific, sub Saharan Africa, America and Europe,

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and studied the common development of agriculture with social structure and cultural forms (Barker and Goucher, 2015).

The Food and Agriculture Organization of the United Nations estimates that by 2050, we will need 60% more food. Agriculture must provide more and more high-quality food, fiber, feed and fuel for human beings in an environmentally, economically and politically sustainable way. And agriculture will become more challenging in the future, and the development and correct implementation of precision agriculture will help to achieve this very important task (Zhang, 2016). The link between agriculture and human needs requires reducing pollution in the areas of air, soil and water, improving food production and related socio-economic issues, with a focus on human health and livelihoods. For sustainalable agriculture, four pillars are land management, resource management, human interface and ecosystem interface. Because of the strong influence of personal values, culture, norms and habits, the human interface may be the most unpredictable and complex (Peattie, 2010). Domestication of plants for thousands of years has led to extreme changes in human diet and social development, prompting people to eat more grains. At present, carbohydrates still account for 60% of human calories (Foster et al., 2003), most of which are consumed by grains such as rice, wheat and corn. Both animals and plants in the area have experienced extensive expansion. Once domestication is successful, it will be scattered from the origin area to another part of World. Domestication began with wheat cultivation in Fertile Crescent areas such as Turkey and spread rapidly throughout Europe (Zohary et al., 2012). With the improvement of domestication, the human diet structure has been accelerated, but it is still changing. Urbanization involves more consumption of polished grain (bran removal). Rice and wheat have been treating for more sugar, more animal products and, more food (Drewnowski and Popkin, 1997). Grain production accounts for a large part of the world's agricultural production. According to FAO, world grain production is expected to reach the target of 3 billion tons by 2050 (Alexandratos and Bruinsma, 2012).

The first challenge facing World Agriculture is to produce enough food to meet the growing world population. It is predicted that the world population will reach 8 billion by 2025. It is expected that in the coming decades, the population in rural areas will decrease, and rapid urbanization will lead to the continued growth of urban population. So far, the income of agricultural activities is low and about 70% of the poor are still rural residents. The second challenge for World Agriculture, therefore, is technical, policy and institutional.

Meeting this challenge will require farmers to have access to domestic and international markets. The third challenge facing World Agriculture is that small-scale farmers pay attention to the long-term management of the natural resources they manage. In the new century, we will create a set of technologies, incentives and policies to encourage development. Around the world, it is vital that most of the fields, Forests and pastures are used by farmers. Agricultural water consumption accounts for more than 70% of the world's fresh water, and there are a lot of biodiversity in the agricultural system. It is estimated that agricultural production can cause 70% of the water area, deforestation of most forests and loss of biodiversity (Zocca et al., 2018). The border between the forest and the desert is affected by agricultural activities. Therefore, the issue of improving our natural resource management is closely related to improving the productivity and profitability of small-holder farmers in developing countries. However, there is a huge pressure on agricultural production systems to cope with increasing demand, climate and soil change. This is mainly due to human interference. The increasing of food production in the case of reduced land per capita and water shortage must be described for humankind (Postel, 1996). With the increase of food production, especially the source of high protein food, how to meet the future needs of the population is facing some challenges (Singh-Ackbarali and Maharaj, 2017); currently entering a new era of agriculture, scientists are developing "intelligent" plants to achieve healthy life to save the future. Human beings always equate their happiness with consumption. Therefore, increasing production has become the competition problem of every country. This, in turn, brings a burden to the environment due to the abuse or overuse of natural resources (Singh, 2018).

Thousands of years of plant domestication have led to extreme changes in the human diet, as well as social development, driving to a greater consumption of grains. Carbohydrates are still serving about 60% of our calories today (Foster et al., 2003). Most of these carbohydrates are consumed as grains (mainly rice, wheat, and maize). Most domesticated plants and animals experience widespread expansion. Once domesticated successfully, these crops and animals expand rapidly and are used in areas where they did not originate. Clearly, the human diet has dramatically altered with increased domestication and is still changing today. Urbanization is involved with greater consumption of polished grains (bran layer removed), where rice and wheat are preferred over grains such as millet, more sugar, more animal products, and most importantly more food is consumed away from home (Drewnowski and Popkin, 1997).

Domestication began with the cultivation of wheat in the Fertile Crescent and rapidly spread all over Europe (Zohary et al., 2012). In contrast, recent analyses suggests that new technology, economic, and environmental factors are the driving trends of increase in local production, manufacturing, and services, a process named "deglobalization" (Hammes, 2016).

Human beings always equate their well-being with consumption. Therefore, every nation rich or poor is in a race to increase production. This in turn is putting a burden on the environment due to misuse or overuse of natural resources. Actually around 60% of the total waste generated from industrial, agricultural, or domestic sectors is biodegradable and can be used for production of economically important plants and nutritionally balanced animal proteins. Vermicomposting is one such technology that synergizes microbial degradation with earthworm's activity for reducing, reusing, and recycling waste materials in a shorter span of time. Mutual action of earthworms and microbes brings faster decomposition as earthworms aerate, condition, fragment, and enhance surface area of the organic matter for microbial action (Singh, 2018).

The interface between human demand and agriculture requires efforts to reduce pollution in the air, soil, and water spheres and improve socioeconomic-related issues in food production with an emphasis on human health and livelihood. The human interface may be the most unpredictable and complex of the four pillars (land management, resource management, human interface, and ecosystem interface) as it is influenced strongly by personal values, culture, norms and habits (Peattie, 2010).

Processing of foods is an important topic since 30% of the overall production of food is lost postharvest (FAO, 2013) because of lack of appropriate technologies and techniques for preservation. In the food industry, the control of unwanted microorganisms is essential and decisive (Stoica et al., 2011). The soil is deposited on food processing equipment and forms films that negatively interact with the processing integrity lines, for example, on the walls of an empty tank and on the internal surface of a heat exchanger (Norton and Tiwari, 2014).

In the food systems, the way of production and distribution, as well as the kind of foods we consume can have a certain effect on the planet where we are living on and the society which we are living in. Air, water, land, climate conditions, and biodiversity are the major driving forces for human well-being and, at the same time, major parts of our lives are exposed to

human activities intentionally. Sustainability of these natural sources plays a primary role in the food systems. However, food system itself also has a primary role for protecting natural sources because of its certain consequences such as greenhouse gas (GHG) emissions, water and soil pollution, and deforestation (Garnett, 2013).

Nowadays, the definition of food security focuses on the access to food rather than food production. In November 1999, The World Food Summit took place with a participation of 185 countries and the European Community for the eradication of hunger. According to definition of World Food Summit (FAO, 1996), food security is met when "all people, at all times, have physical and economic access to sufficient, safe, and nutritious food to meet their dietary needs and food preferences for an active and healthy life." Food and nutrition security are considered as the priorities of food system outcomes and strongly emphasized in the definitions of "sustainable diet" that is comprised of a healthy diet and a healthy environment (Allen and Prosperi, 2016).

Earlier, in 2011, Food and Agricultural Organization (FAO) published a report considering global food losses and food wastes noting that nearly one-third of worldwide food production (1.3 billion ton/year) for human consumption is lost or wasted. The amounts of food loss and waste along the food supply chains, respectively, are 54% of total loss and waste as upstream processes (including production and postharvest) and 46% of total loss and waste as downstream processes (including processing, distribution, and consumption) (FAO, 2011). The European Commission technical report (published in 2010) indicated that around 90 million tonnes of food wastes are generated within European Union (EU) each year.
The percentage breakdown of food wastes according to this report is 39% manufacturing, 42% households, 14% food service/catering, and 5% retail/wholesale (2006 EUROSTAT data and various national sources provided by EU Member States). Based on this study, it is expected that food wastes would reach 126 million ton in 2020 (from about 89 million ton in 2006), without additional prevention policies or activities. From 2006 up to 2020, food waste tonnages are expected to be 3.7 million in EU27 when population increases by nearly 21 million (Otles et al., 2015).

Food supply chains begin from the primary agricultural phase, proceed with manufacturing and retail, and end with household consumption. During this life cycle, food is lost or wasted because of technological, economic, and/or societal reasons. The definitions of "food waste"

and “food loss” within the supply chain have been a subject of disagreement among the related scientists. According to the EU Commission Council Directive, 2008/98/ EC, “waste” is defined as “any substance or object, which the holder discards or intends or is required to discard.” “Food loss” refers to quantitative and qualitative reductions in the amount and value of food. The qualitative loss corresponds to the loss of caloric and nutritive value, loss of quality, and loss of edibility. Quantitative loss refers to the decrease in edible food mass throughout the part of the supply chain that specifically leads to edible food for human consumption. FAO (2015) global voluntary definitional framework defined food loss as the decrease in quantity or quality of food, caused mainly by food production and supply system functioning or its institutional and legal framework. Thereby, “food loss” occurs throughout the food supply chain. Moreover, FAO distinguishes “food waste” as an important part of “food loss,” which refers to the removal of food from the supply chain, which fits for consumption by choice or has been left to spoil or expire as a result of negligence (predominantly but not exclusively) by the final consumer at household level.

Food supply carries a vital importance for human survival. Nevertheless, the protection of natural resources which is tightly coupled with food supply is an inevitable priority in today’s world. The fact of increasing soil, water and air pollution, deforestation, the decline in biodiversity and effects of climate change and in response to all these events, ever-growing human population and the needs for food and energy create a very serious problem to provide continuance of human survive. All these factors constitute certain unsustainability in the global agriculture and food systems, and the generation of huge amount of food waste became a major indicator of this instability.

Food industry has to produce enough food and ensure the food safety while giving rise to a less environmental impact. The improvement of food production efficiency, the prevention of food waste generation, and waste valorization for meeting the increasing demand for chemicals, materials, and fuel are the only solutions to restore this unsustainability. Appropriate waste management strategies including the prevention of unsustainable use of natural sources, huge amount of waste generation, and the recommendation of a more cost-effective and environment friendly disposal system should be a global focus point which is shared by farmers, industrial producers, consumers, and policy makers (Otles and Karta, 2018).

2. Definition of Agriculture

Agriculture is a science, art or practice of growing soil, producing crops and raising animals for marketing. Agriculture is the most basic instinct of human beings, which has a broad definition such as the ability to produce food to meet hunger and the survival of species. The word 'Agriculture' is originated from the Latin word 'Ager' means field and 'Culture' means cultivation. So agriculture is an art of raising living organism from the earth for the use of human being (Alexandratos et al., 2006) We shall understand agriculture as consisting of activities which foster biological processes involving growth and reproduction to provide resources of value. Typically, the resources provided are plants and animals to be used for food and fiber, although agricultural products are also used for many other purposes (Lehmen et al., 1993).

Sustainable agriculture is one of the best practices for environmental sustainability. It maintains the fertility of soil and ecosystem and human's health. It relies on improved ecological processes and cycles of local adaptation, as well as natural biodiversity, rather than the use of synthetic inputs and genetically modified materials. Therefore, farmers must be encourage to engage advance agriculture for future. It has the great potential to contribute to food security and economy (FAO, 2013).

2.1. Agricultural History

World agricultural land is very rich. Some local elders and historians believe that their origin story proves that they have always had agriculture. From past to now agriculture is not yet a major economic activity. Farmers has been doing agriculture activities for survive and support their families. It is very clear that if many of the sub themes that others will emphasize when writing similar works are strictly excluded, and if the book's claims are not so moderate, it will be criticized. Understanding our history also helps us to meet today's local, national and global challenges. Hot issues such as environmental protection, land-use policies and ensuring adequate food supply are not new debates. They come in other times and forms. A deep understanding of the history of agriculture provides the basis for today's agricultural policy (Apple et al., 2015). No matter what the motives of Americans and Europeans are, they are most concerned about making a living. Most newcomers immediately

began to produce their basic products with the initial goal of making a living, but it is expected that surplus products will soon be sold in local or national markets.

2.2. Agricultural Policy

In the past 20 years, the traditional agricultural policy agenda and new policies have been challenged. "Agricultural income" is no longer the main concern although food security is a policy concern after the food price panic at the end of this century, adopting a more westernized diet may lead to a 50% increase in food demand (Huang et al., 2010). The new emergence includes food safety, the provision of environmental and ecosystem services, the role of biotechnology in agricultural production (especially genetically modified organisms, genetically modified organisms), intellectual property rights and biological patents, the use of farmland to produce bioenergy, and the role of the agricultural sector in reducing climate change.

In developing countries, the policy and practice of developing industry at the expense of agriculture has been abandoned, with "development" and "agriculture" as the central policy instead. New policy issues are being addressed in a more flexible institutional environment. In such an environment, there are often conflicts and interactions among regions and institutions based on different values, so policy coordination is needed. This coordination process is called inter agency decision-making. We should encourage participation in the formulation of new agricultural policies. Design and implement policies more effectively. Value balance has become a key feature of the new politics of agriculture and food (World Bank, 2008).

In the past, the research on agricultural decision-making has promoted the development in many political theoretical fields, such as interest groups, policy networks and public policy ideas. The study of these concepts is based on agricultural policy, and often from the agricultural sector to illustrate the theoretical point of view. It makes the agricultural policy department benefit to divide the policy-making process, well-organized policy-making process and agricultural groups with sufficient resources, the government's massive intervention in the market, and the possibility of significant redistribution of income and wealth among economic actors (Daugbjerg and Botterill, 2012).

In order to promote greater agricultural production and linkages with other sectors, more targeted agricultural measures are needed. This may include further integration of food production to provide value-added and market access for food producers in the domestic tourism sector. It is more necessary to provide targeted agricultural funds in order to create more value-added agricultural industries and establish links with services and manufacturing industries. In order to create opportunities that directly benefit the rural population, partnerships among rural agricultural producers, governments and industry must be strengthened. We will provide more targeted agricultural financing, and set up the agricultural and food production insurance market for domestic producers. Invest in agricultural and food infrastructure, such as training and skills development, to improve roads and logistics capabilities, refrigeration facilities, food processing and value-added (Gani and Scrimgeour, 2019).

Agricultural policies that encourage mass production lead to highly concentrated agricultural practices that are likely to lead to environmental degradation. For example, fossil fuels are used to produce and transport chemical fertilizers and pesticides over long distances; then, raw products and finished products are further transported; water sources are also transported to agriculture; used water is often polluted by chemical fertilizers and pesticides, resulting in "dead zones" in the downstream. Consumers' food prices do not include the actual cost of their production. The actual costs include the cost of environmental clean-up, the cost of toxic exposure to human health and the lack of clean water, the cost of fossil fuel overuse, and the cost of food growth for future generations, while agricultural losses will be significantly reduced. Our current agricultural policies run counter to our nutritional, environmental and economic needs. Agricultural policies should not harm the health of the public, especially our children. Nor should it promote or allow our natural environment to continue to deteriorate. A healthy food system should ensure the well-being of consumers and farmers, as well as the producers, processors and distributors on which they depend. Organic and regional food production are promising examples of change. Unhealthy people in unhealthy places cannot produce healthy food. It is the responsibility of the health community to ensure the conditions for people's health. This means participating in agricultural policies to influence better food supply (Jackson et al., 2009). Someone may be a good manager, but being a good politician is another matter. Be able to accept the experience of the past society to guide the future. Agricultural policies are very important for human and environmental health. It is always useful to fully grasp the agricultural and sideline industries and eliminate

the harm. Access to and maintenance of a healthy life should be the goal of agricultural policy. As a person, a society, a country, this is unchangeable. This policy is crucial to the economy, business and industry. People can't understand the value of two things at any time: time and health. It should be such a state that the management of a family in a society is basically similar to that of a country.

3. Conclusion

Although the agricultural structure has changed significantly and dramatically beyond some elements, the agricultural production sector is still composed of agricultural units owned and operated by families. Still most important are inputs such as rainfall, sunlight and temperature. As a result, changes in climate, topography, soil and other agro ecosystems continue to affect production options for crops and livestock. In turn, the changes of these natural factors affect the implementation of management and technology selection. It can be seen that family decentralized farm management has advantage. Capital and other technologies have replaced or improved the impact of these natural changes. The nature and speed of technological changes aimed at influencing agricultural production, as well as more general production options.The change of agricultural structure coincides with the increase of animal raising efficiency and the decrease of production cost. The increase of productivity is mostly due to the increase of production scale and technological innovation (Key et al., 2007).

In the toolbox of public policy analysis, the theory of policy stability is explained through new corporatism, policy network analysis and new system focusing on path dependence. It is proved that it is effective to determine the mechanism of returning stable agricultural policy path over time. It also emphasizes the importance of compartmentalizing policy-making and involves only a limited number of shared ideas and values of interest. The previous wave of policy reform has drawn the attention of policy analysts to the development theory and analysis framework, which can be used to explain these change multi flow models, punctuation equilibrium model, advocacy alliance framework and concept theory. These theories are the same as "stability theory", that is, the concept of conventional decision-making in relatively closed and exclusive subsystems, or networks (Daugbjerg and Botterill, 2012).

Grant (2012) said that by using new modes of action (such as social media), new participants may be able to "debate. As a result, these new actors may successfully launch and promote through policy and institutional reforms. Inter agency coordination links core policy sectors to new policy areas that have not previously been approached by core policy sectors. The lower political cost strategy proposed by such new actors may be to initiate a policy level process in which new policy concerns are addressed by adding new measures to existing core policies. The concept of policy stratification has not been clearly defined, but the definition of institutional stratification by Thelen (2003) may also cover policy stratification. Stratification refers to a "retention of the core (of an institution) while adding amendments through which rules and structures inherited from the past can be synchronized with changes in the normative, social and political environment".

The case study also shows that this is not an easy process, which may lead to the ecological corporatization of policy stratification and the value balance involved in inter agency policy-making. Kay and Ackrill (2012) and Daugbjerg and Botterill (2012) have shown that although this may only have the potential to internalize value conflicts in the short and medium term, value can be balanced through policy stratification. Due to the change of political or economic relations, the value balance has changed. The most stable solution for inter agency policy. Just as Cockfield and Botterill (2012) made decisions when an overall value dominates the policy complex. In the agricultural policy sector, decentralization and inter agency decision-making are becoming increasingly important. It is possible to provide the same theoretical insights as the traditional agricultural policy research in the past. As in the past, contributors to this topic have been firmly involved in the broader theoretical development of political science, enabling them to draw more general lessons from case studies (Daugbjerg and Swinbank, 2012).

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