

**REPUBLIC OF TURKEY
ERCIYES UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED
SCIENCES
DEPARTMENT OF AGRICULTURAL SCIENCE AND
TECHNOLOGIES**

**EFFECTS OF DIETARY OREGANO ESSENTIAL OIL
(OEO) AND DIFFERENT FORMS OF SELENIUM
(ORGANIC, INORGANIC & NANO-Se) ON
PERFORMANCE, MEAT QUALITY AND BLOOD
PARAMETERS OF BROILERS**

**Prepared By
Sami Hammed FARHAN**

**Supervisor
Assoc. Prof. Dr. Mehmet Ulaş ÇINAR
Co-supervisor
Assoc. Prof. Dr. Ebru ÇETİN**

Ph.D. Thesis

**June 2019
KAYSERİ**

**REPUBLIC OF TURKEY
ERCIYES UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED
SCIENCES
DEPARTMENT OF AGRICULTURAL SCIENCE AND
TECHNOLOGIES**

**EFFECTS OF DIETARY OREGANO ESSENTIAL OIL
(OEO) AND DIFFERENT FORMS OF SELENIUM
(ORGANIC, INORGANIC & NANO-Se) ON
PERFORMANCE, MEAT QUALITY AND BLOOD
PARAMETERS OF BROILERS**

(Ph.D. Thesis)

**Prepared By
Sami Hammed FARHAN**

**Supervisor
Assoc. Prof. Dr. Mehmet Ulaş ÇINAR
Co-supervisor
Assoc. Prof. Dr. Ebru ÇETİN**

**This study was supported by Erciyes University Scientific Research
Projects Unit under the code of FDK-2018-7630.**

**June 2019
KAYSERİ**

SCIENTIFIC ETHICS SUITABILITY

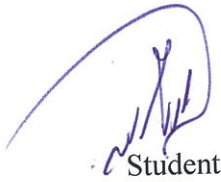
I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all materials and results that are not original to this work.



Sami Hammed FARHAN

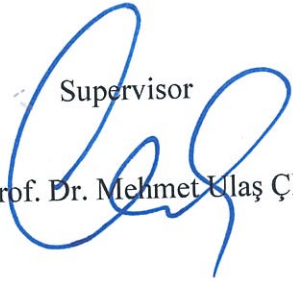
SUITABILITY FOR GUIDE

The Ph.D. thesis entitled “Effects of Dietary Oregano Essential Oil (OEO) and Different Forms of Selenium (Organic, Inorganic & Nano-Se) on Performance, Meat Quality and Blood Parameters of Broilers” has been prepared in accordance with Erciyes University Graduate School of Natural and Applied Sciences Thesis Preparation and Writing Guide.



Student

Sami Hammed FARHAN



Supervisor

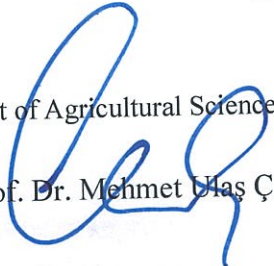
Assoc. Prof. Dr. Mehmet Ulaş ÇINAR

Co-supervisor

Assoc. Prof. Dr. Ebru ÇETİN



Head of the Department of Agricultural Science and Technologies



Assoc. Prof. Dr. Mehmet Ulaş ÇINAR

ACCEPTANCE AND APPROVAL PAGE

This study entitled “Effects of Dietary Oregano Essential Oil (OEO) and Different Forms of Selenium (Organic, Inorganic & Nano-Se) on Performance, Meat Quality and Blood Parameters of Broilers”, submitted by **Sami Hammed FARHAN** under the supervision of Assoc. Prof. Dr. Mehmet Ulaş ÇINAR, has been accepted by the jury as a thesis for the **degree of Doctor of Philosophy** at Erciyes University, the Graduate School of Natural and Applied Sciences, the Department of **Agricultural Science and Technologies**

26.06.2019
(Date of Thesis Defense)

JURY:

Head of the Jury: Prof. Dr. Filiz KARADAŞ

Juror : Prof. Dr. Ahmet ŞAHİN

Juror : Assoc. Prof. Dr. Mehmet Ulaş ÇINAR

Juror : Assoc. Prof. Dr. Ebru ÇETİN

Juror : Asst. Prof. Dr. Osman İBİŞ

APPROVAL:

That the acceptance of this thesis has been approved by the Institute Board with the decision number 04/07/19 and the date of : 2019/38-05


09.07.2019

Director of the Institute
Prof. Dr. Mehmet AKKURT

ACKNOWLEDGEMENTS

I would like to say thanks to my academic supervisor **Assoc. Prof. Mehmet Ulas ÇINAR** for his valuable guidance throughout the study period and my gratitude goes to my co supervisor, **Assoc. Prof. Dr. Ebru ÇETİN** for her help and guidance. I would also like to thank Dean of Faculty of Agriculture **Prof. Dr. Mehmet ARSLAN** and Head of Animal Science Department **Prof. Dr. Yusuf KONCA** and I am appreciative for of all they have done for me. Also, I am most appreciative of the staff and students at Erciyes University where I conducted my project for this thesis, including Head of Applied Agriculture Research Center **Prof. Dr. Savaş SARIÖZKAN**, also **Asst. Prof. Dr. Erkan YILMAZ** from Nanotechnology Research Center, **Assoc. Prof. Dr. Zeki GÖKALP**, **Dr. Hasan Ali İRIK**, **Res. Asst. Mahmut KALİBER**, **Mr. Özgür ÖZMEN**, **Ms. Gül PARA**, **Mr. Ahmed Qasim NAJI**, **Mr. Jawad AL-ABBASSI**, and **Mr. Firas Riyadh JAMEEL**. In addition, my thanks go to **Turkish Government** for fund support and this work was supported by **Research Fund of the Erciyes University**, Kayseri. Thanks, must go to all my colleagues in my office (Agricultural Research office / Ministry of Agriculture) in Iraq, particularly **Dr. Firas M. AL-KHILANI**, **Dr. Waleed Mohammed RAZUKI** and **Sabah AL-SHAMERY**. My express gratitude goes to my close friend **Thamir Hassan RIHAN** and my nephew **Ahmed FAWZI** for their assistance, also to my parents who are deceased (May Allah have mercy on them) for all of the help they gave me throughout my life and the motivation to become a better man, father, husband, student and researcher, and my brothers and sisters for their support. Finally, I would like to thank my wife for her understanding, support, tolerance and her patience and my children, **Rawan**, **Mustafa**, **Sarmad** and **Arslan**. I hope they will have the same opportunity as I have in studying at such a high-profile university in the world.

Sami Hamed FARHAN

Kayseri/ June 2019

**EFFECTS OF DIETARY OREGANO ESSENTIAL OIL (OEO) AND
DIFFERENT FORMS OF SELENIUM (ORGANIC, INORGANIC & NANO-Se)
ON PERFORMANCE, MEAT QUALITY AND BLOOD PARAMETERS OF
BROILERS**

Sami Hammed FARHAN

**Erciyes University, Graduate School of Natural and Applied Sciences
Ph.D Thesis, June 2019**

Thesis Supervisor: Assoc. Prof. Dr. Mehmet Ulaş ÇINAR

Co-supervisor: Assoc. Prof. Dr. Ebru ÇETİN

ABSTRACT

The objective of this study was to determine the effects of dietary oregano essential oil (OEO) and different forms of selenium on performance, meat quality and blood parameters of broilers during the rearing period of 0-6 weeks. A total of 450, one-day-old ROSS 308 broiler chicks were randomly assigned to five groups with six replicates: the non-supplemented basal diet (T1), basal diet + OEO 300 mg/kg (T2), basal diet + organic selenium 0.3 mg/kg (T3), basal diet + inorganic selenium 0.3 mg/kg (T4), basal diet + nano selenium 0.3 mg/kg (T5). Birds fed diets supplemented with OEO, organic or nano selenium achieved better performance than the control group. The protein level of the breast meat was lower in the T4 group compared to the other groups. pH level was higher in the diet with additives after 24 h than the control group. Hemoglobin value, heterophil and heterophil/ lymphocyte ratio in the T5 group was higher than the T2, T3 and T4 group. Albumin and globulin values were higher in the T3, T5 and T2 group than the T1 and T4 group. The GOT and GPT values were higher in the T5 and T4 group than the other groups. TNF- α level in the T4 group was higher compared with others. The IL-2 level in the T2 group was higher compared with other groups. The IL-10 level was higher in the T1, T3 and T4 group compared with other treatments while in the T2 and T5 group were the lower. In conclusion, the study has shown that OEO and different forms of selenium significantly affected the performance, meat quality and some blood parameters in broilers.

Keywords: Broiler, Nutrition, Oregano Essential Oil, Selenium, Nano-Se, performance, blood parameters

**RASYONA KATILAN KEKİK ESANSİYEL YAĞI VE FARKLI
FORMLARDAKİ SELENYUMUN (ORGANİK, İNORGANİK VE NANO-
SELENYUM) KAYNAKLARININ ETLİK PİLİÇLERİN PERFORMANSI, ET
KALİTESİ VE KAN PARAMETRELERİ ÜZERİNE ETKİLERİ**

Sami Hammed FARHAN

**Erciyes Üniversitesi, Fen Bilimleri Enstitüsü
Doktora Tezi, Haziran 2019**

Tez Danışmanı: Doç. Dr. Mehmet Ulaş ÇINAR

İkinci Danışman: Doç. Dr. Ebru ÇETİN

ÖZET

Bu çalışma, broyler yemlerine ilave edilen kekik esansiyel yağı (OEO) ve farklı formlardaki selenyumun (Organik, İnorganik ve nano-Se) 0-6 haftalık yetiştirme döneminde performans, et kalitesi ve bazı kan parametreleri üzerine etkilerini belirlemek amacıyla yapıldı. Bir günlük toplam 450 ROSS 308 broylerler rastgele altı tekrarlı beş gruba ayrıldı: Takviye edilmemiş kontrol rasyonu (T1), bazal rasyon + OEO 300 mg / kg (T2), bazal rasyon + organik Se 0.3 mg / kg (T3), bazal rasyon + inorganik Se 0,3 mg / kg (T4) ve bazal rasyon + nano Se 0,3 mg / kg (T5). Organik kekik esansiyel yağı veya Nano selenyum ile desteklenmiş yemler ile beslenen civcivler kontrol grubundan daha iyi performans göstermişlerdir. Göğüs etinin protein seviyesi T4 grubunda diğer gruplara göre daha düşüktü. Yemlere ilave edilen katkıların, karkas pH düzeyi kontrol grubundan kesimden 24 saat sonra daha yüksek olduğu saptanmıştır. T5 grubundaki hemogloblin değeri, heterofil ve heterofil/ lenfosit oranı T2, T3 ve T4 grubundan daha yüksekti. Abümin ve globulin değeri T3, T5 ve T2 grubunda T1 ve T4 grubundan daha yüksekti. GOT ve GPT değerleri, T5 ve T4 gruplarında diğer gruplara göre daha yüksekti. T4 grubundaki TNF- α düzeyi diğerlerine göre daha yüksekti. T2 grubundaki IL-2 düzeyi diğer gruplara göre daha yüksekti. IL-10 düzeyi, T1, T3 ve T4 grubunda diğer tedavilere kıyasla daha yüksekken, T2 ve T5 grubunda daha düşüktü. Sonuç olarak, bu çalışma, OEO ve farklı selenyum formlarının broylerlerde performansı, et kalitesini ve kan parametrelerini önemli ölçüde etkilediğini göstermiştir.

Anahtar Kelimeler: Broyler, beslenme, kekik esansiyel yağı, selenyum, nono-selenyum, performans, kan parametreleri

CONTENTS

EFFECTS OF DIETARY OREGANO ESSENTIAL OIL (OEO) AND DIFFERENT FORMS OF SELENIUM (ORGANIC, INORGANIC & NANO-Se) ON PERFORMANCE, MEAT QUALITY AND BLOOD PARAMETERS OF BROILERS

SCIENTIFIC ETHICS SUITABILITY	i
SUITABILITY FOR GUIDE.....	ii
ACCEPTANCE AND APPROVAL PAGE	iii
ACKNOWLEDGEMENTS	iv
ABSTRACT.....	v
ÖZET.....	vi
CONTENTS.....	vii
LIST OF ABBREVIATIONS	xi
LIST OF TABLES	xii
LIST OF FIGURES	xiii
 INTRODUCTION.....	 1

CHAPTER 1

LITERATURE REVIEW

1.1. General Outline	6
1.2. Outline of Dietary Oregano Essential Oil (OEO)	6
1.2.1.1. Oregano	7
1.2.1.2. Oregano Structure	7
1.2.2. Mode of Action Dietary Oregano Essential Oil (OEO).....	8
1.2.3. The Effects of Dietary Oregano Essential Oil on Broiler Performance	9
1.2.4. The Effects of Dietary Oregano Essential Oil on Meat Quality	11

1.2.5. The Effects of Dietary Oregano Essential Oil on Immuno-Competence ..	12
1.2.6. The Effect of Dietary Oregano Essential Oil on Blood Parameters	14
1.3. Outline of Dietary Selenium (Organic, Inorganic & Nano-Se) Sources	15
1.3.1.1. Selenium (Se)	16
1.3.1.2. Selenium Structure	17
1.3.2. Metabolism and Action of Dietary Selenium (Organic, Inorganic & Nano-Se)	18
1.3.3. The Effects of Dietary Selenium (Organic, Inorganic & Nano-Se) on Broiler Performance.....	21
1.3.4. The Effects of Dietary Selenium (Organic, Inorganic & Nano-Se) on Meat Quality	25
1.3.5. The Effects of Dietary Selenium (Organic, Inorganic & Nano-Se) on Immuno-Competence.....	26
1.3.6. The Effects of Dietary Selenium (Organic, Inorganic & Nano-Se) on Blood Parameters	27
1.4. Outline the Dietary Oregano Essential Oil (OEO) and Selenium (Organic, Inorganic & Nano-Se) Requirement for Broiler Chickens.	28

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials	30
2.1.1. Place and Time to Carry Out the Experiment.....	30
2.1.2. Source of Chicks	31
2.1.3. Experiment Design	31
2.1.4. Experimental Diets	32
2.1.5. Chicks Management.....	34
2.2. Methods.....	35
2.2.1. Variables Measured	35
2.2.1.1. Body Weight.....	35
2.2.1.2. Weight Gain (g).....	36

2.2.1.3. Feed Intake (g)	36
2.2.1.4 Food Conversion Ratio.....	36
2.2.1.5 Production Index (PI).....	36
2.3. Characteristics of Slaughter.....	36
2.3.1 Relative Weights of Edible Internal Organs.....	37
2.3.2. The Carcass and Carcass Cuts Weight (Hot Weight).....	37
2.3.3 pH.....	38
2.3.4. The Color of the Carcass	38
2.4. Characteristics of Meat.....	39
2.4.1. Cooking Loss (%)	39
2.4.2. Drip Loss (%).....	40
2.4.3. Dry Matter (%).....	40
2.4.4. Humidity (%).....	41
2.4.5. Crude Protein (%).....	41
2.4.6. Crude Ash (%).....	43
2.4.7. Ether Extract (%).....	44
2.5. Blood Profile Analysis.....	45
2.5.1. Packed Cell Volume (PCV) % (Hematocrit)	46
2.5.2. Hemoglobin Concentration (Hb)	46
2.5.3. White Blood Cells (WBC) and H/L %.....	46
2.5.4. Blood Immunity	47
2.5.4.1. Albumin, Globulin, A/G Ratio, GOT, GPT	47
2.5.4.2. IL-2, IL-10 and TNF- α ELISA	48
2.6. Statistical Analysis	49

CHAPTER 3

RESULTS

3.1. Productive Parameters	50
3.1.1. Body Weight.....	50
3.1.2 Body Weight Gain (g).....	52

3.1.3. Feed Consumption (g)	53
3.1.4 Feed Conversion Ratio (%)	54
3.1.5. Production Index (PI)	55
3.2. Carcass Parameters	56
3.2.1. The Carcass and Carcass Cuts Weight (Hot Weight).....	56
3.2.2. Relative Weights of Edible Internal Organs.....	58
3.2.3. Meat Color and pH.....	59
3.3. Meat Quality Characteristics	63
3.4. Immune Competence	65
3.5. Blood Profile	69

CHAPTER 4

DISCUSSION AND CONCLUSION

4.1. Production Parameters	72
4.1.1. Body Weight and Body Weight Gain (g).....	72
4.1.2. Feed Consumption (g), and Feed Conversion Ratio (%).....	75
4.1.3. Mortality Rate and Production Index (PI).....	78
4.2. Carcass Parameters and Relative Weights of Edible Internal Organs.....	80
4.2.1. Carcass Parameters.....	80
4.2.2. Relative Weights of Edible Internal Organs.....	81
4.3. Meat Color and Meat pH Level	82
4.4. Meat Quality Characteristics: (DM, ASH, EE, CP. Moisture, Cooking Loss and Drip Loss %)	83
4.5. Immunity Parameters	85
4.6. Blood Profile	87
CONCLUSIONS	89
REFERENCES.....	91
CURRICULUM VITAE.....	104

LIST OF ABBREVIATIONS

a*	: Redness
AGP	: Antibiotic Growth Promoters
b*	: Yellow
BD	: Basal Diet
BW	: Body Weight
BWG	: Body Weight Gain
CP	: Crude Protein
DM	: Dry Matter
EE	: Ether Extract
FCR	: Feed Conversion Ratio
FI	: Feed Intake
G/DL	: Grams (g) per Deciliter (dL)
L*	: Lighter
Ng/L	: Nanogram/Liter
OEO	: Oregano Essential Oil
PFA	: Phytogenic Feed Additive
Pg/ML	: Picograms Per Milliliter
ppm	: Part Per Million
SBM	: Soybean Meal
Se	: Selenium
U/L	: Units Per Liter

LIST OF TABLES

Table 2.1. Experimental design.....	31
Table 2.2. Ingredients and chemical composition of the basal diet	34
Table 3.1. Body weight (g)	50
Table 3.2. Daily weight gain (g/bird/d) of treatments.....	52
Table 3.3. Feed consumption (g/bird) of treatments	53
Table 3.4. Feed conversion ratio (g feed: g gain)	54
Table 3.5. Effect of diet additives on Mortality % and Production Index	55
Table 3.6. Carcass parts % of treatments	56
Table 3.7. Giblets % of treatments.....	58
Table 3.8. Meat color and pH measurements of treatments.....	59
Table 3.9. Meat quality characteristics of treatments	63
Table 3.10. Immune parameters of treatments.....	65
Table 3.11. Blood parameters measurement of treatments	69

LIST OF FIGURES

Figure 1.1. Essential oil of <i>Origanum vulgare</i> L.	7
Figure 1.2. Chemical structures of oregano	8
Figure 1.1. Selenium (Se) (Adapted from Lenntech).....	16
Figure 1.4. Selenium structure	17
Figure 1.5. Schematic model showing different application of selenium nanoparticles	19
Figure 1.6. Schematic model showcasing the role of selenium nanoparticles in immunomodulation.....	20
Figure 2.1. Experiment field	30
Figure 2.2. Broiler hatchery (Beyza Company)	31
Figure 2.3. Feed mixer (Diets were prepared in a feed factory)	33
Figure 2.4. Cages with drinkers and feeders	35
Figure 2.5. The birds were slaughtered and cleaned	37
Figure 2.6. High precision balance (OHAUS max 410g- readability 0.001gm).....	37
Figure 2.7. pH meter (Mettler Toledo- Swiss) and color detector Chroma-meter Minolta CR-400, Konica Minolta Sensing, Osaka, Japan	38
Figure 2.9. Breast placed in the refrigerator for 24 hours.	40
Figure 2.10. Laboratory oven (12 hours under the temperature of 105 °C.).....	41
Figure 2.11. Small milling machine	42
Figure 2.12. The crude protein processing devices	43
Figure 2.13. Ash oven and desiccator	44
Figure 2.14. Fat extraction devices	45
Figure 2.15. Collect blood samples for analysis	45
Figure 2.16. Blood parameters analysis at Veterinary faculty /Erciyes University.....	46
Figure 2.17. UV-1700 Spectrophotometer, Shimadzu.....	48
Figure 2.18. Microplate washer and microplate reader.....	49
Figure 2.19. Summary procedures of chicken elisa kit (Sunred Company, China).....	49

Figure 3.1.	The effect of additives in diet on body weight at 21 d and 42 d.....	51
Figure 3.2.	The effect of additives in diet on body weight at 1 d, 21 d and 42 d.....	51
Figure 3.3.	The effect of diet additives on body weight gain (1-21 d, 21-42 d and 1-42 d).....	52
Figure 3.4.	The effect of diet additives on feed consumption at 1-21 d, 21-42 d and 1-42 d.	53
Figure 3.5.	The effect of diet additives on FCR ratio at 1-21 d, 21-42 d and 1-42 d.	54
Figure 3.6.	Effect of diet additives on production index.....	55
Figure 3.7.	The effect of diet additives on Carcass yield at 42 d of age	57
Figure 3.8.	The effect of diet additives on carcass parts (breast, thigh, drumstick and back).....	57
Figure 3.9.	The effect of diet additives on carcass parts (neck, wing, and abdominal fat).	58
Figure 3.10.	The effect of diet additives on edible internal organs %.	59
Figure 3.11.	The effect of diet additives on meat color L* in breast and thigh.	60
Figure 3.12.	The effect of diet additives on meat color a* in breast and thigh.....	61
Figure 3.13.	The effect of diet additives on meat color b* in breast and thigh.....	61
Figure 3.14.	The effect of diet additives on meat pH value (0 time) in breast and thigh.	62
Figure 3.15.	The effect of diet additives on meat pH value (after 24 h) in breast and thigh.	62
Figure 3.16.	The effect of diet additives on meat characteristics (DM, CP and cooking loss).....	64
Figure 3.17.	The effect of diet additives on meat characteristics (ash and EE).....	64
Figure 3.18.	The effect of diet additives on meat characteristics (moisture and WHC).....	65
Figure 3.19.	The effect of diet additives on immune parameters (Albumin and Globulin).....	66
Figure 3.1.	The effect of diet additives on immune parameters (AST and ALT).....	66

Figure 3.21. The effect of diet additives on immune parameters (TNF-a (ng/l))	67
Figure 3.22. The effect of diet additives on immune parameters (IL-2 (ng/l))	67
Figure 3.23. The effect of diet additives on immune parameters (IL-10 (pg/ml))	68
Figure 3.24. The effect of diet additives on blood parameters (PCV, Hb, Heterophile, Lymphocyte.)	70
Figure 3.25. The effect of diet additives on blood parameters (Monocyte, Eosinphil, Basophile and H/L Ratio.)	70



INTRODUCTION

Dietary additives like oregano essential oil (OEO) and selenium (organic, inorganic & nano-Se) are key nutrient additives for broiler chickens and are important nutrients as a part of poultry diets. Thus, factors that improve feed utilization and decrease its negative influence on health would be beneficial to the poultry sector; resulting in reduced both feed costs and feed waste and increased broiler immunity. So, diets containing non-chemical additives are recently growing at an increasing rate using in the diet of avian nutrition. After comparing various studied on the issue, it can be surmised that it is possible to use phytogetic additive compounds as an alternative to antibiotics for promoting growth in the diets of broilers. Researchers reported that there are few evidences into how beneficial these compounds can be for improving broiler digestion and the function of their guts. This is because the effectiveness can be dependent upon a number of components. The major factors which can impact their effectiveness include the genetically makeup of the broilers, how the diet is supplemented, and how the supplements are added to the diets and to what levels [1]. The performance of broilers has drastically improved in recent years based on improvements in feed conversion that impact their increased growth rates, with the growth period seeing a drastic reduction to a maximum of five or six weeks. The average body weights of the studied broilers indicate how they develop under normal conditions based on standard environments that promote natural development. This range is 2101.1 grams for Ross 308 broilers through 2154.4 grams for Hubbard Flex broilers after 42 days [2]. The meat from these broilers has received excellent scores in quality and quantity across all markets, with low costs and high quality meat being produced [3].

Nowadays, nutritionists try to ban most forms of antibiotic growth promoters. Some experiments have shown that phytogetic additives as alternatives can improve broiler growth performance and decrease excreta gas emission without any negative side effect

on meat quality. In addition, the nutrition of these birds has a significant impact on poultry meat quality and safety [4]. Also, the natural additives might be used as natural herbal growth promoters for poultry. Similarly, chickens fed on a diet with essential oils from oregano, sage, rosemary, and pepper crude extract (OLES) is suitable for a final body weight and weight gain similar to those observed in broilers supplemented with antibiotic growth promoters [5]. Additionally, medical plants and natural herbal dietary additives are being considered as natural sources and biologically important ingredients. High meat quality comes from more natural rearing conditions that don't constantly use growth promoters for increasing activity and muscle mass growth and decreasing. These conditions produce less stressful animals that are more responsive to their pre-slaughter treatments [6].

Also, additives like manna oligosaccharide (MOS) and probiotic might be enhanced immunoglobulin levels will have a more positive effect on growth performance, production and birds' ability to resist disease [7]. Restricting or banning antibiotics as food additives is growing among many nations because of the health risks associated with them, which has led to an increased research effort regarding alternative and natural additives.

Dietary additives are usually included in poultry diets for two important reasons: preventing diseases and promoting growth. The Law of European Union has banned feed antibiotic growth promoters, due to a risk of possible medication resistance in people pathogenic bacteria [8]. Manafi *et al.* (2016) indicated that phytogetic additives are referred to the herb parts like coriander, cinnamon, thyme, rosemary, garlic, and oregano plus to their forms of essential oils. The valuable impacts of phytogetic additives are that can improve poultry industry [9].

Additionally, Demir *et al.* (2003) reported that there was a decrease in crypt depth in the ileum of birds given dietary natural growth promoters like garlic and thyme, and the energy conserved by the decreased turnover proportion of the epithelial cells could be utilized for lean tissue mass synthesis and might help to do declaration for some improvements seen in body weight gain [10]. Li.*et al.* (2015) showed that phytogetic additives as alternatives can improve broiler growth performance and decrease excreta gas emission without any negative side effect on meat quality [11]. Also, the natural

additives might be used as natural herbal growth promoters for poultry. Diets containing non-chemical additives are recently increasing in popularity, including use in avian nutrition. Nowadays, nutritionists try to ban most forms of antibiotic growth promoters [10]. So, medical plants and natural herbal dietary additives are being considered as natural sources and biologically important ingredients. In addition, medicinal herbs and herbal products have a positive effects on poultry performance [12]. In the same way, it is also possible to accomplish this through reducing gut pathogens to promote a healthy gut and reduce the levels of contaminants present at the time of slaughter. The European Food Safety Authority (EFSA) considers this method an optimal way of ensuring a reduction in contamination in order to limit illnesses carrying over to humans who consume the meat. It is also possible to carry out this task through inhibiting the growth of pathogenic bacteria that accumulates in the metabolic tissues from leftover active elements of the essential oils in the body [1].

Additionally, researchers worldwide are becoming more aware about Se deficiency due to its health effects on people and animals. Almost one billion people around the world complain from health problems such as cancer, cardiovascular and infectious diseases (including HIV) due to getting less selenium than required for optimum protection [13]. Also, animal feed comes from plants whose selenium levels rely on the selenium levels of the soil where they are grown. As a result, there are urgent needs to investigate about Se levels in lands, animals and plants. So, solutions for these problems about selenium deficiency can be accomplished using selenium as food additives. People's health might be enhanced if dietary consumptions of Se are elevated. The consumption of chicken meat is increasing due to popularity of broiler meat and meat content of Se is affected by the composition of poultry diets [14]. Moreover, selenium additives in broiler ration are associated with many positive effects on growth, decreased mortality rates, enhanced FCR, reduction of drip loss, and lipid peroxidation in stored meat. Likewise, many tropical countries have high temperatures which cause financial losses in the broiler industry due to reduced feed intake and feed conversion efficiency. This issue can also be avoided by feeding rations supplemented with selenium [15]. Moreover, heat stress due to an increase in ambient temperature lowers the productivity in the broiler industry [16].

This thesis seeks to answer a practical question which has arisen during recent published articles, defining the using of dietary oregano essential oil and selenium in broiler diets. The improvement in the live body weight of birds which feeds oregano essential oil and selenium could be attributed to some of its biological function such as its role on enzymatic oxidation-reduction, nucleic acid metabolism and in promoting the activity of easily oxidized substances. Moreover, such improvement may be due to the role of oregano essential oil and selenium as an immune stimulant which in turn raises the bird resistance. Additionally, previous studies found that the improved FCR of broilers fed organic Se-supplemented diets could be related to the increased concentrations of the active form of thyroid hormone in the serum of chickens supplemented with organic Se as well as to the immunomodulation properties of Se. Also, improvement in FCR could be related to the better feathering of chickens fed with organic Se supplemented diet. From the results, it is clear that improving FCR may be due to increased dietary protein or energy. Also, a relationship was established between feed intake and FCR. When feed intake decreased, FCR decreased. This is because of the role of selenium and oregano essential oils play in improving FCR. The ability of most semi-purified low selenium diets to maintain growth and health when supplemented with oregano essential oil can be attributed to contamination with selenium and apply the role of selenium which was decrease mortality. Additionally, effect of selenium on meat yield could be due to changes in thyroid hormone metabolism or a result of changes in broiler feathering [17]. Proper feathering is crucial to broilers as it regulates their body temperatures and offers extra skin and muscle protection. Selenium plays a significant role within animals for promoting this feathering, maintaining the integrity of cells, and increasing muscle tissue levels. Thus, Se and OEO as antioxidants protect the biological membranes from oxidative damage and decrease osmotic fragility of erythrocytes due to improved physiological traits. Both additives significantly increased the carcass weight, dressing percentage and carcass parts percentages. The chemical composition in breast meat (such as protein percentage) increased. Also, both additives lead to improved immune systems in broilers by increasing of lymphocytes and H/L ratio.

Most of the previous studies based on using Se forms or oregano forms separately. For this reason, this study used them together in one experiment to make a comparison

between them and know their useful outcome. This decision was made due to the fact that both impact immune competence, oxidation-reduction and broiler performance. The relation between both selenium and oregano in broiler diets contributed to an improvement of carcass composition, especially in increasing the dressing percentage, breast meat gain and decreasing the abdominal fat content. Additionally, it affects the mechanism of the antimicrobial action of oregano (OEO) on *Escherichia coli* and *Bacillus subtilis* by using, Gram-negative and Gram-positive bacterial models. Also, it enhances the chicken's immunity to several diseases including *Escherichia coli* infection, Coccidiosis infectious, bursal disease, and Newcastle disease. Furthermore, both play an important role on enzymatic oxidation-reduction. Additionally, both Selenium and oregano lead to an increased appetite of birds for increased feed intake. Moreover, it can be seen clearly at NRC 1994 guide (National Research Council 1994) [18] that the requirement of Selenium for poultry throughout the growth age is (0.15 ppm) [18]. However, 0.30 ppm is considered as the maximum acceptable level of selenium supplementation [19]. Additionally, oregano essential oil can be used in diet around 300 mg/kg [20]. Also, Peng *et al* (2016) are used 300 mg/kg or more in poultry diets [21].

Within that context, the objective of this study is to determine the effect of dietary amount of oregano essential oil (OEO) and selenium (Organic, Inorganic & Nano-Se) in broiler diets (rearing period 0-6 weeks of age) on performance, meat quality, and immuno-competence and blood parameters of broilers. A total number of 450 ROSS 308 chicks at one day-old have been weighed and allocated randomly to 30 pens (15 birds /pen) and hosed flooring pens for 6 weeks. Period (1-42 days of age) fed five dietary treatments with each treatment being applied to six replicates of 15 birds. Broilers fed corn-soybean meal-based diets with five treatments (groups) with or without additives (control group). Broiler chickens fed experimental diets and the study conducted in 30 floor pens on litter. Bird performances have been evaluated by measuring body weight gain, feed intake and feed conversion. In addition, blood parameters, blood immunity and Meat quality had been measured. The diets offered *ad libitum* to all experimental birds. Finally, from the results of this study it is possible to make the conclusions and recommendations

CHAPTER 1

LITERATURE REVIEW

1.1. General Outline

The literature review is presented in four major parts. Firstly, an outline of dietary oregano essential oil (OEO) and selenium (Organic, Inorganic & Nano-Se) in broiler diets, and the importance of supplementation. Secondly, dietary oregano essential oil and selenium (Organic, Inorganic & Nano-Se) absorption and metabolism are explained. Thirdly, the type of oregano essential oil (OEO) and selenium (Organic, Inorganic & Nano-Se) sources and their impacts on broiler digestion, performance, immune competence and blood profile are described. Finally, the Dietary oregano essential oil (OEO) and selenium (Organic, Inorganic & Nano-Se) requirement for broiler chickens is outlined.

1.2. Outline of Dietary Oregano Essential Oil (OEO)

Phytogenic additives have shown a positive impact, yet it is crucial to provide continued study into their effects for use in poultry diets [1]. In addition, substances obtained from plants are of high interest to researchers because of the potential antioxidant and antimicrobial effects. Additionally, natural alternatives are sought because of rising consumer concerns of the potential harmful effects of chemical compounds that they have. The banning of many antibiotics has led to a revival of several previously subdued diseases. Because of these concerns, researchers are working hard seeking natural substitutes [22]. Most published reports have focused on searching for natural alternatives with the same as valuable effects to AGPs (antibiotic growth promoters) for animal production. For example, herbal feed additives, such as essential oils, are natural and residue free, and they can serve as potential replacements for AGPs which will limit the use of antibiotic growth promoters as the feed supplementation agents in animal nutrition [23]. Essential oils are considered ethereal or volatile oils, which are scented

oily liquids produced from vegetal material (flowers, fruits, bark, twigs, buds, seeds, roots, leaves, herbs and wood). They can be obtained by enfleurage, extraction, expression or fermentation, but the most commonly commercial method for production of essential oils is steam distillation [24]. The main constituents of oregano essential oil extracted from *Origanum* genus are carvacrol and thymol, together representative up to (85%) of the essential oil [24]. Oregano is a bushy perennial mint with the scientific name of *Origanum vulgare*, whose leaves are used as a seasoning and a source of scented oil also that are called *Origanum* or a wild marjoram [24].

1.2.1.1. Oregano

Oregano's scientific name is *Origanum vulgare* L (Figure 1.1.). It is both a medicinal plant and an aroma plant which originates from the Mediterranean area. It contains a different group of biologically active compounds that have great antimicrobial and anti-oxidant effects. These compounds are thymol, carvacrol, γ -terpinene and p-cymene (Figure 1.2.) [25]. Similarly, oregano is a flowering plant in the mint family. Oils derived from *Origanum vulgare* are known antimicrobials that are used for extending foods' shelf-lives through a reduction in microbes and fatty acids going spoiled, both common instances that cause food to spoil.



Figure 1.2. Essential oil of *Origanum vulgare* L. (Adapted from Zane Hellas Company [113])

1.2.1.2. Oregano Structure

The major constituents of *Origanum vulgare* are Carvacrol, β -fenchyl alcohol, thymol, and γ -terpinene (Figure 1.2). It also contains: alkaloids, flavonoids, saponins, anthraquinones, tannins (catechin and gallic acid), glycosides, coumarins, cardiac glycoside, phenolic acids, anthocyanins, leucoanthocyanins, starch, and steroids.

Classification of *Origanum vulgare* L.

Kingdom	: Plantae
Order	: Lamiales
Family	: Lamiaceae (Labiata)
Genus	: <i>Origanum</i>
Species	: <i>Origanum vulgare</i> L.
English Name	: Oregano
Parts Used	: Whole Plant

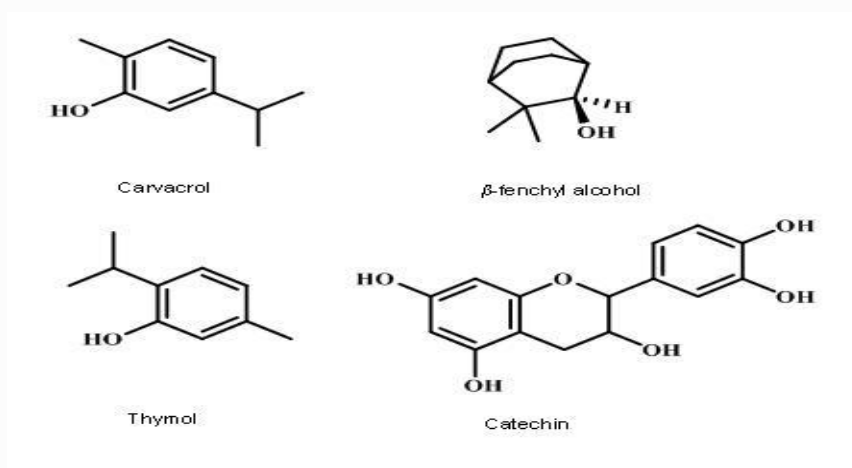


Figure 1.3. Chemical structures of oregano Adapted from Folk Medicine Sindh [114])

1.2.2. Mode of Action Dietary Oregano Essential Oil (OEO)

The important antimicrobial effects of essential oils have been described above. The apparatus of oregano's antimicrobial effect on *Escherichia coli* and *Bacillus subtilis* is used by the Gram-negative and Gram-positive bacterial models, respectively. The oils as well as their major components are capable of inducing cell lysis. Scanning electronic microscope observations revealed that both the cell walls and membranes of the treated bacteria were significantly damaged [26]. Also, studies related to this antimicrobial effect from essential oils derived from oregano and also from cinnamon and savory on the cellular membranes and cells were limited to the pH and ATP concentrations, cell

constituent releases, and the observation of electronic microcopies conducted on the cells after the essential oils (at MICs) contacted *E. coli* O157:H7 and *Listeria monocytogenes*. *E. coli* O157:H7 and *L. monocytogenes*, two pathogenic foodborne bacteria, were used as gram-negative and gram-positive bacterial models, respectively. Treatment with these essential oils at their MICs affected the membrane integrity of bacteria and induced depletion of the intracellular ATP concentration [27]. Additionally, the highest efficacy against all the tested strains were shown when testing the oregano essential oil. EO of basil and bergamot were active against the Gram-positive bacteria (*S. aureus* and *B. subtilis*), while perilla EO strongly inhibited the growth of yeast (*S. cerevisiae*) [28]. Furthermore, oregano essential oil (OEO) against *Pseudomonas aeruginosa* and *Staphylococcus aureus* was assessed by using a groundbreaking technique, so OEO as considered as a substance with high inhibition and a minimal flavor impact. Inhibition is due to damage in membrane integrity, which further affects pH homeostasis and equilibrium of inorganic ions [29]. Essential oils originating from plants have anti-microbial activity and have toxic effects in poultry only when administered at extremely high doses. Antioxidant activity and hypocholesterolemia effects have been reported in chickens [30]. In addition, dietary addition of EO showed a decreased *E. coli* population in ileo-cecal digesta and a significant increase in certain digestive enzyme [31].

1.2.3. The Effects of Dietary Oregano Essential Oil on Broiler Performance

First of all, the dietary oregano essential oil (OEO) effects on poultry performance may be a promising alternative to antibiotic growth promoters [21]. In order to reach consumers market needs, it is crucial to constantly update the birds' production characteristics (birth weight / mother age / slaughtering age & weight / average batch weight / yield & each cuts weight) [32]. Similarly, most research articles consider OEO useful for improving growth performance [33]. As a result, there is an urgent need to make more research into using oregano essential oil (OEO) in animal diets and trying to obtain significant results. Mohiti-Asli and Ghanaatparast-Rashti (2015) reported that OEO supplementation in avian diets has substantial effects on body weight gain (BWG) and feed conversion particularly in age (22-28 d) [34]. Also, using supplemented rations in female turkeys between 43-84 day / age led to an improved feed conversion efficiency [25]. Moreover, dietary oregano oil in broiler feed can enhance feed

conversion ratios and BWG even with broilers that have been infected with *Eimeria tenella* [35]. Additionally, there were increases of BWG around 16 percent over the control group and approximately 8 percent over the antibiotic group and improved FCR in broiler diets containing an essential oil mix produced from oregano, clove and anise [36]. A previous study reported improvements in BWG and FCR ($p < 0.05$) in broiler fed diet supplemented with either 10, 15, 20, 25 or 30 g/kg of thyme or oregano, although feed intake was increased ($p < 0.001$) only in diet contain oregano [37]. Also, dietary supplementation with phytogetic products (an equal mixture of thymol plus carvacrol; T+C) had a higher ($p < 0.01$) BW gain and feed efficiency at 42d, resulting in enhanced growth performances [38]. Similarly, phytogetic feed additives (PFA) comprised of a blend of essential oils from oregano, anise and citrus in broiler diets increased the BWG, decreased the feed intake (FI), and improved FCR. These beneficial effects were noticed mainly in the finisher growth period [39]. Additionally, broiler diets with essential oil supplementation (essential oil derived from oregano, anis and citrus peel) improved the BWG and FCR compared with the control group, showing signs of its potential use as a natural growth promoter and viable alternative to antibiotics [39]. Moreover, broilers given diets supplemented with enzymes or essential oils, alone or in combination, tended to have a higher weight gain than broilers fed a control diet without enzymes or essential oils at the first week of life [40].

In addition, OEO dose can result in a positive impact on carcass traits and poultry growth performance [21]. Alp *et al.* outlined that using OEO in bird diets can improve growth performance at early age clearly but there is no impacts on carcass traits [25]. A dose of OEO 300 mg/kg in broiler diet can improve FCR at 21-42 d and 1- 42 d compared to the control group [20]. Dietary treatments containing 24 mg/kg or 48 mg/kg of an essential oil mixture displayed an improved feed intake and FCR, as well as a significantly reduced mortality rate [41]. Similarly, diets supplemented with different levels of turmeric and thyme powders increased chicks' body weights and much higher feed intake and lower FCR compared to the control groups at 42 days of age ($p > 0.05$) [42].

Conversely to positive effects, Kirkpinar *et al.* (2011) indicted that oregano additives can decrease body weight at 42 d in poultry, with no significant impact on FCR and feed intake [43]. Additionally, some studies outlined that researchers found no

significant effects from essential oil on animal performance[31]. So, improvements in poultry performance rely on types of plant and their essential oils. For broiler diets supplemented with garlic, quip onion, and thyme, there was no influence on BW gain, feed consumption and FCR from 0 to 14d of age or 0 to 42d [44]. Similarly, dietary supplements of OEO (50 and 100 mg/kg of feed) in broiler diet had no effects on broiler performance [45]. Moreover, there were no significant effects from dietary treatments containing 24 mg/kg or 48 mg/kg of an essential oil mixture on body weight of the broilers at 21 and 42 days [41]. Also, dietary supplementation with oregano essential oil had no significant effect on growth performance (LBW and FCR were not affected) [46].

1.2.4. The Effects of Dietary Oregano Essential Oil on Meat Quality

Producing broilers on a massive scale has already been successfully accomplished. The focus has thus shifted towards providing better quality meats through the modification of the broiler's characteristics to affect such properties as: water holding capacity, shear force, drip loss, cook loss, pH, shelf life, collagen content, protein, and fat binding capacity. These factors are indispensable for processes involved in the manufacture of value added meat products [47]. In addition, the meat's color is an extremely important feature that plays a large role in how it is commercially accepted. Consumers will not accept meats if the color is even slightly different from what they expect. As such, it is crucial to understand the full brevity by which color of the processed meat is impacted by supplementing their diets with additives in order to maintain a consumer friendly status quo [48]. Moreover, the most common methods used to assess the water hold capacities (WHC) include a measurement of the meat's weight fluctuations that are caused by lost moisture from gravimetric force, cooking, of mechanical force. WHC in meat is complicated and is influenced by its muscle tissues' chemical and structural characteristics because they reflect the postmortem change from muscles into meats. Higher WHC counts in raw meat allow it to retain more marinade, have an improved protein function, and produce better yield after cooking and processing [49]. Additionally, a partial effect on meat quality that was observed found that supplementation of broiler diets with oregano did not affect drip loss and pH 24 and L* at any age of slaughter. The fact that the effect of oregano essential oil on a* and b* values expressed an inconstant behavior probably means that it could be a dose or

genetic (hybrid) dependent effect [46]. Recently, the proportion of poultry marketed as complete carcasses has declined due to increased consumer demands for cut-up carcass parts and further-processed poultry products [49]. As such, people around the world are more aware of carcass cut parts and their quality. The color of the meat, as mentioned above, is crucial and can often relate to how fresh the consumer perceives it to be [47]. Similarly, color defects of raw and cooked poultry meat have been a problem for the poultry industry for many years. Color defects of note include looking: pale, soft, and exudative [48]. Oregano plus garlic oil supplementation can lead to a significantly decreased L* value [50].

As a general rule, the yield of edible offal in chickens is low. Yield improvement is beneficial for consumers, both quantitatively and qualitatively and may lead to consumers reconsidering the inclusion of these products in their diet, making it more versatile [51]. So, feed additives might improve the yield of edible offal. Dietary oregano essential oil in poultry feed has no significant impact on carcass traits [25]; [20]. Broiler carcass gains and relative weights of ileum, colon, ceca, gizzard, duodenum, jejunum, proventriculus, liver, pancreas, spleen, bursa and heart are not effected by dietary oregano essential oil in poultry diets [43]. Also, dietary oregano and garlic oil supplementation did not affect carcass yields, the relative weight of carcass parts, breast and thigh meat composition, pH or b* value of breast meat [50].

1.2.5. The Effects of Dietary Oregano Essential Oil on Immuno-Competence

Oregano is known for its antimicrobial, anti-fungal insecticidal and antioxidant properties [52]. The supplementation of enzymes together with oregano essential oil at both levels in diets is likely to be more effective in terms of performance, nutrient digestibility, enzyme activities and immune system efficiency [40]. Dietary oregano essential oil in poultry diets has significant effects on poultry health. Using a dosage of 500 ppm OEO can reduce oocysts per gram feces (OPG) and can prevent of coccidiosis in poultry [34]. In the study by Peng *et al.* (2016), it was determined that OEO in avian diets can improve intestinal health [21]. Also, a diet supplemented by oregano essential oil in broiler feed can decrease OPG counts in excreta and slightly increase serum IgG (using as indicator to examine anticoccidial effects). As a result of their study, it is considered as a substitute for normal anticoccidial additives in bird feed [20].

Additionally, *Streptococcus*, *Lactobacillus* spp., total organism and Coliform bacteria which calculations in the ileum were not affected by adding dietary OEO in poultry diet while, *Clostridium* counts were significantly affected and decreased [43]. Moreover, thyme oil can improve immune response as well as intestinal barrier integrity and antioxidant status [53]. Also, oregano has an elevated IgA concentration in duodenal mucosa. On the other hand, broiler diets with essential oil supplementation (essential oil derived from oregano, anis and citrus peel) were unaffected in terms of immunoglobulin G (IgG) concentration [39]. Moreover, when supplementing sow diets with OEO, there was no impact ($p > 0.05$) on the concentration of immunoglobulin in piglets post-suckling. There was also no effect ($p > 0.05$) on T lymphocytes, on their subpopulation percentages, and on the activity of natural killer cells activity when the supplementation took place during gestation or lactation [54]. Also, observing non-treated chickens with OEO demonstrated a significant change in AST, ALT, and kidney parameters (uric acid and creatinine). However, for the broilers treated with the challenges, there was a significant change ($p < 0.05$) in the values compared to the negative control and groups treated with the phytobiotic essential oils and ciprofloxacin [55]. In addition, broilers receiving turmeric and thyme powders had lower uric acid, total cholesterol, HDL, LDL and triglyceride concentrations compared to the control groups. This information shows that the addition of turmeric and thyme powders increased antibodies for Newcastle and Influenza titers [42]. Additionally, broilers fed diets supplemented with oregano showed a higher white blood cell count ($p < 0.1$) and a significant increase of lymphocytes ($p = 0.044$) [56]. Similarly, chickens fed a diet of (infected + essential oils of thyme, anise and others (oregano, carvacol, yucca extract and cinnamaldehyde)) had a significant increase in blood protein and globulin ($p < 0.05$) in birds during the first four weeks [57]. On the other hand, significant changes were not observed in the subpopulations of CD3, CD4, CD8, IgM and IgG positive lymphocytes with OEO compared to control chickens [58]. Additionally, chickens fed a diet with essential oils from oregano, sage, rosemary, and pepper crude extract (OLES) showed no significant difference in serum TP, albumin, globulins, and albumin/globulins ratio [5]. Another study indicated that weaned pigs feeding on a diet supplemented with OEO has increased lymphocyte transformation and leucocyte phagocytosis rates, as well as the levels of IgA, IgM, C3 and C4 in blood ($p < 0.05$) [59]. Some researchers reported that treating using phytobiotics or ciprofloxacin displayed a significant reduction ($p < 0.05$) in the presence

of serum albumin. It has been noted that there was a drastic increase ($p < 0.05$) when combining the treatments, in the serum total protein and globulin presence in comparison with the other groups. The ratio of albumin-to-globulin was less in birds either challenged or treated than the challenged birds without treatment. The lowest recordings came in the groups treated with the essential oils derived from phytobiotics, ciprofloxacin, and combining these two [55]

1.2.6. The Effect of Dietary Oregano Essential Oil on Blood Parameters

Bampidis *et al.* (2005) reported that cholesterol ranges are similar in all treatments with OEO or without [25]. In addition, the concentrations of serum cholesterol, viscosity and triglyceride were not affected by OEO additives to broiler chicken diets [43]. Furthermore, there was a slight increase in the serum IgG levels of broilers fed the oregano oil diet [20]. Additionally, chicken fed diets added with (enzyme or thymol plus carvacrol) showed significantly reduced readings of total cholesterol, TP and albumin significantly ($p < 0.05$) at 40d [38]. Additionally, birds fed a supplemented diet with oregano showed higher white blood cell counts ($p < 0.1$) and that of lymphocytes increased significantly ($p = 0.044$) [56]. Similarly, chicks fed a diet with thyme essential oil 200 mg/kg indicated that heterophile had significantly lower numbers and lymphocyte had higher numbers from white blood cell counts compared with the control [60]. Chickens fed a diet with (100mg/kg rosemary VO plus 100mg/kg oregano VO (50:50%)) showed that the hemoglobin (Hb) and hematocrit (PCV) values and the heterophile/lymphocyte (H/L) ratio increased in the blood samples [61]. Also, chickens fed on diets with thymol and carvacrol showed that RBC and WBC, hematocrit and hemoglobin were not influenced by treatments, but the heterophile and heterophile to lymphocyte ratio decreased ($p < 0.05$) [62]. Additionally, broilers fed on a diet with Thyme (*Thymus vulgaris*) improved hematocrit percentage and hemoglobin concentration, but not significantly [63].

On the other hand, chickens fed a diet with oregano oil extracts did not display significant changes in account numbers of peripheral white blood cells compared to control chickens. Also, the level of plasma immunoglobulins significantly decreased in chickens fed diet supplemented with oregano oil at day 29 of age [58].

1.3. Outline of Dietary Selenium (Organic, Inorganic & Nano-Se) Sources

Selenium (Se) is critical to many selenoproteins, mainly those that relate to the body's antioxidant systems. As such, poultry requires it in order to maintain a healthy life, which leads to better quality meats, and also as a micronutrient necessary for regular growth. Its common biological arrangements include sulphur-based amino acids, such as selenomethionine and selenocysteine. In addition to the benefits already mentioned, selenium is also crucial towards regulating glutathione peroxidase, maintaining the thyroid hormones, enhancing vitamin E, and improving both the immune and reproductive systems [64]. It is quite a rare element, with a lower prevalence on the planet than silver, present mostly as derivatives from methyl [65]. When uncombined selenium is located, it is usually in one of the 40 minerals that contain as much as 30 percent of it, usually in combination with metals such as lead, zinc, and copper. These are mostly found in Russia, Canada, Bolivia, and the United States, being released both naturally and as a result of human activity. The element of selenium, its natural form, cannot be produced nor destroyed. That said, it can change forms. Most human exposure to selenium comes by way of food or water, but it can also come from soil and air that contains traces of it. Within foods, it is mostly found in meats, cereals, and grains [66]. Because of its importance to the body regulation systems mentioned above, it is highly recommended that living creatures absorb selenium for the promotion of optimal health [12]. This is also because selenium can target areas related to the growth of tumors, resistance to drugs, angiogenesis, and metastasis. Through clinical experimentations of low-doses, conflicting results have been found thus far [67]. Several diseases are linked to deficiencies of it, including: cancer, immune system diseases, Alzheimer's, thyroid conditions, and more [68].

Because of all of these natural benefits, the biologically produce Nano-selenium is a hopeful facet of modern food supplementation, particularly in adding it to the diets of livestock and fish [69].

Nowadays, identification of several selenocysteine-containing proteins, encoded by twenty five people genes, gives data on the molecular and biochemical basis of beneficial and possible adverse effects of this trace element [68].

Cereals are important components in the diets of animals and human diets. Low levels of selenium in animals come from low levels in the soil where the plants are grown that their feed is derived from. The risk deficiency is higher in big animals like cattle and sheep than for smaller animals such as pigs and poultry because of the low dependence on soil Se [65]. Moreover, dietary Se is a critical additive for the activity of virtually all activities of the immune system [12]. Cai *et al.* (2012) reported that all experiment indexes clearly demonstrated that (0.3 to 0.5 mg /kg) is recommended to be the optimal supplementation level of Nano Selenium and that the maximum supplementation of (Nano Se) should not exceed (1.0 mg / kg) in broiler birds [12]. With regards to poultry dietary needs, there are two main sources of selenium: inorganic (selenite and selenate) and organic (selenomethionine, known as SeMet). Organic selenium comprises the majority of food additives, with SeMet being larger than 50 percent of the total used. However, in commercial additives, selenite and selenate are much more commonplace over the last four decades [70]. Although using selenium to improve broiler performance has been extensively covered by the research, there is a major lacking in studies related to how it affects their immune systems and adds an antioxidant effect to the tissues [71].

1.3.1.1. Selenium (Se)

Selenium (Se) is a trace element (figure 1.3) and considered as essential in animal diets and has a big role related to the animal industry, disease prevention and fertility [12]. The element has been known for more than two centuries after being discovered by the Swedish chemist Berzelius [72]. Also, selenium's biological activities have been known for nearly 100 years. Selenium deficiency is still a big problem for both humans and animals [65].



Figure 1.4. Selenium (Se) (Adapted from Lenntech [115])

1.3.1.2. Selenium Structure

Selenium has different forms including organic (selenocysteine, selenomethionine), inorganic (selenite, selenate), and Nano-Se, with the element's structure shown in Figure 1.4 below. Payne (2004) indicated that dietary inorganic and organic Selenium has the same results in broiler performance [73]. Also, Payne and Southern (2005) found that sodium selenite is an inorganic form of selenium used as a primary source in poultry diets. The organic selenium is considered ideal for these diets. Such Se sources include: SM (selenomethionine), SC (selenocysteine), SCH (selenium chlorella) and SY (se-enriched Yeast). Both organic and inorganic Se are important dietary sources of Selenium. Soils usually contain Se in its inorganic form (selenite, selenate) that flora (plant of particular region or time) can convert to form organic Se (selenocysteine, selenomethionine) and their methylated derivatives [65]. Because of its importance to human and animal diets, further study should be carried out regarding Selenium-enriched alga *Chlorella* applied in commercially produced premixes as a potential source of organic selenium form [75]. Moreover, Haug *et al.* (2007) reported that there are some lands have low concentration of Selenium in the soil, such as some areas in Sub-Saharan Africa, Asia and Latin America which causes Se-deficiency in agricultural crops [13]. As a result, Se levels might be low in animal diets in these regions.

Selenium Structure

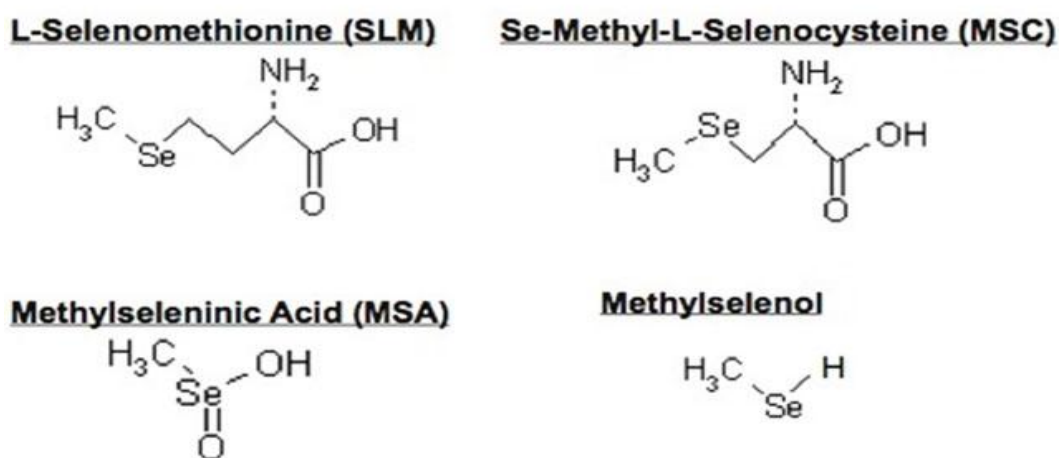


Figure 1.5. Selenium structure [67].

The NRC (1994) report informs that the minimum level for selenium supplementation of broilers is (0.15 mg/kg diet). However, there is an important opinion from researchers in the animal industries NRC which said that the minimum recommendation cannot be sufficient to prevent production losses because of selenium scarcity syndromes. So, continued research into substitute selenium sources and alternative selenium supplementation levels is highly recommended [12]. In addition, Placha *et al.* (2014) and Rayman (2000) reported that Selenium (Se) is considered as a trace element which is naturally found in many foods and is available as a dietary supplement. Selenium is critical for the important roles it plays in metabolism, reproduction, protection from oxidative damage and infection and DNA synthesis [53]; [66]. Research has also revealed major pros to using organic selenium in poultry feed. This is because SeMet is much better retained within the body of the broilers because they cannot synthesize it. Thus, it can boost stressors in conditions when they need extra selenium due to being stored in the muscles and continuing to provide the intended antioxidizing effect that can counteract these stresses. As such, the broiler industry is actively seeking methods to include organic selenium in commercial products [72].

1.3.2. Metabolism and Action of Dietary Selenium (Organic, Inorganic & Nano-Se)

Dietary selenium plays an important role in many animal functions such as: reproduction, antioxidation, digestion, antimicrobial benefits, preventing diseases such as cancer and fatty liver diseases (Figure1.5) [69]. Also, selenium has been recognized as crucial to combating the oxidative stresses and regulating cellular growth, as well as modifying the cellular signal systems that relate to numerous processes within the broiler bodies. As such, it is thus crucial towards providing a retaliatory effect towards stresses and various diseases [72]. In seeking to produce a commercial organic selenium product, broiler industries worldwide have begun using premixes around 0.2 to 0.3 mg/kg in the diet [72]. As mentioned above, selenium in the body at the right levels promotes the thyroid hormone synthesis [76]. Selenium was also recognized as being crucial to many enzyme functions, including selenocysteine and selenomethionine as amino acids. It was also discovered as showing tumorigenesis in many animals and having an effect towards reducing cancers through limiting the growth of tumors through autocorrecting nutrition deficiencies that promote tumors. As such, because of this and the antioxidant properties, selenium supplementation is highly desirable [77].



Figure 1.6. Schematic model showing different application of selenium nanoparticles (Adapted from Sarkar *et al.* 2015 [69])

Current studies have shown a link between selenium and reducing DNA damage as well as oxidative stress, with further information producing the connection of selenoproteins in reducing cancer risks. These anti-cancerous effects come from the selenium metabolites of methyl selenol that are produced by elements of γ -glutamyl-selenomethyl-SeCys and selenomethyl-SeCys. Beyond the cancer fighting properties, evidence also points to progression and metastasis benefits [78]. Additionally, the new discovery of numerous separate selenocysteine-containing proteins, encoded by 25 human genes, establishes data related to the molecular and biochemical basis of beneficial impacts from selenium. The thyroid gland has the highest selenium content per mass unit. Selenoproteins involved in cellular antioxidative defense systems and redox control, including the glutathione peroxidase (GPx) and thioredoxin reductase (TxnRd), are involved in protection of the thyroid gland from excess hydrogen peroxide and reactive oxygen species produced by the follicles for biosynthesis of thyroid hormones [68]. Additionally, Nano-Se has a big role in the immune system, as shown in Figure (1.6.) [69].

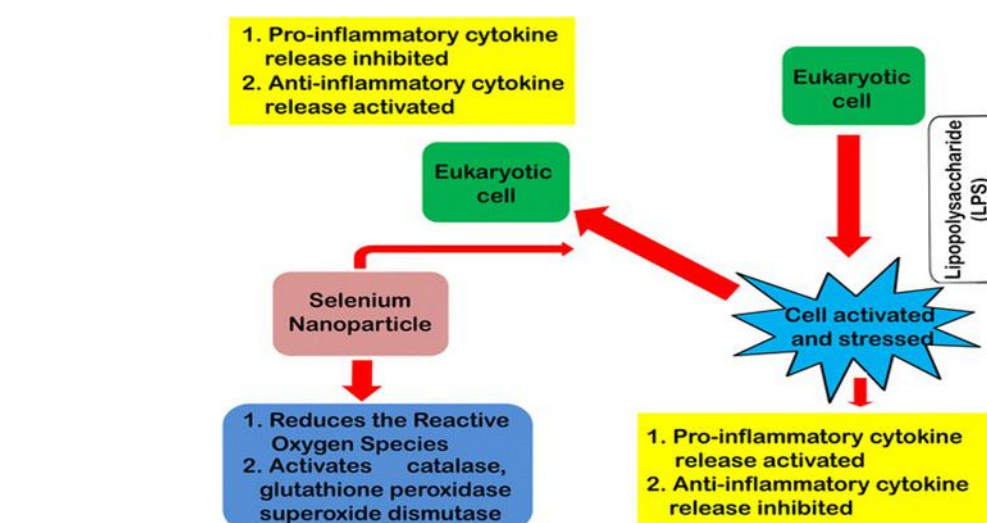


Figure 1.7. Schematic model showcasing the role of selenium nanoparticles in immunomodulation (Adapted from Sarkar et al. 2015 [69])

Three levels of commercially sold selenium products are sold globally. The first, that has been commercially available since 1970, is now considered outdated and comprises the inorganic selenium of mostly selenite and selenate. This type has helped the livestock industries remove selenium deficiency, but with their value has diminished that of today's precision nutrition concepts. The only reason they remain in use is because they are cheap to produce and consume. As for the second type, they include selenium yeast and SeMet, and have come into prominence over the last two decades. OH SeMet is the third type, which was designed to combine the benefits of several elements of the second generation, such as the stability of selenium yeast and the active compounds found in SeMet. Thus, it remains crucial to further develop the Nano selenium productions using contemporary molecular methodologies in order to understand the metabolization process of these particles in the bodies of humans and the livestock (such as poultry relevant to this study) they consume. In particular, it is necessary to understand how this metabolism impacts the body's synthesizing of selenoproteins, the highest biological activity using selenium [72]. The most important applications in this area are Nano minerals, they are characterized by a particle size of 1 to 100 nm (nanometer= 1×10^{-7}) some of these are stable at high temperatures and pressure levels [79]. Moreover, several types of Nano minerals and components are able to bind and destroy pathogens and toxins. One such example is Nano silver, which has

displayed an impressive antimicrobial characteristic [80]. Additionally, using these Nano minerals and components, such as those from selenium, zinc, and chromium in animal diets have displayed strong evidence towards improving health, their production, and the product quality of items derived from them. Research has pointed towards nanoparticles having a better absorption than other materials, which drastically raises the quality of the products derived from the animals who consume them. More importantly, the meats produced from these animals are able to combat mineral deficiencies of the humans who consume them, which is a crucial element of their continued use. It should be stated, however, that for selenium like many other substances, there is a fine line between deficiency and excess, which both of these extremes being dangerous. As such, the research into the overall health benefits for the human consumers of these meats is necessary in order to determine whether Nano selenium is desirable for food supplementation [81].

1.3.3. The Effects of Dietary Selenium (Organic, Inorganic & Nano-Se) on Broiler Performance

Rajashree *et al.* (2014) demonstrated a clear decrease in the number of bird mortality with treatment diets supplemented Se-yeast (0.5 mg/kg) [82]. Moreover, Djordjevic *et al.* (2016) reported that organic selenium contributed to improving carcass composition, especially in increasing the dressing percentage, breast meat gain and decreasing the abdominal fat content [83]. Additionally, performance traits higher ($p \leq 0.05$) live weight of broiler chickens was recorded in the experimental groups with organic Selenium compared to the control group. However, there were no significant differences between the groups in feed conversion, mortality, carcass traits and dressing percentage [84]. Moreover, the body weight of broilers fed organic selenium were increased compared to no selenium or inorganic selenium treatment groups, and the combination of inorganic and organic Se was no more effective than organic-Se alone [85]. Another study showed that the Se supplement significantly affected BW at 21 ($p \leq 0.001$) and 35 days of age ($p \leq 0.05$), with the highest BW values reached at 21 d of age in broilers fed 0.3 mg/kg of SS (1050 g) and at 35 d in dietary treatments with 0.15 mg/kg of SP (2 122 g) and 0.3 mg/kg of SCH (2 116 g). At the same time, no significant differences among dietary treatments were determined in FCR [75]. In addition, FCR improved with organic-Se supplementation and combination of inorganic and organic Se being superior

to inorganic-Se only treatment. Also, carcass weight, yields of viscera, feet, thigh and neck were higher in organic-Se treated birds. However, chickens fed a supplemented diet with inorganic-Se showed higher breast meat drip loss. This proves that organic-Se was used more efficiently for performance in fast growing, high yielding broiler chickens [85]. Also, there was no difference in chicken performance between the birds fed inorganic or organic forms of Se, despite an improvement in feathering of the organic Se group. Birds fed 0.3 ppm of organic Se had decreased drip loss from breast meat [17]. Additionally, the supplementation of the diet with Se-Chlorella (0.3mg/kg) increased live weight at 21 and 42 days ($p < 0.05$) compared to the control diet [86]. In addition, dietary supplementation of organic Se sources in male broilers (Ross $\times$ Ross 308) diet significantly improved average daily gain (ADG), gain: feed ratio and European production efficiency factor ($p < 0.05$) compared to birds fed on diets supplemented with inorganic source [71].

In contrast, supplemented broiler diets with (0.0, 0.3, 0.5, 1.0, or 2.0 mg/kg diet) of Nano-Se was shown to have no effects on broiler performance ($p > 0.05$) [12]. Additionally, research into their nutrition indicated that there was no impact ($p > 0.05$) using organic Selenium supplementation in different levels (0, 400, 300, 200, or 100 $\mu\text{g/kg}$ diet) on BW gain and feed conversion, relative weight of liver and abdominal fat [15]. Also, different sources of Se (Nano-selenium 0.3 mg/kg, INO: Inorganic selenium 0.3 mg/kg, ORG: Organic selenium 0.3 mg/kg) had no effect on daily gain, feed intake and FCR ($p > 0.05$) [87]. Furthermore, the dietary selenium supplement had no impact on these important parameters: meat derived from the breast and thigh; fat share of the heart, abdomen, and gizzard; and carcass weight. However, there was a significant increase ($p \leq 0.05$) in the liver weights for broilers fed 0.3 mg/kg of SS (3.8 percent) compared to SCH at the two levels of selenium presence (3 and 3.1 percent) [75]. Another study showed that the selenium levels had no impact ($p > 0.05$) on the following weight characteristics: Maryland, dressed, breast, and eviscerated weights. The organic selenium source increased the dressed weight as the organic selenium feed increased the Maryland and eviscerated weights ($p > 0.05$). Although this is true, and the overall yield of breast meat was also increased by raising the amounts of organic selenium, it should be concluded that the study revealed that inorganic selenium in the form of sodium selenite displayed the exact opposite result [64]. Similarly, research into

how selenium is processed in the body has revealed that organic selenium is a natural substance that is seen in many selenoamino acids common food for poultry usually consume, which implies an adaptation of these birds to digest it, explaining why organic and inorganic selenium is received differently [70]. Also, selenium from organic or inorganic sources (0.1, 0.2, or 0.3 ppm) did not affect ($p > 0.05$) the growth performance of broilers at 42 d of age [88]. Moreover, Broilers were fed 1, 2, 4, or 8 ppm Se in their diet did not show a change in BW and feed efficiency by dietary selenium levels [89]. Also, daily gain, feed intake, gain: feed, eviscerated and chill weights, carcass yield, breast weight, and moisture loss from the breast were not affected ($p > 0.05$) by Se supplementation [90] .

Interestingly, Ryu *et al.* (2005) showed that broiler performance was not improved by inorganic-Se might be over doses. There was, though, a drop in how much food was consumed each day ($p < 0.01$) when selenium was added at 6 mg/kg and 9 mg/kg, especially after three weeks, which implies toxicosis being developed [91]. Moreover, the results of experiments on broiler chickens fed Se revealed information about Nano selenium being transferred from the intestinal lumen into the body at a higher rate ($p < 0.05$) when compared to selenite, with Nano selenium also being retained in the intestine for a shorter period of time ($p < 0.05$) when compared to the same selenite. As such, it can be deduced that there is a larger gap between optimum levels and toxic levels when using Nano selenium compared to sodium selenite, with the former also having a much better retention rate [92]. The Federal Drug Administration of the American government accepted the use of selenite and selenate supplements used for both swine and poultry in the mid-1970s. Since this time, extensive research into their use has led to the conclusions that inorganic selenium is not beneficial in the diets of poultry [70];[72]. Further research into how sodium selenite interacts with ascorbic acid on a chemical level in the food, premix, or the gut has provided a connection between less selenite and the elemental selenium, which is impossible for chickens to digest [93]. In addition, recent studies have revealed the damaging effects that sodium selenite at 0.3 ppm has on chicken guts [94]. Also, Cai *et al.* (2012b) indicted that during the periods from 0 to 21 d, 22 to 42 d, and 0 to 42 d, BWG, FI and FCR did not differ between treatments fed diet with Nano-Se (0.00, 0.30, 0.50, 1.00 and 2.00mg/kg) [95]. Similarly, supplementing the diet with 0.3 ppm mineral Se (Sodium selenite) or organic Se (Se

enriched yeast) had no significant effect on BW, BWG and FI after observations at 21 and 42 days. However, total FCR (1-42 days) was significantly improved in broiler fed organic-Se compared to other groups [96]. Similarly, the final BW and daily BWG values were not affected by the dietary treatments, with no noticeable difference between group one and group two supplemented with sodium selenite and selenium yeast and control [97]. Likewise, in broiler diets that treated with A=0.10 mg/kg (Sodium selenite); B=0.25mg/kg (Sodium selenite); C=0.10mg/kg (Sel-Plex 50); D=0.25mg/kg (Sel-Plex 50), there was no difference in body weight at 38d between all treatments affected by the level and source of selenium [64]. Moreover, broiler fed treated with different Se forms has caused reduction in feed intake by a high selenium concentration in feed resulted in an improved feed conversion ratio (FCR) as result of improved feather score ($p < 0.001$) at d 37 [64]. Some research has suggested that improvement in feed conversion ratio could be related to the better feathering of chickens fed with a diet supplemented with organic Se [96]. Similarly, organic selenium 0.3 ppm (Se-enriched yeas) had reduced feed conversion when compared with inorganic selenium 0.3 ppm ($p < 0.05$) in broiler [98]. Likewise, broiler fed treated with different Se forms has caused reduction in feed intake by a high selenium concentration in feed resulted in an improved feed conversion ratio (FCR) [64]. Furthermore, in the experimentation, a significant difference ($p < 0.05$) was recorded in FCR of Inorganic-Se 0.2mg/kg) and organic-Se 0.2mg/kg compared to control while no significant differences ($p > 0.05$) in FCR between Inorganic-Se and organic-Se treatment broiler chickens [97].

Moreover, researchers around the world are becoming aware of mortality rates and trying to achieve a lower rate by adding feed additives to improve immunity system. In a study on the topic, mortality was not significantly altered by selenium supplementation whatever the Se forms [96]. Similarly, the survival rate of Inorganic-Se 0.2mg/kg) and organic-Se 0.2mg/kg and the control were not affected by the dietary treatments [97]. Further evidence was provided from another study where 308 Ross broilers were fed a diet treated with 0 (control), 0.15 or 0.30 mg of selenium/kg in the form of sodium selenite (SS), Selenium enriched yeast (Sel-Plex (SP)) and selenium-enriched alga Chlorella (SCH). The results showed that there is no significant differences among dietary treatments were determined mortality it was under 5% [75].

Also, for the feed supplemented with A= 0.10mg/kg (Sodium selenite), B=0.25mg/kg (Sodium selenite), C=0.10mg/kg (Sel-Plex 50), and D=0.25mg/kg (Sel-Plex 50), mortality was not affected by the level and source of selenium [64]. In contrast, the organic selenium could decrease survival rate by 0.93% ($p < 0.05$) [98].

1.3.4. The Effects of Dietary Selenium (Organic, Inorganic & Nano-Se) on Meat Quality

There has been a drastic shift in how poultry meat is produced and sold in recent years, with parts of these birds being often preferred to buying an entire carcass. Beyond this, there has also been an increase in pre-made and processed chicken products, such as breaded chicken, nuggets, and more. That said, these products are still only as good as the quality of the meat used to produce them [99]. Furthermore, there is an increasing awareness about meat selenium contain the organic selenium supplement (SP, SCH) (Organic-Se) being effectively absorbed into muscles of chickens contrary to SS (Inorganic-Se) [75]. Also, birds receiving inorganic selenium retained less (4.25mg/kg) than those on the selenium yeast treatment (1.32 mg/kg) [64]. Additionally, the main characteristics of the meat which are considered include: pH, color, and water holding capacity [100]. In addition, Nano-Se supplementation linearly and quadratically ($P < 0.01$) reduced the drip loss percentage [95]. Similarly, entire carcass drip-loss was significantly reduced in broilers fed organic-Se (0.3 ppm) when compared with the control and inorganic-Se supplementation groups [96]. Similarly it has been reported in another research; broilers receiving sodium selenite (Inorganic-Se) had a higher 24 h drip loss ($p < 0.001$) than those given the organic source (1.12% vs 0.87%) [64].

There are many factors which impact meat quality. The most significant are the feed quality and some characteristics that occur before and after slaughter, as indicated by Bowker (2017). Numerous interacting antemortem and postmortem factors can influence WHC characteristics in the final meat product. Biochemical and structural changes within the muscle tissue during the conversion of muscle to meat greatly impact WHC [49]. Also, Se (0.3mg/kg) supplementation did not affect ($p > 0.05$) the protein and fat contents in dry matter of meat in broiler chickens [86].

Dietary Nano-Se in broiler diets with different levels has no effects on meat color [12]. Also, Cai *et al.* (2012) reported that when dietary Nano-Se level increased as result

muscle selenium content enriched ($p < 0.01$), and the highest reached value at (2.0 mg/kg) [12]. Additionally, broiler feed supplemented with organic se 0.5 mg /kg of feed can impact contents of (Se) in the (thigh muscle tissue) significantly ($p < 0.05$) and enrich of total n3 polyunsaturated fatty acids from (5.27- 6.25%), and decrease of total monounsaturated fatty acids from (39.60- 36.74%) in the lipids of thigh muscle tissue. Increasing Se contents in broiler feed caused a reduction in thiobarbituric acid reactive ingredients significantly, and increasing of linolenic acid and total n-3 polyunsaturated fatty acids significantly in thigh muscle tissue of chickens [102]. Kralik *et al.* (2013) found decreased amounts of monounsaturated fatty acids in the lipids of thigh muscle from 39.60% to 36.74% with supplemented with amount of 0.5 mg Se [101].

It has been reported that, the meat's red color degree a^* (redness) of breast meat and leg muscle were increased by 13.98 ($p < 0.05$) and 20.83% ($p < 0.05$), respectively in broiler fed different form of Selenium (organic or Inorganic Se) [98]. Moreover, Organic-Se can improve chicken carcass composition, particularly in dressing ratio, breast meat yield and reducing the abdominal fat [83]. In contrast, the broiler's meat color, as expressed by (L^* , a^* , and b^*) were not affected by different dietary supplementations with selenium (8 ppm Se) [89]. Similarly, no differences in muscle meat color in broiler fed diet contain different level of Nano-Se (0.00, 0.30, 0.50, 1.00 and 2.00mg/kg) [95]. Furthermore, broilers fed different form of Selenium (organic or Inorganic) of dietary selenium did not affect pH values and sheer force of meat significantly ($p > 0.05$) Also, the drip loss of breast meat and leg muscle were decreased by 13.57 ($p < 0.05$) and 24.92% ($p < 0.05$), respectively. This indicates that Organic-Se was better than inorganic selenium supplementation in broiler diet [98].

1.3.5. The Effects of Dietary Selenium (Organic, Inorganic & Nano-Se) on Immuno-Competence

Rayman (2000) said that selenium deficiency leads to insufficient cell and humoral immune-competence responses in avian and human [66]. In addition, using Nano-Se in avian diets in different levels has no significant on organ index of immunity such as thymus, spleen and bursa fabricius [12]. Moreover, Se supplementation to chicken feed was not affected ($p > 0.05$) on Newcastle disease vaccination, the ratios between lymphocytes and heterophyls and lymphoid organs relative weight such as bursa

fabricius, spleen, and thymus. While, the cell-mediated immunity (lymphocyte proliferation ratio) and antioxidant improved significantly [15]. In addition, dietary supplementation of 0.4 mg/kg Se from an organic source resulted in better production performance and immune system response in broilers and Immunoglobulin and hypersensitivity were enhanced by increasing supplemental concentration of Se and using organic sources of Se rather than inorganic Se ($p < 0.05$) [71]. Moreover, selenium elevated glutathione peroxidase (GPx) function in blood and liver and also provided thioredoxin decline (TrxR) function in duodenal mucosa, kidney and in the liver [53]. Additionally, broilers fed Nano-Se 0.30 mg/kg showed a significant difference for IgG and IgM (ANOVA, $p < 0.01$), with the highest IgG and IgM levels [95]. Further research showed that supplementation with organic and Nano-Se increased IgM and IgG concentration compared to the other groups in oxidative and nonoxidative condition. Groups which received Nano-Se at oxidative and non-oxidative condition showed a significant difference in IgM concentration compared to the control group ($p < 0.05$) [87]. Moreover, Selenium-deficiency-induced liver damage is related to oxidative stress, and the alternative transcription of cytokines has been linked to liver disease. The experimental results showed that IL-1, IL-6, IL-8, IL-17, IL-4, IL-10 and IFN-g in Se-deficient chicken liver and serum were dramatically higher than the normal control group, and that Selenoprotein W (SelW) regulated inflammation-related cytokines during H₂O₂-dependent chicken liver damage [103].

1.3.6. The Effects of Dietary Selenium (Organic, Inorganic & Nano-Se) on Blood Parameters

Cai *et al.* (2012) showed that in rearing broilers 0-42d with Nano-Se (0.30 mg/kg) in diet supplemented was high effect while broilers fed (2.0 mg/kg diet) of Nano-Se was presented the worst in blood parameters, there was significant impacts on glutathione, free radical inhibition, glutathione peroxidase activity, contents of IgM, and malondialdehyde in serum, on glutathione peroxidase activity. Furthermore, using dietary organic selenium in bird diets leads to decrease lipid peroxidation in plasma [12]. However, dietary organic selenium caused increasing actions of glutathione peroxidase in plasma and glutathione reductase ($p < 0.01$) [15]. Also, poultry who received inorganic Se displayed higher activities in the red cell GSH-Px (200.7IU/gHb) compared to those receiving the organic variety, which was directly related to the source

of selenium used [64]. In addition, dietary selenium in avian diets has a positive effect on triglycerides level and plasma cholesterol by reducing their levels [83]. However, variation in concentration of Se with Se (0, 100, 200, 300, or 400 µg/kg diet) supplementation in diet did not influence ($p > 0.05$) the heterophil to lymphocyte (HL) ratio in broilers [15]. Also, increasing organic selenium supply from 0.1 to 0.25mg/kg numerically elevated the hemoglobin levels from 7.36 to 7.99 g/dl. Inorganic selenium, on the other hand, tended to reduce circulating hemoglobin concentration from 7.53 to 7.36 g/dl when the dietary level was increased from 0.1to 0.25 mg/kg, respectively so organic selenium was superior to the inorganic form in the improvement of Hb levels [64]. Immunoglobulins in serum, such as IgA, IgG, and IgM, are involved with humoral immunity, the effects of sodium selenite against aflatoxin B1 (AFB1) so, the contents of serum IgA and IgG in the AFB1 group were significantly decreased ($p < 0.01$) from 7 to 21 days of age, compared with the control group. In third group (+Se group III) (0.3mg/kgAFB1+0.6mg/ kg Se), the contents were markedly higher ($p < 0.01$) than those in the AFB1 group from 7 to 21 days of age [104].

1.4. Outline the Dietary Oregano Essential Oil (OEO) and Selenium (Organic, Inorganic & Nano-Se) Requirement for Broiler Chickens.

Selenium deficiency is connected to a number of diseases and illnesses that have been largely nullified in the global poultry industries. It is still possible to find farm chickens displaying lessened reproductive and productive performance because of selenium deficiencies, leading to weakened antioxidant responses based on stress. This increased response to stress largely stems from the using of genetic influences to improve the sizes and growth rates of chickens. As such, it becomes severely important to prevent selenium deficiency in order to optimize all of the relevant features whilst retaining an increased rate of growth and improved meat quality [72]. Cai *et al.* (2012) reported that all investigated indexes indicated that (0.3 to 0.5 mg/kg) is the best supplementation level of Nano Selenium, and the maximum level of supplementation of Nano-Se should not exceed (1.0 mg/ kg) in broiler diets [12]. Additionally, using Organic-Se 0.5mg/kg in chicken diet can improve chicken performance [102]. Also, it can be seen clearly at NRC 1994 guide (National Research Council, 1994) that the requirement of Selenium for poultry throughout the growth age is (0.15 ppm). However, 0.30 ppm is considered as the maximum acceptable level of selenium supplementation[19]. On the other hand,

oregano essential oil can be used in diet around 300mg/kg [20]. Also, research from Peng *et al* (2016) used 300 mg/kg or more in poultry diets[21]. Moreover, it can be shown from the experiments that diets can contain *Thymus vulgaris* essential oil (EO) 0.5 g /kg in diet and Se 0.4 mg /kg derived from sodium selenite in diet [53]. Additionally, the amount of selenium used in diets can be around 0.3 mg/kg as inorganic selenium (sodium selenite) or organic selenium (selenium methionine) in chicken diets [83]. In another study, Heindl *et al.* (2010) used 308 Ross broilers in his experiment which fed them a diet treated with Se 0 (control), 0.15 or 0.30 mg /kg in the form of sodium selenite (SS). The results discovered a benefit of the addition of sodium selenite (Inorganic-Se) at both levels of 0.15 and 0.30 mg /kg as having the same effect [75].

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

Animal Care and Use

The study was approved by the University of Erciyes Care and Ethics Committee and accordance with the Turkish Code of Practice for the use of animal for scientific purpose.

2.1.1. Place and Time to Carry Out the Experiment

The experiment was conducted in the poultry field of Animal Production Department, Faculty of Agriculture, University of Erciyes, Kayseri (figure 2.1). The field experiment was conducted for six weeks between 24.6.2018 to 04.08.2018



Figure 2.1. Experiment field

2.1.2. Source of Chicks

The chicks were obtained from broiler hatchery (Beyza Hatchery Company) (figure 2.2), a total number of four hundred fifty (ROSS 308) chicks at one day-old were used in the experiment.



Figure 2.2. Broiler hatchery (Beyza Hatchery Company)

2.1.3. Experiment Design

The chicks were weighed and allocated randomly to 30 pens (15 birds/pen) and housed in cages for 6 weeks. They were chosen based on similar physical appearance and body weight, before being distributed randomly for different treatments. The broiler chicks with lower and higher body weights than average was excluded.

Five treatments with six replicates and 15 Chicks each (Table 2.1)

Treatment Numbers = 5 and 6 cages for each treatment

Total cages= $6 \times 5 = 30$, $30 \times 15 = 450$ Chicks

Treatment 1 / Control 0% Additives

Treatment 2 / basal diet+ Oregano essential oil 300 mg/kg

Treatment 3 / basal diet + Organic Selenium 0.3 mg/kg

Treatment 4 / basal diet + Inorganic Selenium 0.3 mg/kg

Treatment 5 / basal diet + Nano Selenium 0.3

Table 2.1. Experimental design

No	Treatment Diets	Numbers of birds						Total
1	Control basal diet + 0% Additives	15	15	15	15	15	15	90
2	basal diet t+ Oregano essential oil 300 mg/kg	15	15	15	15	15	15	90
3	basal diet + Organic Selenium 0.3 mg/kg	15	15	15	15	15	15	90
4	basal diet + Inorganic Selenium 0.3 mg/kg	15	15	15	15	15	15	90
5	basal diet + Nano Selenium 0.3 mg/kg	15	15	15	15	15	15	90

2.1.4. Experimental Diets

The feed materials obtained from the Faculty of Agriculture / Erciyes University / Kayseri. The diets were prepared according to the proportions found in the diet and were crushed and mixed. Diets were prepared in a feed mixer in the poultry field, Animal production Department, Faculty of Agriculture, Erciyes University (Figure 2.3). Starter diets were fed from 1 to 21 days and had an AME and crude protein content of 3200 Kcal/kg and 23%, respectively. Finisher diets were fed from 21 to 42 days and had an AME and crude protein content of 3200 Kcal/kg and 20%, respectively (Table 2.3). organic Se, mainly in the form of selenomethionine (SeMet), Inorganic-Se in the form sodium selenite and Nano-Se synthesized from sodium selenite by a particle size of 1 to 100 nm (nanometer= 1×10^{-9} Meter). The diet treatments contented of the basal diet include: non-supplemented control diet T1, basal diet+ oregano essential oil (OEO) 300 mg/kg T2, basal diet + organic se 0.3 mg/kg T3, basal diet + Inorganic Selenium 0.3 mg/kg T4 and basal diet + Nano se 0.3 mg/kg T5, for the starter period (0 to 21 d) and for the finisher period (22 to 42 d). There are five dietary treatments, with each treatment being applied to six replicates. Nano-selenium was made in Nanotechnology Research Center/Turkey. “A sample of 2.53 g of ascorbic acid was dissolved in 25 mL of distilled water. In another beaker, a 1.58 g of sodium selenate was dissolved in 150 mL of distilled water. Magnetic fish was placed into a 500 mL glass flask and the flask was put on the magnetic stirrer. The rotation speed was set to 200 rpm. The ascorbic acid solution was put into the flask and sodium selenite solution was added. The color quickly turns to tile red. The 2 mL of concentrated sulfuric acid was added dropwise into the mixture which continues to rotate. With the addition of sulfuric acid, nano-shaped selenium particles begin to form. After further stirring for a few minutes, 200 mL of distilled water was added. The resulting mixture was stirred at 600 rpm for 24

hours in a light-free environment. Nano-selenium particles in the flask were allowed to sediment for 5 minutes. The excess liquid which remains on top of the sediment in the bottom was carefully poured into the waste system (approximately 250-300 mL). The remaining liquid and sediment were transferred to centrifuge tubes and centrifuged. Red-colored nano selenium particles combined in a single tube were centrifuged by washing 2 times with distilled water. It was then allowed to dry at 90 ° C and the resulting solid was homogenized by milling”.



Figure 2.3. Feed mixer (Diets were prepared in a feed factory)

Table 2.2. Ingredients and chemical composition of the basal diet

Item	Starter phase (d 1 to 21)	Grower phase (d 22 to 42)
Ingredient, %		
Corn	52.52	63.34
Soybean meal	37.09	28.49
Meat-bone meal	4.00	4.00
Vegetable oil	4.72	3.10
Dicalcium phosphate	0.48	-
Limestone	0.45	0.53
Sodium chloride	0.32	0.23
Vitamin-mineral premix	0.20	0.20
Antioxidant	0.10	0.10
Nutrient content, %		
ME (kcal/kg)	3,200	3,200
CP	23.00	20.00
Calcium	1.00	0.90
Total phosphorus	0.72	0.60
Methionine	0.50	0.38
Lysine	1.28	1.04

2.1.5. Chicks Management

A semi-controlled environment floor-shed were used throughout the 6 weeks study in two period; starter period (0-21day) and finisher period (22-42 day). A semi-controlled environment floor-shed maintained initially at 32-33 °C, and then reduced daily to 23 °C until the end of the experiment. From 1 to 7 day old, the experimental chicks were brooded with a 100-watt ball lamp in each pen (23h light +1h dark) and equipped with small drinkers and feeders. After 8 days of age, the screens were removed and the birds had access to the automatic drinkers and feeders for the remainder of the study (Figure 2.4)



Figure 2.4. Cages with drinkers and feeders

2.2. Methods

2.2.1. Variables Measured

Throughout the study, 4 different variables were accessed. First, they included: broiler performances (body weight (g/bird), body weight gain (g/bird/day), feed intake (g/bird/day), feed conversion ratio (feed in g/ weight gain in g). Measurements were made at 1, 7, 14, 21, 28, 35, and 42 days of age, with the Production index (PI) done in the end the experiment. Secondly, meat quality was measured. Thirdly, immune competence measurement was recorded. Finally, blood profile has been measured.

2.2.1.1. Body Weight

The birds in each experimental cage were collected in a plastic bucket and weighed for body weight (gram) at 1, 7, 14, 21, 28, 35, 42 days of age. Body weight for individual birds was calculated with the following formula:

$$\text{Body weight (g)} = \text{Total body weight (g)} / \text{total birds in a cage}$$

2.2.1.2. Weight Gain (g)

The daily growth rate of individual birds was calculated from the differences between weight at 7 , 14 , 21 , 28 , 35 , and 42 days with the initial weight as the following formula.

$$\text{Weight gain (g)} = \text{Present body weight (g)} - \text{initial body weight (g)}$$

2.2.1.3. Feed Intake (g)

Feed consumption for each experimental unit was accessed at 7 , 14 , 21 , 28 , 35 , and 42 days. The records are presented on a daily basis (g/bird/day). The feed intake was calculated by the difference between total feed given (g) and remaining feed in the feeders on the measurement day.

$$\text{Feed intake (g)} = \text{Total feed given (g)} - \text{remaining feed (g)} / \text{Number of days}$$

2.2.1.4 Food Conversion Ratio

Feed conversion ratio of birds in individual cages was calculated by using the rate of body weight gain and feed intake figures at 7, 14, 21, 28, 35, 42 days as the formula.

$$\text{FCR} = \text{Average feed intake (g)} / \text{Average body weight gain (g)}$$

2.2.1.5 Production Index (PI)

Production index (PI) for all treatments will be measured according to equation below:

$$\text{Production index (PI)} = \text{Live ability (\%)} \times \text{average daily gain (g)} / \text{feed conversion ratio} \times 10.$$

2.3. Characteristics of Slaughter

At the end of the experiment (42 days), 4 birds / repeaters (2 males + 2 female) were randomly taken from each cage. The birds were individually weighed by an electronic scale and then slaughtered and cleaned, before the internal viscera were isolated and the carcasses were weighed (figure 2.5).



Figure 2.5. The birds were slaughtered and cleaned

2.3.1 Relative Weights of Edible Internal Organs

The internal edible giblets (heart, liver and gizzard) of each slaughter were separated from the rest of the viscera and then weighed using a High precision balance (OHAUS max 410 g- readability 0.001gm) (Figure 2.6) and the relative weights were determined.



Figure 2.6. High precision balance (OHAUS max 410g- readability 0.001gm)

2.3.2. The Carcass and Carcass Cuts Weight (Hot Weight)

The carcass was weighed after cleaning and removing the viscera and then cut them to carcass parts (neck, wings, breast, thigh, drumstick, back) before being weighed. Meat

samples were stored at 4C° in refrigerator for 24h after slaughter, before the meat analysis was performed.

2.3.3 pH

The pH of the meat was measured using distilled water and a pH meter (Mettler Toledo S220, Greifensee, Switzerland) (Figure 2.7) through the Breast and Thigh. The pH was recorded for hot (at 0 hour) and cold (placed in the refrigerator for 24 hours) from 2 locations and left side of all samples. To determine pH, the probe was inserted into the center of the breast muscle 0.5 to 1 cm below the surface of the muscle and then the pH value was read.



Figure 2.7. pH meter (Mettler Toledo- Swiss) and color detector Chroma-meter Minolta CR-400, Konica Minolta Sensing, Osaka, Japan

2.3.4. The Color of the Carcass

The color of the meat was obtained using a laboratory color detector, where the color was taken for Meat from 24 h after the slaughter. Breast and thigh (from 1 point and left side of all) breast and thigh. Meat color was determined by using a Chroma Meter CR-400, Japan (figure 2.7); with a color measuring area of 50 mm in diameter in CIE color system $L^*a^*b^*$. The L^* value represents lightness, ranging from 0 for black to 100 for ideal white. The a^* and b^* are color coordinates ($+a^*$ = redness, $-a^*$ = green, $+b^*$ =

yellow, $-b^* = \text{blue}$). The device was calibrated using a Minolta calibration tile ($L^* = 93.8$, $a^* = 0.3136$, $b^* = 0.3192$).

2.4. Characteristics of Meat

2.4.1. Cooking Loss (%)

The samples of the breast meat from 24h after the slaughter were weighed in different weights after being naturalized and placed in closed plastic bags. Meat samples pre-weighed and boiled at $80\text{ }^{\circ}\text{C}$ for 1 hour. Cooked meat samples were cooled and dried and re-weighed (figure 2.8), and then the extracted loss of cooking was measured according to the following equation

$$\text{Cooking loss} = \text{weight after cooking} / \text{weight before cooking} * 100$$

$$\text{Cooking loss percentage\%} = 100 - \text{cooking loss.}$$


Figure 2.8. Cooking loss measurement (water bath GFL 1086)

2.4.2. Drip Loss (%)

Breast meat from 24 h after slaughter samples were pre-weighed and placed in closed plastic bags and hung with a rope at 4°C for 24h. After 24h, the breast meat stored in the refrigerator was reweighed (Figure 2.9) and then extracted. The loss of refrigerating was measured according to the following equation

$$\text{Drip lose} = \text{Weight after refrigerating} / \text{weight before refrigerating} * 100$$



Figure 2.9. Breast placed in the refrigerator for 24 hours.

2.4.3. Dry Matter (%)

The dry matter samples (24h after slaughter) were determined according to AOAC (2001) [105].

Samples of the breast were weighed in different weights after being naturalized and placed in different sizes of sulfonic cups, where the weight of these cups was measured and then placed in the laboratory oven cups + breast samples for 12 hours under the temperature of 105 °C. (Figure 2.10).

And after extraction, the weight of the meat was measured after drying according to the following equation:

$\text{Weight of meat after drying} = \text{total weight of meat and cup} - \text{cup weight}$
--

After obtaining the weight of dry meat, the dry matter ratio was measured according to the following equation:

$\text{Dry matter ratio\%} = \text{weight of meat after drying} / \text{weight of meat before drying} * 100$
--



Figure 2.10. Laboratory oven (12 hours under the temperature of 105 °C)

2.4.4. Humidity (%)

Humidity was obtained using the following equation:

$\text{Humidity ratio \%} = 100 - \text{Dry matter ratio}$
--

2.4.5. Crude Protein (%)

The crude protein of meat samples (24h after slaughter) were determined according to AOAC (2001) [105].

It can be seen that protein was obtained using the kjeldahl protein analysis method, with the use of a burning device and distillation device.

After drying the samples in the oven, as mentioned above, the samples were grinded by a small milling machine, and then (0.200 g) (figure 2.11) of each sample was weighed and placed in a glass tube. Then, 1 kjeldahl tablet was placed in each tube (each tablet weighed 5 g) and 10 ml of sulfuric acid (H_2SO_4) .was used per tube [105].

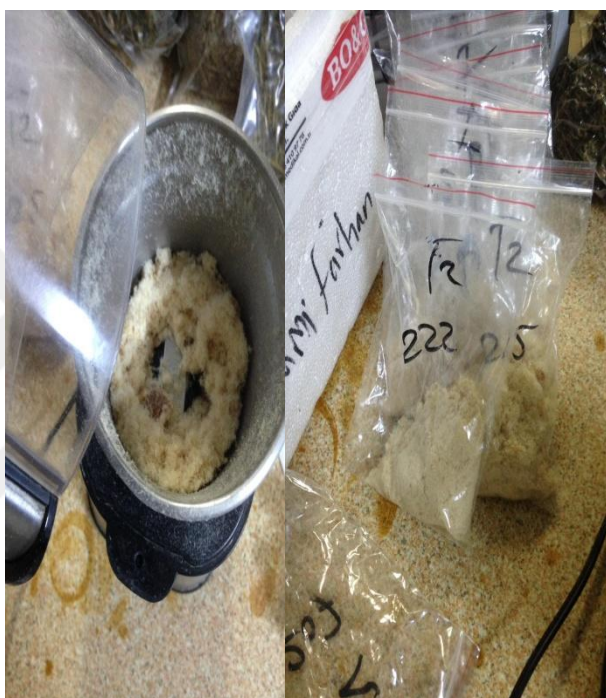


Figure 2.11. Small milling machine

The tubes were placed in a special device called a burner. This device contains a container in which the batteries are placed vertically under different temperatures. The temperature of the device is gradually raised by a button in the device as shown below:

150 °C- 30 minutes

200 °C- 30 minutes

250 °C- 30 minutes

300 °C-30 minutes

350 °C- 60 minutes until the color of the solution in the tube turns green. (Figure 2.12)

The distillation process was then carried out using distilled water and sodium hydroxide by the distillation device, where the tube is placed in the device using 10 ml of the boric acid, which is placed in the receptacle flask in the distillation device and (the process of distillation of one tube takes 4 minutes). Where the color of the boric acid turns from red to light blue, the receiver flask with the light blue solution was removed and calibrated with several drops of 0.1 HCl acid until the color turned to light pink and the reading was recorded. (Figure 2.12)



Figure 2.12. The crude protein processing devices

The crude protein ratio was then obtained according to the following equation:

$$\text{C.P \%} = [0.014 \times 0.1 \times 100 \times [\text{titration value} - 0.25 / \text{sample weight}]] \times 6.25$$

2.4.6. Crude Ash (%)

The ash content of meat samples (24h after slaughter) were determined according to AOAC (2001).[105]

After drying and grinding the samples as previously mentioned and showed by Figure 2.11, the samples were weighed (0.980-1000 mg) and placed in small ceramic jars with

different weights, where the weights of these jars were taken and then placed in the Ash Oven under a temperature of 600 m for 3 hours. When the jars were removed, they were left to cool. After that, they were placed in the Glassware (a tighten glass container) that prevents the entry and exit of air. Then, the jars' weights were taken with the ash (Figure 2.13) and the ash ratio was obtained using the following equation:-

$$\text{Ash\%} = \text{ash weight} / \text{sample weight before incineration} * 100$$

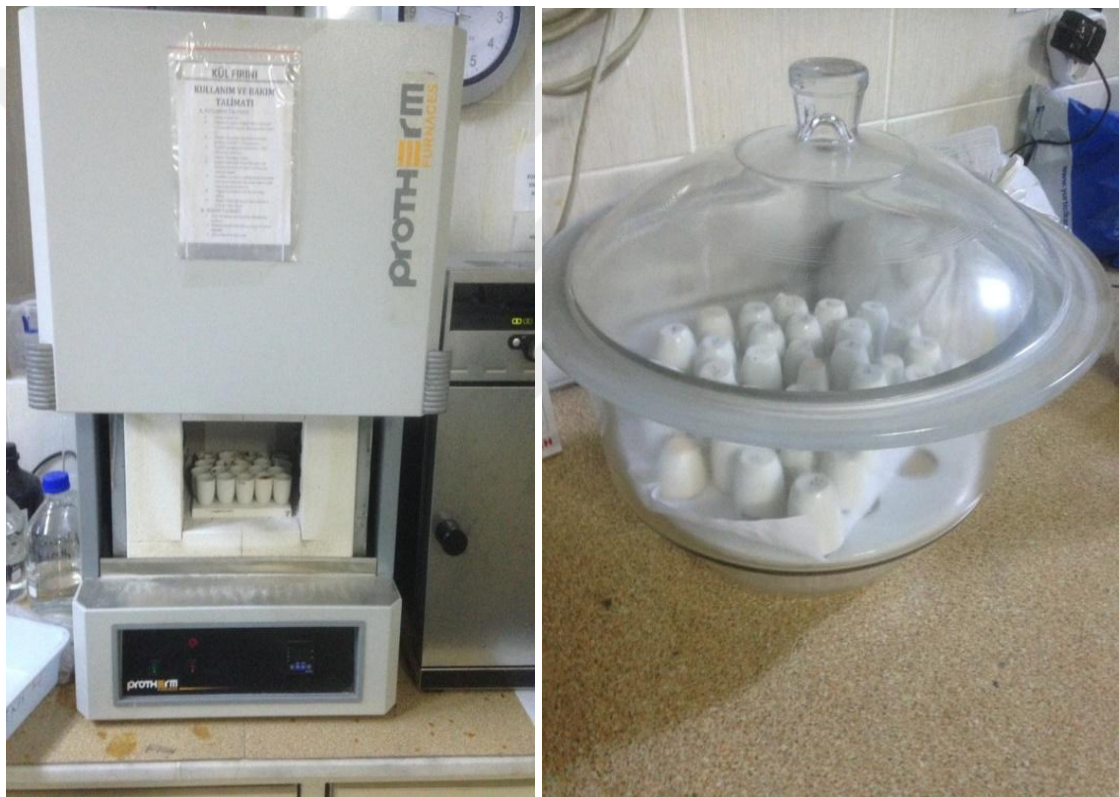


Figure 2.13. Ash oven and desiccator

2.4.7. Ether Extract (%)

Fat content in the meat samples were determined by using a Soxhlet extractor (Velp SER 148/6, Velp Scientifica, Milano, Italy) according to method described by Honikel Karl (1998) [106]. Fat has been determined by Soxhlat extraction using petroleum ether (Figure 2.14).



Figure 2.14. Fat extraction devices

2.5. Blood Profile Analysis

Four blood samples per cage (120 birds) were obtained by venipuncture of the neck vein at 42 days for blood biochemical analysis. Then, the blood samples were kept on ice and transferred to the laboratory where they were centrifuged at 1500 g for 10 min. The serums were removed and stored at -20 °C until analyzed (Figure 2.15.). Blood parameters (PCV, Hb, WBC) were analyzed at Veterinary faculty/ Erciyes University (Figure 2.16.).



Figure 2.15. Collect blood samples for analysis



Figure 2.16. Blood parameters analysis at Veterinary faculty /Erciyes University

2.5.1. Packed Cell Volume (PCV) % (Hematocrit)

The PCV was determined by the method described by Benjamin [107]. The micro hematocrit tube was filled up to the mark with the well mixed heparinized blood and then the capillary tubes were placed in the centrifuge for 5 minutes at 1500 rpm. Then, the tubes were removed and the percent values of PCV were recorded directly using the special ruler (this was done at 42 days of age) at VET faculty /Erciyes University (Figure 2.16.).

2.5.2. Hemoglobin Concentration (Hb)

The determination of hemoglobin concentration was done using the Spectrophotometer apparatus and Hematocrit solution which has been specialized for hemoglobin concentration measurement. The blood was pulled by special pipette to 0.5 and mixed with 5 milliliters of the solution and left for 5 min until it become constant, and then it has been recorded by the Spectrophotometer apparatus according to Jain [108]. This was done at the VET faculty /Erciyes University (Figure 2.16.).

2.5.3. White Blood Cells (WBC) and H/L %

At 42 days of age, blood was obtained via wing vein (brachial vein) and was collected in tubes containing acid citrate dextrose (ACD) as anticoagulant. Two drops of blood were placed on the slide, and blood smear was prepared using Geimsa stain according to Cross et al. [109]. All slides were coded and the ratio of white blood cells (WBC), such as Lymphocytes%, Heterophile%, Monophile%, Esonophile%, Basophile%, H/L, was

counted to a total number of 100 cells per slide. This was carried out at the VET faculty/Erciyes University (Figure 2.16.).

2.5.4. Blood Immunity

In this research the blood immunity measurement was conducted by measuring Albumin, Globulin, A/G Ratio, GOT, GPT, IL-2, IL-10 and TNF-2 α ELISA in serum of broiler blood, as illustrated below.

2.5.4.1. Albumin, Globulin, A/G Ratio, GOT, GPT

Albumin is typically measured in blood serum and is the majority portion of serum protein. The liver produces it using amino acids that are obtained from food. Albumin is beneficial towards the body removing waste, regulating osmotic pressure, and in the transportation of nutrients. Instances of liver failure, myelomas, fevers, infections, malnutrition and other diseases usually reflect lessened levels of albumin in the body. Albumin was measured using the BT3000 Plus Otoanalyzer.

Globulin is a larger protein than albumin and is important for its immunologic responses especially its gamma portion (IgA, IgG, IgM and IgE). Globulin has many diverse functions such as serving as the carrier of some hormones, lipids, metals and antibodies and can be measured in blood serum. When chronic infections, liver disease, rheumatoid arthritis, myelomas and lupus are present, elevated levels are seen. It has been observed that lower levels are present in immune compromised animals, poor dietary habits, Malabsorption and liver or kidney disease. A/G Ratio (Albumin/Globulin Ratio) is an important indicator of disease states although a high level is not considered clinically significant.

GOT (AST) and GPT (ALT): The glutamic oxaloacetic transaminase is GOT also known AST (aspartate aminotransferase) and glutamic pyruvic transaminase is GPT also known ALT (alanine aminotransferase) which are liver blood test (enzyme activities) can be measured in blood serum. In viral hepatitis and other forms of liver disease, there is an association with hepatic necrosis the level of GPT (ALT) at elevated levels, even before the clinical signs and symptoms of disease appear. Noticing these levels can help diagnose body sickness. GOT and GPT were measured by using UV-

1700 Pharmasec, UV-Visible spectrophotometer, Shimadzu (Figure 2.17) at the Veterinary Faculty/Erciyes University (Figure 2.16.).



Figure 2.17. UV-1700 Spectrophotometer, Shimadzu

2.5.4.2. IL-2, IL-10 and TNF- α ELISA

Serum concentrations of IL-2, IL-10 and TNF- 2α were measured by ELISA (enzyme-linked immunosorbent assay) in all treatment groups. Measurement of concentrations of IL-2, IL-10 and TNF- 2α in serum was performed using commercially available kits for the level of cytokines. Immune parameters were measured by IL-2, IL-10 and TNF- 2α ELISA following routine immunization from blood serum. This included specific and quantitative measurements of disease-related proteins.

Analyses of TNF- α , IL-2 and IL-10 in the blood serum were performed by the enzyme immunoassay (EIA) method using a microplate washer (W206, EMP Medical, China) and microplate reader (M201, EMP Medical, China) (Figure 2.18) in the Laboratory of Animal Physiology and Endocrinology at Erciyes University, Faculty of Agriculture, Department of Animal Science. TNF- α (Sunred, 201-16-0030, China), IL-2 (Sunred, 201-16-0014, China) and IL-10 (Sunred, 201-16-0011, China) concentrations were determined using species-specific commercial ELISA kits. Assay ranges of the kits were 1-300 ng/L, 0.15-400 ng/L and 0.5-120 pg/mL for TNF- α , IL-2 and IL-10, respectively. The Eliza analyses were measured by following the kits' guide (Sunred Company, China) and the summary procedures of chicken Elisa kit in (Figure 2.19) at the laboratory of Animal Science Department Erciyes University.



Figure 2.18. Microplate washer and microplate reader

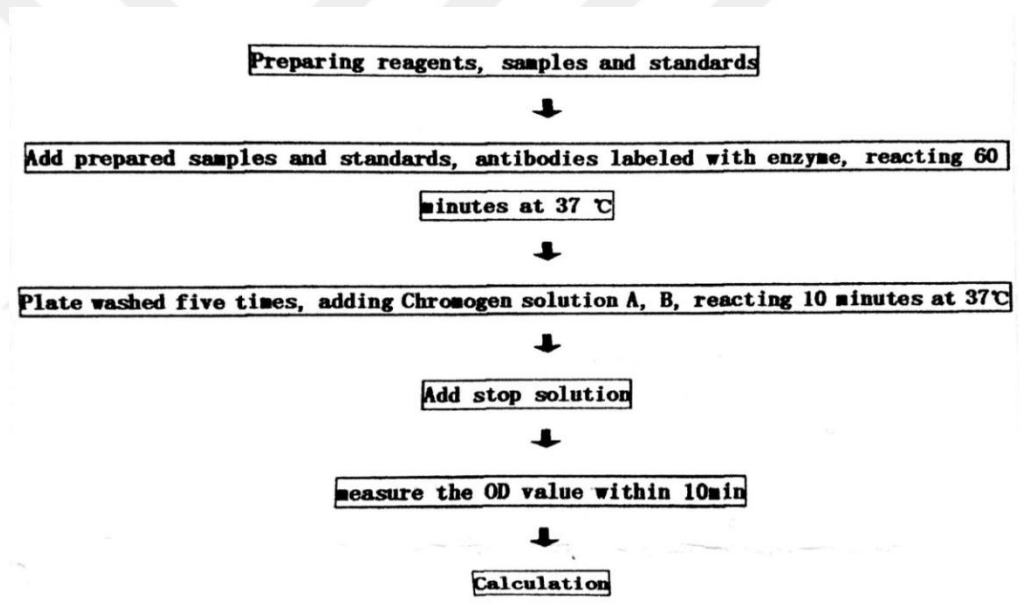


Figure 2.19. Summary procedures of chicken elisa kit (Sunred Company, China)
(Adapted from chicken kit instruction, Sunred company -China)

2.6. Statistical Analysis

The experiment was conducted as a completely randomized design. Data were analyzed by GLM procedure of SAS. Duncan's multiple range tests were used to compare differences among treatment means (SAS, Institute, 1992). Values were considered statistically at $p < 0.05$.

CHAPTER 3

RESULTS

3.1. Productive Parameters

3.1.1. Body Weight

The body weight of the experimental birds during the study was measured at different age and is presented in the Table 3.1.

Table 3.1. Body weight (g)

Traits	Treatments ¹						
	T1	T2	T3	T4	T5	Pooled SEM	<i>p</i> -value
Body weight (g)							
1 d	43.4 ^{ab}	43.9 ^a	43.5 ^{ab}	42.4 ^b	43.9 ^a	0.40	0.0366
21 d	721 ^c	805 ^b	801 ^b	714 ^c	826 ^a	6.21	0.0001
42 d	1844 ^c	2014 ^{ab}	2035 ^b	1824 ^c	2121 ^a	25.08	0.0001

a-c Mean within the same row with no common superscripts are differ significantly ($P < 0.05$).

¹T1 =Control no added selenium or oregano essential oil, T2= diet+Oregano essential oil 300 mg/kg, T3= diet+ Organic Selenium 0.3 mg/kg, T4=diet+ Inorganic Selenium 0.3 mg/kg, T5= diet+ Nano Selenium 0.3 mg/kg.

The results showed that the BW was affected by dietary treatment. Chicks fed diet supplemented with organic, oregano or Nano selenium achieved better performance than control and non-organic selenium, as shown in Table (3.1). Also, in Figure (3.1) and (3.5) body weight differences are explained clearly.

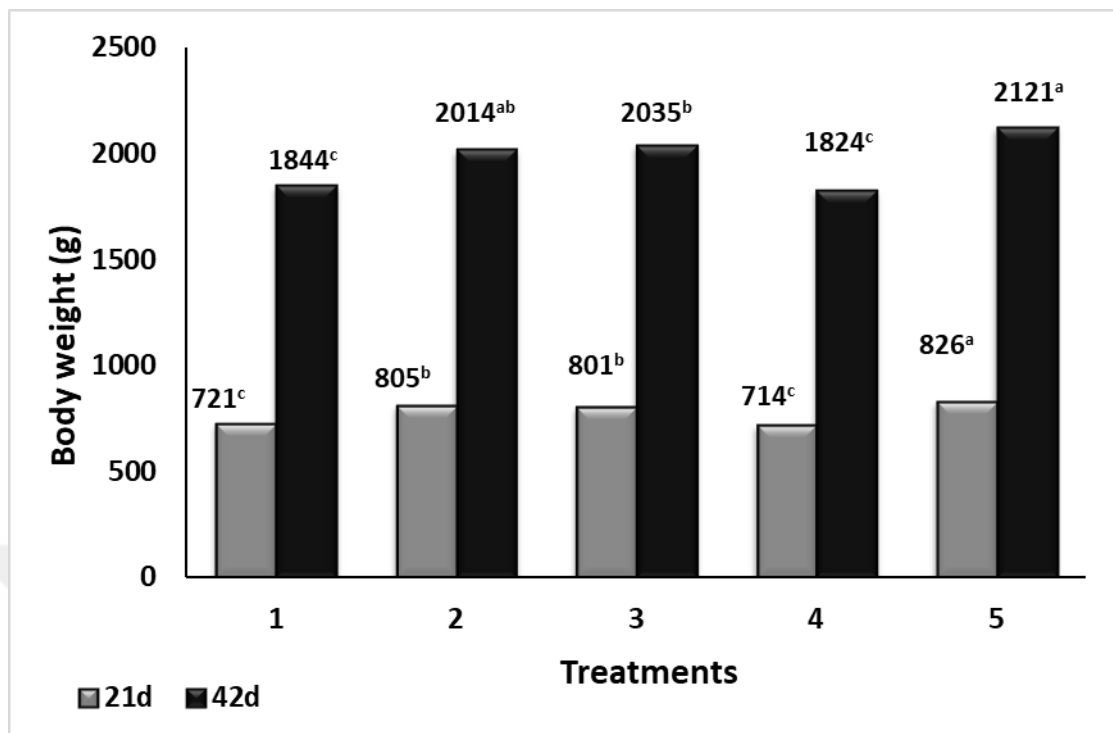


Figure 3.2. The effect of additives in diet on body weight at 21 d and 42 d.

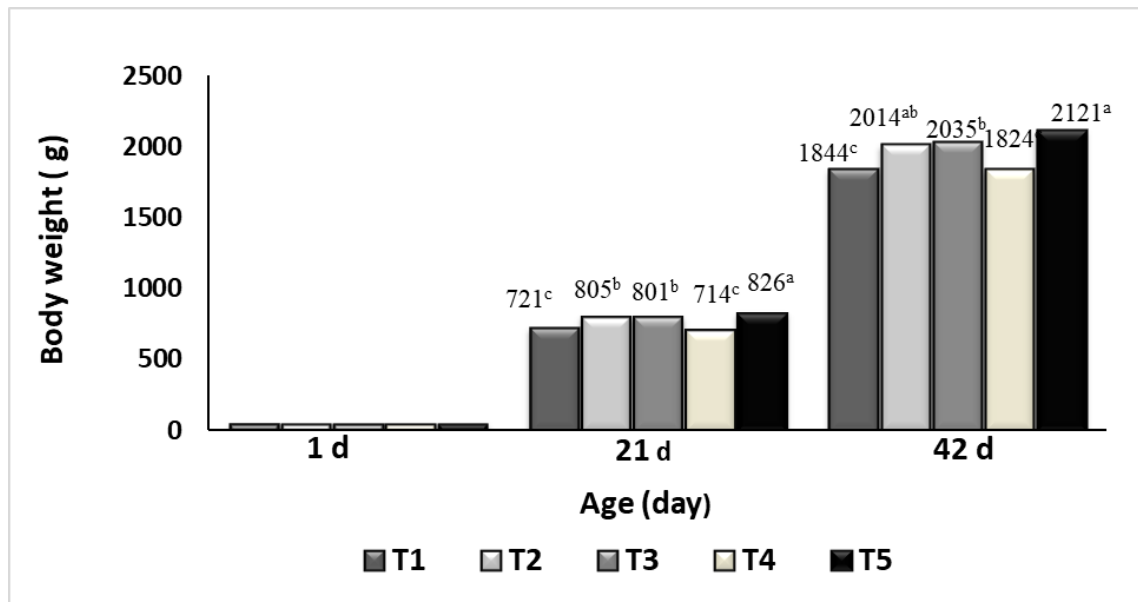


Figure 3.3. The effect of additives in diet on body weight at 1 d, 21 d and 42 d.

3.1.2 Body Weight Gain (g)

Body Weight gain during the study is shown in the Table 3.2

Table 3.2. Daily weight gain (g/bird/d) of treatments

Traits	Treatments ¹						
	T1	T2	T3	T4	T5	Pooled SEM	p-value
Daily weight gain (g/bird/d)							
1 to 21 d	32.3 ^c	36.2 ^b	36.1 ^b	32.0 ^c	37.7 ^a	0.24	0.0001
21 to 42d	53.5 ^b	61.8 ^a	58.8 ^a	52.8 ^b	61.6 ^a	1.56	0.0001
1to 42 d	42.9 ^c	49.0 ^{ab}	47.4 ^b	42.2 ^c	49.5 ^a	0.60	0.0001

a-c Mean within the same row with no common superscripts are differ significantly (P<0.05).

¹T1 =control no added selenium or oregano essential oil, T2= diet +Oregano essential oil 300 mg/kg, T3= diet+ Organic Selenium 0.3 mg/kg, T4=diet+ Inorganic Selenium 0.3 mg/kg, T5= diet+ Nano Selenium 0.3 mg/kg.

The results showed that the WG was affected by dietary treatment. Chicks fed diet supplemented with organic, oregano or nano selenium achieved better performance than control and non-organic selenium, as shown in table (3.2) and Figure (3.3).

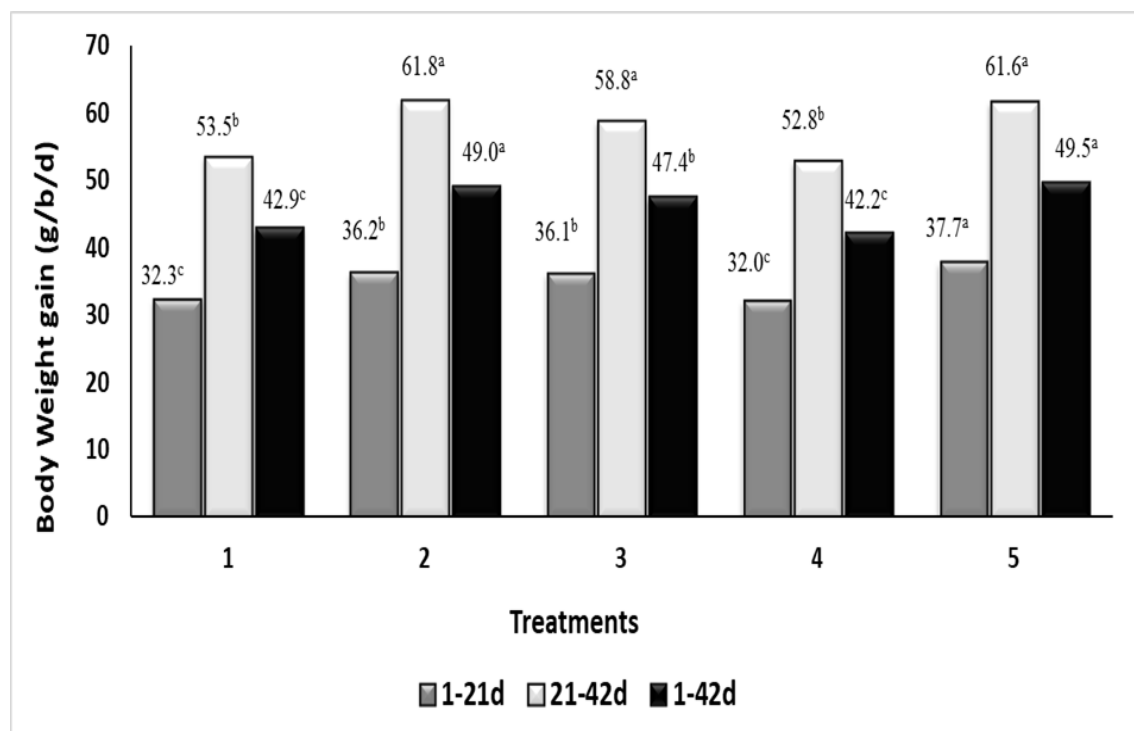


Figure 3.4. The effect of diet additives on body weight gain (1-21 d, 21-42 d and 1-42 d)

3.1.3. Feed Consumption (g)

Feed consumption during the experiment was recorded. The data were documented weekly for six consecutive weeks. The overall feed intake is described in Table (3.3).

Table 3.3. Feed consumption (g/bird) of treatments

Traits	Treatments ¹						
	T1	T2	T3	T4	T5	Pooled SEM	P- value
Feed consumption (g/bird)							
1to 21 d	44.4 ^b	48.1 ^a	47.2 ^a	43.5 ^b	48.0 ^a	0.47	0.0001
21 to 42d	102.0 ^b	117.2 ^a	111.6 ^{ab}	102.7 ^b	116.9 ^a	1.81	0.0029
1to 42 d	73.2 ^b	82.7 ^a	79.4 ^a	73.1 ^b	82.5 ^a	1.07	0.0004

a-c Mean within the same row with no common superscripts are differ significantly (P<0.05).

¹T1 =control no added selenium or oregano essential oil, T2= diet + Oregano essential oil 300 mg/kg, T3= diet+ Organic Selenium 0.3 mg/kg, T4=diet+ Inorganic Selenium 0.3 mg/kg, T5= diet+ Nano Selenium 0.3 mg/kg.

The results showed that the feed intake was affected by dietary treatment. Chicks fed diet supplemented with organic, oregano or Nano selenium achieved better performance than control and non-organic selenium, shown in Table (3.3) and Figure (3.4).

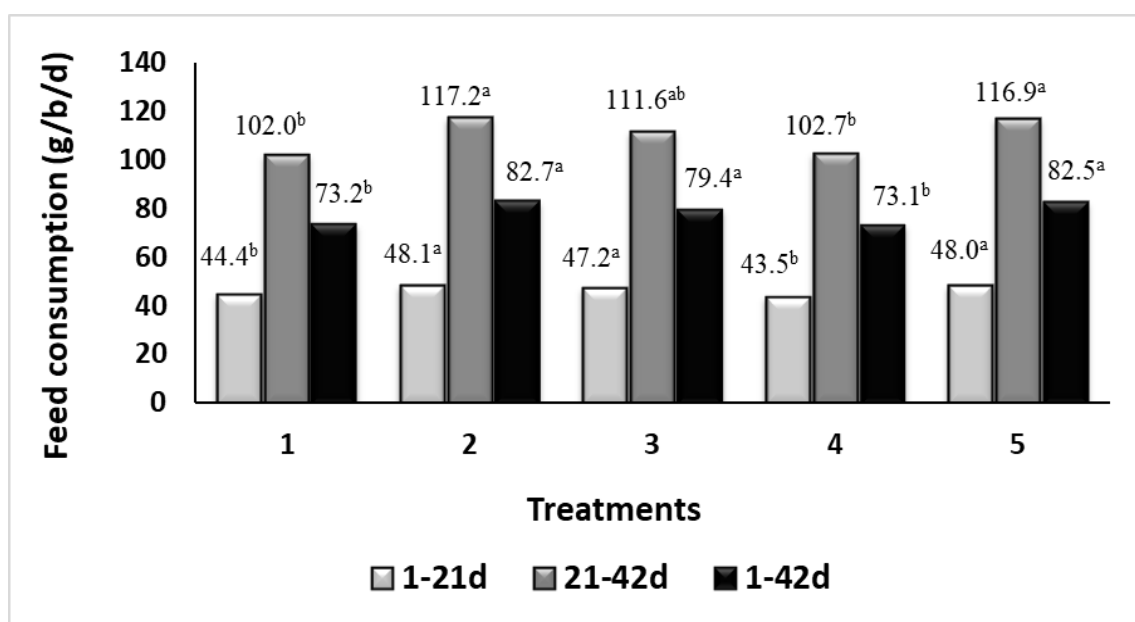


Figure 3.5. The effect of diet additives on feed consumption at 1-21 d, 21-42 d and 1-42 d.

3.1.4 Feed Conversion Ratio (%)

The data of FCR% during the six weeks of the study are presented in Table (3.4).

Table 3.4. Feed conversion ratio (g feed: g gain)

Traits	Treatments ¹						
	T1	T2	T3	T4	T5	Pooled SEM	<i>p</i> -value
Feed Conversion Ratio (g feed: g gain)							
1 to 21 d	1.37 ^a	1.33 ^b	1.31 ^{bc}	1.36 ^a	1.29 ^c	0.007	0.0001
21 to 42d	1.91 ^{ab}	1.90 ^b	1.90 ^b	1.94 ^a	1.90 ^b	0.006	0.1049
1 to 42 d	1.71 ^{ab}	1.69 ^{bc}	1.67 ^c	1.72 ^a	1.67 ^c	0.005	0.0003

a-c Mean within the same row with no common superscripts are differ significantly ($P < 0.05$).

¹T1 =control no added selenium or oregano essential oil, T2= diet+Oregano essential oil 300 mg/kg, T3= diet+ Organic Selenium 0.3 mg/kg, T4=diet+ Inorganic Selenium 0.3 mg/kg, T5= diet+ Nano Selenium 0.3 mg/kg.

The results showed that the FCR was affected by dietary treatment. Chicks fed diet supplemented with organic, oregano or Nano selenium achieved better performance than control and non-organic selenium, shown in Table (3.4) and Figure (3.5).

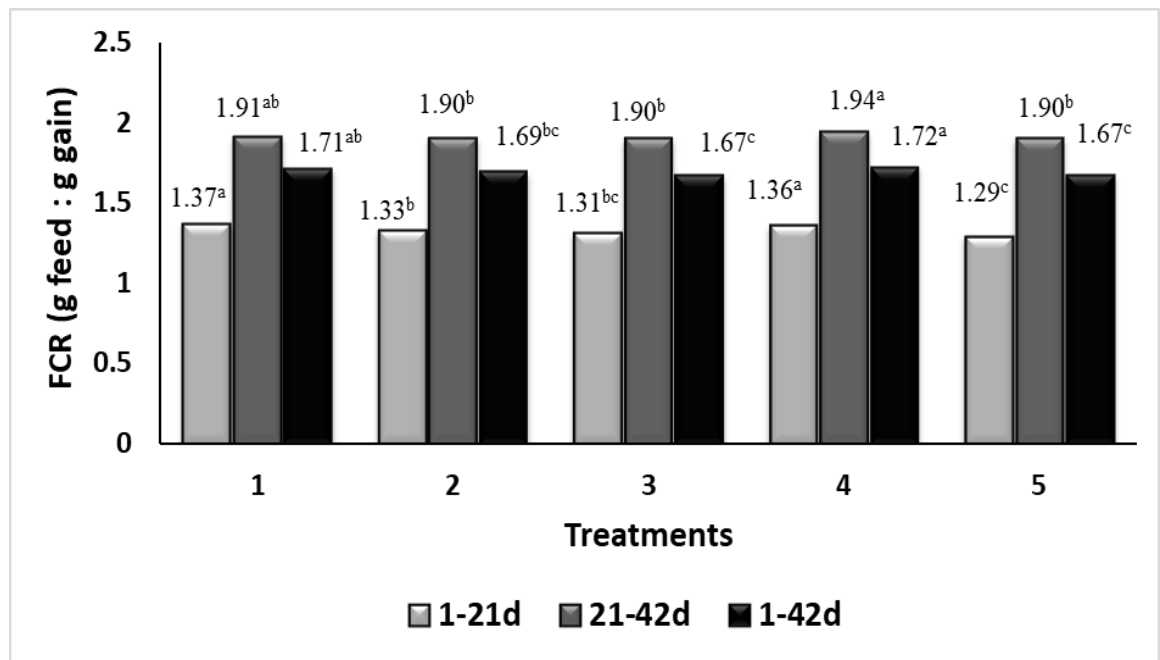


Figure 3.6. The effect of diet additives on FCR ratio at 1-21 d, 21-42 d and 1-42 d.

3.1.5. Production Index (PI)

The data of (PI) of the study are presented in Table (3.5.) To compare the flocks of broilers in a uniform way, often a production index is calculated. This index is calculated with a formula, that takes into account the following: - growth per day, feed conversion and mortality rate. Normally the production index of a flock of broilers runs somewhere between 300 and 350 points. The current experiment showed that the mortality rate was 0%, with the Production Index shown below in table (3.5).

Table 3.5. Effect of diet additives on Mortality % and Production Index

Traits	Treatments ¹						
	T1	T2	T3	T4	T5	Pooled SEM	<i>p</i> -value
Mortality %	0	0	0	0	0		
Production Index PI	250 ^b	290 ^a	283 ^a	245 ^b	296 ^a	4.63	<0.0001

a-c Mean within the same row with no common superscripts are differ significantly ($P < 0.05$).

¹T1 =control no added selenium or oregano essential oil, T2= diet +Oregano essential oil 300 mg/kg, T3= diet+ Organic Selenium 0.3 mg/kg, T4=diet+ Inorganic Selenium 0.3 mg/kg, T5= diet+ Nano Selenium 0.3 mg/kg.

Production Index is the highest in T5, T2 and T3 comparing with T1 and T4 as shown in Table (3.5) and in Figure (3.6).

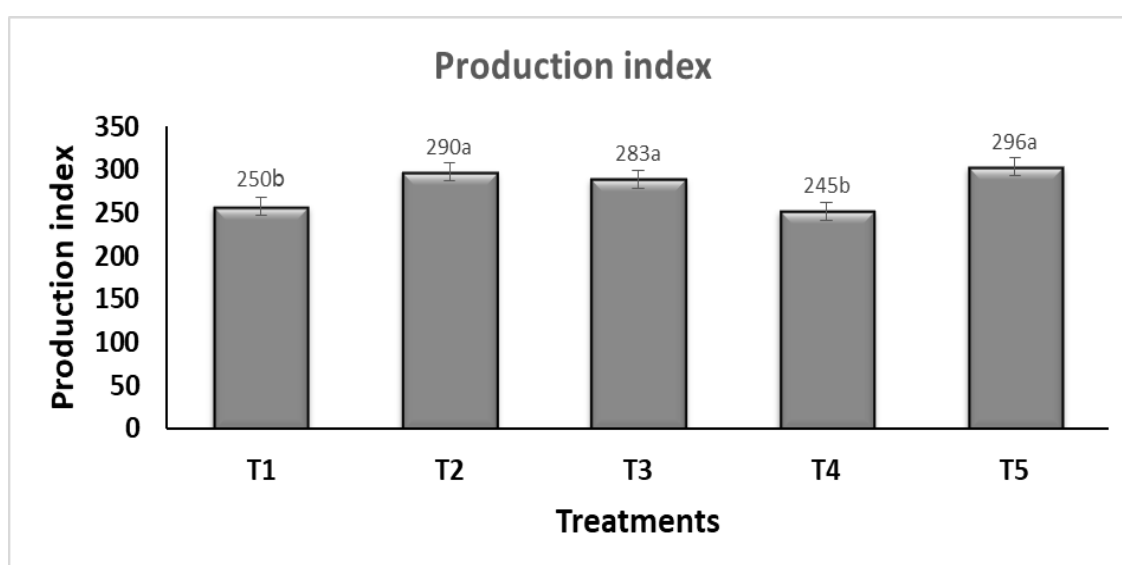


Figure 3.7. Effect of diet additives on production index

3.2. Carcass Parameters

3.2.1. The Carcass and Carcass Cuts Weight (Hot Weight)

Carcass-cut parts at 42 age is shown in the Table (3.6.)

Table 3.6. Carcass parts % of treatments

Traits	Treatments ¹						
	T1	T2	T3	T4	T5	Pooled SEM	p-value
Carcass-cut parts %							
Carcass	71.90 ^b	72.64 ^{ab}	73.37 ^a	72.43 ^b	73.28 ^a	0.13	0.0007
Breast	23.19 ^{ab}	23.41 ^{ab}	23.83 ^a	22.53 ^b	23.16 ^a	0.15	0.0963
Thigh	11.16	10.72	11.08	10.80	10.73	0.07	0.2098
Drumsticks	9.01 ^c	9.42 ^{bc}	9.43 ^{bc}	9.94 ^a	9.79 ^{ab}	0.07	0.0013
Back	15.41	15.44	15.47	15.18	15.47	0.10	0.8904
Nick	4.31 ^b	4.58 ^{ab}	4.93 ^a	4.91 ^a	4.93 ^a	0.08	0.0399
Wing	7.92	7.86	7.50	7.87	7.81	0.8	0.4833
Abdominal fat	0.86 ^c	1.20 ^{ab}	1.09 ^b	1.17 ^{ab}	1.32 ^a	0.03	<0.0001

a-c Mean within the same row with no common superscripts are differ significantly (P<0.05).

¹T1 =control no added selenium or oregano essential oil, T2= diet+Oregano essential oil 300 mg/kg, T3= diet+ Organic Selenium 0.3 mg/kg, T4=diet+ Inorganic Selenium 0.3 mg/kg, T5= diet+Nano Selenium 0.3 mg/kg.

The results showed that the carcass cut parts were affected by dietary treatment. Chicks fed diet supplemented with organic, oregano or nano selenium achieved better performance than control, as shown in Table (3.6) and Figures (3.7), (3.8) and (3.9).

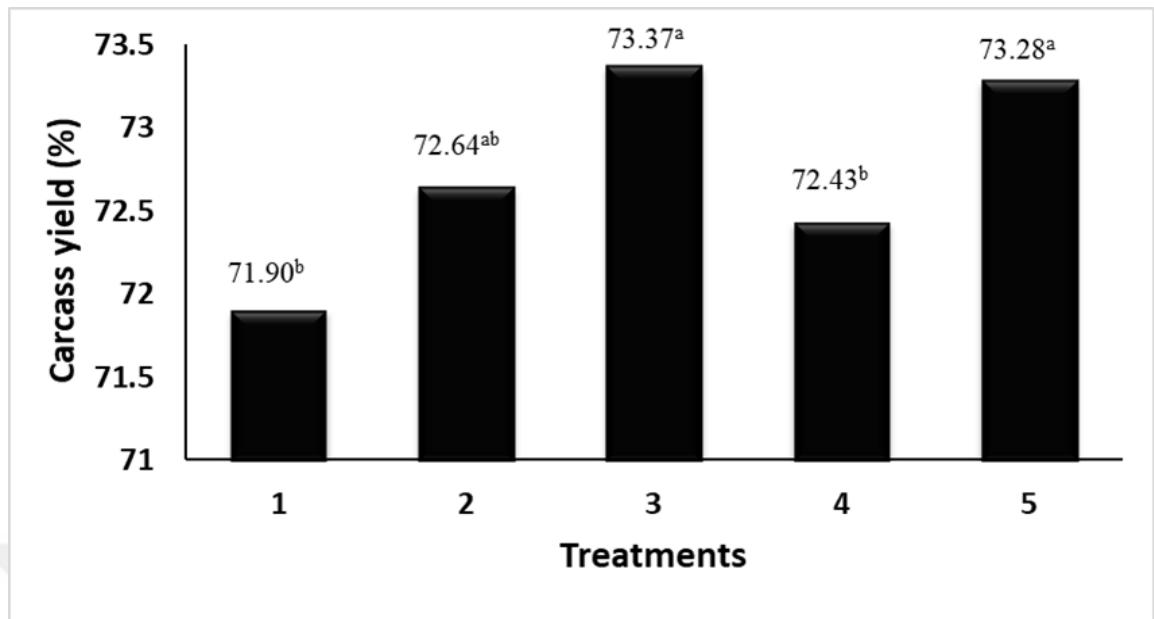


Figure 3.8. The effect of diet additives on Carcass yield at 42 d of age

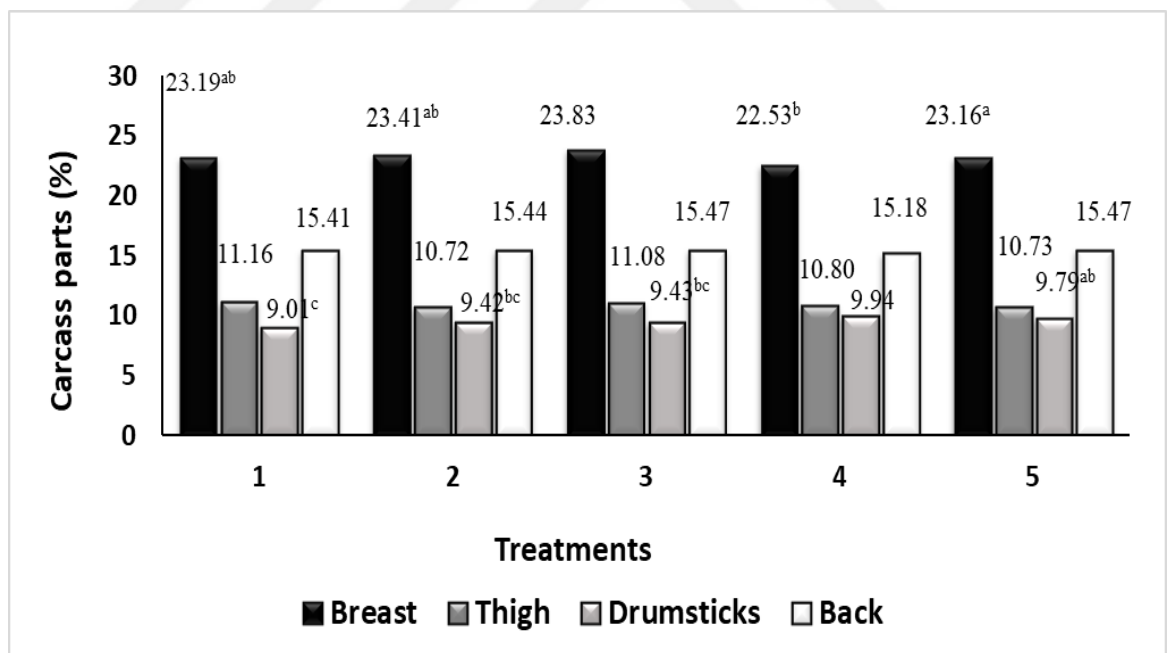


Figure 3.9. The effect of diet additives on carcass parts (breast, thigh, drumstick and back).

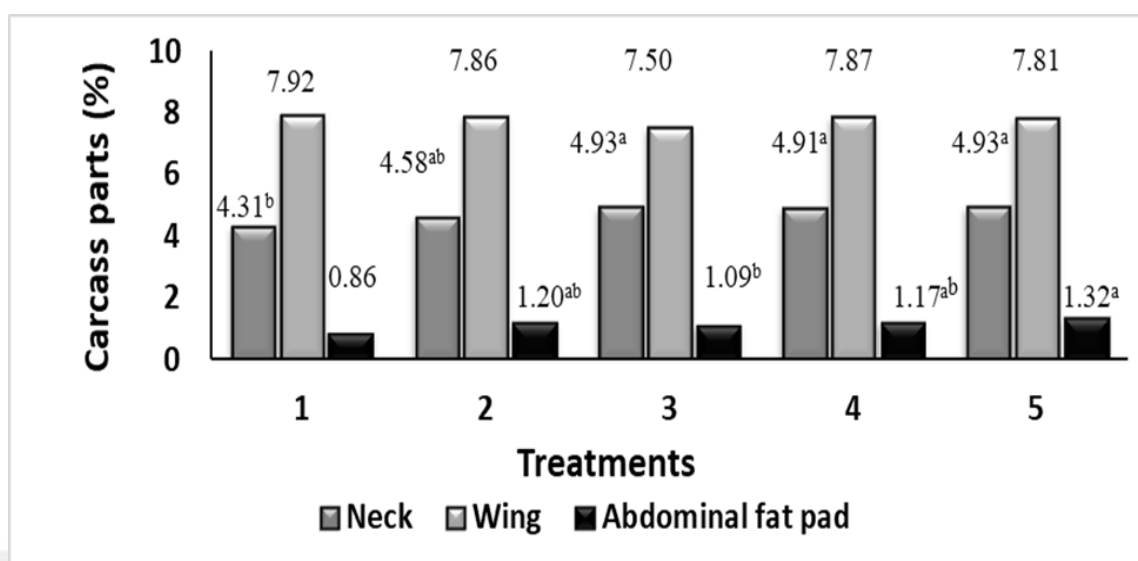


Figure 3.10. The effect of diet additives on carcass parts (neck, wing, and abdominal fat).

3.2.2. Relative Weights of Edible Internal Organs

An edible internal organ at 42 day of age is shown in the Table (3.7.)

Table 3.7. Giblets % of treatments

Traits	Treatments ¹						
	T1	T2	T3	T4	T5	Pooled SEM	<i>p</i> -value
Giblets %							
Heart	0.48	0.42	0.45	0.45	0.45	0.01	0.2086
Liver	1.78	1.70	1.82	1.86	1.79	0.02	0.2477
Gizzard	1.70 ^{ab}	1.5 ^{9b}	1.68 ^{ab}	1.79 ^a	1.55 ^b	0.02	0.0314

a-c Mean within the same row with no common superscripts are differ significantly ($p < 0.05$).

¹T1 =control no added selenium or oregano essential oil, T2= diet +Oregano essential oil 300 mg/kg, T3= diet+ Organic Selenium 0.3 mg/kg, T4=diet+ Inorganic Selenium 0.3 mg/kg, T5= diet+ Nano Selenium 0.3 mg/kg.

Table 3.7 and Figure (3.10) show the output results: There was no significant effect of treatments on Heart% and liver%. However, there was a significant effect on gizzard%. The highest gizzard% was in T4, which was recorded at 1.79% in comparison with others treatments.

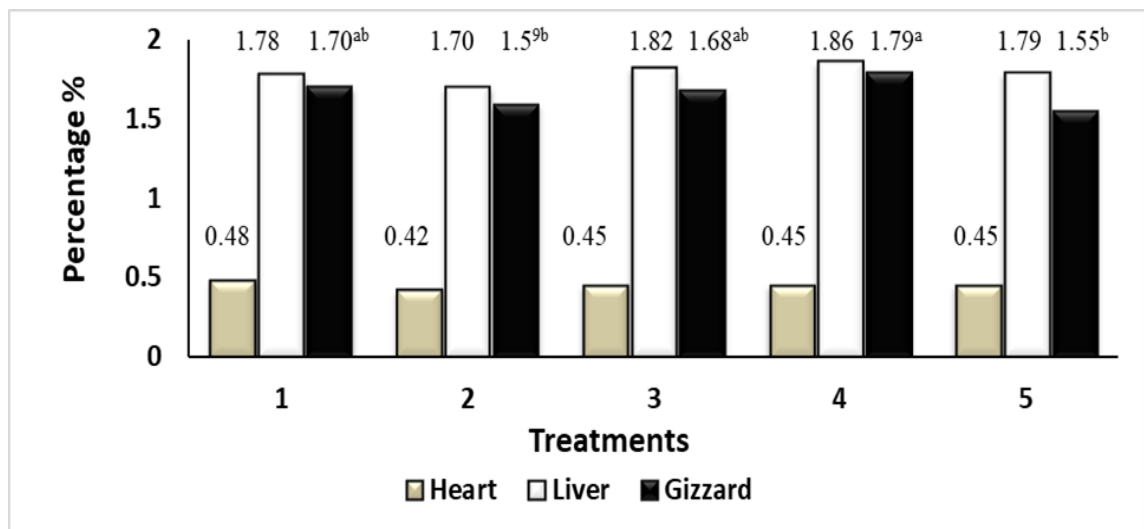


Figure 3.11. The effect of diet additives on edible internal organs %.

3.2.3. Meat Color and pH

Influence of diet additives on Meat color and pH are shown in Table (3.8.).

Table 3.8. Meat color and pH measurements of treatments

Traits	Treatments ¹						
	T1	T2	T3	T4	T5	Pooled SEM	<i>p</i> -value
L*							
Breast	55.03 ^b	54.18 ^b	56.11 ^{ab}	58.17 ^a	54.31 ^b	0.45	0.0290
Thigh	53.69 ^b	51.77 ^b	52.35 ^b	56.27 ^a	53.00 ^b	0.32	<0.0001
a*							
Breast	3.75 ^{ab}	3.61 ^{ab}	2.86 ^b	4.20 ^a	4.17 ^a	0.16	0.0422
Thigh	3.63	3.65	3.23	4.00	3.66	0.11	0.2826
b*							
Breast	6.59 ^{abc}	5.88 ^c	6.40 ^{bc}	7.65 ^a	7.20 ^{ab}	0.18	0.0235
Thigh	5.46 ^{ab}	4.83 ^b	5.76 ^{ab}	5.34 ^{ab}	6.28 ^a	0.15	0.0307
pH0							
Breast	5.71	5.73	5.73	5.73	5.72	0.01	0.9833
Thigh	6.21	6.30	6.29	6.20	6.25	0.02	0.4785
pH24							
Breast	5.76 ^b	5.85 ^a	5.84 ^a	5.82 ^{ab}	5.81 ^{ab}	0.01	0.0708
Thigh	6.22 ^b	6.36 ^a	6.43 ^a	6.34 ^a	6.33 ^a	0.02	0.0032

a-c Mean within the same row with no common superscripts are differ significantly (P<0.05). L*= Lightness, a*= Redness, b*= Yellowness

¹T1 =control no added selenium or oregano essential oil, T2= diet+Oregano essential oil 300 mg/kg, T3= diet+ Organic Selenium 0.3 mg/kg, T4=diet+ Inorganic Selenium 0.3 mg/kg, T5= diet+ Nano Selenium 0.3 mg/kg.

a*, =redness, -a*, = green, b*, = yellow, -b* = blue, L*,=lighter, -L*,=darker

Table containing output results:

- 1- The highest level of L*color was noticed in T4 compared with those of T1, T2 and T5, as shown in Figure (3.11).
- 2- The a* and b*color in the breast was greater in T4 than T1 while other treatments were not different from each other, as shown in Figures (3.12) and (3.13).
- 3- The highest level of L*color in the thigh was noticed in T4 compared with other treatments, as shown in Figure (3.11).
- 4- A significant difference was shown in a* color in the thigh of the T4 group compared with the T3, shown in Figure (3.12).
- 5- PH level was highest after 24 h in diet with additives than control, as shown in Figure (3.15).

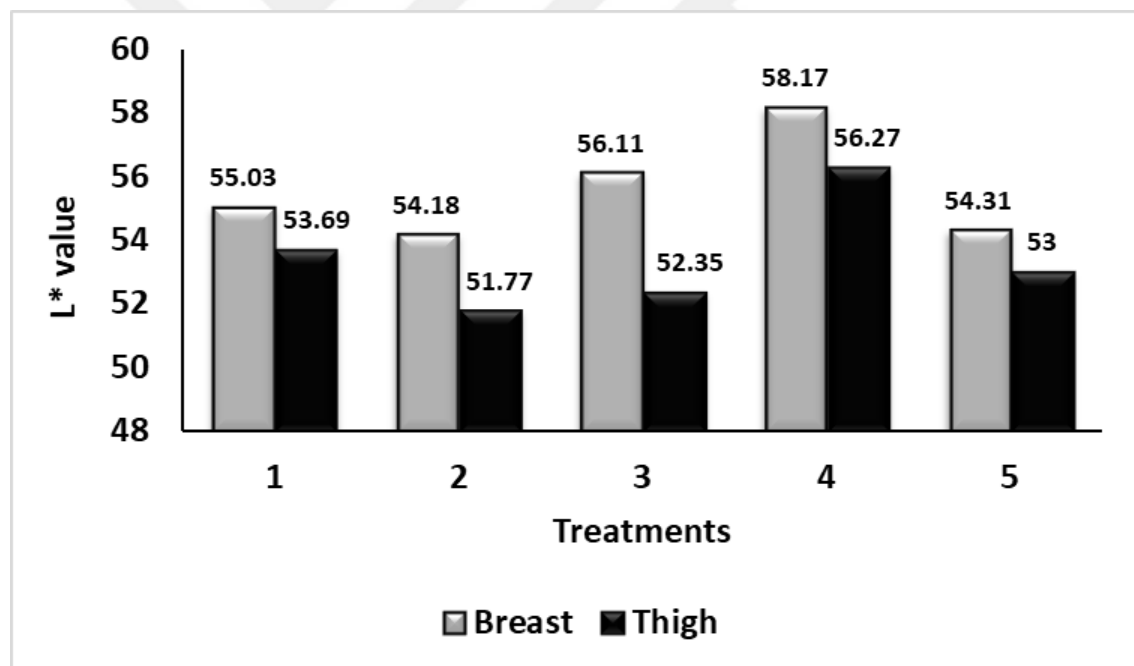


Figure 3.12. The effect of diet additives on meat color L* in breast and thigh.

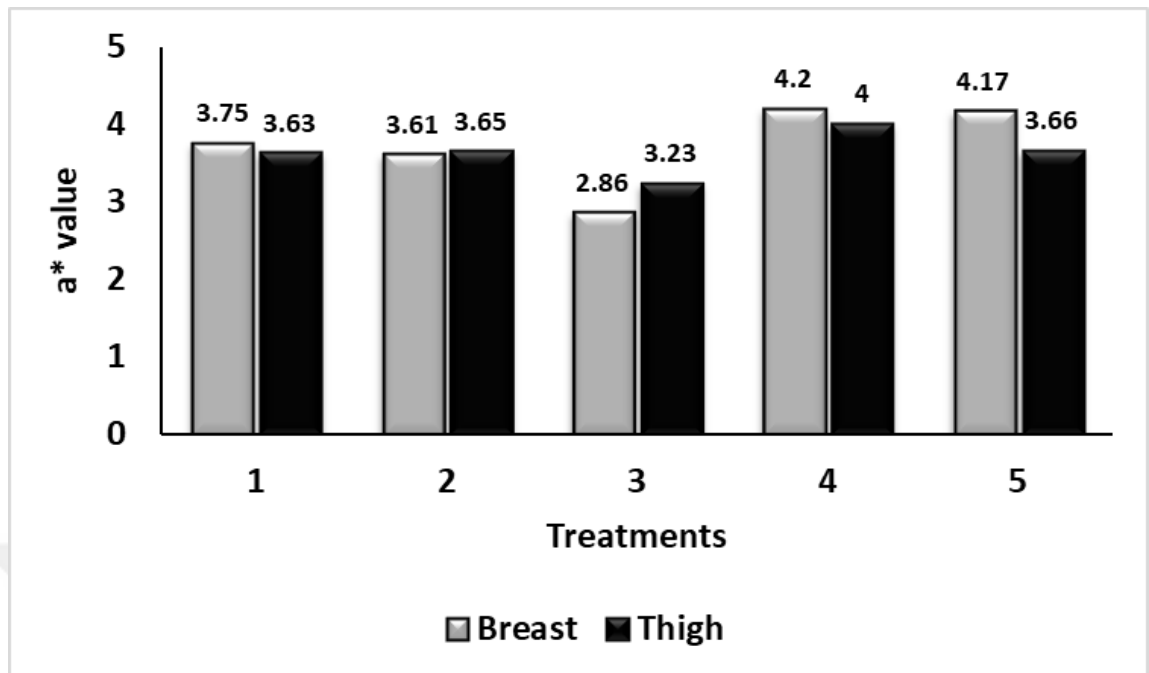


Figure 3.13. The effect of diet additives on meat color a* in breast and thigh.

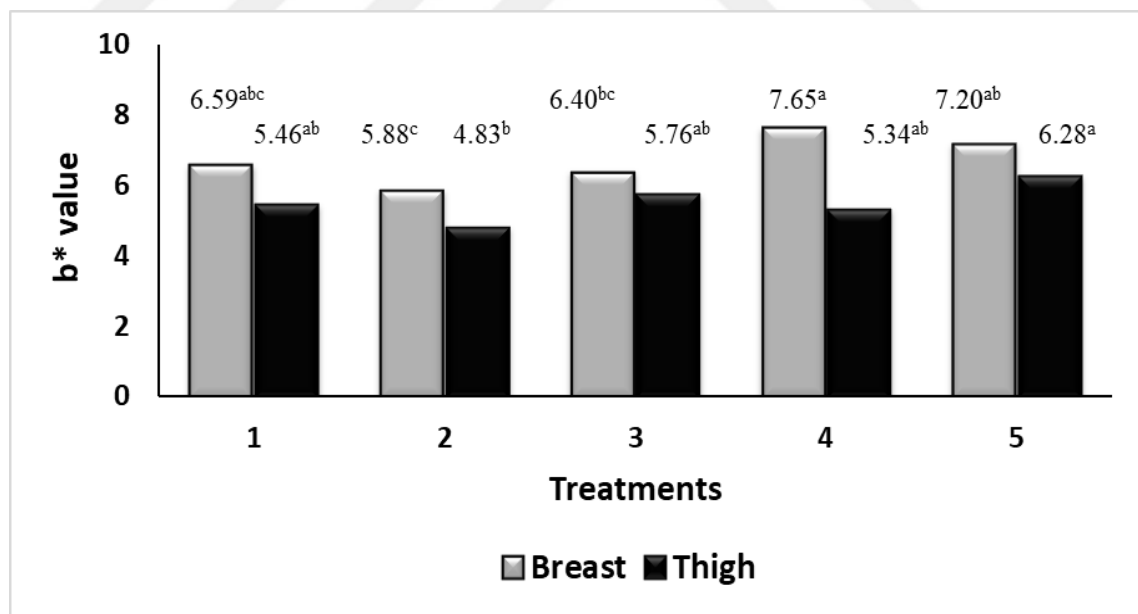


Figure 3.14. The effect of diet additives on meat color b* in breast and thigh.

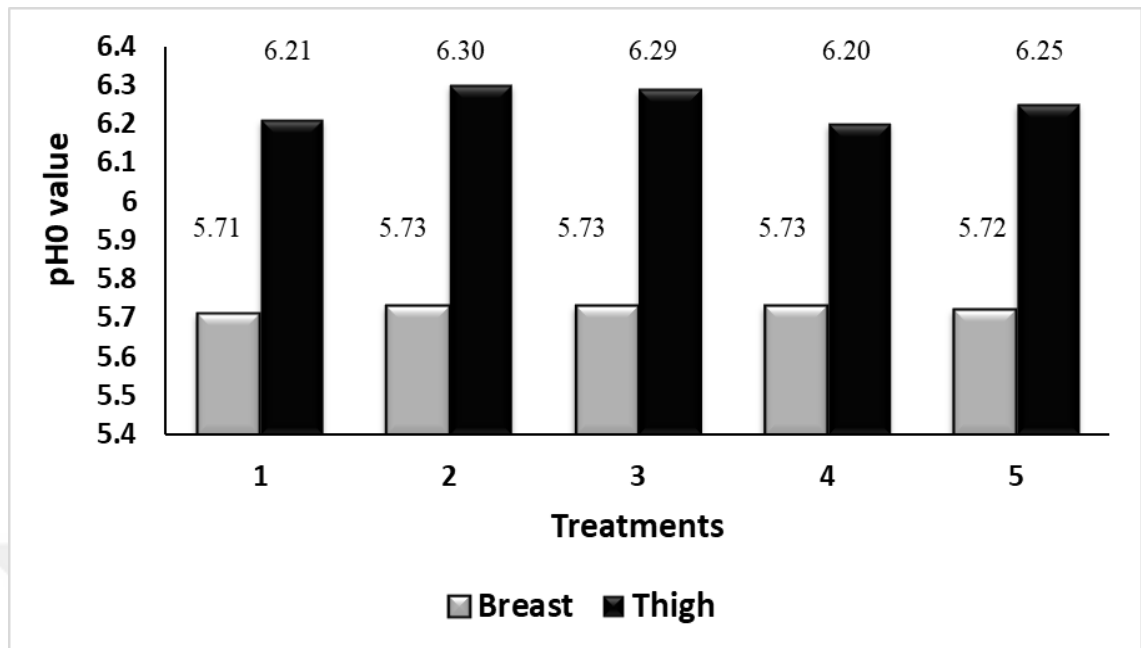


Figure 3.15. The effect of diet additives on meat pH value (0 time) in breast and thigh.

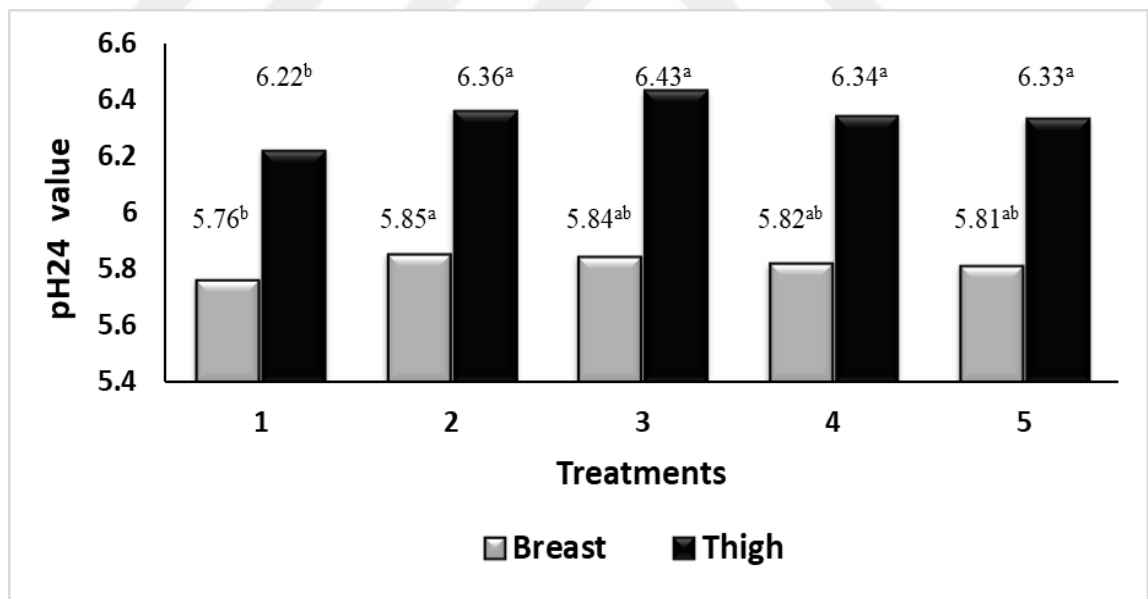


Figure 3.16. The effect of diet additives on meat pH value (after 24 h) in breast and thigh.

3.3. Meat Quality Characteristics

The effect of feed additives on Meat Quality Characteristics (DM, ASH, EE, CP, Moisture and Cooking (%)) of meat are shown at Table (3.9.).

Table 3.9. Meat quality characteristics of treatments

Traits	Treatments ¹						
	T1	T2	T3	T4	T5	Pooled SEM	p-value
DM (%)	25.9	25.9	25.98	25.93	25.96	0.10	0.9954
ASH (%)	1.53 ^a	1.26 ^b	1.37 ^b	1.41 ^{ab}	1.38 ^{ab}	0.02	0.0114
EE (%)	3.2	3.4	3.51	3.34	3.48	0.06	0.5374
CP (%)	21.90 ^a	22.02 ^a	21.92 ^a	20.14 ^b	22.24 ^a	0.13	<0.0001
Moisture (%)	74.1	74.29	74.13	74.07	74.03	0.09	0.9259
Cooking loss (%)	32.8	33.17	34.24	34.09	34.9	0.37	0.3871

a-c Mean within the same row with no common superscripts are differ significantly (P<0.05). DM (%) =Dry matter, EE (%)= Ether Extract, CP (%)= Crude Protein

1T1 =control no added selenium or oregano essential oil, T2= diet +Oregano essential oil 300 mg/kg, T3= diet+ Organic Selenium 0.3 mg/kg, T4=diet+ Inorganic Selenium 0.3 mg/kg, T5= diet+ Nano Selenium 0.3 mg/kg.

Table containing the output results:

1-The results showed that the breast ash was significantly greater in T1 than T2 and T3. This result is shown in Figure (3.17).

2- CP% level was significantly lower in the breast in the bird of T4 compared with other treatments. This result is shown in Figure (3.16).

3- The other parameters were not significantly different due to diet supplementation. This result is shown in Figures (3.16), (3.17) and (3.18).

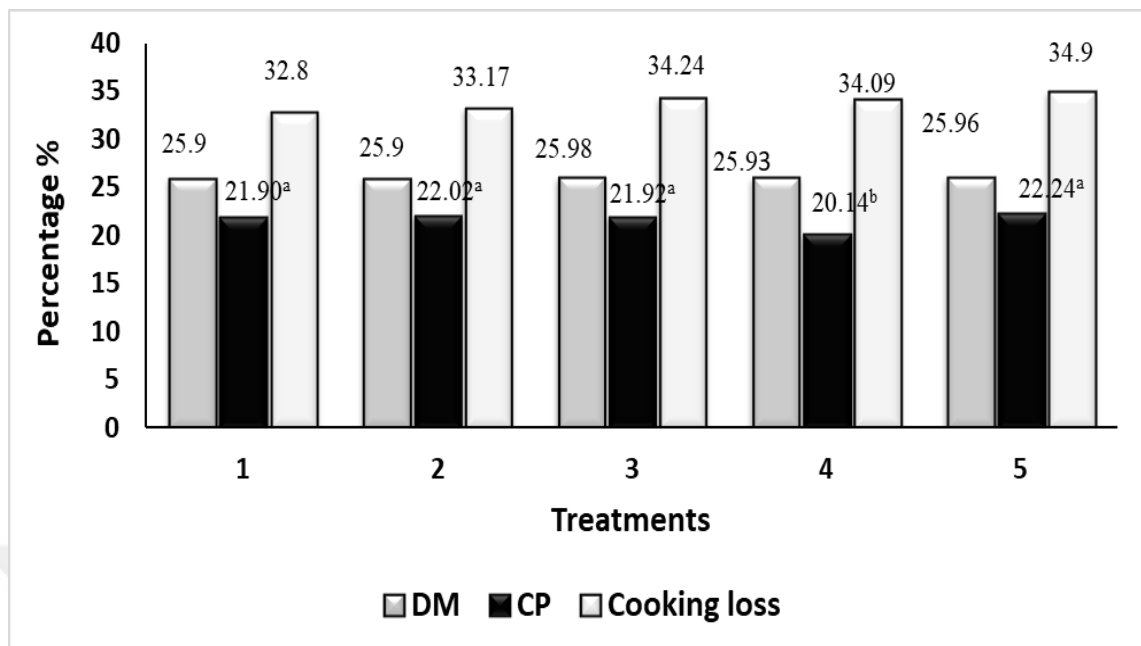


Figure 3.17. The effect of diet additives on meat characteristics (DM, CP and cooking loss)

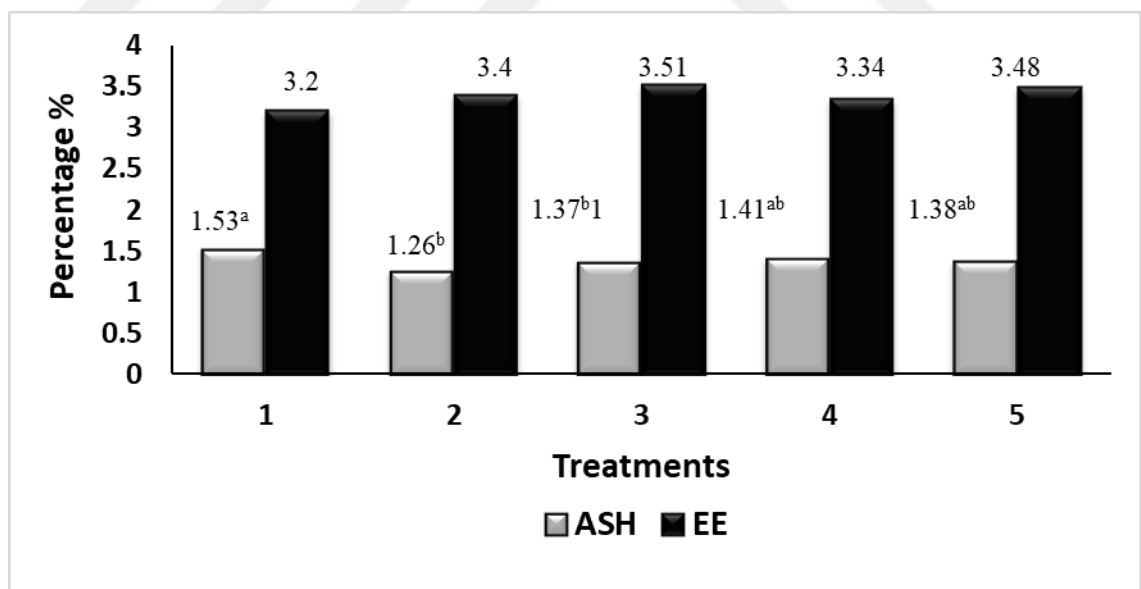


Figure 3.18. The effect of diet additives on meat characteristics (ash and EE).

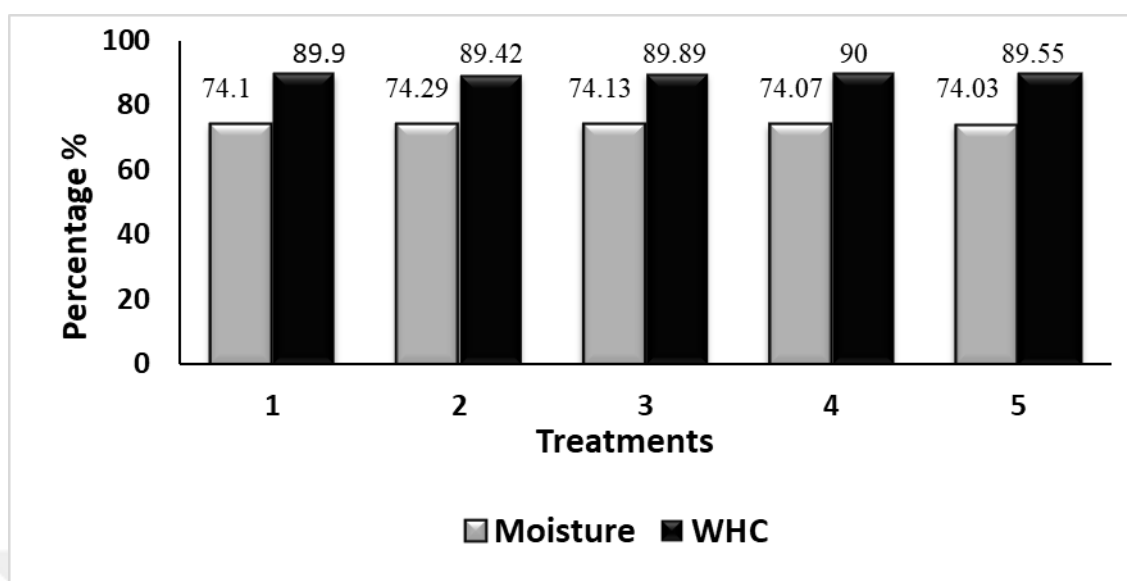


Figure 3.19. The effect of diet additives on meat characteristics (moisture and WHC).

3.4. Immune Competence

The effect of feed additives on immune parameters is shown at Table (3.9.).

Table 3.10. Immune parameters of treatments

Traits	Treatments ¹						
	T1	T2	T3	T4	T5	Pooled SEM	<i>p</i> -value
Blood parameters							
Albumin (g/dl)	0.86 ^b	0.92 ^{ab}	0.99 ^a	0.87 ^b	0.94 ^{ab}	0.01	0.0551
Globulin (g/dl)	1.38 ^b	1.49 ^{ab}	1.58 ^a	1.41 ^b	1.49 ^{ab}	0.02	0.0651
A:G Ratio	62.38	62.18	62.53	63.04	62.88	0.21	0.6735
GOT-AST (U/L)	266.8 ^b	203.7 ^b	223.1 ^b	805.1 ^a	911.8 ^a	43.21	<0.0001
GOP-ALT (U/L)	27.13 ^b	28.10 ^b	28.10 ^b	44.56 ^b	90.19 ^a	4.51	<0.0001
TNF-2a (ng/L)	25.92	35.18	30.88	36.67	35.18	4.89	0.5196
IL-2 (ng/L)	3.64	5.19	4.29	4.09	3.76	0.47	0.3340
IL-10 (pg/Ml)	20.08	17.55	20.04	19.41	16.52	2.33	0.7556

a-c Mean within the same row with no common superscripts are differ significantly ($P < 0.05$).

DM (%) =Dry matter, EE (%)= Ether Extract, CP (%)= Crude Protein

¹T1 =control no added selenium or oregano essential oil, T2= diet +Oregano essential oil 300 mg/kg, T3= diet+ Organic Selenium 0.3 mg/kg, T4=diet+ Inorganic Selenium 0.3 mg/kg, T5= diet+ Nano Selenium 0.3 mg/kg.

Table contained output results

Albumin (g/dl) was highest in T3, T5 and T2, respectively than T1 and T4. Globulin (g/dl) was highest in T3, T5, and T2 when compared with other treatments, as shown in Figure (4.19). For results of A:G Ratio, there was no significant difference among treatments. GOT-AST (U/L and GOP-ALT (U/L) were highest in T5 and T4 compared with others, as shown in Figure (3.20). TNF-a (ng/L) was highest in T4 comparison with other treatments and this result shown in Figure (3.21). IL-2 (ng/L) was highest in T2 compared with other treatments, as shown in Figure (3.22). IL-10 (pg/ml) was highest in T1, T3, and T4 compared with other treatments, while T2 and T5 was the lowest, as shown in Figure (3.23).

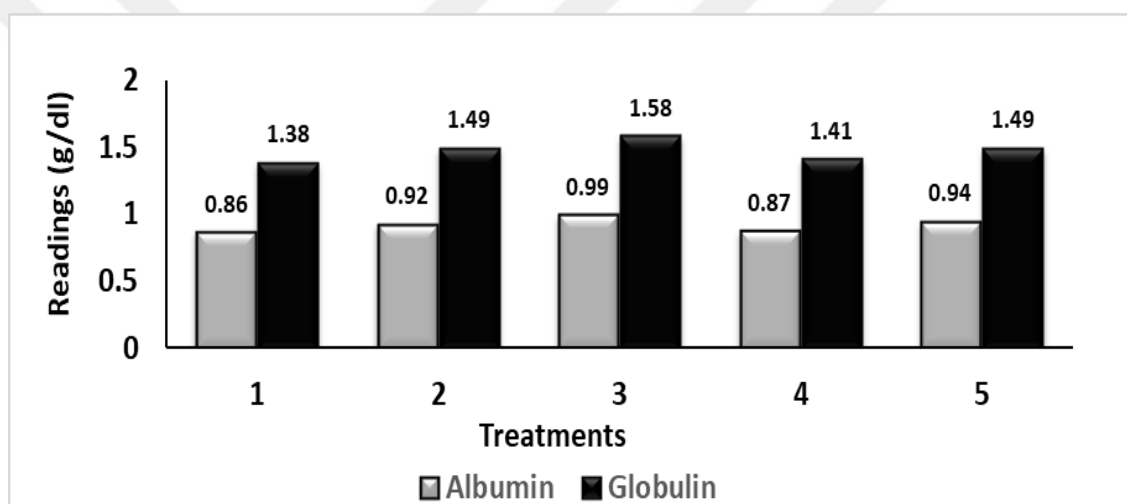


Figure 3.20. The effect of diet additives on immune parameters (Albumin and Globulin)

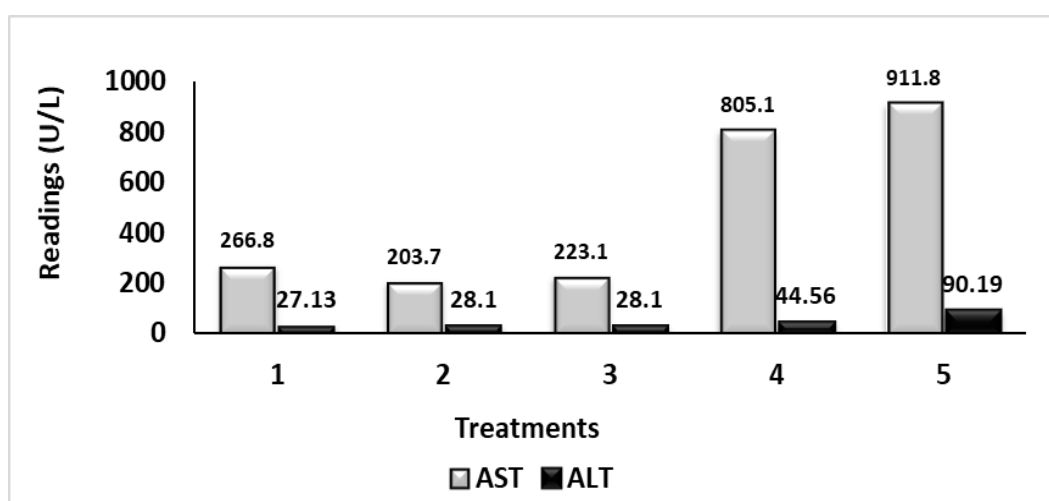


Figure 3.21. The effect of diet additives on immune parameters (AST and ALT)

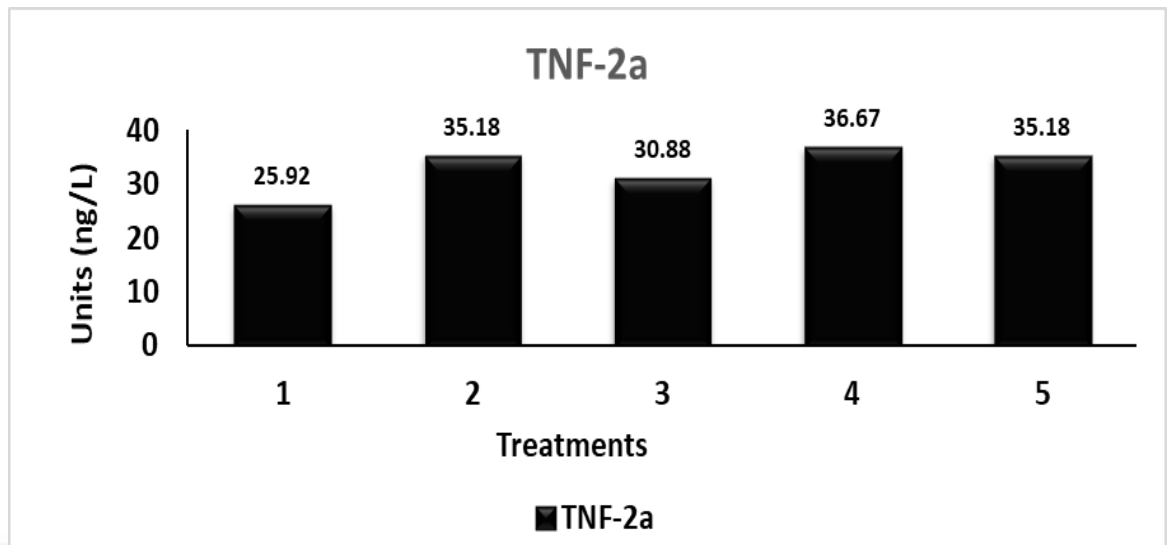


Figure 3.22. The effect of diet additives on immune parameters (TNF-a (ng/l))

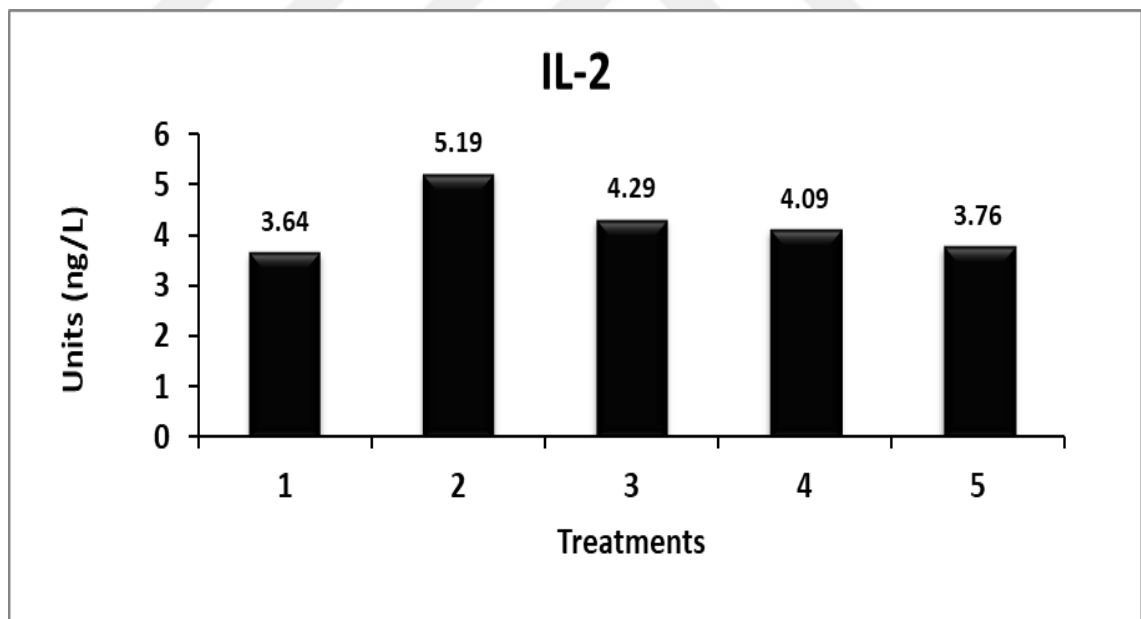


Figure 3.23. The effect of diet additives on immune parameters (IL-2 (ng/l))

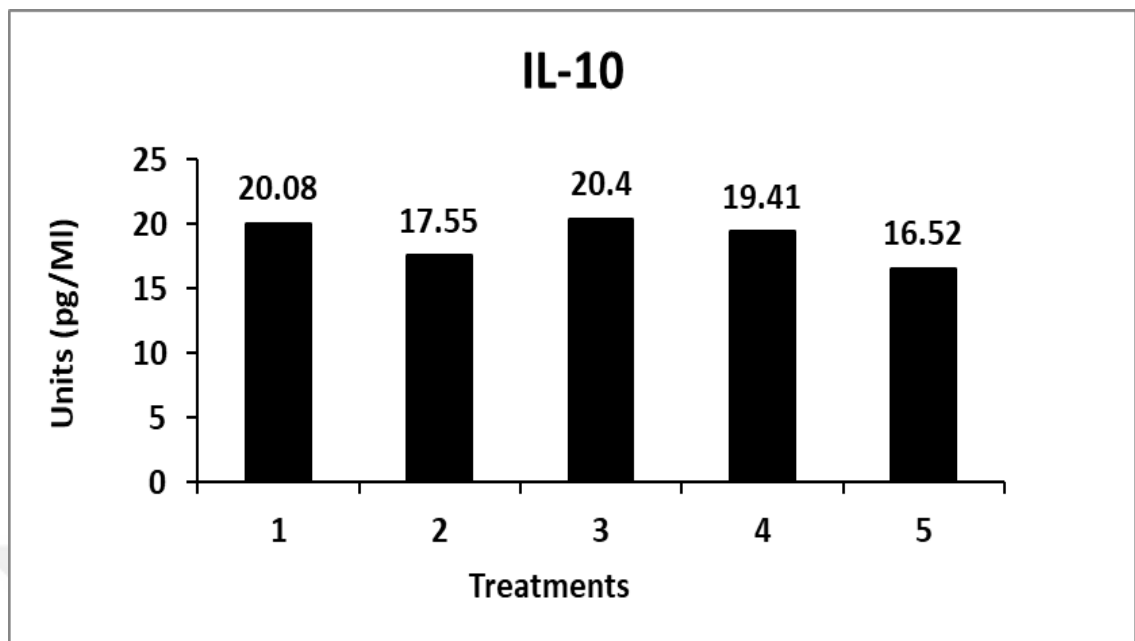


Figure 3.24. The effect of diet additives on immune parameters (IL-10 (pg/ml))

3.5. Blood Profile

The effects of different diets on Blood Profile was shown at Table (3.10).

Table 3.11. Blood parameters measurement of treatments

Traits	Treatments ¹						
	T1	T2	T3	T4	T5	Pooled SEM	<i>p</i> -value
Blood parameters							
PCV	31.43	31.34	32.15	30.93	31.40	0.42	0.9295
Hb	9.45 ^a	8.54 ^b	9.07 ^{ab}	9.05 ^{ab}	9.43 ^a	0.10	0.0347
Heterophil	36.45 ^a	32.91 ^b	35.41 ^a	35.70 ^a	37.37 ^a	0.37	0.0016
Lymphocyte	54.16	55.20	55.0	54.50	53.62	0.41	0.7482
Monocyte	1.08	1.66	1.37	1.20	1.54	0.11	0.4404
Eosinophil	3.08	3.79	2.95	2.87	3.08	0.18	0.5274
Basophil	5.20	6.41	5.25	5.70	4.79	0.30	0.4970
H/L Ratio	0.67 ^a	0.60 ^b	0.65 ^{ab}	0.66 ^{ab}	0.70 ^a	0.01	0.0209

a-c Mean within the same row with no common superscripts are differ significantly ($P < 0.05$).

¹T1 =control no added selenium or oregano essential oil, T2= diet+ Oregano essential oil 300 mg/kg, T3= diet+ Organic Selenium 0.3 mg/kg, T4=diet+ Inorganic Selenium 0.3 mg/kg, T5= diet+ Nano Selenium 0.3 mg/kg.

Table contained output results

1-Hb value in the T5 group was higher than T2, T3 and T4 group as shown in Figure (3.23).

2-Heterophil in the T5 group was higher than T2, T3 and T4 as shown in Figure (3.23).

3-H/L Ratio was higher in the T5 group than T2, T3 and T4 as shown in Figure (3.24).

The other parameters were not different due to diet supplementation, as shown in Figures (3.23) and (3.24).

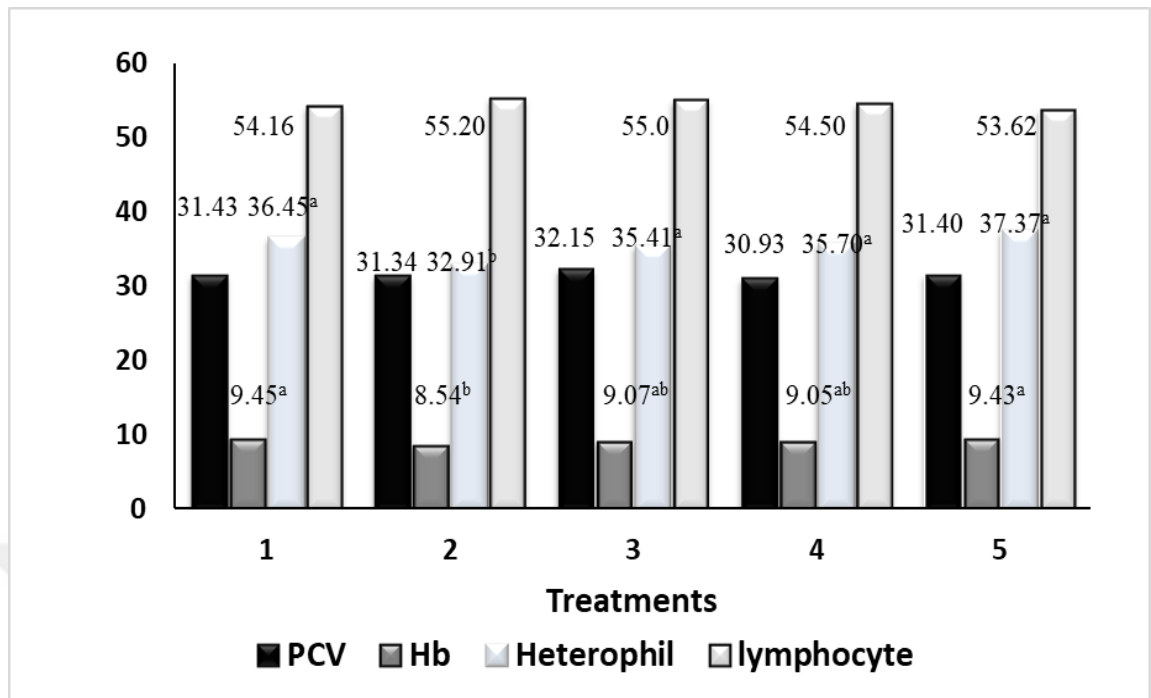


Figure 3.25. The effect of diet additives on blood parameters (PCV, Hb, Heterophile, Lymphocyte.)

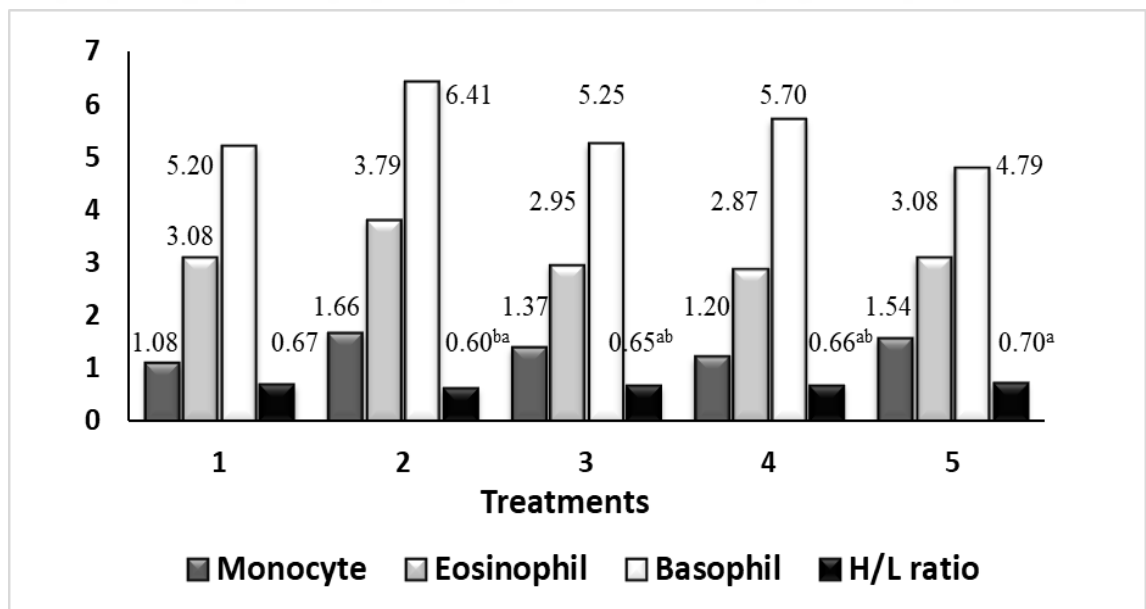


Figure 3.26. The effect of diet additives on blood parameters (Monocyte, Eosinphil, Basophile and H/L Ratio.)

CHAPTER 4

DISCUSSION AND CONCLUSION

Dietary additives are used in poultry feed for both preventing diseases and promoting growth. Dietary additives like oregano essential oil (OEO) and selenium (organic, inorganic & nano-Se) are important for poultry feed by improving feed utilization and increase immunity. They can reduce both feed costs and feed waste and increase broiler immunity. It has been indicated that medicinal plant additives can be used, such as the herb parts like coriander, cinnamon, thyme, rosemary, garlic, and oregano and their essential oil forms (Manafi *et al.* (2016). The valuable impacts of phytogetic additives are that activation poultry industry through reducing medication costs [9]. Moreover, selenium additives in broiler diet are associated with many positive effects on growth performance, decreasing mortality, improving FCR, reducing drip loss and lipid peroxidation during meat storage. Likewise, most tropical countries with hotter temperatures experience financial losses in the chicken industry because of reduced feed intake and feed conversion efficiency. However, it is possible to avoid this problem by feeding diets supplemented with selenium [15]. This means that the supplementation of some feed additives like oregano essential oil or selenium in poultry diet may contribute to both broiler performance and immune system activity. Therefore current study examined the effect of different feed additives such as oregano essential oil and different sources of Se in diets on performance, meat quality, immunity and blood profile of broilers.

The overall results indicated that reasonable differences in performance, meat and blood parameters due to feeding additive supplementation were recorded.

4.1. Production Parameters

4.1.1. Body Weight and Body Weight Gain (g)

The inclusion of oregano essential oil and selenium (organic and nano-Se) to the diet positively affected body weight and body weight gain. These indications were observed at day 21 and 42.

The experimental results showed that chickens fed on a diet supplemented with oregano essential oil 300 mg/kg achieved high body weight 805gm at 21d and 2014 gm at 42d and body weight gains 36.2 gm from 1- 21d and 61.8gm from 21-42d and 49gm from 1-42d, which is more than the control, as shown in Tables (4.1) and (4.2). This finding is similar to the result reported by Peng *et al.* (2016) who showed an increase in BW and BWG in the diet with OEO 300mg/kg. Also, this result was in agreement with the findings of Mohiti-Asli and Ghanaatparast-Rashti (2015) who found that OEO supplementation in bird diets has significant effects on BW gain and feed conversion ratio particularly in age of 22-28 days [34]. This finding is also similar to result of Giannenas *et al.* (2003), who reported that dietary oregano oil in broiler ration can improve body weight gains even with broiler infected with *Eimeria tenella* [35]. Additionally, Ertas *et al.* (2005) mentioned that there were increased in BWG around 16 % over the control group [36]. This result was in agreement with Abdel-Wareth *et al.* (2012) who reported that broiler fed diet supplemented with oregano either 10, 15, 20, 25 or 30 g/kg of feed increased body weight and feed consumption [37]. Our findings are in agreement with those of Mountzouris *et al.* (2011) who reported that broiler diet with essential oil supplementation (essential oil derived from oregano, anis and citrus peel) improved body weight gain (BWG) and feed intake (FI) compared with the control group [39]. This result also was in agreement with the findings of Hashemipour *et al.* (2016) who indicated that dietary supplementation with phytogenic product (equal mixture of thymol plus carvacrol; T+C) had a higher ($p < 0.01$) BW gain at 42d [38]. More evidence was shown in the recent research by Bakhshalinejad *et al.* (2018) who showed that dietary supplementation of organic Se sources in male broilers (Ross $\times$ Ross 308) diet significantly improved average daily gain (ADG), gain: feed ratio and European production efficiency factor ($P < 0.05$) compared to birds fed on diets supplemented with inorganic source [71].

This result was in contrast with the report by Kirkpinar *et al.* (2011) who reported there was no improvement on BW, BWG by adding OEO into the diet and it could decrease body weights at 42 d [43]. Similarly, the report by Jang *et al.* (2007), who indicated that adding essential oil in animal diets, had no effect on animal performances [31]. Also, Botsoglou *et al.* (2002) who reported dietary OEO (50 and 100 mg/kg of feed) in broiler diet had no effects on broiler performances [110] and Bozkurt *et al.* (2011) who indicated that no significant effects of dietary treatment containing 24 mg/kg or 48 mg/kg of an essential oil mixture on body weight of the broilers at 21 and 42 days [41]. Deligeorgis *et al.* (2010) also reported that oregano essential oil had no significant effect on body weight [46].

The chickens achieved the highest body weight and body weight gain compared to other diet when fed with selenium (organic and Nano-Se). Broiler fed diet supplemented with organic-Se 0.3mg/kg and Nano-Se 0.3mg/ kg achieved higher body weight 801 gm, 826gm at 21d and 2035 gm, 2121 gm at 42d, respectively, and body weight gain at 36.1gm, 37.7 from 1- 21d and 58.8 gm, 61.6gm from 21-42d and 47.4gm, 49.5gm from 1-42d, respectively which is more than control diet, as shown in Tables (4.1) and (4.2).

Our findings are in agreement with the results of Surai *et al.* (2006) who reported the natural occurrence of organic selenium in the poultry diet, implying an adaptation from these birds accepted the organic Se forms, which explains why they had difficulty retaining and metabolizing inorganic selenium [70]. Also, this result was in agreement with Upton *et al.* (2008) who reported Body weight of broilers fed organic selenium were increased compared to No Selenium or inorganic selenium treatment groups [85]. Also, our findings were similar to Heindl *et al.* (2010) who indicated the highest values BW were reached in dietary treatments with 0.15 mg/kg of SP (2 122 g) and 0.3 mg/kg of SCH (2 116 g) [75]. Moreover, our current results are consistent with Dokoupilová *et al.* (2018) who said the diet with organic-Se (Se-Chlorella) (0.3mg/kg) increased live weight at 21 and 42 days ($p < 0.05$)[86]. More evidence for this was found in the recent research by Bakhshalinejad *et al.* (2018) who showed that dietary supplementation of organic Se sources in male broilers (Ross × Ross 308) diet significantly improved average daily gain (ADG), gain: feed ratio and European production efficiency factor ($p < 0.05$) compared to birds fed on diets supplemented with inorganic source [71].

This result was contrary with the findings by Yoon *et al.* (2007) who reported that Selenium from organic or inorganic sources (0.1, 0.2, or 0.3 ppm) supplementation did not affect ($p > 0.05$) the growth performance of broilers at 42 d of age [88]. Also, this report was supported by Cai *et al.* (2012b) indicated that during the periods from 0 to 21 d, 22 to 42 d, and 0 to 42 d, BWG not differ between treatments fed diet with Nano-Se (0.00, 0.30, 0.50, 1.00 and 2.00 mg/kg) [12]. Additionally, the results were in contrast with those of Deniz *et al.* (2005) who indicated that the supplementation of the diet with 0.3 ppm mineral Se (Sodium selenite) or organic Se (Se enriched yeast) respectively had no significant effect on BW, BWG observed on days 21 and 42 [96]. Moreover, our findings are in disagreement with those of the findings by Wang and Xu (2008) who said final BW, Daily BWG were not affected by the dietary treatments and had no difference between group one and group two supplemented with sodium selenite and selenium yeast and control [97]. Also, our results were in disagreement with the findings of by Choct *et al.* (2004) who reported that there was no difference in body weight at d 38 between all treatments affected by the level and source of selenium [64].

Broilers fed a diet supplemented with inorganic-Se 0.3mg/kg achieved lower body weight 714 gm at 21d and 1824 gm at 42d and body weight gain 32 gm from 1- 21d and 52.8 gm from 21-42d and 42.2 gm from 1-42d, which is compared with the control diet and other treatments in Tables (3.1) and (3.2). This result was in agreement with Ryu *et al.* (2005) who indicated that broilers were fed with 1, 2, 4, or 8 ppm Se (Sodium selenite) different Se level in diet, BW and feed efficiency were not influenced by dietary selenium levels and broiler performance was not improved by Inorganic-Se due to developing toxicities [89] as he cited from Echevarria *et al.* (1988) reported that there was a decline in daily feed intake ($p < 0.01$) at 6 and 9 mg/kg added Se, particularly at 3 wk, suggesting developing toxicosis [91]. Also, similar results were observed by Wang and Xu (2008) who reported that Final BW, Daily BWG were not affected by the dietary treatments with sodium selenite (inorganic-Se) [97]. Further agreement was found in the research by Surai *et al.* (2006) and Surai *et al.* (2017) who reported on the various benefits of using organic selenium and the negative effects of using the inorganic variety [70] [72]. Further evidence was found in the study by Gosetti *et al.* (2007) who outlined how the interactions between sodium selenite and ascorbic acids on the chemical level in the food, premix, and guts of the broilers reduce

the quantities of selenium as an element, which is impossible for chickens to digest [93]. In addition, this result was in agreement with the findings of Attia *et al.* (2010) who reported that Inorganic-Se (sodium selenite) at 0.3 ppm can damage the gut structure in chickens and it can lead to improper digestion [94]. It can be seen clearly using Inorganic-Se has a negative effect on broiler performance. Based on both the feed conversion and rates of survival, organic selenium proved much more beneficial and financially responsible than inorganic. The results found in this research agreed with those of Choct *et al.* (2004) in terms of both broiler growth and the effectiveness of the feedstuffs [111]. The cause might be that organic-Se could cross through intestine and enter into blood by active transport, while inorganic selenium was absorbed by passive diffusion [98]. The results of this study concluded the previously held notions of how selenium is absorbed and used in the body: organic selenium uses the pathways of the amino acid movement within the small intestine and inorganic involves passive absorption [87];[92].

The results showed that, it was clear that adding feed additives can improve BW and BWG.

4.1.2. Feed Consumption (g), and Feed Conversion Ratio (%)

In this study, adding oregano essential oil and selenium (organic and nano-Se) to the diet positively affected feed intake and feed conversion ratio. These indications were accessed from 1- 21d, 21- 42d and 1-42d.

Broiler fed diet supplemented with oregano essential oil 300 mg/kg achieved feed consumption 48.1 g /d (1- 21 d), 117.2 g/d (21- 42 d) and 82.7 g/d (1- 42 d). Feed conversion ratio of 1.33 from 1- 21d and 1.90 from 21-42 d and 1.69 from 1- 42d which achieved a positive effect on feed utilization in comparison with control diet, shown in Tables (3.3) and (3.4). This finding was similar to the result reported by Giannenas *et al.* (2003) who reported that dietary oregano oil in broiler ration can enhance feed conversion ratios [35]. Also, Ertas *et al.* (2005) supports this result and indicated that improving FCR in broiler diet contained essential oil mix derived from Oregano, Clove and Anise [36]. Our results are supported by Abdel-Wareth *et al.* (2012) who reported that improvements in FCR ($p < 0.05$) in broiler fed diet supplemented with either 10, 15, 20, 25 or 30 g/kg of thyme or oregano, although feed intake was increased ($p <$

0.001) only in the diet containing oregano [37]. The findings of this investigation was supported by further evidence of study of Mountzouris *et al.* (2011) who outlined that broiler diet with essential oil supplementation (essential oil derived from oregano, anis and citrus peel) could improve FCR comparing with the control group [39]. Also, these results are supported by Alp *et al.* (2012) who reported that dose OEO 300 mg/kg in broiler diet could improve FCR at 21-42 d and 1-42 d of age comparing with control group [20]. Similar evidence is supported by Bozkurt *et al.* (2011) who reported dietary treatment containing 24 mg/kg or 48 mg/kg of an essential oil mixture has been improved feed intake and FCR [41].

The results of this study have opposite views with some authors in the literature who found non-significant results by adding oregano essential oil to diet. Such a study was that of Kirkpınar *et al.* (2011) who indicted oregano additives had no significant impacts on FCR and feed intake [43]. These findings were different from the results of Botsoglou *et al.* (2002) who reported that dietary OEO (50 and 100 mg/kg of feed) in broiler diet has no effects on broiler performances [45]. Also, Deligeorgis *et al.* (2010) who reported that dietary supplementation with oregano essential oil had no significant effect on LBW and FCR [46].

Broilers fed a diet supplemented with organic-Se 0.3 mg/kg and Nano-Se 0.3mg / kg achieved feed consumption was recorded as 47.2 g /d (1- 21d), 111.6 g/d (21- 42d) and 79.4g / d (1- 42 d); 48 gm / d (1- 21d), 116.9 g/d (21- 42d) and 82.5 g/d (1- 42d) respectively. Feed conversion ratio 1.31 from 1- 21d and 1.90 from 21-42d and 1.67 from 1- 42d; 1.29 from 1- 21d and 1.90 from 21-42d and 1.67 from 1- 42d, respectively was calculated as in this research . This achieved a positive effect on feed utilization in comparison with T1 control, as shown in Tables (3.3) and (3.4). This finding was similar to the result by Upton *et al.* (2008) who reported that FCR improved with organic-Se supplementation and combination of inorganic and organic Se being superior to inorganic-Se only treatment [85]. Similarly, our findings were supported by Deniz *et al.* (2005) who reported that total FCR (1-42 days) was significantly improved in broiler fed organic-Se comparing with other groups [96]. However, Choct *et al.* (2004) outlined that broiler fed treated with different Se forms had caused a reduction in feed intake by a high selenium concentration in feed resulted in an improved feed conversion ratio (FCR) as result of improved feather score ($p < 0.001$) at d 37 [111]. Further support for

our result by Yang (2012) who reported that organic selenium 0.3 ppm (Se-enriched yeas) had reduced feed conversion when compared with inorganic selenium 0.3 ppm (sodium selenite) ($p < 0.05$) in broilers [98]. Similarly, Wang and Xu (2008) who reported that there was a significant difference ($p < 0.05$) in FCR of group inorganic-Se 0.2 mg/kg) and organic-Se 0.2mg/kg respectively, compared with the control [97]. Surely, some researcher suggested that improvement in feed conversion ratio could be related to the better feathering of chickens fed with a diet supplemented with organic Se [96].

Contrary to our findings, some researchers reported that there was non-significant differences when adding Selenium on FI and FCR, such as the work by Sevcikova *et al.* (2006) who showed there were no significant differences between control diet group and (organic-Se +diet) group in feed conversion [84]. Also, Cai *et al.* (2012) who indicated that supplemented broiler diets with (0.0, 0.3, 0.5, 1.0, or 2.0 mg/kg diet) of Nano-Se had no effects on broiler performance ($p > 0.05$) [12]. Further opposite findings are found in the work by Rama Rao *et al.* (2013) who reported that no impact ($p > 0.05$) using organic Selenium supplementation in different levels (0, 400, 300, 200, or 100 μ g/kg diet) on FCR [15]. Moreover, Yoon *et al.* (2007) reported that supplementation of Se from organic or inorganic sources at different levels 0.1, 0.2, or 0.3 ppm did not affect ($p > 0.05$) the growth performance of broilers at 42 d of age [88].

Also, Boostani *et al.* (2015) reported that 0.3 mg/kg different sources of Se (Nano, inorganic or organic selenium) had no effect on daily gain, feed intake and FCR ($p > 0.05$) [87].

Broilers fed a diet supplemented with inorganic-Se 0.3 mg/kg achieved feed consumption 43.5 g /d (1- 21d), 102.7 g/d (21- 42d) and 73.1 g/d (1- 42 d). Feed conversion ratio 1.36 from 1- 21d and 1.94 from 21-42d and 1.72 from 1- 42d which achieved a negative effect on feed utilization in comparison with other treatments (T1, T2, T3, T5), in Tables (3.3) and (3.4). These findings are in agreement with results of Ryu *et al.* (2005) who reported that broilers were fed 1, 2, 4, or 8 ppm Se (sodium selenite) (inorganic-Se) in diet, BW and feed efficiency were not influenced by dietary selenium levels and broiler performance was not improved by inorganic-Se due to developing toxicities [89], as he cited from Echevarria *et al.* (1988) who said there was a decline in daily feed intake ($p < 0.01$) at 6 and 9 mg/kg added Se, particularly at 3 wk

of age, suggesting developing toxicosis [91]. Similar supports to my results come by Upton *et al.* (2008) who indicated that FCR improved with organic-Se supplementation and combination of inorganic and organic Se being superior to (inorganic-Se only) treatment [85].

As above results showed that there are many circumstances are affected adding feed additives like types, forms and amounts to broiler diets. Therefore, it should be carefully adding to chicken diets. So householders or chicken producers do not just follow NRC guide and it might be not enough since some researchers have indicated, and they should update their information depend on new researches. The results of this present study clearly indicated that the OEO, organic-Se and Nano-Se supplemented diet improved the FCR of broiler chickens, thus dietary supplementation could be necessary. The result of this present study Selenium has a large number of biological functions in the broilers. This finding was supported by Wang and Xu (2008) who reported that important action of antioxidant effect because of its forms selenocysteine, part of the active center of the glutathione peroxidase enzyme [97]. Also, the current study demonstrated that different Se source (inorganic and organic) treated with supplemented diet could improve the FCR. Moreover, organic forms gave better results than inorganic forms (sodium selenite).

4.1.3. Mortality Rate and Production Index (PI)

To compare the flocks of broilers in a uniform way, often a production index is calculated. This index is calculated with a formula, which takes into account the following: Growth per day, feed conversion and mortality rate. Normally the production index of a flock of broilers runs somewhere between 300 and 350 points. Growth and feed conversion have been calculated in Tables 3.2. and 3.3. Mortality in this study was zero (Table 3.5) which indicates the effect of adding oregano essential oil and selenium (organic, inorganic and nano-Se) to the diet on total mortality rate. There were no significant differences in mortality rate among experimented treatments. Adding additives to diet might prevent clinical signs of diseases and maintain growth and health [4]; [5]; [7]. The index is not suitable to compare flocks in different countries, as circumstances between countries (feed quality, housing, climate, etc.) are too variable for a good comparison. Within an integration or within a production area, it can be a very valuable tool to compare different flocks .

Our findings are in agreement with those of Bozkurt *et al.* (2011) who reported that dietary treatment containing 24 mg/kg or 48 mg/kg of an essential oil mixture has been and reduced mortality significantly [41]. Similarly, Rajashree *et al.* (2014) outlined that it was clearly decreased in a number of mortalities in birds with treatment diets supplemented Se-yeast (0.5 mg/kg) [82]. Further support to our findings come by Mountzouris *et al.* (2011) who reported that broiler diet with essential oil supplementation (essential oil derived from oregano, anis and citrus peel) could be considered as a potential natural growth promoter, and may be used as an alternative to antibiotic[39]. Also, Heindl *et al.* (2010) reported that 308 Ross broilers were fed a diet treated with 0 (control), 0.15 or 0.30 mg of selenium/kg in the form of sodium selenite (SS), Selenium enriched yeast (Sel-Plex (SP)) and selenium-enriched alga *Chlorella* (SCH) shown that there was no significant differences among dietary treatments related mortality [75].

The inclusion of oregano essential oil and selenium (organic and nano-Se) to the diet positively affected Production Index (PI), while the inclusion of selenium (inorganic) to the diet negatively affected the Production Index (PI). These indications were accessed on day 42.

Broilers fed a diet supplemented with kg nano selenium 0.3mg/kg, Organic selenium 0.3mg/kg and oregano essential oil 300 mg/kg achieved high Production Index (PI) which are 296, 283 and 290 respectively. PI increased in treatment T2, T3, and T5 due to the higher body weight and more feed consumption, and optimal FCR was achieved by using additives above and the best way in poultry feeding and management. However, control diet and diet supplemented with inorganic-Se (0.3mg/kg) were lower Production Index (PI) 250, 245, respectively.

In this study, there was a new finding T3 organic-Se 0.3mg/kg and T5 Nano-Se 0.3mg/kg which achieved a positive effect on feed utilization in comparison with control, diet supplemented with Oregano essential oil and diet supplemented with Inorganic-Se, shown in Tables (3.3) and (3.4). So organic-Se 0.3mg/kg and Nano-Se 0.3mg/kg was used more efficiently for performance in fast growing, high yielding broiler chickens, feed intake, FCR. Researchers around the world are aware about mortality rates and try to achieve lower figures by adding feed additives to improve immune system and

elevate Production Index (PI). So, Oregano essential oil (OEO) and selenium (organic-Se 0.3mg/kg and nano-Se 0.3mg/ kg) showed better results in this study.

4.2. Carcass Parameters and Relative Weights of Edible Internal Organs

4.2.1. Carcass Parameters

In this study, supplementation of oregano essential oil and selenium (organic and nano-Se) to the diet of broilers showed that the carcass cut parts were affected by dietary treatment. Chicks fed diet supplemented with organic, oregano or nano selenium achieved better performance than control and this accessed at 42d as below.

Broilers fed a diet supplemented with oregano essential oil 300 mg/kg (T2) achieved better performance in carcass cut parts than control, Carcas 72.64% , Breast 23.41% , Thigh 10.72%, Drumsticks 9.42%, Back 15.44%, Nick 4.58%,Wing 86%, Abdominal fat 1.20%, which is more than the control diet (Table4.6). This finding was similar to the results reported by Peng *et al.* (2016) who reported that adding Oregano essential oil to diet can give a positive impact on carcass traits and poultry growth performance [21]. Also, Hashemipour *et al.* (2016) showed that dietary supplementation with phytogenic product (equal mixture of thymol plus carvacrol) can enhance poultry performances [38]. More evidence to support our findings come from Cai *et al.* (2012a) who reported that medicinal herbs and herbal products had positive effects on poultry performance [12].

In contrast, some authors had different findings, such as Bampidis *et al.* (2005) who reported that adding OEO to bird diets did not had any impacts on carcass traits [25]. Also, Jang *et al.* (2007) reported that there was no significant effects from essential oil on animal performance [31]. More disagreement came from Kirkpinar *et al.* (2014) who reported that dietary oregano and garlic oil supplementation did not affect carcass yields and the relative weight of carcass parts [50].

Broilers fed a diet supplemented with organic-Se 0.3mg/kg (T3) and nano-Se 0.3mg/ kg (T5) achieved better carcass cut parts than control. Carcass cut parts were as above (Table 4.6).

These findings were similar to the results reported by Djordjevic *et al.* (2016) who reported that organic selenium contributed in improvement of carcass composition, especially in increasing the dressing percentage, breast meat gain and decreasing the abdominal fat content [83]. Also, more evidence to support our results come from Upton *et al.* (2008) who reported that carcass weight, yields of viscera, feet, leg and thigh and neck were higher in organic-Se treated birds [85]. More evidence comes from Choct *et al.* (2004) who reported that there were interactions between selenium level and source. Increasing the inclusion level of organic selenium improved breast yield and maryland weight, whereas sodium selenite (Inorganic-Se) had the opposite effect [64].

On the other hand, the results were in contrast with those of Sevcikova *et al.* (2006) who reported that there were no significant differences between the groups with or without organic-Se in feed conversion, mortality, carcass traits and dressing percentage [84]. Also, Heindl *et al.* (2010) who reported that the dietary selenium supplement did not influence the characteristics of carcass analysis such as carcass weight, breast meat, thigh meat, heart, gizzard and abdominal fat share and dressing percentage [75].

Broiler fed basal diet (T1) and supplemented diet with inorganic-Se 0.3 mg/kg (T4) achieved a lower percentage of carcass cut parts than other treatment which were achieved as above (Table4.6).

: Our findings are in agreement with those of Ryu *et al.* (2005) who indicted that broiler performance was not improved by Inorganic-Se due to developing toxicities [89].

Generally, Carcass% in diet T2 was higher than other treatments and T3, T5 higher than T1 and T4. Cut parts such as Thigh%, Back%, and wing% have no significant differences among treatments. However, Breast% in T3 was the highest than other treatments. Additionally, Drumstick%, Nick% abdominal fat % in diet T1 were lower than others.

4.2.2. Relative Weights of Edible Internal Organs

The inclusion of oregano essential oil and selenium (organic, inorganic and nano-Se) to the diet had no effect on edible internal organs percentage (Heart and liver%) except gizzard % which have effected significantly by T4 (Inorganic-Se) and has highest percentage (1.79 %) in comparison with other treatments and the lowest percentage in

Gizzard were T2 (OEO) and T5 (Nano-Se) which were 1.55%,1.5% respectively, these indications were accessed at 42 d (table 4.7). Our results might show that T4 (inorganic-Se) is caused by developing toxicosis and reduced digestion [89] ; [91].

Our current results are consistent with Kirkpinar *et al.* (2011) who outlined that dietary oregano essential oil in poultry diets had no affected broiler Carcass gains and relative weights of ileum, colon, ceca, gizzard, duodenum, jejunum, proventriculus, liver, pancreas, spleen, bursa and heart [43]. More evidence by Heindl *et al* who said the dietary selenium supplement did not influence the characteristics of carcass analysis and edible organs such as heart and gizzard except liver significantly ($p \leq 0.05$) higher in broiler fed diet with Inorganic-Se (0.3 mg/kg of SS) [75].

4.3. Meat Color and Meat pH Level

The inclusion of oregano essential oil and different forms of selenium (organic, inorganic and nano-Se) to the diet positively affected meat quality characteristics. These indications were recorded at 42 day of age.

a*,b*,L* indicate to a*, =redness, -a*, = green, b*, = yellow, -b* = blue, L*,=lighter, -L*,=darker

The Higher level of L* color in breast meat was recorded in T4 (diet + inorganic-Se) 58.17 compared with those of T1 (basal diet) 55.03; T2 (OEO) 54.18; T3 (diet + organic-Se) 56.11, and T5(diet + nano-Se) 54.31 (Table 4.8.).

The results are consistent with those described by Kirkpinar *et al.* (2014) who reported that oregano plus garlic oil supplementation significantly lead to decreased the L* value. T2 (diet + oregano essential oil) less than T4 [50]. Further evidence that supports our findings comes from Deligeorgis *et al.* (2010) who outlined that supplementation of broiler diets with oregano did not affect and L* at any age of slaughter [46].

The a* and b* color in the breast meat were greater in T4 (a* 4.20a), (b*7.65a) than T1 (a* 3.75), (b*6.59) while other treatments were not different from each other Table4.8. Similar results were reported by Kirkpinar *et al.* (2014) who reported that dietary oregano and garlic oil supplementation did not affect b* value of breast meat [50]. More evidence to support our findings was found in reports of Yang (2012) who outlined that

supplementation of broiler fed by different form of selenium (organic or inorganic Se) increased the meat red color degree a^* (redness) of breast meat and leg muscle as 13.98 and 20.83% ($p < 0.05$), respectively [98].

In contrast, a study by Cai *et al.* (2012) reported that dietary nano-se in broiler diets with different levels of nano-Se (0.00, 0.30, 0.50, 1.00 and 2.00 mg/kg) had no effects on meat color [95]. Also, Ryu *et al.* (2005) said meat color of the broilers, as expressed by (L^* , a^* , and b^*) were not affected by different dietary supplementations with selenium [89].

The Higher level of L^* color in the thigh was noticed in T4 (56.27) compared with other treatments T1 (53.69), T2 (51.77), T3 (52.35), T5 (53.00) (table 4.8).

The significant difference was shown in a^* color in the thigh of the T4 group (4.20) compared with the T3 group (2.86). Table 3.8.

pH level after slaughtering at time 0 there was no significant effect of the inclusion of oregano essential oil and selenium (organic, inorganic and Nano-Se) to the diet on pH level. While pH level highest after 24 h in diet with additives T2 (breast pH5.85, thigh pH6.36); T3 (breast pH5.84, thigh pH6.43); T4 (breast pH5.82, thigh pH6.34) and T5 (breast pH5.81, thigh pH6.33) than control (breast pH5.76, thigh pH 6.2), as shown in Table (4.8). This finding was in contrast with the results by Deligeorgis *et al.* (2010) who reported that supplementation of broiler diets with oregano did not affect pH 24 at any age of slaughter [46] also; the report by Kirkpinar *et al.* (2014) who indicated that dietary oregano and garlic oil supplementation did not affect pH of breast meat [50] and the result by Yang (2012) who reported that broiler fed different form of selenium (organic or inorganic) of dietary selenium did not affect pH values [98].

4.4. Meat Quality Characteristics: (DM, ASH, EE, CP. Moisture, Cooking Loss and Drip Loss %)

The inclusion of oregano essential oil and selenium (organic, inorganic and nano-Se) to the diet have affected some of meat quality characteristics: DM, ASH, EE, CP. Moisture, Cooking Loss and Drip Loss % these indications were recorded at 42 day of age.

The result showed that the breast ash was significantly greater in Broiler fed T1 basal diet (1.53%) than T2 basal diet + oregano essential oil 300 mg/kg 1.26% and T3 basal diet + organic-Se 1.37 %. (Table 3.9.)

Reduction in protein% in the breast in the birds fed T4 (20.14%) compared with other treatments. This result is consistent with those described by Ryu *et al.* (2005) and Echevarria *et al.* (1988) reported that broiler performance was not improved by inorganic-Se due to developing toxicities [89];[91]. More recent results by Dokoupilová *et al.* (2018) reported that Se (0.3mg/kg) supplementation did not affect ($p > 0.05$) the protein and fat contents in dry matter of meat in broiler chickens [86].

The rest traits (DM, EE, Moisture, Cooking Loss and Drip Loss %) were not different due to diet supplementation. Table 3.9, shows that our current results are consistent with Deligeorgis *et al.* (2010) who reported that supplementation of broiler diets with oregano did not affect drip loss [46]. More evidence to support our finding by Bampidis *et al.* (2005) who reported that dietary oregano essential oil in poultry feed has no significant impact on carcass traits [25].

These findings are different from the results of Peng *et al.* (2016) who reported that OEO dose can give a positive impact on carcass traits [21]. Other author like Upton *et al.* (2008) outlined that inorganic-Se supplanted diets had higher drip loss of breast meat [85]. More previous result by Perić *et al.* (2009) reported that birds fed 0.3 ppm of organic Se had decreased drip loss % from breast meat [17]. More results by Cai *et al.* (2012b) indicated that nano-Se supplementation linearly and quadratically ($p < 0.01$) reduced the drip loss percentage [12]. Additionally, Deniz *et al.* (2005) reported that whole carcass drip-loss was significantly reduced in broiler which fed by organic-Se (0.3 ppm) compare with control and inorganic-Se supplemented groups [96]. More previous findings by Choct *et al.* (2004) who outlined that broilers receiving sodium selenite (inorganic-Se) had a higher 24 h drip loss ($p < 0.001$) than those given the organic source (1.12% vs 0.87%) [111]. Also Yang (2012) who reported that the drip loss of breast meat and leg muscle were significantly decreased by 13.57 and 24.92% ($p < 0.05$), respectively by adding organic-Se compare to inorganic selenium supplemented diets of broiler [98].

It can be concluded from this result that feed additives supplementation of diets for broilers is effective to increase OEO and Se contents of broiler meat and improving meat quality parameters. Furthermore, dietary supplementation is effective to enhance the oxidative stability of chicken meat during storage in a refrigerator [112].

4.5. Immunity Parameters

The inclusion of oregano essential oil and selenium (organic and nano-Se) to the diet positively affected immunity competence. These indications were recorded at day 42.

Broilers fed a diet supplemented with oregano essential oil 300 mg/kg achieved high Albumin 0.99 (g/dl) and Globulin 1.49 (g/dl) in serum at 42d which more than control diet Table (4.10). However, A/G ration, GOT (AST) and GOP (ALT) have not been affected by oregano essential oil additives. Additionally, IL-2 is the highest in T2 which was 5.19 (ng/L) comparisons with other treatments while IL-10 is the lowest in T2 which was 17.55 (pg /ML) Table (3.10).

This result was in agreement with the findings of Abudabos *et al* (2018) who said that chickens fed with essential oil of thyme, nise and others (oregano, carvacol, yucca extract and cinnamaldehyde) had shown that blood protein and Globulin levels were increased[57]. Also, Misirlioglu (2010) reported that oregano essential oil affects the immune system significantly [40]. Also, Placha *et al.* (2014) reported that thyme oil can improve immune response [53]. On the other hand, these findings are different from the results of some researchers like Ariza-Nieto *et al.* (2011) who reported that OEO additives did not affect immunoglobulin levels [54]. Also, Traesel *et al.* (2011) outlined that chickens fed different form of essential oils (such as oregano, rosemary, sage and pepper crude extract) did not show any significant effects on Albumin, Globulin and A/G Ratio [5]. Similarly, Abd El-Ghany *et al* (2014) reported that birds fed with organum vulgare or ciprofloxacin had showed that serum albumin concentration reduced and combined treatment shown elevation of serum total protein, globulin and A/G Ratio levels were lower in different treatments groups compare to control [55].

The result of GOT (AST) and GOP (ALT) disagree with Abd El-Ghany & Ismail (2014) who outlined that chicks treated with OEO achieved the lowest levels of GOT (AST) and GOP (ALT) compared with other treatments [55].

The chickens achieved the highest Albumin and Globulin at 42d compared to control diet when fed with selenium (organic and nano-Se). Broilers fed a diet supplemented with organic-Se 0.3mg/kg and Nano-Se 0.3mg/ kg achieved higher Albumin 0.94 (g/dl) and Globulin 1.49 (g/dl) in serum at 42d which more than control diet Table (4.10). While, A:G ration were not affected by selenium additives. Also, chickens fed a Nano-Se 0.3mg/ kg achieved the highest GOT (AST) and GPT (ALT) which were 911.8 (U/L) and 90.19 (U/L) respectively at 42d compared to control diet. However, T1, T2 and T3 have no significant effect.

Generally, our current results are consistent with Rayman (2000) who reported that selenium deficiency leads to insufficient cell and humoral immune competence responses in avian [66]. Also, similar results were reported by Bakhshalinejad *et al* (2018) said that broiler fed with organic selenium 0.4 mg/kg in diet showed better immune system response and enhanced immunoglobulin and hypersensitivity [71]. Also, Boostani *et al* (2015) said supplementation birds with organic-Se and Nano-Se showed increases in immunoglobulin at oxidative and non-oxidative condition compared with control [87].

On the other hand, these findings are different from the results of Cai *et al.* (2012) and Rama Rao *et al.* (2013) who reported that selenium supplementations have no effect on organ index immunity and immune system[12];[15].

TNF-a and IL-10 are the highest in T4 (Inorganic-Se 0.3mg/ kg) which was 36.67 (ng/L) and 19.41 (pg/ml) Comparison with other result .also IL-10 in T1 and T3 achieved 20.08 (pg/ml), 20.04 (pg/ml) respectively while T5 is the lowest result 16.52 (pg/ml) Table (3.10).

Similar results were reported by Yao *et al.* (2015) who outlined that IL-1, IL-6, IL-8, IL-17, IL-4, IL-10 and IFN-g in Selenium deficient chicken liver and serum were dramatically higher than control group and Se regulated inflammation related cytokines during H2O2 dependent chicken liver damage [103].

According to the results, organic Se could actively improve the humoral immunity and this result approved by Peng *et al.* (2014) who reported that it can improve the immune system through an alleviation of the bursal histopathological lesions and a reduction of

apoptotic cells in the bursa. As such, selenium may be connected with a lowered activity of the mitochondria-mediated apoptotic pathway [104].

4.6. Blood Profile

The inclusion of oregano essential oil and selenium (organic, inorganic and nano-Se) to the diet has affected Blood Profile (PCV, Hb, Heterophil, Lymphocyte, Monocyte, Eosinophil, Basophil, H/L Ratio) these indications were recorded at 42 d as below.

Hemoglobin (Hb) in broiler fed T5 was (9.43) and it was highest than T2 (8.54), T3 (9.07) and T4 (9.05) in table 4.10

Heterophil in T5 basal diet+ Nano-Se (37.37) highest than T2 basal diet+ oregano essential oil (32.91) , T3 basal diet+ organic-Se (35.41) and T4 basal diet +inorganic-Se(35.70) while T1 control Heterophil (36.45) and Lymphocyte (55.20) Table 4.10

H/L Ratio in T5 basal diet+ Nano-Se (0.70) highest than T2 basal diet+ oregano essential oil (0.60), T3 basal diet+ organic-Se (0.65) and T4 basal diet +inorganic-Se (0.66) Table 4.10

The rest traits (PCV, Lymphocyte, Monocyte, Eosinophil, and Basophil) were not differences due to diet supplementation. (Table 3.10)

This result is consistent with those described by Najafi and Torki (2010) who reported that chicks fed diet with thyme essential oil at 200 mg/kg level indicated that heterophile had significantly lower numbers and lymphocyte had higher numbers from white blood cell counts compared with control [60]. Also, Paulsen *et al.* (2013) who indicated that birds supplemented the diet with oregano showed white blood cell count higher ($p < 0.1$) and that of lymphocytes increased significantly ($p = 0.044$) [56]. Additionally, similar results were observed by Choct *et al.* (2004) who reported that organic selenium was superior to the inorganic form in the improvement of Hb levels in bird blood samples [111].

In contrast, these findings are different from the results of Taylor *et al.* (2012) who outlined that chickens fed diet with (100 mg/kg rosemary VO plus 100mg/kg oregano VO (50:50%) showed that the haemoglobin (Hb) and haematocrit (PCV) values and the

heterophile/lymphocyte (H/L) ratio increased in the blood samples [61]. Also, Saadat Shad *et al.* (2016) reported that chickens fed on thymol and carvacrol added diets showed that RBC and WBC, hematocrit and hemoglobin were not influenced by treatments, but heterophile and heterophile to lymphocyte ratio decreased ($p < 0.05$) [62]. Similar results were reported by Revajová *et al.* (2010) who indicated that chickens fed a diet with oregano oil extracts did not significant changes in account numbers of peripheral white blood cells compared to control [58]. Moreover, Rama Rao *et al.* (2013) who said birds fed diet with Se (0, 100, 200, 300, or 400 $\mu\text{g/kg}$ diet) did not influence ($p > 0.05$) the heterophil to lymphocyte (HL) ratio in broilers [15].



CONCLUSIONS

This study can be concluded that using feed additives like dietary oregano essential oil and selenium (Organic, Inorganic & Nano-Se) in broiler diets can be benefits to poultry industry as below.

- 1- Oregano essential oil level of 300 mg/kg and dietary selenium (organic& nano-Se) level of 0.3 mg/kg have positive effects on performance and recommended as it provides an adequate safety margin for feed formulation.
- 2- This was most evident with the diet that contained level of 0.3 mg/kg inorganic Se which had significantly reduced performance due to might be high dose and may be can damage the gut structure. While, using sodium selenite in the nano form has the best potential because it binds, removes toxins, limits pathogens, and allows synthesize selenoproteins from selenium.
- 3- The improvement in broiler performances due to dietary additive supplementation as found in the present study is in agreement with the results of many researchers. However, they do contradict some scientists who disagree with present results. This is because these agreements or disagreements came from using different types or amounts of feed additives and from conducting experiments under different conditions.
- 4- Preventing poultry diseases by increasing immunity and reduced mortality.
- 5- Increasing feed utilization will lead to decrease feed waste and saving money.
- 6- Avoid using chemical drugs and rearing poultry under healthy environment.
- 7- Rearing poultry with organic additives lead to increase in meat quality and poultry production also avoid side effects from chemical additives.

8- Follow the Law of European Union which banned feed antibiotic growth promoters, due to a risk of possible medication resistance in people pathogenic bacteria as a result can export poultry products under this law easily.



REFERENCES

1. Puvača, N., Stanačev, V., Glamočić, D., Lević, J., Perić, L., Stanačev, V., Milić, D., 2013. Beneficial effects of phytoadditives in broiler nutrition. **World's Poultry Science Journal**, **69** (1): 27–34.
2. Kokoszyński, D., Bernacki, Z., Saleh, M., Stęczn, K., Binkowska, M., 2017. Body Conformation and Internal Organs. **Brazilian Journal of Poultry Science**, **19** (1): 47–52.
3. De Oliveira, J., Avanço, S V., Garcia-Neto, M., Ponsano, EHG. , 2016 . Composition of broilers meat. **The Journal of Applied Poultry Research**, **25** (2): 173–181.
4. Mir, NA., Tyagi, PK., Biswas, AK., Tyagi, PK., Mandal, AB., Wani, MA., 2018. Performance and meat quality of broiler chicken fed a ration containing flaxseed meal and higher dietary lysine levels, **The Journal of Agricultural Science**, **156** (2): 291–299.
5. Traesel, CK., Wolkme, P., Schmidt, C., Silva, CB., Paim, FC., Rosa, AP., 2011. Serum biochemical profile and performance of broiler chickens fed diets containing essential oils and pepper, **Comp Clinical Pathology**, **20** (1): 453-460
6. Castellini, C., Mugnai, C., Dal Bosco, A., 2002. Effect of organic production system on broiler carcass and meat quality, **Meat Science**, **60** (3):219-25
7. Cetin, N., Goclo, BK., Cetin, E., 2005. The effects of probiotic and mannanoligosaccharide on some haematological and immunological parameters in turkeys, **Journal of Veterinary Medicine. A, Physiology, Pathology, Cinical Medicine**, **52** (6):263-267.
8. Buchanan, NP., Hott, JM., Cutlip, SE., Rack, AL., Asame,r A., Moritz, JS., 2008. The effects of a natural antibiotic alternative and a natural growth promoter feed additive on broiler performance and carcass quality, **The Journal of Applied Poultry Research**, **17** (2): 202–210.
9. Manafi, M., Hedayati, M., Khalaji, S., 2016. Effectiveness of phytogenic feed additive as alternative to bacitracin methylene disalicylate on hematological parameters, intestinal histomorphology and microbial population and production performance of Japanese Quails, **Asian-Australasian Journal of Animal Sciences**, **29** (9): 1300–1308.

10. Demir, E., Sarica, Ş., Özcan, M a., SuiMez, M., 2003. The use of natural feed additives as alternatives for an antibiotic growth promoter in broiler diets, **British Poultry Science**, **44** (1): 44–45
11. Li. HL., Zhao, PY., Lei, Y., Hossain, MM., Kim, IH., 2015. Phytoncide, phytogenic feed additive as an alternative to conventional antibiotics, improved growth performance and decreased excreta gas emission without adverse effect on meat quality in broiler chickens. **Livest Science**, **181**(1): 1–6.
12. Cai, SJ., Wu, CX., Gong, LM., Song, T., Wu, H., Zhang, LY., 2012. Effects of nano-selenium on performance, meat quality, immune function, oxidation resistance, and tissue selenium content in broilers. **Poultry Science**, **91** (10): 2532–2539.
13. Haug, A., Graham, RD., Christophersen, OA., Lyons, GH ., 2007. How to use the world's scarce selenium resources efficiently to increase the selenium concentration in food. **Microbial Ecology in Health Disease Journal**, **19** (4) : 209–228
14. Haug, A., Eich-Greatorex, S., Bernhoft, A., Wold, JP., Hetland, H., Christophersen OA, 2007. Effect of dietary selenium and omega-3 fatty acids on muscle composition and quality in broilers. **Lipids in Health and Disease**, **6**(29) :1–9.
15. Rama Rao, SV., Prakash ,B., Raju, MVLN., Panda, AK., Poonam, S., Murthy, OK., 2013. Effect of supplementing organic selenium on performance, carcass traits, oxidative parameters and immune responses in commercial broiler chickens. **Asian-Australasian Journal of Animal Science**, **26** (2) : 247–252.
16. Yan, F., Seo, J-K., Ogbeifun, OOE., Cheng, H-W., Brad Kim, YH., Kim, H-W., 2018. Breast Meat Quality and Protein Functionality of Broilers with Different Probiotic Levels and Cyclic Heat Challenge Exposure. **Meat and Muscle Biology**, **1** (1) : 81-89.
17. Perić, L., Milošević, N., Žikić, D., Kanački, Z., Džinić, N., Nollet, L., 2009. Effect of selenium sources on performance and meat characteristics of broiler chickens. **Journal of Applied Poultry Research**, **18** (3): 403–409.

18. National Research Council, NRC., 1994. Nutrient Requirements of Poultry: Ninth Revised Edition, NATIONAL ACADEMY PRESS. USA
19. Tayeb, IT., Qader, GK., 2012. Effect of Feed Supplementation of Selenium and Vitamin E on Production Performance and Some Hematological Parameters of Broiler. **KSU Journal of Nature and Science**, **15** (3): 46-56 .
20. Alp, M., Midilli , M., Kocaba, N., Yilmaz , H., Turan, N., Gargili, A., 2012. The effects of dietary oregano essential oil on live performance, carcass yield, serum immunoglobulin G level, and oocyst count in broilers. **Journal of Applied Poultry Research** , **21** (1): 630–636.
21. Peng, QY., Li ,JD., Li, Z., Duan, ZY., Wu, YP.,2016. Effects of dietary supplementation with oregano essential oil on growth performance, carcass traits and jejunal morphology in broiler chickens. **Animal Feed Science and Technology**, **214** (0377-8401): 148–153.
22. Çetin, E., Silici, S., Çetin, N., Güçlü, BK., 2010. Effects of diets containing different concentrations of propolis on hematological and immunological variables in laying hens. **Poultry Science**, **89** (8): 1703–1708.
23. Corduk, M., Sarica, S., Yarim, GF., 2013. Effects of oregano or red pepper essential oil supplementation to diets for broiler chicks with delayed feeding after hatching. 1. Performance and microbial population. **Journal of Applied Poultry Research**, **22** (4): 738–749.
24. Burt, S., 2004. Essential oils: their antibacterial properties and potential applications in foods. **International Journal of Food Microbiology**, **94** (3): 223–253.
25. Bampidis, VA., Christodoulou, V., Florou-Paneri, P., Christaki, E., Chatzopoulou, PS., Tsiligianni, T., 2005. Effect of dietary dried oregano leaves on growth performance, carcass characteristics and serum cholesterol of female early maturing turkeys. **British Poultry Science**, **46** (5): 595–601.
26. Rhayour, K., Bouchikhi, T., Tantaoui-Elaraki, A., Sendide, K., Remmal, A., 2003. The Mechanism of Bactericidal Action of Oregano and Clove Essential Oils and of their Phenolic Major Components on Escherichia coli and Bacillus subtilis. **Journal of Essential Oil Research**, **15** (5): 286–292.

27. Oussalah, M., Caillet, S., Lacroix, M., 2006. Mechanism of action of Spanish oregano, Chinese cinnamon, and savory essential oils against cell membranes and walls of *Escherichia coli* O157:H7 and *Listeria monocytogenes*. **Journal of Food Protection**, **69** (5): 1046–1055.
28. Lv, F., Liang, H., Yuan, Q., Li, C., 2011. In vitro antimicrobial effects and mechanism of action of selected plant essential oil combinations against four food-related microorganisms. **Food Research International**, **44** (9): 3057–3064.
29. Lambert, RJW., Skandamis, PN., Coote, PJ., Nychas, GJE., 2001. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. **Journal of Applied Microbiology**, **91** (1): 453–462.
30. Lee, KW., Everts, H., Beynen, AC., 2004. Essential oils in broiler nutrition. **International Journal of Poultry Science**, **3** (12): 738–752
31. Jang, IS., Ko, YH., Kang, SY., Lee, CY., 2006. Effect of a commercial essential oil on growth performance, digestive enzyme activity and intestinal microflora population in broiler chickens. **Animal Feed Science and Technology**, **134** (3): 304–315.
32. Soares, C. E., Dahlke, F., Netto, D. P., Scussel, V. M., 2017. Chicken (*Gallus gallus domesticus* L.) Cuts Yield Specifics of Cobb 500 Slow and Hubbard Flex Hybrids. **Food and Public Health**, **7** (1): 23–28.
33. Khattak, F., Ronchi, A., Castelli, P., Sparks, N., 2014. Effects of natural blend of essential oil on growth performance, blood biochemistry, cecal morphology, and carcass quality of broiler chickens. **Poult Science**, **93** (1): 132–137.
34. Mohiti-Asli, M., Ghanaatparast-Rashti, M., 2015. Dietary oregano essential oil alleviates experimentally induced coccidiosis in broilers. **Preventive Veterinary Medicine**, **120** (2): 195–202.
35. Giannenas, I., Florou-Paneri, P., Papazahariadou, M., Christaki, E., Botsoglou, N A., Spais, A B., 2003. Effect of dietary supplementation with oregano essential oil on performance of broilers after experimental infection with *Eimeria tenella*. **Archiv Fur Tierernahrung**, **57** (2): 99–106.

36. Ertas, ON., Güler, T., Çiftçi, M., Dalkiliç, B., Gülcihan Simsek, Ü., 2005. The effect of an essential oil mix derived from oregano, clove and anise on broiler performance. **International Journal of Poultry Science**, **4** (11): 879-884
37. Abdel-Wareth , AAA., Kehraus, S., Hippenstiel, F., Südekum, KH., 2012. Effects of thyme and oregano on growth performance of broilers from 4 to 42 days of age and on microbial counts in crop, small intestine and caecum of 42-day-old broilers. **Animal Feed Science and Technology**, **178** (1): 198–202.
38. Hashemipou,r H., Khaksar, V., Rubio, LA., Veldkamp, T., van Krimpen, MM., 2016 Effect of feed supplementation with a thymol plus carvacrol mixture, in combination or not with an NSP-degrading enzyme, on productive and physiological parameters of broilers fed on wheat-based diets. **Animal Feed Science and Technology**, **211** (0377-8401): 117–131.
39. Mountzouris. KC., Paraskevas, V., Tsirtsikos, P., Palamidi, I., Steiner, T., Schatzmayr, G., 2011. Assessment of a phytogenic feed additive effect on broiler growth performance, nutrient digestibility and caecal microflora composition. **Animal Feed Science and Technology**, **168** (1): 223–231
40. Basmacioğlu Malayoğlu, H., Baysal, Ş., Misirlioğlu, Z., Polat, M., Yilmaz, H., Turan, N., 2010. Effects of oregano essential oil with or without feed enzymes on growth performance, digestive enzyme, nutrient digestibility, lipid metabolism and immune response of broilers fed on wheat–soybean meal diets. **British Poultry Science**, **51** (1): 67–80.
41. Çabuk, M., Bozkurt, M., Alçiçek, A., Akbaş, Y., Küçükyılmaz, K., 2006. Effect of a herbal essential oil mixture on growth and internal organ weight of broilers from young and old breeder flocks. **South African Journal of Animal Science**, **36** (2): 135-141.
42. Fallah, R., Mirzaei, E., 2016. Effect of Dietary Inclusion of Turmeric and Thyme powders on performance, blood parameters and immune system of broiler chickens. **Journal of Livestock Science**, **7** (2277-6214): 180–186.
43. Kirkpinar, F., Ünlü, HB., Özdemir, G., 2011. Effects of oregano and garlic essential oils on performance, carcase, organ and blood characteristics and intestinal microflora of broilers. **Livestock Science**, **137** (1/3): 219–225.

44. Demir, E., Sarica, Ş., Özcan, M A., SuiMez, M., 2003. The use of natural feed additives as alternatives for an antibiotic growth promoter in broiler diets. **British Poultry Science**, **44** (1): 44–45.
45. Botsoglou, NA., Florou-Paneri, P., Christaki, E., Fletouris, DJ., Spais, AB., 2002. Effect of dietary oregano essential oil on performance of chickens and on iron-induced lipid oxidation of breast, thigh and abdominal fat tissues. **British Poultry Science**, **43** (2): 223-230.
46. Symeon, GK., Zintilas, C., Ayoutanti, A., Bizelis, JA., Deligeorgis, SG., 2009. Effect of dietary oregano essential oil supplementation for an extensive fattening period on growth performance and breast meat quality of female medium-growing broilers. **Canadian Journal of Animal Science**, **89** (1): 331–334.
47. Mir, NA., Rafiq, A., Kumar, F., Singh, V., Shukla, V., 2017. Determinants of broiler chicken meat quality and factors affecting them: a review. **Journal of Food Science and Technology**, **54** (10): 2997–3009.
48. Qiao, M., Fletcher, DL., Smith, DP., Northcutt, JK., 2001. The effect of broiler breast meat color on pH, moisture, water-holding capacity, and emulsification capacity. **Poultry Science**, **80** (5): 676–680.
49. Bowker. B., 2017. Developments in Our Understanding of Water-Holding Capacity. Poultry Quality Evaluation, Chapter: 4. United States Department of Agriculture, Athens, GA, United States 77-113pp
50. Kirkpinar, F., Ünlü, HB., Serdaroğlu, M., Turp, GY., 2014. Effects of dietary oregano and garlic essential oils on carcass characteristics, meat composition, colour, pH and sensory quality of broiler meat. **British Poultry Science**, **55** (2): 157–166.
51. Abdullah, F.A.A., Buchtova, H., 2016. Comparison of qualitative and quantitative properties of the wings, necks and offal of chicken broilers from organic and conventional production systems. **Veterinarni Medicina**, **61**(11): 643–651.
52. Sabino, M., Capomaccio, S., Cappelli, K., Verini-Supplizi, A., Bomba, L., Ajmone-Marsan, P., Cobellis, G., Olivieri, O., Pieramati, C., Trabalza-Marinucci, M., 2018. Oregano dietary supplementation modifies the liver transcriptome profile in broilers: RNASeq analysis. **Research in Veterinary Science**, **117** (1): 85–91.

53. Placha, I., Takacova, J., Ryzner, M., Cobanova, K., Laukova, A., Stropfova, V., Venglovska, K., Faix, S., 2014. Effect of thyme essential oil and selenium on intestine integrity and antioxidant status of broilers. **British Poultry Science**, **55** (1):105-114
54. Ariza-Nieto, C., Bandrick, M., Baidoo, SK., Anil, L., Molitor, TW., Hathaway, MR., 2011. Effect of dietary supplementation of oregano essential oils to sows on colostrum and milk composition, growth pattern and immune status of suckling pigs. **Journal of Animal Science**, **89**(4):1079-1089.
55. Abd El-Ghany, WA., Ismail, M., 2014. Tackling experimental colisepticaemia in broiler chickens using phytobiotic essential oils and antibiotic alone or in combination. **Iranian Journal of Veterinary Research**, **15** (2): 110–115.
56. Steletr, k., Frahm, J., Paulsen, J., Berk, A., Kleinwächter, M., Selmar, D., Dänicke, S., 2013. Effects of oregano on performance and immunomodulating factors in weaned piglets. **Archives of Animal Nutrition**, **67** (6): 461–476.
57. Abudabos, AM., Alyemni, AH., Dafalla, YM., Khan, RU., 2018. The effect of phytogenics on growth traits, blood biochemical and intestinal histology in broiler chickens exposed to *Clostridium perfringens* challenge. **Journal of Applied Animal Research**, **46** (1): 691–695.
58. Revajová, V., Pist, J., Levkut, M., Marcin, A., Levkutová, M., Influence of oregano and salvia extracts on lymphocyte subpopulation and functional activity of blood phagocytes and lymphocytes in chickens. **Food and Agricultural Immunology**, **21** (4): 307–316.
59. Li, SY., Ru, YJ., Liu, M., Xu, B., Péron, A., Shi, XG., 2012. The effect of essential oils on performance, immunity and gut microbial population in weaner pigs. **Livestock Science**, **145** (1-3): 119-123
60. Najafi, P., Torki, M., 2010. Performance, blood metabolites and immunocompetence of broiler chicks fed diets included essential oils of medicinal herbs. **Journal of Animal and Veterinary Advances**, **9** (7): 1164–1168.
61. Yesilbag, P., Gezen, SS., Biricik, H., Bulbul, T., 2012. Effect of a rosemary and oregano volatile oil mixture on performance, lipid oxidation of meat and haematological parameters in Pharaoh quails. **British Poultry Science**, **53** (1): 37–41.

62. Saadat Shad, H., Mazhari, M., Esmaeilipour, O., Khosravinia, H., 2016. Effects of thymol and carvacrol on productive performance, antioxidant enzyme activity and certain blood metabolites in heat stressed broilers. **Iranian Journal of Veterinary Research**, **6** (1): 195–202.
63. Rahimi, S., Teymouri Zadeh, Z., Karimi Torshizi, MA., Omidbaigi, R., Rokni, H., 2011. Effect of the three herbal extracts on growth performance, immune system, blood factors and intestinal selected bacterial population in broiler chickens. **Journal of Agricultural Science and Technology**, **13** (4): 527–539.
64. Choct, M., Naylor, AJ., Reinke, N., 2004. Selenium supplementation affects broiler growth performance, meat yield and feather coverage. **British Poultry Science**, **45** (5): 677–683.
65. Suchý, P., Straková, E., Herzig, I, 2014. Selenium in poultry nutrition. **Czech Journal of Animal Science**, **59** (11): 495–503.
66. Rayman, MP., 2000. The importance of selenium to human health. **The Lancet Rheumatology**, **356** (9225): 233–241.
67. Zakharia, Y., Bhattacharya, A., Rustum, YM., 2018. Selenium targets resistance biomarkers enhancing efficacy while reducing toxicity of anti-cancer drugs: preclinical and clinical development .**Oncotarget**, **9** (12): 10765–10783.
68. Köhrle, J., Gartner, R., 2009. Selenium and the thyroid. **Best Practice & Research Clinical Endocrinology & Metabolism**, **23** (6): 815–827.
69. Sarkar, B., Bhattacharjee, S., Daware ,A., Tribedi, P., Krishnani, KK., Minhas, PS., 2015. Selenium Nanoparticles for Stress-Resilient Fish and Livestock . **Nanoscale Research Letters**, **10** (371): 1-14.
70. Surai, PF., Karadas, F., Pappas, AC., Sparks, NHC., 2006. Effect of organic selenium in quail diet on its accumulation in tissues and transfer to the progeny. **British Poultry Science**, **47** (2): 65–72.
71. Bakhshalinejad, R., Akbari Moghaddam Kakhki. R., Zoidis, E., 2018. Effects of different dietary sources and levels of selenium supplements on growth performance, antioxidant status and immune parameters in Ross 308 broiler chickens. **British Poultry Science**, **59** (1): 81–91.

72. Surai, PF., Kochish, IL., Fisinin, VI., Velichko, OA., 2017. Selenium in Poultry Nutrition: from Sodium Selenite to Organic Selenium Sources. **The Journal of Poultry Science**, 55 (2): 79-93.
73. Payne, RL., 2004. The effects of inorganic and organic selenium sources on growth performance, carcass traits, tissue mineral concentrations, and enzyme activity in poultry. Louisiana State University, Agricultural and Mechanical Colleg , Phd thesis, USA, 96.
74. Payne, RL., Southern, LL., 2005. Comparison of inorganic and organic selenium sources for broilers. **Poultry Science**, 84 (6): 898–902.
75. Heindl, J., Ledvinka, Z., Englmaierová, M., Zita, L., Tůmová, E., 2010. The effect of dietary selenium sources and levels on performance, selenium content in muscle and glutathione peroxidase activity in broiler chickens. **Czech Journal Animal Science**, 55 (12): 572–578.
76. Zimmermann, MB, Köhrle, J., 2002. The Impact of Iron and Selenium Deficiencies on Iodine and Thyroid Metabolism: Biochemistry and Relevance to Public Health. **Thyroid Journal Program**, 12 (10): 867–878.
77. Combs, GF., Gray, WP., 1988 Chemopreventive agents: Selenium. **Pharmacology and Therapeutics**, 97 (3) 179–192.
78. Rayman, MP., 2005. Selenium in cancer prevention: a review of the evidence and mechanism of action. **Proceedings of the Nutrition Society**, 64 (4): 527–542.
79. Stoimenov, PK., Klinger, RL., Marchin, GL., Klabunde, KJ., 2002. Metal oxide nanoparticles as bactericidal agents. **Langmuir**, 18 (17): 6679–6686.
80. Pineda, L., Sawosz, E., Lauridsen, C., Engberg, RM., Elnif, J., Hotowy, A., Sawosz, F., Chwalibog, A., 2012. Influence of in ovo injection and subsequent provision of silver nanoparticles on growth performance, microbial profile, and immune status of broiler chickens. **Open Access Animal Physiology**, 4 (2012): 1-8
81. Konkol, D., Wojnarowski, K., 2018. The Use of Nanominerals in Animal Nutrition as a Way to Improve the Composition and Quality of Animal Products. **Journal of Chemistry**, 2018 (ID 5927058): 1–7.

82. Rajashree, K., Muthukumar, T., Karthikeyan, N., 2014. Comparative study of the effects of organic selenium on hen performance and productivity of broiler breeders. **British Poultry Science**, **55** (3): 367–374.
83. Djordjevic, S., Omerovic, I., Stolic, N., Milosevic, B., 2016. Effect of Organic Selenium in Broiler Diet on Slaughter Traits and Plasma Lipids. **Journal of Agriculture and Veterinary Science**, **9** (5): 71–73.
84. Sevcikova, S., Skřivan, M., Dlouhá, G., Koucký, M., 2006. The effect of selenium source on the performance and meat quality of broiler chickens. **Czech Journal Animal Science**, **51**(10): 449–457.
85. Upton, JR., Edens, FW., Ferket, PR., 2008. Selenium yeast effect on broiler performance. **International Journal of Poultry Science**, **7** (8): 798–805.
86. Dokoupilová, A., Zita, L., Skřivan, M., Heindl, J., Ševčíková, S., Dlouhá, G., 2008. Effect of dietary selenium sources on growth performance, breast muscle selenium, glutathione peroxidase activity and oxidative stability in broilers. **Czech Journal of Animal Science**, **53** (6): 265–269.
87. Boostani, A., Sadeghi, AA., Mousavi, SN., Chamani, M., Kashan, N., 2015. Effects of organic, inorganic, and nano-Se on growth performance, antioxidant capacity, cellular and humoral immune responses in broiler chickens exposed to oxidative stress. **Livestock Science** , **178** (1): 330-336.
88. Yoon, I., Werner, TM., Butler, JM., 2007. Effect of source and concentration of selenium on growth performance and selenium retention in broiler chickens. **Poultry Science**, **86** (4): 727–730.
89. Ryu, YC., Rhee, MS., Lee, KM., Kim, BC., 2005. Effects of different levels of dietary supplemental selenium on performance, lipid oxidation, and color stability of broiler chicks. **Poultry Science**, **84** (5): 809–815.
90. Payne, RL., Southern,LL.,2005. Comparison of inorganic and organic selenium sources for broilers. **Poultry Science**, **84** (6): 898–902.
91. Echevarria, MG., Henry, PR., Ammerman, CB., Rao, P V., Miles, RD., 1988. Estimation of the relative bioavailability of inorganic selenium sources for poultry. 2. Tissue uptake of selenium from high dietary selenium concentrations. **Poultry Science**, **67** (11): 1585-1592

92. Hu, CH., Li, YL., Xiong, L., Zhang, HM., Song, J., Xia, MS., 2012. Comparative effects of nano elemental selenium and sodium selenite on selenium retention in broiler chickens. **Animal Feed Science and Technology**, **177** (3-4): 204–210.
93. Gosetti, F., Palma, P., Gianotti, V., Aigotti, R., Baiocchi, C., Polati, S., Aigotti, R., Baiocchi, C., Gennaro, MC., 2007. Speciation of selenium in diet supplements by HPLC–MS/MS methods. **Food Chemistry**, **105** (4): 1738–1747
94. Attia, YA., Abdalah, AA., Zeweil, HS., Bovera, F., Tag El-Din, AA., Araft, MA., 2010. Effect of inorganic or organic selenium supplementation on productive performance, egg quality and some physiological traits of dual-purpose breeding hens. **Czech Journal of Animal Science**, **55**(11): 505-519.
95. Cai, S., Wu, CX., Gong, LM., Song, T., Wu, H., Zhang, LY., 2012. Effects of nano-selenium on performance, meat quality, immune function, oxidation resistance, and tissue selenium content in broilers. **Poultry Science**, **91** (10): 2532-2539.
96. Deniz, G., Gezen, SS., Turkmen, II., 2005. Effects of two supplemental dietary selenium sources (mineral and organic) on broiler performance and drip-loss. **Revue de Medecine Veterinaire**, **156** (8-9): 423–426.
97. Wang, YB., Xu, BH., 2007. Effect of different selenium source (sodium selenite and selenium yeast) on broiler chickens. **Animal Feed Science and Technology**, **144** (3-4): 306–314.
98. Yang, Y. R., Meng, F.C., Wang, P., Jiang, Y.P., Yin, Q.Q., Chang, J., Zuo, R.Y., Zheng, Q.H., Liu, J.X., 2012. Effect of organic and inorganic selenium supplementation on growth performance, meat quality and antioxidant property of broilers. **African Journal of Biotechnology**, **11** (12): 3031–3036.
99. Rocha Garcia, CE., Freitas, LW., Schwingel, A.W., Farias, RM., Caldara, FR., Gabriel, A.M A., Graciano, JD., Komiyama, C.M., Almeida Paz, ICL., 2010. Incidence and Physical Properties of PSE Chicken Meat in a Commercial Processing Plant. **Brazilian Journal of Poultry Science**, **12** (4): 233 - 237.

100. Le Bihan, DE., Millet, N., Remignon, H., 1999. Broiler meat quality: effect of selection for increased carcass quality and estimates of genetic parameters. **Poultry Science**, **78** (6): 822–826.
101. Kralik, Z., Kralik, G., Biazik, E., Straková, E., Suchý, P., 2013. Effects of organic selenium in broiler feed on the content of selenium and fatty acid profile in lipids of thigh muscle tissue. **Acta Veterinaria Brno**, **82** (3): 277–282.
102. Kralik, Z., Kralik, G., Grčević, M., Suchý, P., Straková, E., 2012. Effects of increased content of organic selenium in feed on the selenium content and fatty acid profile in broiler breast muscle. **Acta Veterinaria Brno**, **81** (1): 31–35.
103. Jiang, Z-H., Khoso, PA., Yao, H-D., Zhang, Z-W., Zhang, X-Y., Xu, SW., 2015. SelW regulates inflammation-related cytokines in response to H₂O₂ in Se-deficient chicken liver. **Royal Society of Chemistry Advances**, **5** (47): 37896–37905.
104. Chen, K., Fang, J., Peng, X., Cui, H., Chen, J., Wang, F., Chen, Z., Zuo, Z., Deng, J., Lai, W., Zho, Y., 2014. Effect of selenium supplementation on aflatoxin B₁-induced histopathological lesions and apoptosis in bursa of Fabricius in broilers. **Food and Chemical Toxicology**, **74** (1): 91–97.
105. Helrich, K., 1990. Association of Official Analytical Chemists(AOAC). The Executive Director Office of The Federal Register, Washington, 771 PP.
106. Karl, O.H., 1998. Reference methods for the assessment of physical characteristics of meat. **Meat Science**, **49** (4):447-57.
107. Benjamin, M.M., 1978. Outline of Veterinary Clinical Pathology, 3rd. Iowa State University Press, USA, 351 PP.
108. Jain, N. C., 1986. Schalm's Veterinary Hematology. Lea and Febiger, 4th edition. Philadelphia, USA. 1221PP.
109. Gross, W. B., Siegel, H. S., 1983. Evaluation of the Heterophil / Lymphocyte Ratio as a Measure of Stress in Chickens. **Avian Diseases**, **27** (4): 972–979.
110. Botsoglou, NA., Florou-Paneri, P., Christaki, E., Fletouris, D., Spais, AB., 2002. Effect of dietary oregano essential oil on performance of chickens and on iron-induced lipid oxidation of breast, thigh and abdominal fat tissues. **British Poultry Science**, **43**(2): 223–230.

111. Choct, M., Naylor, AJ., Reinke, N., 2004. Selenium supplementation affects broiler growth performance, meat yield and feather coverage. **British Poultry Science**, **45** (5): 677-683.
112. Dlouhá, G., Ševčíková, S., Dokoupilová, A., Zita, L., Heindl, J., Skřivan, M., 2008. Effect of dietary selenium sources on growth performance, breast muscle selenium, glutathione peroxidase activity and oxidative stability in broilers. **Czech Journal of Animal Science**, **53** (6): 265–269.
113. Gaur, S., Kuhlenschmidt, TB., Kuhlenschmidt, MS., Andrade, JE., 2018. Effect of oregano essential oil and carvacrol on *Cryptosporidium parvum* infectivity in HCT-8 cells. (Web page: <https://www.zanehellas.com>), (Date accessed: February 2019).
114. Choudhary, I., 2016. *Origanum Vulgare* L. (web page: http://folkmedsindh.com.pk/origanum_vulgare), (Date accessed: February 2019).
115. Lenntech, BV., 20019. Selenium -Se (Wep page: <http://www.lenntech.com/-periodic/elements/se.htm>), (Dateaccesssed February 2019).r

CURRICULUM VITAE

Name and Surname: Sami Hammed FARHAN

Nationality: Iraqi

Birth Date and Place: 01-06-1973, Iraq

E-mail: samihammedfarhan1@gmail.com

EDUCATION

Degree	Institute	Date of Graduate
MSc	Queensland University Animal studies / Poultry Nutrition Australia	2012
BSc	Baghdad University College of Agriculture/ Animal Resources Iraq	1995

Language:-

Arabic, English, Turkish

Hobbies:

Sports, Traveling

Interesting in:-

Animal science, Poultry Nutrition, Feed additives