



Republic of Iraq
Ministry of Higher Education and Scientific Research
University of Kerbala
College of Veterinary Medicine
Department of Veterinary Public Health

**Effect of Replace Whole Flaxseed in Reduced Energy Diet on
Broiler Chicken Performance and Gene Expression under
Heat Stress**

A thesis Submitted to the Council of the Faculty of Veterinary Medicine /
University of Kerbala in Partial Fulfillment of the Requirement for the
Master Degree of Science in Veterinary Medicine /Veterinary Public
Health.

BY

Harith Modher Jawad

Supervised by

Prof. Dr.

Yasser Jamal Jamel

2024 A.D

1446A.H

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

((فَلْيَنْظُرِ الْإِنْسَانُ إِلَى طَعَامِهِ))

سُورَةُ عَبَسَ الْآيَةُ (٢٤)

سورة عبس الآية (٢٤)

Supervisor Certificate

I certify that this thesis (**Effect of Replace Whole Flaxseed in Reduced Energy Diet on Broiler Chicken Performance and Gene Expression under Heat Stress**) has been prepared by **Harith Modher Jawad** under my supervision at the college of Veterinary Medicine, University of Kerbala in partial fulfillment of the requirements for the Degree of Master in the Sciences of Veterinary Medicine in Veterinary Public health.



Supervisors

Prof. Dr. Yasser Jamal Jamel

uco / 1 / A

College of Veterinary Medicine

University of Kerbala

The recommendation of the Department

In the view of the above recommendation, I forward this thesis for
Scientific discussion by the examining committee



Prof. Dr. Ihab Ghazi Mahdi

Vice Dean for Postgraduate studies and scientific Affairs

College of Veterinary Medicine

University of Kerbala

/ /

Committee Certification

We, the examining committee, certify that after reading this thesis entitled **(Effect of Replace Whole Flaxseed in Reduced Energy Diet on Broiler Chickens Performance and Gene Expression under Heat Stress)** presented by **Harith Modher Jawad** from the department of **(Veterinary Public health)** and we have examined the student in its contents .we have found that it is adequate for the Degree of master in science of Veterinary Medicine veterinary public health.



Prof. Dr. Kadhim Saleh Kadhim

College of Veterinary Medicine
University of Kerbala
(Chairman)



Prof. Dr. Abbas Fakhri Husseim AL-Machi

College of Science
University of Kerbala
(Member)




Asst. Prof. Dr. Mohanad Falhi Hamood

College of Veterinary Medicine
University of Baghdad
(Member)




Prof. Dr. Yasser Jamal Jamel

College of Veterinary Medicine
University of Kerbala
(Member and Supervisor)



Asst. Prof. Dr. Ali Redha Abid

Head of Veterinary Public
Health Department



Asst. Prof. Dr. Mohammed Assad Saleh AL-Kaabi

Dean of College of
Veterinary Medicine

Certification of Linguistic Evaluator

I certify that thesis entitled (**Effect of Replace Whole Flaxseed in Reduced Energy Diet on Broiler Chickens Performance and Gene Expression under Heat Stress**) for the student (**Harith Modher Jawad**) was linguistically reviewed by me and the necessary correction has been made. Thus, it is linguistically ready for examination.

Linguistic Evaluator Signature
Name



Asst. Prof. Dr. Ganim Jwaid Idnan AL-Asieedy Ph.D

Declaration

I hereby declare that this thesis is my original work except for equations and citations, which have been fully acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at University of Kerbala or other institutions.

Harith Modher Jwad

/ / 2024

Dedication

To my:

Father's soul, to the one I am so proud to carry his name.

Kind and virtuous mother.

To the one who helped me reach this day, my older brother Yarab.

Sisters and brothers.

My friends. Life partner, my beloved wife.

Those who own my heart, Siraj and Jawad, my children.

I would like to thank everyone who supported me to successfully complete my research.

Harith Modher Jwad

/ / 2024

Acknowledgments

Thanks and a special gratitude to **Allah** for His entire blessing, during pursuit of our academic career goals. I would like to thank the Dean of the College of Veterinary Medicine, **Assistant Professor Muhammad Asaad saleh**. My deepest thanks and gratitude to my research supervisor, **Professor Dr. Yasser Jamal Jamil**, for the advice and information he provided me during my studies, and who did not hold back any information. Thanks and gratitude to the Head the Department of Public Health, **Assistant Professor Dr. Ali Reza Abed**, and thanks and gratitude to **Professor Dr. Kadhim Saleh Kadhim, Professor Dr. Mona AL-Amili**. Thanks and gratitude to all the professors of the College of Veterinary Medicine my esteemed professors who kindly provided me with advice and counsel. Thanks to all my colleagues.

Harith Modher Jwad

/ / 2024

Summery

Adapting to rising global temperatures while maintaining production efficiency is an important emerging challenge for the poultry industry. Under hot temperatures especially in Iraq, birds reduce their feed intake, and this is the main factor explaining the degradation of bird performance. This experiment, was conducted to determine the effects of whole flaxseed in a reduced energy diet on broiler performance parameters, like body weight (BW), weight gain (WG), feed intake (FI), feed conversion ratio (FCR), lipids profile, meat quality, liver function, immunity, IGF_1 gene expression in liver tissue was quantitated using real-time quantitative PCR, intestinal morphology of broiler chickens and carcass characteristics, under heat stress conditions.

A total of 250 straight- run one-day old (Ross 308) broiler chicks were divided randomly to five groups each group (50/birds) with 2 replicates. Each replicate was subdivided to 25 birds/pen. The control negative treatment (T1) fed corn-soybean diet. The second treatment control positive (T2) fed corn-soybean diet under heat stress. The third treatment (T3) fed flaxseed-corn- soybean diet by using of flaxseed 7.5% g / kg in starter and grower 10% g/ kg under heat stress. The fourth treatment (T4) fed flaxseed _ reduced energy (200 KCL) diet by using of flaxseed 7.5% g / kg in starter and grower 10% g/ kg under heat stress. The fifth treatment (T5) fed reduced energy diet (200 KCL) under heat stress. The experiment period was five weeks. This study was carried out in a private brooding house, from 27 December 2023 to 31 January 2024. Blood samples were collected on end of experiment in day 35 of the experiment. A significant ($P \leq 0.05$) were observed among treatments in weekly BW, WG, FI and F.C.R. The highest significant increase ($P \leq 0.05$) in productive traits (BW, WG) in the (T3) and (T4) groups that feed with flaxseed. However, FI and FCR were decrease

significantly ($P \leq 0.05$). Meat quality enhanced in the T3 and T4 groups by increase the omega-3 meat content [α -linolenic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] were increase significantly ($P \leq 0.05$) by using GC mass technique in the (T3) and (T4) compared with the control fed with corn-soybean diet. The lipid profile enhanced significantly ($P \leq 0.05$) in the (T3) and (T4) groups compared with the control. The cholesterol, triglyceride, LDL, vLDL were decrease significantly ($P \leq 0.05$) in the (T3) and (T4) compared with the control. However, HDL was increase significantly ($P \leq 0.05$). The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity was decrease significantly ($P \leq 0.05$) in the (T3) and (T4) compared with the control. The immune status against ND and IBD virus enhanced significant ($P \leq 0.05$) in the (T3) and (T4) groups compared with the control. The groups (T3) and (T4) increased the IGF1 gene expression in the liver compared with the control treatment ($P \leq 0.05$). Significant increases ($P \leq 0.05$) were obtained in villus height, crypt depth, villus width and villus area in (T3) and (T4) compared to the control group. In conclusion, adding whole flaxseeds and flaxseed _ reduced energy diet at rate 7.5 percentage of whole flaxseed in starter and grower 10 in broiler chicken diet lead to improve growth performance, healthy meat enriched with omega-3 (ALA, EPA, DHA) by using GC mass technique. Additionally, reducing using oil and corn in diet.

In summary, using whole flaxseed the source of omega-3 FAs may enhance the performance of broiler chickens and elevate the omega-3 level in their meat. The intake of whole flaxseed enhanced the expression of the liver IGF-1 gene, which is crucial for the differentiation and metabolism of myogenic cell lines across different species. Insulin-like growth factors (IGFs) regulate somatic and muscular development in broiler chickens. The inclusion of whole flaxseed in a reduced-calorie diet led to elevated

expression of the IFG-1 gene, improved performance, and enhanced intestinal structure and mucin activity. Implementing a reduced calorie diet will lower feed expenses and is ideal under heat stress conditions. Our results correspond with the guidelines provided by Aviagen (Nutrition Specification, 2022).

Table of Contents

No.	Subject	Page No.
	Summary	I
	Table of Contents	IV
	List of Tables	VIII
	List of Figures	X
	List of Abbreviations	XI
	Chapter One/ Introduction	
1.	Introduction	1
	Chapter Two / Review of the Related Literature	
2	Review of the Related Literature	5
2.1	Heat stress	5
2.1.1	Impact of heat stress on poultry production efficiency	7
2.1.2	Impact of heat stress on immunity responses of poultry	8
2.1.3	Impact of heat stress on the inflammation	9
2.1.4	Impact of heat stress on oxidative status in poultry	11
2.1.5	Impact of heat stress on intestinal integrity and gut microbiota of poultry	13
2.1.6	Impact of heat stress on the quality of poultry meat	13
2.2	Flaxseed	14
2.2.1	Flaxseed content	15
2.2.2	Health Advantages of flaxseed	18

2.2.3	Influence of flaxseed on broiler chickens productive performance	19
2.2.4	Influence of flaxseed on meat quality of broiler chickens	20
2.2.5	Influence of flaxseed on lipid profile of broiler chickens	21
2.2.6	Impact of flaxseed on immunity and Anti-Oxidative Properties of broiler chickens	22
2.2.7	Impact of flaxseed on intestinal histology of broiler chickens	24
	Chapter Three / Methodology	
3.1	Experiment design	26
3.2	Nutrients used in the experiment	29
3.3	Poultry House Preparation	20
3.4	Vaccination programs	31
3.5	Instruments & equipment	32
3.6	Laboratory chemicals and reagents	32
3.7	Parameters studied	33
3.7.1	Productive performance	33
3.7.1.1	Weekly mean body weight	33
3.7.1.2	Weekly mean weight gain	33

3.7.1.3	Weekly feed intake	33
3.7.1.4	Feed conversion Ratio	34
3.7.2	Hematological Sampling	34
3.7.3	Biochemical parameters	34
3.7.3.1	Estimation of serum Cholesterol concentration	35
3.7.3.2	Estimation of serum Triglyceride concentration	35
3.7.3.3	Estimation of serum HDL-Cholesterol concentration	35
3.7.3.4	Estimation of serum LDL-Cholesterol concentration	35
3.7.3.5	Estimation of serum vLDL-Cholesterol concentration	35
3.7.3.6	Serum glutamic oxaloacetic transaminase (AST) activity determination	36
3.7.3.7	Serum alkaline phosphatase (ALT) activity determination	36
3.7.3.8	Immunological tests	36
3.8	Estimation of Omega-3 content in the meat	36
3.9	Gene expression	36
3.10	Histological analysis	36
3.11	Measuring of edible organs weight	37
3.12	Statistical analysis	37

	Chapter Four / Results and Discussion	
4	Results and Discussion	39
4.1	Productive performance of broiler chickens	39
4.2	Impact of whole flaxseed and reduced energy diet on omega-3 content in the meat under heat stress	43
4.3	Biochemical parameters	46
4.3.1	Impact of whole flaxseed and reduced energy based diet on serum lipid profile broiler chickens under heat stress	46
4.3.2	Impact of whole flaxseed and reduced energy based diet on liver function of broiler chickens	48
4.3.3	Impact of whole flaxseed and reduced energy diet on immunological of broiler chickens.	50
4.4	Impact of whole flaxseed in reduced energy diet on the intestinal, and Mucin activity of Broiler Chickens.	52
4.5	Impact of whole flaxseed and reduced energy based diet on IGF_1 Gene expression of broiler chickens	67
4-6	Impact of whole flaxseed and reduced energy based diet on carcass characterization in broiler chickens	70
	Chapter Five	
	Conclusion and Recommendations	
5.1	Conclusion	75
5.2	Recommendation	76
6	References	78

List of Tables

Tables No.	Title	Page No
2.1	Flaxseed content	16
3.1	Ingredients and nutrients composition of broiler starter diets from 1-15 days.	29
3.2	Ingredients and nutrients composition of broiler grower from 16-35 days	23
3.3	Program of vaccination	31
3.4	Instruments and equipment with their origin.	32
3.5	Kits and their suppliers	33
4.1	Effect of whole flaxseed and reduced energy diet on live body weight	39
4.2	Effect of whole flaxseed and reduced energy diet on weight gain of broiler chickens	40
4.3	Effect of whole flaxseed and reduced energy diet on feed intake of broiler chickens	40
4.4	Effect of whole flaxseed and reduced energy diet on feed conversion ratio of broiler chickens	41
4.5	Effect of whole flaxseed and reduced energy diet Omega-3 content in the meat of broiler chickens.	44
4.6	Effect of whole flaxseed and reduced energy diet on serum lipid profile of broiler chickens.	47
4.7	Effect of whole flaxseed and reduced energy diet on liver function of broiler chickens.	49
4.8	Impact of whole flaxseed and reduced energy diet on immunological of broiler chickens.	51
4.9	Effect of whole flaxseed and reduced energy diet on intestinal histology of broiler chickens.	53
4.10	Effect of whole flaxseed and reduced energy based diet on IGF_1 gene expression of broiler chickens.	67
4.11	Effect of whole flaxseed and reduced energy based diet on carcass characteristic.	70

List of Figures

3.1	Experimental design	27
3.2	Analyzed parameters	28
4.1	T1: Photomicrograph of intestine section (duodenum), and mucin activity	54
4.2	T1: Photomicrograph of intestine section (jejunum), and mucin activity	54
4.3	T1:Photomicrograph of intestine section (ileum), and mucin activity	55
4.4	T2:Photomicrograph of intestine section (duodenum), and mucin activity	55
4.5	T2e :Photomicrograph of intestine section (jejunum), and mucin activity	56
4.6	T2:Photomicrograph of intestine section (ileum), and mucin activity	56
4.7	T3 :Photomicrograph of intestine section (duodenal), and mucin activity	57
4.8	T3: Photomicrograph of intestine section (jejunal) and mucin activity	57
4.9	T3: Photomicrograph of intestine section (ileum) and mucin activity	58
4.10	T4: Photomicrograph of intestine section (jejunum) and mucin activity	58
4.11	T4 :Photomicrograph of intestine section (jejunum) and mucin activity	59
4.12	T4 :Photomicrograph of intestine section (ileum), and mucin activity	59

4.13	T5 :Photomicrograph of intestine section (duodenum), and mucin activity	60
4.14	T5 :Photomicrograph of intestine section (jejunum), and mucin activity	60
4.15	T5 Photomicrograph of intestine section (ileum), and mucin activity	61
4.16	Fold change comparison among the groups expressed IGF-1 gene. This shows significant upregulation of the (T3) and (t4) groups compared with control groups.	69

List of abbreviations

ALA	Alpha-linolenic acid
ALT	Alanine transaminase
AST	aspartate aminotransferase
BW	Body weight
DHA	Docosahexaenoic acid
EPA	Eicosapronic acid
FAs	Fatty acids
FCR	Feed conversion ratio
FI	feed intake
FO	Fish oil
PUFA	Polyunsaturated fatty acid
GC	Gas chromatograph
HDL	high density lipoprotein
HS	heat stress
HSF	heat shock factor
HSP	heat shock proteins
LDL	low density lipoprotein
N-3	Omega-3
N-6	Omega-6
PCR	Polymerase chain reaction
ROS	reactive oxygen species
S.D	Standard Deviation
S.E	Standard Error
vLDL	Very low density lipoprotein

Chapter One

Introduction

Introduction

In commercial environments, feed bills can constitute about 70% of the total costs related to broiler production. Thus, any decrease in feed expenses that preserves the health and performance of broilers will directly enhance the profitability of broiler production cycles (Georganas *et al.*, 2023). Heat stress occurs when heat production exceeds the animal's capacity to dissipate heat, and chickens may suffer from hyperthermia when exposed to high ambient temperatures.

Heat stress (HS) has a significant impact on global poultry production and lowers producer profits (Liu *et al.*, 2020). Numerous harmful physiological reactions, including impaired immune function, endocrine abnormalities, and reproductive complications, are brought on by heat stress (HS) (Waheed *et al.*, 2020; Mirzaei *et al.*, 2022). Poultry productivity is adversely affected by heat stress. (Abioja and Abiona, 2021; Uyanga *et al.*, 2023). It lowers feed intake and promotes weight gain, which lowers productivity and raises mortality rates. (Liu *et al.*, 2020). HS influences antioxidant activity and the production of free radicals, which include reactive oxygen and nitrogen species that are very harmful and can interact with essential macromolecules including fatty acids and amino acids (Hu *et al.*, 2019). Animal performance is thought to be adversely affected by a brief state of oxidative stress (Surai *et al.*, 2019).

Flaxseed (*Linum usitatissimum*) from the family Linaceae, produces small flat seeds that contains approximately 40% lipids, 20% proteins, 10–20% fibre, 4% ash and 6% moisture, although the chemical composition differs among varieties and environmental conditions (Kauser *et al.*, 2024).

Flaxseed, abundant in omega-3 FAs, ALA and fiber, is utilized in daily diets to enhance bodily health due to the bioactive properties of its

constituents, which exhibit anti-inflammatory effects, antioxidant capabilities, and lipid-modulating functions (Lu *et al.*, 2020). Omega-3 polyunsaturated fatty acids (omega-3 PUFA), are linked to numerous health benefits for the prevention of human diseases. The dietary enrichment of animal meat and eggs with omega-3 PUFAs may enhance the intake of these fatty acids.

In the chicken industry, particularly in recent years, the escalation of feed ingredient prices, especially those contributing energy, which constitutes around 65% of diet costs, has prompted the investigation of techniques to mitigate feed production expenses. Since they are the most expensive components of the diet, one tactic is to reduce the amounts of metabolizable energy (ME) and crude protein (CP). As a result, several experiments were conducted to improve the development performance of broiler chicks and to reduce costs by reducing the proportion of specific energy components. (Cozannet *et al.*, 2021).

Furthermore, diminishing protein or calorie levels in the diet may decrease nitrogen excretion and mitigate ammonia emissions, thereby contributing to a reduction in environmental effect (Liu *et al.*, 2024). Consequently, reduced-energy diets may diminish heat increase, result in lower fat deposition percentage in the carcass, and subsequently reduce feed and meat costs (Jameel, 2018).

Aims of the stud

The current study will be aimed at using an alternative source of energy and protein by using whole flaxseed in reduced energy diet and studying their effect on productivity performance, intestinal morphology and gene expression subjected under heat stress of broiler chickens by studying of:

1. Effect on productive performance: body weight, weight gain, feed intake, feed conversion ratio.
2. Omega 3 content in the meat by using GC.
3. Effect on liver function: ALT and AST.
4. Effect on lipid profile: cholesterol, triglyceride, HDL, LDL, vLDL.
5. Effect on gene expression (IGF-1).
6. Effect on intestinal morphology villus height, crypt depth, villus width, villus area and mucin activity.

Chapter Two

Review of the Related Literature

2. Review of the related literature

2.1 Heat stress

Stress is a state in which an organism endeavors to reestablish homeostasis, induced by factors or challenges that disturb the body's regular functioning. Poultry, similar to all animals, are frequently subjected to several stressors, social and environmental elements (Scanes, 2016).

An imbalance between the net energy transfer from the animal's body to its environment and the heat energy the animal produces results in heat stress. A combination of environmental factors (such as sunlight, thermal radiation, air temperature, humidity, and movement) and animal characteristics (such as species, metabolic rate, and thermoregulatory processes) may cause this imbalance. Environmental stresses that are particularly detrimental to animal agriculture include heat stress. (Sugiharto, 2020).

Heat stress in poultry production can be described as acute (short and sudden periods of extremely high temperatures) or chronic (extended periods of increased environmental temperatures) (Kpomasse *et al.*, 2021). Both forms of HS can lead to considerable physiological complications, immunological suppression, and dysbiosis of the gut microbiota (Chang *et al.*, 2020; Wasti *et al.*, 2021). The most favorable temperature range (thermo-neutral zone) to enhance broiler performance and health are 33–32°C for the 1st, 32–28°C for the 2nd, 28–26°C for the 3rd, 26–24°C for the 4th, 18–24°C for the 5th and 18–24°C for 6th weeks of age (Cassuce *et al.*, 2013).

Numerous researchers have documented a variety of intervention techniques to lessen the negative effects of HS in the last ten years (Goel *et al.*, 2021). In certain situations, significant and diverse efforts are required to lessen the negative effects of heat stress. Housing, management

techniques, nutrition, genetic selection, early thermal conditioning (EHC), and reduced breeding density are some of the tactics (Toth *et al.*, 2021).

Heat stress impairs normal physiological function, suppress immune system, causes respiratory alkalosis, decreases feed intake and consequently decreased body weight and quantity and quality of eggs (Awad *et al.*, 2020). And also, gut integrity consist of enterocytes and tight junctions is usually negatively influenced by HS, and it causes of immune inflammation takes place due to the penetration of microbes arising from challenged gut health leading to disease infections and eventually raising food safety issues (Zhang *et al.*, 2017).

Chickens lack sweat glands, preventing them from thermoregulating as humans do. Chickens, owing to their heightened susceptibility to heat stress, may seek cooler areas within the dwelling to evade elevated ambient temperatures. Chickens employ many adaptive strategies to cope with heat stress. Chickens regulate their body temperature by spreading their wings, distancing them from their bodies, molting their feathers, and reducing their mobility. Chickens typically consume more water, extend resting periods, and decrease feed consumption (Nawab *et al.*, 2018).

Chickens employ physiological responses as primary defense mechanisms to counteract heat stress, reacting directly to external stimuli. To manage, HS, chickens undertake several measures to regulate body temperature. The Featherless areas of the body often serve as thermal windows that control heat loss. These responses are deemed inadequate if the environmental temperature is excessively elevated (Shakeri & Le, 2022).

2.1.1 Impact of heat stress on poultry production efficiency.

The development rate indicates the managerial and ecological effects on an animal's productivity. In tropical and subtropical locations regions, considerable variations in biological processes negatively affect animal growth traits, including differences in dietary components such as water, protein, minerals and energy metabolism. Multiple studies have shown heat stress adversely affects chicken physiology, health, immunity, and production performance. In high ambient temperatures, chickens prioritize survival over other factors (Sun *et al.*, 2023). Moreover, elevated ambient temperatures can result in increased death rates, welfare concerns and diminished feed consumption. (Zhang *et al.*, 2017).

Heat stress elevates the heat load on chickens, resulting in diminished feed consumption as a behavioral adaptation to lower heat generation (Rostagno, 2020). Stressed chickens struggle to regulate the equilibrium between created and dissipated body heat. Furthermore, HS diminishes feeding efficiency, nutritional digestion, metabolism, and absorption, hence decreasing food availability to cells and physiological functions (Ringseis and Eder, 2022).

Researchers indicate that HS diminishes feeding efficiency, nutritional digestion, metabolism, and absorption, hence decreasing food availability to cells and physiological functions. The decrease in feed intake caused by heat stress may result in diminished energy available for tissue development and disruption of biological functions inside the body's cellular systems. Moreover, HS causes intestinal dysfunction by impairing intestinal architecture and diminishing villus height, both of which are critical for nutrient absorption (Deng *et al.*, 2023).

Research indicates that heat stress causes hormonal imbalances, including changes in thyroid hormones, in broiler chickens. In animals, thyroid hormones are necessary for growth, metabolism, and other critical biological processes; however, heat stress can upset this equilibrium, resulting in inadequate growth (Beckford *et al.*, 2020).

Some studies indicate hormonal imbalances; such as changes in thyroid hormones in broiler chickens after exposure to heat stress. Thyroid hormones are essential for growth, metabolism, and other important biological events in animals; However, HS can upset its balance leading to poor growth (Beckford *et al.*, 2020). Furthermore, chronic heat stress leads to increased liver index (weight and liver enzymes), abdominal fat, and fat accumulation, which reduces the availability of nutrition needed for growth and development (Oladinde *et al.*, 2023). Researchers found that HS stimulates the up regulation of fat synthesis genes and the down regulation of genes related to lipolysis in broiler chickens (Li *et al.*, 2023)

2.1.2 Impact of heat stress on the immune responses of poultry

High temperature stress can affect hormonal stress markers such cortisol. Additionally, the sympathetic-adrenal–medullary (SAM) and hypothalamic-pituitary-adrenal (HPA) axis are activated to maintain redox homeostasis and immune functionality in response to stress (Sejian *et al.*, 2021). During acute periods of HS, the production of cortisol hormone may serve as a trigger for the immune system. However, in case of chronic stress, the release of cortisol has been linked to immune inhibition (Sun *et al.*, 2023).

The animal is more susceptible to diseases and immune challenges when its immune function is suppressed. Of the dual immune reactions, the adaptive and innate response, the adaptive reaction is more multifaceted and

involves prolonged immunological challenge (Mahasneh *et al.*, 2024). Serum immunoglobulin G (IgG) and secretory immunoglobulin (SIgA) were significantly decreased in heat stressed broilers (Li *et al.*, 2023).

SIgA and IgG are 2 main kinds of immunoglobulin in chickens that show a role in maintaining immunity. SIgA has a significant role in protecting and regulating intestinal mucosal health by unraveling the external environment from the inside of the body, limiting the entry of microbes and mucosal antigens into the delicate mucosal barriers. B cells, and directly part to an immune response including neutralization of viruses and toxins form the IgG (Liu *et al.*, 2024).

Numerous studies have reported the harmful effects of HS on immunological responses. Heat stress is responsible for inhibiting the phagocytic activity of leukocytes and suppressing the biosynthesis of B and T lymphocytes (Li *et al.*, 2023). The heterophil to lymphocyte ratio (H/L ratio) is a popular signal of stress in broilers (Al-Murrani *et al.*, 2006). It has been observed that the H/L ratio was augmented in broiler exposed to HS, indicating immune dysfunction. Altan *et al.* (2003) and Nofal *et al.* (2015) discovered that high ambient temperature significantly increased the H/L and basophil ratios while decreasing hematocrit in birds. Inversely, Mashaly *et al.* (2004) recognized that high ambient temperature reduced the H/L ratio in table egg laying hens in addition to decay the actions and quantities of leukocytes.

2.1.3 Impact of heat stress on inflammation.

Heat stress leads to reduced growth rates and a weakened immune system in the majority of the poultry. In addition to that, in circumstances where the animal, is exposed to stress, both pro- and anti-inflammatory cytokines are released from different excrete of various immune tissues and

could play crucial roles in modulating the immune status of broiler. Generally, pro-inflammatory mediators facilitate inflammatory destruction, while anti-inflammatory mediators mitigate inflammation and stimulate the healing process during the environmental stimuli (Bamias *et al.*, 2014).

Some studies reported, Interleukin-10 (IL-10) is a critical anti-inflammatory mediator involved in the inflammatory response. That IL-10 is one of the most significant cytokines associated with numerous pathophysiological circumstances, where it constrains the production of pro-inflammatory mediators. On the other hand, tumor necrosis factor alpha (TNF- α) is a pro-inflammatory mediator, that is widely considered in animal models. Where it is considered, TNF- α is an early and important mediator of hepatic damage). It is well established that HS is a significant environmental factor responsible for liver damage. Therefore, the elevation of TNF- α level in the liver or serum may contribute to liver dysfunction (Hoek and Pastorino, 2002).

Immune modulatory mediators may lessen the negative effects of heat stress by altering the activity of the gut microbiota, controlling cytokine responses, and raising antioxidant levels. Prior research has shown that HS adversely impacts the relative mass and functions of immunological organs (thymus, liver, and spleen) in mice, specifically concerning leukocytes and immunoglobulins (Abdelnour *et al.*, 2019).

According to earlier research, heat stress increases TLR4 and NF- κ B expression (Cheng *et al.*, 2019). NF- κ B is a key intracellular signaling protein that controls the transcription of multiple genes related to apoptosis, inflammation, and cellular progress (Liu *et al.*, 2012). In vitro studies, it has show that NF- κ B has a protective function against heat shock-induced cellular death through its interactions with heat shock protein 27 (HSP27), reactive oxygen species (ROS), and mitogen-activated protein kinases

(MAPKs). The inflammatory signaling system's activation may significantly alter innate immunity brought on by heat stress and initiate an inflammatory reaction. (Lampros *et al.*, 2022).

2.1.4 Impact of heat stress on oxidative state in poultry

One common environmental issue that causes increased oxidative stress in biological systems is heat stress. This situation could lead to a discrepancy between the body's defense mechanisms and reactive oxygen species (ROS). Problems with biological components like DNA, proteins, and lipids could arise from this imbalance. Reactive oxygen species (ROS) are naturally produced by animal cells during metabolic processes, particularly during thermogenesis, via the electron transport chain in the mitochondria (Wang *et al.*, 2023). To fight dangerous substances, the immune system generates high concentrations of specific reactive oxygen species, such as nitric oxide and superoxide radicals. Ion transport and cytokine synthesis depend on reactive oxygen species. (Bilal *et al.*, 2021).

During heat stress, the demand for cellular energy increases, leading to higher production of mitochondrial ROS, to prevent oxidative damage, the body relies on its antioxidant system to neutralize ROS. The main antioxidant enzymes secreted by the body are catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx). However, prolonged heat stress can denature these enzymes, causing tissue damage and cell lesions (Calik *et al.*, 2022). Heat stress elevates the cellular ROS levels in broiler chickens and impairs the effectiveness of the antioxidant system, As a result, the enzymatic antioxidant activities decrease. In broilers, HS leads to significant reductions in various antioxidant elements, such as GPx, SOD, and CAT, the total antioxidant capacity and nuclear muscle factor erythroid 2-related factor 2 (Nrf2), while instantaneously aggregate muscle Kelch-like

ECH-associated protein 1 (Keap1) transcript and the levels of malondialdehyde (MDA) (Hidayat *et al.*, 2023).

A key factor leading to the production of ROS is the outcome of the respiratory chain, which occurs within the inner mitochondrial membrane. Here, the electron transport chain complexes within the mitochondria transfer electrons to oxygen; the Nrf2-mediated antioxidant response pathway maintains cellular redox homeostasis by inducing the transcription of a variety of cytoprotective genes (Lu *et al.*, 2023). Du *et al.* (2023) stated that HS displayed a less SOD level in jejunal tissues and a greater MDA aggregation in serum, hepatic and intestinal tissues in stressed broilers. When the level of ROS increases, cellular molecules such as enzymes, phospholipids, and side chains of polyunsaturated fatty acids (PUFAs), and nucleic acids will lead to modification in the permeability and fluidity of cellular membranes, and eventually in fluctuations in cell function and structure (Hashem *et al.*, 2021).

Deng *et al.* (2023) concluded that the levels of OS indicators (such as MDA and H₂O₂) increased in serum under HS condition. Recently, multiple studies have indicated that phytochemicals can ameliorate OS in various syndrome and stress conditions and reduce lipid peroxidation in liver-renal tissue in heat-exposed broilers these phytochemicals may diminish the damage of antioxidant enzymes activity (SOD, GPX, and CAT) induced by HS via constraining NF- κ B stimulation to reduce ROS production (Abd El-Hack *et al.*, 2020).

2.1.5 Impact of heat stress on intestinal integrity and gut

The diverse microbial populations in the intestine are essential for feed degradation, nutrient absorption and the augmentation of immune system development and function, while the intestine acts as barrier, expelling poisons, pathogenic bacteria and infectious agents. Exposure to extreme temperatures compromises intestinal health, leading to reduced nutritional absorption, weakened immunity, and intestinal dysfunction (Rostagno, 2020).

The effectiveness of intercellular connections is closely linked, to the digestive tract's epithelial cells. The multiplexes maintain the integrity of the epithelial barrier and are vital for intestinal health. (Gieryńska *et al.*, 2022). The gut epithelial connection multiplex comprises desmosomes, adherens junctions, gap junctions, and tight junctions, interconnected by keratin filaments, with adherens junctions positioned beneath tight junctions to facilitate intracellular communication (Raya-Sandino *et al.*, 2021).

Because heat stress weakened tight junctions, luminal pollutants were able to enter the bloodstream (Yang *et al.*, 2021). As a result, chronic systemic inflammation brought on by leaky gut lowers broiler disease resistance. (Zhang *et al.*, 2022). HS together promotes intestinal dysfunction via modifying tight junction proteins, leading to diminished food absorption and, subsequently, growth retardation in broilers.

2.1.6 Impact of heat stress on the quality of poultry meat:

In recent years, the quality of meat and food safety have emerged as prominent issues in the global chicken industry (Lara & Rostagno, 2013). Exposure of chickens to elevated temperatures during rearing can result in inferior meat quality. During and after slaughter, the pH is known to drop quickly due to the conversion of glycogen to lactic acid. Additionally,

because muscle has a reduced capacity to hold water, the denaturation of sarcoplasmic proteins and a lower pH result in meat that is pale, mushy, and exudative. While high body temperatures increase carbon dioxide exhalation, enhance corticosteroid hormone secretion, and disrupt the structure and function of enzymes that regulate sarcoplasmic calcium levels in muscles, prolonged panting during heat stress causes metabolic acidosis in skeletal muscle (Zaboli *et al.*, 2019).

According to a study, HS causes a significant change in the carcass composition of broiler chickens, specifically a reduction in lean tissue, particularly breast yield, while also increasing fat content. (Zampiga *et al.*, 2021).

Various research suggests that rearing hens during severe heat waves may negatively affect food safety. A study suggested that pathogen shedding on poultry farms might be associated with stress conditions, especially heat stress (Pawar *et al.*, 2016). Heat stress causes broiler chickens to have less desirable meat qualities. (Kim *et al.*, 2017). Additionally, it has been observed that the quality of meat decreases when broilers are transported from farms to processing facilities in hot weather. (Lara & Rostagno, 2013).

2.2 Flaxseed

Flaxseeds are among the most ancient crops produced for the extraction of oil and fiber, alongside wheat and barley are regarded as one of the earliest crops utilized by humans for fundamental life necessities and agricultural modernization (Rahman and Hoque, 2023). Archaeological evidence suggests that flax has been farmed from about 6000 BC and is regarded as one of the most ancient and significant advantageous agriculture products (Oybak Dönmez & Hüryilmaz, 2024).

Flaxseed (*Linum usitatissimum*) belongs to the Linaceae family and use an annual herb having blue flowering, which produces flat, oval, and pointed seed consisting of an embryo with two cotyledons embedded by the endosperm. The genus *Linum* in the family Linaceae consists of about 230 species, which are divided into six sections based on morphological characteristics *Linum*, *Linastrum*, *Dasylinum*, *Syllinum*, *Cliococca*, and *Cathartolinum* (Dribnenki *et al.*, 2007).

Flaxseeds have a flat and oval shape with a pointed tip. Compared to sesame seeds, they are slightly larger at about 4-6 mm in size. The seeds are brittle and chewy and have a pleasant nutty taste (Hirdyani and Sharak, 2015). The color of flaxseeds varies depending on the variety from dark yellow to light yellow (Bechlin *et al.*, 2019). The color is determined by the amount of pigment present in the outer shell (the more pigment, the darker the seed). The most common type of flax that is also rich in omega-3 fatty acid - alpha-linolenic acid (ALA) is brown flax while yellow flax is mainly known in two types - omega-rich ALA like brown flax and yellow flax. (Linola), which has a low ALA content and has been possess a varying oil profile (Dribnenki *et al.*, 2007).

The main parts that make up flax seeds are three parts: Embryo (4%), cortex (36%), and cotyledon (55%). The structure contains a thick layer of seed coat and a thin layer of endosperm. The seed weighs about 5 mg and is about 4 to 6 mm long (Daun *et al.*, 2003).

2.2.1 Flaxseed content

Over the past few years, there has been interest in feeding whole flaxseed to poultry, as the addition of flaxseed would increase the total

omega-3 fat content of the carcass. Flaxseed contains, as shown in the table below (2-1) (Leeson & Summers, 2009).

Table (2-1) flaxseed content

Dry Matter 1	90.0	Methionine	0.41
Crude Protein	22.0	Methionine + Cystine	0.82
Metabolizable Energy:		Lysine	0.89
kcal/kg)	35001-42002	Tryptophan	0.29
(MJ/kg)	14.64–17.60	Threonine	0.82
Calcium	0.25	Arginine	2.10
Av. Phosphorus	0.17	Dig Methionine	0.281-0.352
Sodium	0.08 2	Dig Meth + Cys	0.56-0.70
Chloride	0.05 0	Dig Lysine	0.64-0.78
Potassium	1.20	Dig Tryptophan	0.25-0.27
Selenium (ppm)	0.11	Dig Threonine	0.53-0.67
Fat	34.0	Dig Arginine	1.49-1.93
Linoleic acid	5.2		
Crude Fiber	6.0		

Brown flaxseed is used as livestock feed and as a source of dietary fiber (Faintuch *et al.*, 2011). Flaxseeds contain many bioactive compounds, dietary fiber, polyphenols, lignans, vitamins (C, A, E, and F), and minerals (Heimbach, 2009). Oils, protein and dietary fiber are abundant in flax seeds but the starch content is present in lesser quantity. Typically, whole flaxseeds consist of 29-41% fat, 21-31% protein, 21-36% dietary fiber, 1% simple sugars, 3-4% ash, and 4-8% water content. Geographical region, harvest time, environment, conditions, soil conditions, processing method, and variation in varieties alter the chemical composition and oil profile of flax seeds (Coskuner and Karababa, 2007).

Flaxseed oil is an essential resource for α -linolenic acid (Tonon *et al.*, 2011) possessing the ability in reducing low-density lipoprotein primary factors (Kajla *et al.*, 2015). Flaxseed consists of about 55% meal and 45% lipid, 75% of lipid is held in cotyledons and leftover 22% and 3% exist inside the embryo and seed coat (Daun *et al.*, 2003). Flaxseed oil consists of free

fatty acids (0.1%), neutral lipids (98%), phospholipids (0.9%), and oleoresin or coated protein (1.3%) (Daun *et al.*, 2003). On a nutritional basis, flaxseed lipids are of interest due to their stability (Campos *et al.*, 2019).

Flaxseeds are abundant in omega-3 fatty acids. Omega-3 fatty acids are polyunsaturated fatty acids (PUFA) characterized by the presence of the first double bond at the third (Ω) carbon from the methyl terminus of the molecule. The predominant omega-3 polyunsaturated fatty acids (PUFAs) in the diets of individuals from industrialized nations are α -linolenic acid (ALA; 18:3 omega-3), eicosapentaenoic acid (EPA; 20:5 omega-3), and docosahexaenoic acid (DHA; 22:6 omega-3) (Maki and Dicklin, 2019). Alpha-linolenic acid is essential for mammals and birds, as it must be consumed and cannot be generated endogenously. Through desaturation and elongation, alpha-linolenic acid (ALA) can be converted into long-chain fatty acids (FA), such as docosahexaenoic acid (DHA, C22:6omega-3), which are essential for growth and health. (Simopoulos, 2011).

Another significant category of fatty acids is omega-6 fatty acids (n-6 fatty acids), with linoleic acid (LA, C18:2n-6) serving as the precursor. OMEGA-3 and n-6 fatty acids directly compete for the same enzymes necessary for the formation of long-chain fatty acids. This competition may lead to issues when one group's consumption of fatty acids surpasses that of another (Simopoulos, 2011). Vegetable oils, including soybean, corn, and safflower oils, predominantly consist of n-6 fatty acids, although omega-3 fatty acids can be obtained from specific vegetable oils like flaxseed and chia, as well as from marine sources such as algae and oily fish. (Simopoulos, 2011).

The contemporary poultry diet comprises corn, which is abundant in n-6 fatty acids but deficient in omega-3 fatty acids, resulting in a notable

scarcity of these essential fatty acids. Soybean oil (SO) has a substantial proportion of polyunsaturated fatty acids (PUFAs), comprising approximately 53.2% n-6 fatty acids and 7.8% omega-3 fatty acids, in addition to 24% monounsaturated fatty acids and 15% saturated fatty acids. (Mohsen *et al.*, 2019). Consequently, chicken Dietary regimens are significantly lacking in omega-3 fatty acids concentration.

2.2.2 Health Advantages of Flaxseed.

Flax is a botanical food that offers beneficial lipids, antioxidants, and dietary fiber. Flax can be purchased as seeds, oil, powder, or capsules. Supplements containing flaxseed are used to reduce constipation and reduce risks associated with diabetes, cholesterol, heart disease, cancer, and other illnesses. (Shim *et al.*, 2015). Beneficial elements found in flaxseed include lignans, antioxidants, fiber, protein, and polyunsaturated fatty acids, particularly ALA. (Rubilar *et al.*, 2010).

Flaxseeds contain compounds called lignans that may reduce the risk of developing certain cancers, such as colon, ovarian, prostate, and breast cancer. Flaxseed (30 g/day) consumption may reduce the risk of breast cancer by preventing tumor growth. (Lowcock, Cotterchio, and Boucher, 2013). Gut bacteria into mammalian lignans, namely enterolactone and enterodiol, convert the lignans in flaxseeds. These substances produce physiological reactions that are comparable to those of estrogen. (Chang *et al.*, 2019).

Antioxidants, particularly lignans, which are polyphenols connected to insoluble flaxseed fiber, are abundant in flaxseed. Dietary antioxidants, like lignan, reduce the harm caused by free radicals, which are linked to a number of illnesses and conditions. (De Silva & Alcorn, 2019).

2.2.3 Influence of flaxseed on broiler chicken performance.

Flaxseed is considered a vital energy feed ingredient in broiler diets due to its substantial amount of oil (35–45%) and advantageous omega-3 p PUFA, particularly Alpha-linolenic acid (ALA; 45–52 percent) (Gheorghet *et al.*, 2020). According to Anjum *et al.* (2013), flaxseed is a substantial source of protein (20–30%), essential amino acids, dietary fiber, minerals, and vitamins. Studies show that broiler feeds containing 5–15% extruded flaxseed improve growth performance (Mridula *et al.*, 2015).

Zhang *et al.* (2022) determined that differences in body weight (BW), weight gain (WG), feed intake (FI), feed conversion ratio (FCR), and breast muscle weight of ducks on a conventional diet compared to those supplemented with flaxseed oil were statistically insignificant. A 4-week dose of flaxseed oil resulted in an increase in the mass of duck thigh muscles. The slaughter efficiency of the ducks was unaffected by flaxseed oil.

Effect of various soybean oil and fish oil dietary levels on growing quail's live body weight and weight gain over predetermined times. Body weight increased from week 1 to week 3, and there was no statistically significant difference ($P \geq 0.05$) between the various oil sources and the control in live body weight at week 3. When compared to the other experimental groups, the avian groups that were fed meals containing FO at all dosages at five weeks of age showed noticeably higher live weight ($P = 0.001$). The FO-supplemented groups experienced a significantly higher ($P \leq 0.001$) increase in body weight from weeks 1 to 5 and from weeks 3 to 5 than the other groups. (Ahmed *et al.*, 2024).

The experimental diets did not influence body weight, weight gain, feed consumption, or feed-to-gain ratio during the starting phase (1–11 days) ($P > 0.05$). During the growth phase, in comparison to the Control, diet

flaxseed and diet organic Cr showed lower weight gain and a higher feed-to-gain ratio ($P < 0.05$). No significant impact of experimental diets on feed intake was noted during the growth phase (12–21 days) ($P > 0.05$). During the finishing phase (22–42 days), the feed-to-gain ratio was superior in the flax seed diet compared to the control and organic chromium diet ($P < 0.05$), although no significant effects were noted on weight growth and feed intake ($P > 0.05$). Throughout the overall period (1–42 days), the feed-to-gain ratio was superior in the Control group compared to Diet 1 and Diet 2 ($P < 0.05$) (Fraz *et al.*, 2023).

2.2.4 Influence of flaxseed on the meat quality of broiler Chickens

There is an imbalance in the ratio of n-6 to omega-3 polyunsaturated fatty acids (PUFA) and a notable decline in omega-3 PUFA in human diets. While the recommended range is 1 to 4:1, the current ratio of n-6 to omega-3 fatty acids is approximately 10 to 20:1. The main source of omega-3 PUFA, marine fish, is consumed less frequently, which is the reason for the decrease in omega-3 PUFA consumption. The creation of suitable functional foods with altered PUFA content, which are known to have beneficial physiological effects and nutritional advantages, may be a workable solution to this problem. Poultry meat enhanced with omega-3 PUFA could provide a more readily available source of these acids for human consumption. (Galobart *et al.*, 2001).

No correlation was detected between the quality of duck meat and the quantity of flaxseed oil. The quality attributes of meat, such as color, pH, and tenderness of breast and thigh muscles, were unaffected by extended flaxseed oil consumption ($P \geq 0.05$). A 4-week feeding period increased the water content in the thigh muscle of ducks ($P \leq 0.05$), whereas other

nutritional indicators of meat quality, such as proteins, fat, and collagen, showed no significant alterations ($P > 0.05$) (Zhang *et al.*, 2023).

2.2.5 Influence of flaxseed on the lipid profile of broiler chickens

The incorporation of fat in chicken feed is a standard technique vital for fulfilling caloric requirements, promoting the absorption of fat-soluble vitamins, improving feed structure and increasing palatability. Chickens on oil-enriched diets exhibit superior performance compared to those on oil-free diets, albeit having similar nutritional content (Baião & Lara, 2005). Incorporating dietary oils abundant in n-3 fatty acids can significantly alter the fatty acid profile of chicken meat, hence enhancing its nutritional quality (Sierzant *et al.*, 2022).

Researchers indicates that meals rich in polyunsaturated fatty acids lower serum total cholesterol levels. Additional research indicated that broilers consuming diets with lipid sources high in polyunsaturated fatty acids (PUFA) exhibited reduced plasma cholesterol levels compared to those fed diets rich in saturated fatty acids (SFA) (Febel *et al.*, 2008). Compared to broilers fed a diet enriched with lard, the plasma total cholesterol levels of broilers fed diets supplemented with sunflower oil, soybean oil, or linseed oil were significantly lower. Cholesterol levels in the serum of hens consuming FO diets were markedly lower than those in chickens on lard (LF) diets, with the lowest levels observed in chickens fed the FO + pumpkin seeds (PS) diet (Meineri *et al.*, 2018).

In comparison to hens on a lard diet, the addition of perilla oil markedly TG and TCH, while having HDL-C; nevertheless, the levels of LDL cholesterol (LDL-C) in plasma were dramatically diminished by both the perilla oil (PO) and peanut oil (PG) diets. The hepatic levels of

triglycerides (TG) and total cholesterol (TCH) were significantly reduced ($P < 0.05$) in chickens consuming diets supplemented with perilla oil. The activity of the hepatic lipogenic enzyme FAS was dramatically reduced in hens fed perilla oil diets compared to the control diet, by 17.88% (PO), 40.1% (PA), and 50.7% (PG), but the activity of HSL remained unaffected (Cui *et al.*, 2019).

Blood lipid profile indicators were considerably altered by the sources and amounts of dietary oils. Compared to the oil-free control diet, the addition of SO and FO to quail diets at all concentrations decreased LDL ($P = 0.009$) and total cholesterol ($P = 0.014$). Blood triglyceride and vLDL levels were significantly ($P = 0.012$) lowered by varying amounts of FO in quail diets when compared to the control group; however, the values in SO-supplemented groups fell somewhere in between those of the control and FO-inclusive groups. In comparison to the other quail groups (control, 1% SO, and 1% FO), the blood serum concentrations of HDL were significantly higher ($P < 0.001$) in quails fed diets containing 1.5% or 2% SO or FO at all levels. Including 2% FO in the diet produced the best (Ahmed *et al.*, 2024).

2.2.6 Impact of flaxseed on the immune response and antioxidant properties in broiler chickens:

The addition of fatty acids influences the immunological and oxidative condition in hens. It can regulate the immune system via both cellular and humoral immunological responses. Omega fatty acids affect the proliferation, maturation, functioning, and cytokine production of lymphocytes, heterophils, and splenocytes, as well as the synthesis of antibodies such as IgM and IgG. The neutralization of oxidants and the augmentation of antioxidant levels, whether directly or indirectly through fatty acids, mitigate the danger of oxidative stress. The immune response and

oxidative mechanisms are interconnected and mutually affect one other; hence, modifying one can influence the other (Huo *et al.*, 2019, Zollitsch *et al.*, 1997).

Changxing *et al.* (2019) showed that adding fish oil at levels lower than 35 g/kg raised the antibody titers in hens, indicating that a diet high in omega-3 PUFA can change the immune response in chickens. Compared to laying hens that consumed oil rich in n-6 PUFAs (maize oil), those that consumed oils rich in omega-3 PUFAs (fish oil or flaxseed oil) had higher antibody levels. Abdulla *et al.* (2017) demonstrated that, at 42 days of age, dietary omega-3 PUFA (fish or flaxseed oil) significantly enhanced humoral immunity ($p < 0.05$), as evidenced by higher antibody titers against Newcastle disease virus (NDV) in comparison to the control diet.

Al-Khalifa *et al.* (2012) investigated how fatty acid supplementation affected the immune system. Chicks fed omega-3 fatty acid-enriched diets showed a 50–75% decrease in serum and immunological tissue levels of arachidonic acid (AA) (C20:4n-6). However, EPA (C20:5omega-3) and DHA (C20:6omega-3) concentrations increased, suggesting an effect on the immune system. According to the study, moderate intake of omega-3 polyunsaturated fatty acids enhances antioxidative processes, particularly raising laying hens' glutathione peroxidase (GSH-Px) activity and lowering lipid peroxidation in serum and belly fat. (Hamosh, 2007; Velmurugan *et al.*, 2018). When added to Japanese quail diets at a 20 g/kg concentration, fish and flaxseed oils significantly increased GSH-Px activity and total antioxidant capacity while lowering blood levels of thiobarbituric acid reactive chemicals in comparison to the negative control. (Abdulla *et al.*, 2017). Swanson *et al.* (2012) demonstrated that the addition of FiO and FO had a slight but statistically significant ($P < 0.05$) impact on the levels of malondialdehyde (MDA) in broiler chickens.

2.2.7 Impact of Flaxseed on the Intestinal Histology of Broiler Chickens.

In birds, the duodenum and jejunum segments of the small intestine are where nutritional absorption mostly takes place. The gastrointestinal tract's mucosal surface area and the epithelium's functional properties determine how well nutrients are absorbed in the intestine. (Stillhart *et al.*, 2020).

The experimental group's jejunum and duodenum histological characteristics showed crypt cells, mucous cells in the villi, and epithelial cells that resembled those of the control group. According to the jejunum's histomorphometric analysis, broiler chickens' crypt depth, villus height, and villus height/crypt depth ratio were all significantly ($p < 0.001$) improved when flaxseed meal was added to their baseline diet. In comparison to the control groups, the treated groups' jejuna exhibited a marked increase in the enzymatic activity of alpha-amylase and maltase (Popescu *et al.*, 2021).

Huerta *et al.* (2022) demonstrated that the inter-villus distance with respect to the group supplied omega-3 fatty acids during the initial phase was consistently reduced when additional omega-3 fatty acids were added to the grower diet. In broilers, optimal intestinal development improves gastrointestinal health and food absorption, which promotes growth, including muscle development, and may lower the prevalence of muscular myopathies.

Chapter Three

Methodology

3. Methodology

3.1. Experimental design

This study was carried out in a private brooding house, from 27 December 2023 to 31 January 2024. Chicks were obtained from a commercial hatchery in Karbala Governorate (Al-Baz hatchery). Two hundred and fifty, one-day-old Ross 308 broiler chicks unsexed, were divided into five groups (50 chicks/group), with two replicates for each group. Each replicate was 25 birds/pen, and the experimental treatments were as follows:

1. The negative control group (T1) were fed a corn-soybean diet exclusively, without heat stress (HS).
2. The positive control group (T2), were fed a corn-soybean diet under heat stress (HS).
3. The treatment group (T3) provided with a basal diet supplemented with flaxseed (7.5 % in starting feed and 10% in grower feed) under heat stress.
4. The treatment group (T4) provided with a basal diet + flaxseed (7.5 % in starting feed and 10% in grower feed) with reduced energy diet under heat stress.
5. The treatment group (T5), fed with reduced energy diet (200 Kcal/Kg) under heat stress.

The trial period was five weeks. Feed and water were provided along the study. Special programs were used for bird vaccination and health care as recommended in the grill. Figure (3-1) shows the design of the experiment and the study criteria. The lighting was a 23:1 cycle of light and dark. The chick was exposed to heat stress starting from the first day by increasing the temperature up to 3 ° C above normal. Ehere the negative control group

tractor degree was 34_35 ° C on the first day and every 7 days we reduce the temperature by two degrees Celsius until the end of the experiment and throughout the day using a gas-heating incubator. Flaxseeds were purchased from the markets an Indian origin and were dark brown in color.

Experimental design

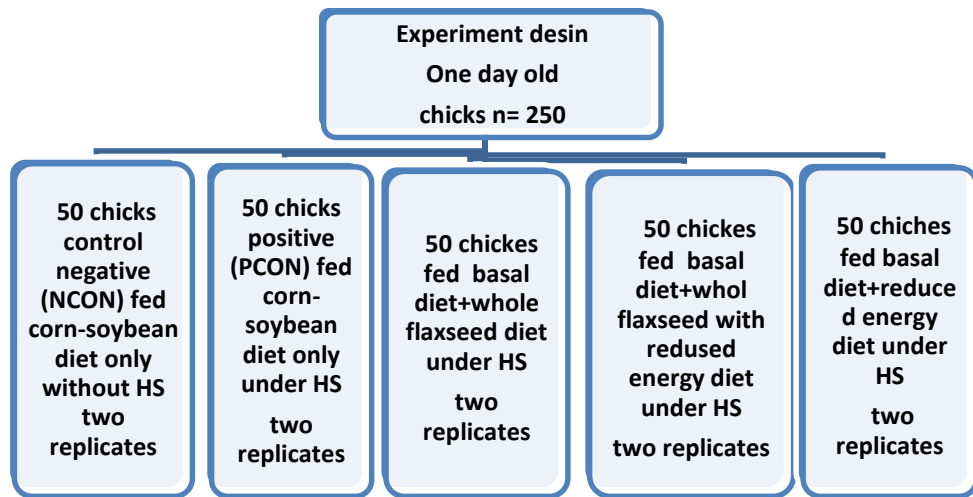


Figure 3-1: Experimental design

Analyzed Parameters

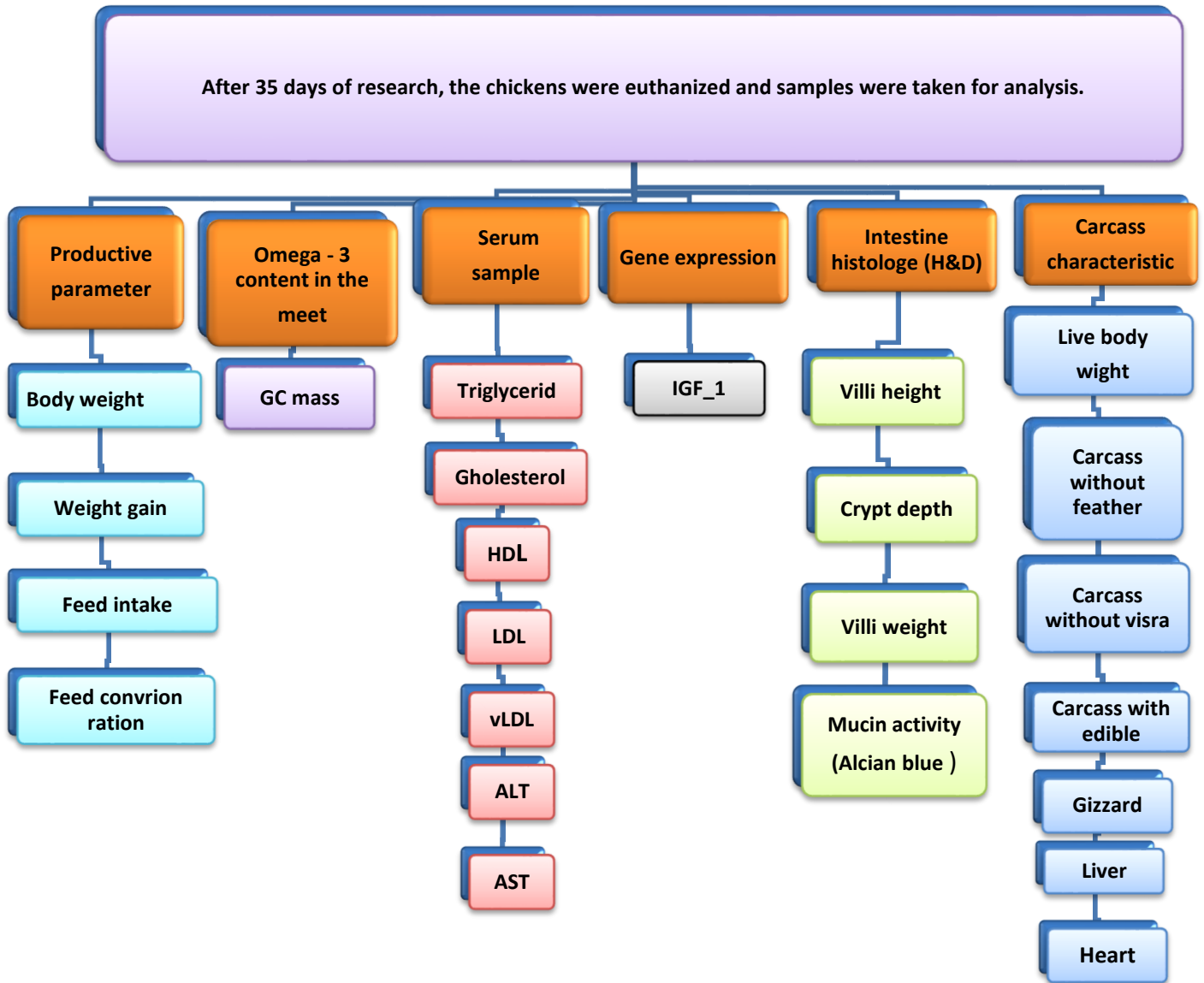


Figure 3-2: Analyzed Parameters.

3.2 Nutrients use in the experiment

Table (3-1) Composition of ingredients and nutrients in broiler starting diets for the first 15 days.

Ingredient (%)	T1 & T2	T3	T4	T5
Yellow corn	52.1	49.2	47	51.2
Soybean meal	40.2	37.1	37.2	40.5
Flaxseed ¹	0	7.5	7.5	0
Vegetable oil	2.9	1.4	0	0
Limestone	1.6	1.6	1.6	1.6
MCP ²	0.6	0.6	0.6	0.6
Premix ³	2.5	2.5	2,5	2,5
Antitoxin	0.1	0.1	0.1	0.1
Sand	0	0	3.5	3.5
Total	100	100	100	100
Calculated chemical composition				
energy	2900	2900	2700	2700
Crude protein (%)	23	23	23	23
Calcium	0.96	0.96	0.96	0.96
Av. Phosphorus	0.54	0.54	0.54	0.54
Av. Lysine	1.3	1.3	1.3	1.3
Av. Methionine	0.65	0.65	0.65	0.65
Av. Threonine	0.87	0.87	0.87	0.87
Electrolyte	289	289	289	289

1. Flaxseed: Ingredients in Table (2-1) page 16.

2. MCP1 GREENPHOSP/22.7 % (Mono Calicium phosphate) cal 16% from, ADANA, Turkey imal feed company) .

3. Composition of Premix2: vitamin, 6,000,000 IU; vitaminD3,1,500,000 IU; vitamin E, 15,000mg; riboflavin, 3,00mg; pantothenic acid, 7,000mg; nicotinic acid, 25,000mg; folic acid, 500mg; vitamin B12, 15,000mg (Vit-VORM 6/1.5· supplid by Animedica, Horb, Germany). 4 composition of trace elements premix supplid per kilogram of premix: Mn, 120,000 mg; Zn, 80,000 mg; Fe, 90,000; Cu, 15,000 mg; I, 1,600 MG; Se, 500 mg (Spu"r Elevor SGI, supplid by Animedica, Horb, Germany).

Table (3-2) Composition of ingredients and nutrients in broiler grower from 16 to 35 days.

Ingredient (%)	T1 & T2	T3	T4	T5
Yellow corn	59.1	55.2	53.7	60.3
Soybean meal	32.6	28.4	29	32.4
Flaxseed ¹	0	10	10	0
Vegetable oil	4	2.1	0	0
Limestone	1.3	1.3	1.3	1.3
MCP ²	0.4	0.4	0.4	0.4
Premix ³	2.5	2.5	2.5	2.5
Antitoxin	0.1	0.1	0.1	0.1
Sand	0	0	3	3
Total	100			
Calculated chemical composition				
energy	3057	3075	2875	2875
Crude protein (%)	20	20	20	20
Calcium	0.96	0.96	0.96	0.96
Av. Phosphorus	0.54	0.54	0.54	0.54
Av. Lysine	1.24	1.24	1.24	1.24
Av. Methionine	0.63	0.63	0.63	0.63
Av. Threonine	0.82	0.82	0.82	0.82
Electrolyte	280	280	280	280

1. Flaxseed: Ingredients in Table (2-1) page 16.

2. MCP1 GREENPHOSP/22.7 % (Mono Calcium phosphate) cal 16% from, ADANA, Turkey (imal feed company) .

3. Composition of Premix2: vitamin, 6,000,000 IU; vitamin D3, 1,500,000 IU; vitamin E, 15,000mg; riboflavin, 3,000mg; pantothenic acid, 7,000mg; nicotinic acid, 25,000mg; folic acid, 500mg; vitamin B12, 15,000mg (Vit-VORM 6/1.5, supplied by Animedica, Horb, Germany). 4 composition of trace elements premix supplied per kilogram of premix: Mn, 120,000 mg; Zn, 80,000 mg; Fe, 90,000; Cu, 15,000 mg; I, 1,600 MG; Se, 500 mg (Spu"r Elevor SGI, supplied by Animedica, Horb, Germany).

3.3 Poultry house preparation

Following the disinfection of the walls, flooring and ceiling with clean water and Glutaraldehyde 150g (15%), Cocobenzyl dimethyl ammonium chloride 100g (10%). Subsequently, after a three-day period, all windows were opened and ventilation was activated to ensure the total removal of

harmful gasses before the chicks' arrival. All feeders and water dispensers were meticulously cleaned and disinfected, and the experimental house was partitioned with wire mesh into ten equal-sized pens (1.5 m x 1.5 m). All experimental treatments were supplied with appropriate litter (wood shavings), and ventilation and illumination were regulated in accordance with the Aviagen guidelines (Aviagen, 2022) for Ross 308 broiler chickens.

3.4 Vaccination programs

This study's vaccination protocol utilized live attenuated vaccines for Newcastle disease (ND), Infectious Bronchitis (IB), and Infectious Bursal Disease (IBD). The vaccination timetable was as follows: On the initial day of life (in the hatchery), chicks were inoculated with Volvac and Lasota strain vaccines via the ocular route against infectious bronchitis (IB) and Newcastle disease (ND), and subcutaneously injected in the neck with Stain 1055 EIDS vaccine for infectious bursal disease (IBD). On day 14, the chickens were administered the Izovac CLONE vaccination against Newcastle Disease via drinking water following a 2-hour water deprivation interval. The procedure was subsequently performed on day 24 utilizing the identical vaccine and administration strategy for ND. Table (3-3) presents the immunization programs (Aviagen, 2016).

Table 3-3 presents the immunization schedule

Age of chicks (days)	Illness	Vaccine classification	Administrative route
1	ND+IB+ IBD ND+H9N1	Volvac®LaSota stain EIDS 10 ^{6.5} , 10 ^{8.5} , 10 ^{8.5} 10 ⁷ , 10 ^{6.5}	Injection Eye drop
14	ND	Izovac CLONE 30 10 ⁶	consuming water
24	ND	Izovac CLONE 30 10 ⁶	consuming water

3.5 Instruments and equipment

The apparatus and instruments utilized in this study are detailed in Table 3-4, along with their respective sources or suppliers.

Table (3-4) enumerating instruments and equipment beside their origins.

No.	Apparatus and Instruments	origin
1	Centrifuge	Japanese
2	Eppendorf tubes and tips	China
3	Disposable gloves	China
4	Deposable syringe (1, 2, 3) cc	China
5	Cooler box	China
6	Gel tube	China laboratory
7	Graduated glass pipettes of sizes 2 cc, 5 cc, and 10 cc	Silber ® -Brand/Germany
8	Refrigerator	Beko ® Turkey
9	Kind-12 multiple channels of communication pipette	Transferpette ®-bRAND /Germany
10	Sensitive electrical balance	Mettler, Switzerland
11	Spectrophotometer	EMCLAB
12	Single channel pipette (micropipette 1-50) microliter)	Sterile glass tube with anti-coagulant EDTA (K3)
13	Test tube rack(stainless steel)	Test tube rack(stainless steel)
14	Medical cotton	labtech Korea
15	Water bath	The Turkey
16	ELIZA printer	Epson/ japan
17	ELIZA reader	Biotek/ USA

3.6 Laboratory `chemicals and reagents:

All laboratory chemicals and reagents were used in this study are listed in table (3-5) with their supplier.

Table (3-5) shows kits and their suppliers

No.	Chemical Suppliers	Suppliers
1.	Newcastle and IBD disease virus antibody test kit	Pro FLOK Kit
2.	Serum aspartate aminotransferase (AST) Kit	Cormay Kit
3.	Serum alanine aminotransferase (ALT) Kit	Cormay Kit
4	Serum cholesterol Kit	Cormay Kit
5	Serum HDL-Cholesterol Kit	Cormay Kit
6	Serum LDL-Cholesterol Kit	Cormay Kit
7	Serum triglyceride Kit	Cormay Kit

3.7 Parameters studied

3.7.1 Productive performance

Al-Fayadh and Naji (1989) assessed weekly output characteristics

3.7.1.1 Weekly mean body weight (BW) (g/birds)

The weekly average body weight was calculated using a calibrated scale by dividing the cumulative weight of all chicks in each replicate by the total number of chicks.

3.7.1.2 Weekly mean weight gains (WG) (g/birds).

Weekly mean body weight gain for each replicate was calculated by substrate recording the Body weight gain at the end of the week and depending on the following equation:

Mean weekly weight gain= mean body weight at the end of the week- mean body weight at the beginning of the week.

3.7.1.3 Weekly mean Feed Intake (FI) (g / bird).

The feed intake was calculated every week depending on weighting the remaining feed at end of the week from the total feed that offered at the beginning of the same week with into consideration taking the number of the dead chicks and number days of feeding.

3.7.1.4 Weekly mean Feed Conversion Ratio (F.C.R) %.

Feed Conversion Ratio was calculated weekly for each group up to the end of experiment. Was reported the equation for measurement of FCR.

$$\text{FCR} = \frac{\text{mean weekly feed intake (gm)}}{\text{mean weekly body weight gain (gm)}}$$

3.7.2 Hematological Sampling.

All blood samples were collected (5cc) at days 35 of age from six (6) birds in each group randomly were obtained from the wing vein in a test tube with EDTA anticoagulant and without anticoagulant. Tubes without anticoagulant were allowed to clot and centrifuged for 10 minute/ 3000 rpm to collect serum. Serum was collected and stored in deep freeze (-20) until analysis. Blood serum was used to determine liver enzymes and lipid profile at 35 days.

3.7.3 Biochemical Parameters

3.7.3.1 Estimation of serum cholesterol levels (mg/dL).

The cholesterol concentration was assessed with the Cormay kit manufactured by PZ CORMAY S.A. Company. Following enzymatic hydrolysis and oxidation, cholesterol is quantified in the presence of phenol and peroxidase, where hydrogen peroxide and 4-aminoantipyrine react to generate quinoneimine, the indicator (Fasce, 1982), as illustrated in appendix (I).

3.7.3.2 Serum Estimation Triglyceride concentration (mg/dL).

The Cormay triglyceride kit, produced by PZ CORMAY S.A. Company, was used to measure the triglyceride concentration. As shown in appendix (II), it undergoes enzymatic hydrolysis to glycerol in accordance with the process described by Fossati and Prencipe (1982).

3.7.3.3 Assessment of serum HDL-Cholesterol levels (mg/dL).

The level of HDL-Cholesterol was evaluated using the Cormay HDL kit produced by PZ CORMAY S.A. The supernatant contains high-density lipoprotein (HDL). HDL-cholesterol is quantified spectrophotometrically by the coupled technique described by Grove (1979), as shown in appendix (III).

3.7.3.4 Estimation of serum LDL-Cholesterol concentration (mg/dL).

As shown in appendix (IV), LDL-C was measured using the Cormay LDL kit produced by PZ CORMAY S.A. (Alan, 2006).

3.7.3.5 Estimation of serum very low-density lipoprotein cholesterol concentration (mg/dL).

vLDL-C was quantified utilizing the equation established by Friedwald in 1972.

$$vLDL = TG/5.$$

3.7.3.6 Assessment of serum glutamic oxaloacetic transaminase (AST) activity.

The serum glutamic oxaloacetic transaminase (AST) activity was assessed utilizing the Cormay kit supplied by PZ CORMAY S.A. (Tietz, 1995). Consult Appendix (V).

3.7.3.7 Assessment of serum alkaline phosphatase (ALT) activity.

Serum Alkaline Phosphatase (ALT) was measured using the Cormay Kit produced by PZ CORMAY S.A. (Soldin *et al.*, 2003), as demonstrated in Appendix (VI).

3.7.3.8 Immunological tests

Serum Immunological tests were done by using ELISA kit as showed in appendix (10).

3.8 Assessment of Omega-3 Fatty Acid Levels in Meat.

Gas chromatography (GC) was used to assess the meat's omega-3 fatty acid content. in accordance with Appendix (VII).

3.9 Gene Expression.

The expression of the insulin-like growth factor 1 gene (IGF-1) from liver tissues was quantified as detailed in Appendix (VIII).

3.10 Histopathological Analysis.

The histology of the intestine was assessed as illustrated in Appendix (IX).

3.11 Measurement of Edible Organ Weight.

The weight of the organs was assessed post-mortem and after evacuation from the body using an electronic scale, and compared with the body weight.

3.12 Statistical analysis.

SPSS 22.0 software was used to analyze the data using the general linear model (GLM) method and one-way ANOVA (Corp, 2011). Using a "protected" Duncan's analysis at the 0.05 significance level, four treatment means were determined.

Chapter Four

Results and Discussion

4. Results and Discussion

4.1 Productive performance of broiler chickens

The results of current study showed an increase significant ($P \leq 0.05$) in the live body weight (BW) and weight gain (WG). The highest significant increase in productive traits (BW) in the (T3) (2178.80 g) and (T4) (2019.70g), (WG) (T3) (2126.86 G) and (T4) (1975.16) groups that feed whole flaxseed and whole flaxseed in reduced energy diet from one day to 35 days. However, FI (3020.06 g) (T3), (3042.7g) (T4) and FCR (1.41) (T3), (1.52) (T4) were decrease significantly ($P \leq 0.05$). The result showed in tables (4-1, 4-2, 4-3 and 4-4).

Table (4-1) Impact of whole flaxseed in reduced energy diet on the BW (g) of broiler chickens

(Mean \pm SD):

Groups Weeks	T1	T2	T3	T4	T5
Day1	43.56 \pm 0.57 A	43.60 \pm 1.29 A	43.58 \pm 1.31 A	43.58 \pm 1.31 A	43.96 \pm 0.74 A
Week1	183.6 \pm 2.67 BC	178.52 \pm 4.92 C	192.58 \pm 3.75 A	188.66 \pm 3.83 AB	183.04 \pm 8.61 BC
Week2	416.44 \pm 5.73 B	399.14 \pm 6.60 C	445.50 \pm 6.59 A	447.60 \pm 7.05 A	447.63 \pm 9.14 A
Week3	907.22 \pm 6.75 C	818.42 \pm 17.28 D	1020.58 \pm 6.09 A	982.71 \pm 37.28 B	904.60 \pm 15.97 C
Week4	1419.68 \pm 3.14 C	1304.23 \pm 8.16 D	1557.24 \pm 8.85 A	1508.40 \pm 7.22 B	1413.55 \pm 13.35 C
Week5	1931.44 \pm 2,49 C	1798.40 \pm 10.72 D	2178.80 \pm 46.34 A	2019.70 \pm 12.44 B	1923.67 \pm 7.99 C

Different letters among treatment show significant difference ($p \leq 0.05$). The control treatment (T1) received a basal diet without additives. (T2) fed basal diet without additives under heat stress; (T3) included whole flaxseed (7.5 % in starting feed and 10% in grower feed) during heat stress; (T4) incorporated whole flaxseed in reduced energy diet (7.5 % in starting feed and 10% in grower feed) during heat stress; (T5) consisted of reduced energy diet during heat stress.

Table (4-2) Effect of flaxseed in a low-energy diet on WG (g) of Broiler Chickens, (Mean \pm SD):

Groups	T1	T2	T3	T4	T5
Week1	140.61 \pm 2.93 BC	134.92 \pm 4.57 C	148.04 \pm 3.69 A	144.12 \pm 3.72 AB	139.08 \pm 8.91 BC
Week2	232.84 \pm 5.22 B	220.62 \pm 9.22 C	252.92 \pm 8.63 A	258.94 \pm 7.80 A	264.59 \pm 14.43 A
Week3	490.78 \pm 8.86 C	419.28 \pm 21.99 E	574.68 \pm 4.89 A	535.11 \pm 39.68 B	456.96 \pm 20.33 D
Week4	512.46 \pm 11.15 AB	485.81 \pm 24.39 B	529.66 \pm 15.65 A	525.68 \pm 39.67 A	508.95 \pm 14.78 AB
Week5	511.76 \pm 4.43 B	499.59 \pm 9.49 B	621.56 \pm 44.24 A	511.29 \pm 10.98 B	510.12 \pm 16.18 B
WG cum	1888.45 \pm 2.30 C	1760.22 \pm 12.08 D	2126.86 \pm 43.60 A	1975.16 \pm 12.27 B	1897.71 \pm 8.32 C

Different letters among treatment show significant difference ($p \leq 0.05$). The control treatment (T1) received a basal diet without additives. (T2) fed basal diet without additives under heat stress; (T3) included whole flaxseed (7.5 % in starting feed and 10% in grower feed) during heat stress; (T4) incorporated whole flaxseed in reduced energy diet (7.5 % in starting feed and 10% in grower feed) during heat stress; (T5) consisted of reduced energy diet during heat stress.

Table (4-3) Impact of whole flaxseed in a reduced energy diet on FI (g) in Broiler Chickens (Mean \pm SD):

Groups	T1	T2	T3	T4	T5
Week1	175.29 \pm .78 A	169.30 \pm .42 B	169.30 \pm .42 B	171.80 \pm .00 AB	173.18 \pm 7.87 AB
Week2	349.00 \pm 5.62 A	338.62 \pm 1.46 C	341.58 \pm 3.93 BC	344.40 \pm .00 B	344.85 \pm .00 B
Week3	801.22 \pm 1.14 A	722.26 \pm 2.23 C	743.96 \pm 11.11 B	745.80 \pm .00 B	740.90 \pm .00 B
Week4	898.54 \pm 14.81 A	832.90 \pm .00 B	839.42 \pm 15.04 B	842.54 \pm 18.25 B	849.12 \pm 11.59 B
Week5	933 \pm 8.41 A	919.72 \pm 3.59 D	925.8 \pm 4.44 BC	920.6 \pm 5 CD	929.92 \pm 3.24 AB
FI cum	3157.05 \pm 16.9 A	2982.8 \pm 4.83 C	3020.06 \pm 16.25 B	3042.7 \pm 47.85 B	3037.97 \pm 16.3 B

Different letters among treatment show significant difference ($p \leq 0.05$). The control treatment (T1) received a basal diet without additives. (T2) fed basal diet without additives under heat stress; (T3) included whole flaxseed (7.5 % in starting feed and 10% in grower feed) during heat stress; (T4)

incorporated whole flaxseed in reduced energy diet (7.5 % in starting feed and 10% in grower feed) during heat stress; (T5) consisted of reduced energy diet during heat stress.

Table (4-4) Effect of whole flaxseed in a reduced energy diet on the FCR of broiler chickens (Mean±SD):

Groups \ Weeks	T1	T2	T3	T4	T5
Week1	1.24±.030 A	1.25±.04 A	1.14±0.02 B	1.19±0.03 B	1.24±0.07 A
Week2	1.50±.05 A	1.53±.06 A	1.35±.03 B	1.33±.03 B	1.17±.20 C
Week3	1.63±.16 B	1.72±.09 A	1.29±.0.02 D	1.40±.10 C	1.62±.07 B
Week4	1.75±0.02 A	1.71±0.08 A	1.58±0.03 B	1.61±0.14 B	1.66±0.04 AB
Week5	1.82±0.01 A	1.84±0.03 A	1.49±0.12 B	1.80±0.03 A	1.82±0.06 A
mean	1.65±0.03 A	1.68±0.03 A	1.41±0.03 D	1.52±0.02 C	1.59±0.04 B

Different letters among treatment show significant difference ($p \leq 0.05$). The control treatment (T1) received a basal diet without additives. (T2) fed basal diet without additives under heat stress; (T3) included whole flaxseed (7.5 % in starting feed and 10% in grower feed) during heat stress; (T4) incorporated whole flaxseed in reduced energy diet (7.5 % in starting feed and 10% in grower feed) during heat stress; (T5) consisted of reduced energy diet during heat stress.

The increment of production performance may be due to, the addition of whole flaxseed in poultry feed. That diet contains a high percentage of oil in the form long-chain polyunsaturated fatty acids as these oil exert many advantages such as increasing the total metabolism, improving the breakdown and absorption of lipoproteins. In addition to their fatty acid (FA), content. Oils can improve palatability, energy utilization and vitamin absorption, as well as increase the absorption of all nutrients by reducing the rate of passage of food consumed through the digestive system. Our result agreement with (Ahmed *et al.*, 2024) who found that adding of supplementing growing quail diets flaxseed oils up to 2% could yield favorable effects on growth performance.

The enhancement productive traits may be due to enhance IGF-1 gene expression shown in table (4-9). It is also known that IGF-1 has an important role in carbohydrate, fat, and protein metabolism in several tissues, e.g. muscle, fat, liver and stimulate proliferation, differentiation and metabolism of myogenic cell lines from different species. The IGFs regulate body and muscle growth in chickens. Diets with elevated omega-3 PUFA content might affect IGF-1 expression since IGF1 mRNA level in liver and muscle greatly depends on nutritional status. Our result agreements with (Szalai *et al.* 2021) who reported that the use of diet enriched with omega-3 PUFA led to enhance of the IGF-1 expression.

The improvement of productivity traits might be due to, the use of whole flaxseed in poultry feed that led to enhance the gene expression of MUC2 gene. This gene works enhancement of mucin density in all regions of the intestine this leads to an increase in the of villus length, area and this evidence in enhancing of intestinal health, where leads to improve in metabolic, absorption and leads to an increase in body weight, as shown in the figures (4.6 to 4.12). Our result agreements with (Obaid, 2023) who reported that use whole flaxseed in poultry feed the enhance of the gene expression of MUC2 gene.

Omega-3 might enhance the release of serotonin and dopamine, two crucial neurotransmitters that influence mood (Whittle *et al.*, 2024); Animals may become more relaxed and less stressed as a result.

Reduced inflammation and anti-inflammatory responses have been linked to omega-3's effect on eicosanoid production and synthesis. Therefore, the production of various eicosanoids that are involved in immunological responses and inflammation is greatly impacted by the regulation of omega-6 to omega-3 ratios. (Al-kalo *et al.*, 2024).

The kind of dietary fat and the time of feeding may affect the inflammatory response in broiler hens during a challenge. A diet abundant in omega-3 FAs following hatching may bolster immune system development in broiler chickens by reducing the production of inflammatory eicosanoids, consequently diminishing the incidence of inflammatory disorders, which could enhance avian health and production efficacy in broiler chickens. Our result agreements with (Ghanbari *et al.*, 2024) who reported that use omega-3 FA abundant the immune system and thus reduces inflammatory eicosanoids.

Flaxseed oil exerts positive effects on heat-stressed chickens through its antioxidant properties, which also alleviate other detrimental impacts linked with heat stress (Ansari, 2024). A prior study demonstrated the influence of PUFA on specific metabolic processes in heat-stressed chickens. The results reveal a significant impact on carbohydrate, lipid, protein, and mineral metabolism in heat-stressed fowl. Carbohydrate, lipid, and protein metabolisms demonstrate complex interrelations within the biological system, typically down-regulating fat and carbohydrate metabolism while up-regulating protein metabolism (Elbaz *et al.* 2023).

4.2 Impact of flaxseed and reduced energy diet on omega-3 content in the meat under heat stress.

Omega-3 content in the meat the current study showed increase a significant ($p \leq 0.05$) in Omega-3 meat content, ALA, EPA and DHA were increase, in T3 and T4 respectively groups as compared to the other groups while there is no significant difference among other groups as shown in table (4-5).

Table (4-5) Impact of whole flaxseed and a decreased energy diet on Omega-3 levels in broiler chicken meat (Mean±SD).

Parameters (%)	T1	T2	T3	T4	T5
Thigh					
ALA	8.16±.33 C	7.66±.37 C	11.79±.46 A	10.95±.56 B	7.92±.39 C
DHA	3.53±.20 B	3.02±.08 C	5.07±.14 A	4.98±.39 A	3.17±.27 C
EPA	4.49±.13 C	3.98±.16 D	6.80±.15 A	6.11±.16 B	4.00±.08 D
Breast					
ALA	3.39±.29 B	3.00±.09 C	5.28±.23 A	5.10±.14 A	3.32±.29 B
DHA	2.41±.25 B	2.25±.22 B	4.11±.06 A	4.01±.06 A	2.44±.21 B
EPA	4.19±.06 C	3.85±.18 D	5.39±.11 A	5.16±.13 B	3.99±.14 D

Different letters among treatment show significant difference ($p \leq 0.05$). The control treatment (T1) received a basal diet without additives. (T2) fed basal diet without additives under heat stress; (T3) included whole flaxseed (7.5 % in starting feed and 10% in grower feed) during heat stress; (T4) incorporated whole flaxseed in reduced energy diet (7.5 % in starting feed and 10% in grower feed) during heat stress; (T5) consisted of reduced energy diet during heat stress.

Flaxseed have a low content of saturated FAs (9%), moderate content of monounsaturated FAs (18%) and high content PUFAs (73% omega-3 FAs) (Bartram, 2013). Soybean oil, a prevalent energy source in poultry diets, have a high content of PUFAs (53.2% n-6 FAs) and (7.8% omega-3 FAs), in addition to 15% saturated FAs and 24% monounsaturated FAs (Mohsen, 2019). Consequently, supplementing with flaxseed can improve the concentration of omega-3 FAs and n-6/omega-3 ratio in poultry diets. However, while reducing the ratio of n-6/omega-3 (Saber and Kutlu, 2020).

Whole flaxseed contains 35%–45% oil, mostly comprising over 70% α -linolenic acid, making linseed an essential source of fat in animal feeds, especially for n-3 fatty acid meat enrichment (Moghadam *et al.*, 2017). Dietary linolenic acid can be physiologically transformed into other beneficial n-3 polyunsaturated fatty acids; hence, the composition of carcass

body fat in poultry may be influenced by dietary polyunsaturated fatty acids (Bostami *et al.*, 2017).

The increase meat of omega-3 may be due to the chicke fed diet enriched with omega-3 PUFAs that led to absorbed it from the small intestine. In addition, we found that the total FA level in the pectoral muscles of was lower than that in the thigh muscles. Our result agreements with (Zhang *et al.*, 2023) who reported that use different structures of dietary FA as an effective means to modify the FA profile of broiler chickens.

The improvement in n-3 may be due to the increase in expression of genes related to FA transport and synthesis, such as FKBP5, FABP1, and ELOVL5 in the breast muscle, FA are transported from the cell membrane to sites of TG and phospholipid synthesis or catabolism. Our result agreements with (Zhang *et al.*, 2023), who reported that use ALA from flaxseed oil can either enter the cell directly via FATP1, or it can first bind to the CD36 molecule on the cell membrane, from which it is delivered to FATP1 and transported into the cell. ALA-entering cells may be catalyzed by ACSL1 to undergo esterification, or is transported to organelle membranes via the FABP family.

Seafood, especially fatty fish (such as mackerel, herring, and salmon), is the main dietary source of omega-3 and contains much higher levels than other meat sources. However, for various reasons, including high price, availability, sustainability concerns, allergies, and consumer preferences. It has been reported that omega-3 enriched poultry meat can be used as a substitute for seafood after feeding it flaxseed, which is rich in polyunsaturated fatty acids, as it contains ideal levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Yi *et al.*, 2023).

Flaxseed oil is a prominent source of ALA and substituting dietary lipids with flaxseed oil in broiler diets has proven effective in enhancing meat omega-3 LCPUFA while circumventing the drawbacks associated with marine oils. Chickens inherently contain the hepatic enzymes necessary to metabolize alpha-linolenic acid (ALA, present in certain vegetable oils) into omega-3 polyunsaturated fatty acids (PUFAs). Consequently, a viable strategy for augmenting omega-3 PUFA concentrations in poultry products could involve administering diets rich in ALA (Al-Khalaifah *et al.*, 2021).

Increased n-3 PUFA concentrations lead to decreased n-6: n-3 PUFA ratios in birds fed diets containing whole flaxseed. Our result agreements with (Shahid *et al.*, 2019), who reported that use flaxseed oil increased total n3 linearly in meat, while SFA, MUFA, and n-6 PUFA linearly decreased with the duration of the flaxseed diet.

4.3 Biochemical parameters.

4.3.1 Impact of whole flaxseed and reduced energy diet on the serum lipid profile of broiler chicks under heat stress.

There was a significant decrease ($P \leq 0.05$) in the cholesterol, triglyceride LDL and vLDL concentration at T3 and T4 groups as compared with the control treatments. High-density lipoprotein (HDL) values increased significantly ($P \leq 0.05$) in the (T3) and (T4) as compared to control. Which T3 recorded high value, as shown in table (4-6).

Table (4-6) Effects of flaxseed and a caloric-restricted diet on the serum lipid profile of Broiler chickens (Mean±Sd):

Parameters(mg/dl)	T1	T2	T3	T4	T5
Cholesterol	150.50±4.36 B	162.66±11.39 C	123.26±4.25 A	130.58±5.17 A	154.76±9.16 BC
Triglyceride	49.18±4.90 BC	54.76±3.18 C	35.06±5.07 A	38.52±7.54 A	46.38±3.83 B
HDL	83.04±15.59 B	77.04±15.72 B	130.60±6.40 A	133.78±5.57 A	88.12±20.21 B
LDL	25.86±6.33	26.88±3.44	20.32±7.21	21.46±7.66	23.04±1.38
vLDL	11.72±1.77 B	12.54±2.00 B	8.80±2.12 A	10.30±2.41 AB	10.98±2.29 B

Different letters among treatment show significant difference ($p \leq 0.05$). The control treatment (T1) received a basal diet without additives. (T2) fed basal diet without additives under heat stress; (T3) included whole flaxseed (7.5 % in starting feed and 10% in grower feed) during heat stress; (T4) incorporated whole flaxseed in reduced energy diet (7.5 % in starting feed and 10% in grower feed) during heat stress; (T5) consisted of reduced energy diet during heat stress.

Increase HDL-C levels of serum may be due to use flaxseed. Flaxseed may increase the liver's capacity to produce HDL by influencing the expression of particular genes. Flaxseed reduced hepatic lipogenesis results in reduced plasma triglyceride levels, as triglycerides are transported into the circulation via lipoproteins. Our result agreements with (Ahmed *et al.*, 2024) who reported added flaxseed oil in quail's diet, at 7 weeks of age, showed a significant decrease in serum total cholesterol, triglycerides, LDL, and vLDL, alongside a marked increase in serum HDL levels.

In our study, flaxseed diet resulted in an increase of good cholesterol (HDL) and a linear decrease in bad cholesterol like LDL, VLDL, and TG, that diet enrichment with PUFA has a beneficial effect on serum lipid profile or that increasing n-3 PUFA in the diet of chickens increases HDL and reduces TG. This is in agreement with results (Mridula *et al.*, 2015) indicating that diet Feeding flaxseed beneficial effect on serum lipid profile in poultry, and abdominal fat decreased linearly.

Improvement in lipid profile, may be due to use PUFA where reduces the activity of lipoprotein lipase, an enzyme that hydrolyzes triglycerides from very low-density lipoprotein (vLDL) particles upon tissue arrival. Moreover, polyunsaturated fatty acid promotes bile excretion, with chicken bile displaying elevated levels. This composition allows bile to serve as a reservoir for lipid excretion in chickens. Thus, any method that obstructs intestinal reabsorption or enhances excretion is considered helpful in lowering blood cholesterol levels specifically and lipids in general. This is in agreement with results (Reda *et al.*, 2020), who noted that dietary flaxseed oil significantly decreased the concentration of total cholesterol and triglycerides in Japanese quail blood.

Increased plasma concentrations of HDL while those of albumin, triglycerides, total cholesterol, and LDL decreased may be due to flaxseed was added to the chicken diet. This is in agreement with results (Sokoła-Wysoczańska *et al.*, 2024) indicating that diet feeding flaxseed oil beneficial effect on serum lipid profile in poultry.

4.3.2 Impact of whole flaxseed and reduced energy diet on the liver function of broiler chickens.

Table (4-7) showed the effect of whole flaxseed and reduced energy based diet on liver function enzymes of broilers at 35th days under heat stress. The result revealed a significant difference ($P \leq 0.05$) in AST, and ALT in all groups, T3 and T4 treatments showed a significant decrease ($P \leq 0.05$) in AST and ALT as compared with the control, as shown in table (4-7).

Table (4-7) Impact of whole flaxseed in a reduced energy diet on the liver function of broiler chickens (Mean±SD):

Parameters	T1	T2	T3	T4	T5
AST (U/L)	26.12±2.99 AB	28.46±4.50 B	22.80±3.96 A	24.14±3.23 AB	25.60±3.82 AB
ALT (U/L)	142.80±12.54 B	146.40±13.52 B	82.92±17.49 A	85.44±12.16 A	139.60±10.46 B

Different letters among treatment show significant difference ($p \leq 0.05$). The control treatment (T1) received a basal diet without additives. (T2) fed basal diet without additives under heat stress; (T3) included whole flaxseed (7.5 % in starting feed and 10% in grower feed) during heat stress; (T4) incorporated whole flaxseed in reduced energy diet (7.5 % in starting feed and 10% in grower feed) during heat stress; (T5) consisted of reduced energy diet during heat stress.

Two essential biochemical indicators of hepatic function are ALT and AST. These are intracellular enzymes whose levels rise as a result of cellular damage, including damaged cell membranes and hepatocyte necrosis. Skeletal and cardiac muscles' mitochondria, cytoplasm, and liver all contain AST. (Abu-Zeid *et al.*, 2024). This enzyme is thought to be a crucial catalyst for the biosynthesis of cholesterol. Elevated levels of the biochemical indicators of liver function, serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) indicate hepatic injury.

The improved liver function, by lower AST, ALT. Such effects of these flaxseed are probably due to the anti-radical and antioxidant properties of flaxseed, which which contains omega-3 are possibly helpful in enhancing antioxidants against the oxidative stress that the liver tissue. This is in agreement with results (Haščík *et al.*, 2022) indicating that feeding quail on diets feeding flaxseed oil beneficial effect under heat stress on improved liver function lower AST, ALT.

The improved liver function, result of use flaxseed has anti-radical and antioxidant properties that effectively inhibit lipid peroxidation of the cell membrane, the supplement reduced the blood AST and ALT levels of broiler chickens. This is in agreement with results (Ahmed *et al.*, 2024) indicating

that feeding broiler chickens on diets feeding flaxseed oil preventing liver damage may be connected to changes in ALT and AST activity in hens given it. As a result, adding flaxseed oil to poultry diets maintained normal physiological functions and had no negative effects on hepatic function.

The improved liver function, by decreased AST and ALT levels may be due to marked decreases in IL-6 and TNF- α (pro-inflammatory cytokines) and marked increases in IL-4 and IL-10 (anti-inflammatory cytokines) in the n-3 FAs –treated. In addition, protective effects of PUFAs as indicated by the improvements in immune function and antioxidant status, which protect organ tissues and support their functions. This is in agreement with results (Jameel *et al.*, 2017) noted comparable results in broilers fed on diets containing flaxseed oil and investigated significant improvements in AST and ALT. In addition, (Shahid *et al.*, 2019) stated that feeding Peking duck on linseed-supplemented diets for up to 30 d decreased serum AST linearly.

Transaminase enzymes (AST and ALT) serve as the principal tests for assessing liver function and tissue integrity. Elevated levels of these markers in the bloodstream signify organ impairment. Our research suggested that incorporating whole flaxseed into broiler chick diets enhanced energy levels and improved liver function, as seen by decreased AST and ALT levels (Table 4_7). The effects of these oils are likely due to the antioxidant properties of omega-3 fatty acids, which may enhance antioxidant defenses against oxidative stress that damages liver tissue (Kumari *et al.*, 2017).

4.3.3 Impact of whole flaxseed and reduced energy diet on immunological of broiler chickens.

Immune response was enhancing by increase of Antibody titer against ND and IBD vaccines. Antibody titter was increased significantly (P

≤ 0.05) of ND and IBD in the (T3) and (T4) compared with other groups as show in table (4-8).

Table (4-8) Impact of whole flaxseed in a reduced energy diet on the immunological of broiler chickens (Mean \pm SD)

Parameters	T1	T2	T3	T4	T5
ND	2835 \pm 44.76 C	1925 \pm 35.50 D	3735 \pm 38 A	3252 \pm 15,4 B	2916.3 \pm 25 C
IBD	2150 \pm 190 B	1120 \pm 75,4 D	2735 \pm 205 A	2563 \pm 195 AB	1985 \pm 188 C

Different letters among treatment show significant difference ($p \leq 0.05$). The control treatment (T1) received a basal diet without additives. (T2) fed basal diet without additives under heat stress; (T3) included whole flaxseed (7.5 % in starting feed and 10% in grower feed) during heat stress; (T4) incorporated whole flaxseed in reduced energy diet (7.5 % in starting feed and 10% in grower feed) during heat stress; (T5) consisted of reduced energy diet during heat stress.

The use of whole flaxseed in broiler feed improved the immune status against ND and IBD. This is consistent with the results (Obaid, 2023) indicating that a diet containing 10% whole flaxseed improved the immune status against ND and IBD.

The improvement in immune status as a result of the use of omega-3 unsaturated fatty acids, as they can regulate the immune system through cellular and humoral immune responses. Omega fatty acids affect the proliferation, maturation, function and production of cytokines in lymphocytes, heterogeneous cells and splenocytes, in addition to the synthesis of antibodies such as IgM and IgG. This is in agreement with results (Huo *et al.*, 2019) indicating that diet Feeding omega-3 beneficial effect on the immunological and oxidative condition in hens.

The improvement in immune status because of the use of flaxseed oil, dietary omega-3 PUFA (flaxseed oil) significantly enhanced humoral immunity as evidenced by higher antibody titers against Newcastle disease virus (NDV) in comparison to the control diet. This is in agreement with results (Abdulla

et al., 2017) indicating that Feeding flaxseed oil beneficial effect increase antibody titers against Newcastle disease virus.

The improvement in immune status because of the use of omega-3 fatty acid-enriched diets showed a decrease in serum and immunological tissue levels of arachidonic acid. However, EPA and DHA concentrations increased, suggesting an effect on the immune system. Moderate intake of omega-3 polyunsaturated fatty acids enhances antioxidative processes, particularly raising laying hens' glutathione peroxidase (GSH-Px) activity and lowering lipid peroxidation in serum. This is in agreement with results (Abdulla *et al.*, 2017) indicating that Feeding flaxseed oil beneficial effect increased GSH-Px activity and total antioxidant capacity.

4.4 Impact of whole flaxseed in reduced energy diet on the intestinal, and Mucin activity of Broiler Chickens.

There was increase significant ($P \leq 0.05$) Length, width, and area villi and crypt depth in duodenum. Jejunum and ileum treatment group (T3) and (T4) as compared to control group as shown in table (4- 10) and figure (4-1 to 4-15).

In the study show a significant higher density of mucin in the cytoplasm after stain the sample with Alcian blue stain which stain acidic mucin in the flaxseed (T3) and flaxseed in reduced energy diet (T4) group as compared to the control group as shown in figure (4-4, 4-5 and 4-6).

Table (4-9) Impact of whole flaxseed and a reduced energy diet on the intestinal morphology of broiler chickens (Mean±SE)

Parameters	T1		T2		T3		T4		T5	
	Mean	±S. E	Mean	±S. E	Mean	±S. E	Mean	±S. E	Mean	±S. E
Duodenum										
Villi length(mm)	1.511b	0.089	1.467a	0.083	1.605	0.053	1.584	0.053	1.520 b	0.058
Villi width (mm)	0.080 b	0.004	0.058 a	0.006	0.1081	0.0091	0.106	0.004	0.083 b	0.004
Villi area (mm ²)	0.120 b	0.010	0.084 a	0.011	0.173	0.012	0.168	0.007	0.126 b	0.008
Crypt depth(m m)	0.177b	0.020	0.139 a	0.013	0.211	0.013	0.199	0.019	0.182 b	0.020
Jejunum										
Villi width(m m)	0.124b	0.007	0.101 a	0.014	0.143	0.008	0.138	0.011	0.118b	0.007
Villi length(mm)	1.36b	0.12	0.99a	0.09	1.57	0.08	1.44	0.09	1.32b	0.12
Villi area (mm ²)	0.169b	0.020	0.101a	0.016	0.225	0.019	0.199	0.022	0.156b	0.018
Crypt depth(m m)	0.162 b	0.023	0.129 a	0.009	0.204	0.010	0.178c	0.025	0.166 b	0.023
Ileum										
Villi length(mm)	0.85b	0.14	0.70 a	0.07	1.04	0.06	0.91 c	0.12	0.85b	0.08
Villi width (mm)	0.087 b	0.010	0.065 a	0.012	0.106	0.009	0.107	0.007	0.089 b	0.008
Villi area (mm ²)	0.074 b	0.015	0.046 a	0.010	0.111	0.013	0.097	0.013	0.076 b	0.014
Crypt depth(m m)	0.153 ^b	0.025	0.115 ^a	0.011	0.194	0.013	0.162 ^c	0.025	0.155 ^b	0.024

Different letters among treatment show significant difference ($p \leq 0.05$). The control treatment (T1) received a basal diet without additives. (T2) fed basal diet without additives under heat stress; (T3) included whole flaxseed (7.5 % in starting feed and 10% in grower feed) during heat stress; (T4) incorporated whole flaxseed in reduced energy diet (7.5 % in starting feed and 10% in grower feed) during heat stress; (T5) consisted of reduced energy diet during heat stress.

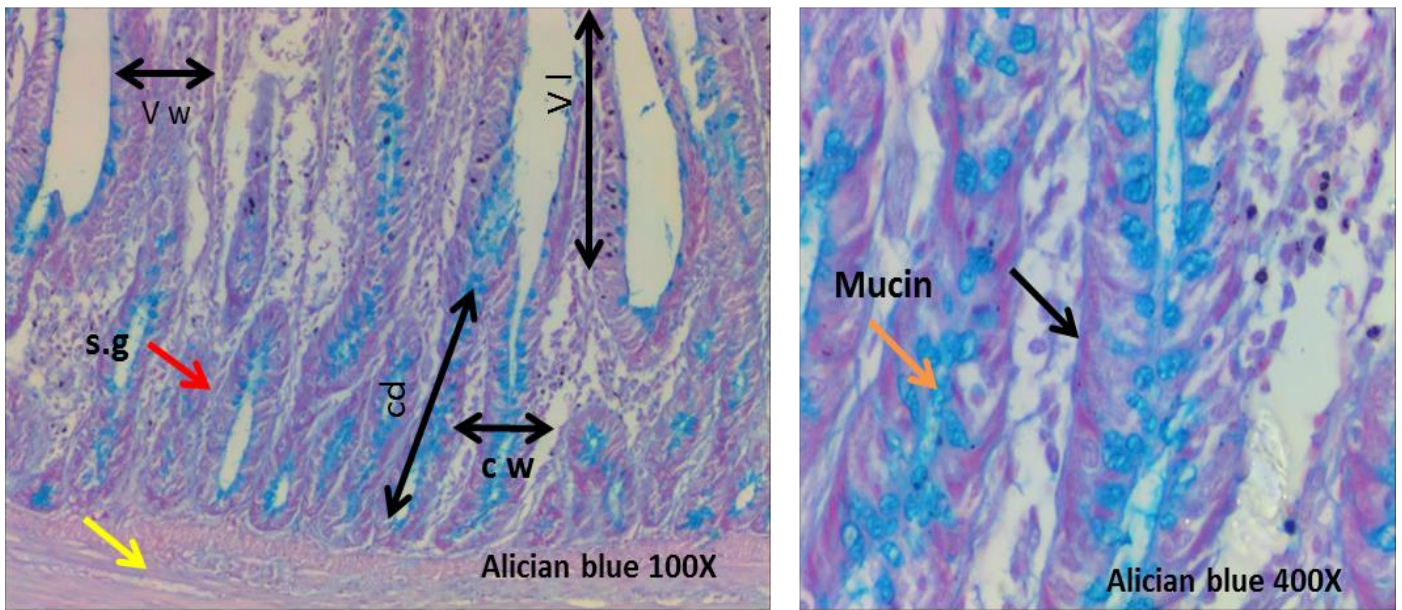


Figure (4.1) : T1: Photomicrograph of intestine section (duodenum), from a control birds group showing, histological architecture of the tubular wall which consists, of tunica mucosa, significant regular of sub mucosa connective tissue represented of cord entire villi (length villi black arrow VI; width villi V w) with mucin was observed in cytoplasm of secretory epithelial cells of crypt of Lieberkuhn (orang arrow)and villus epithelial cells glands (red arrow), so show up (crept depth cd ; crept width c w), and tunica muscles layer (yellow arrow)

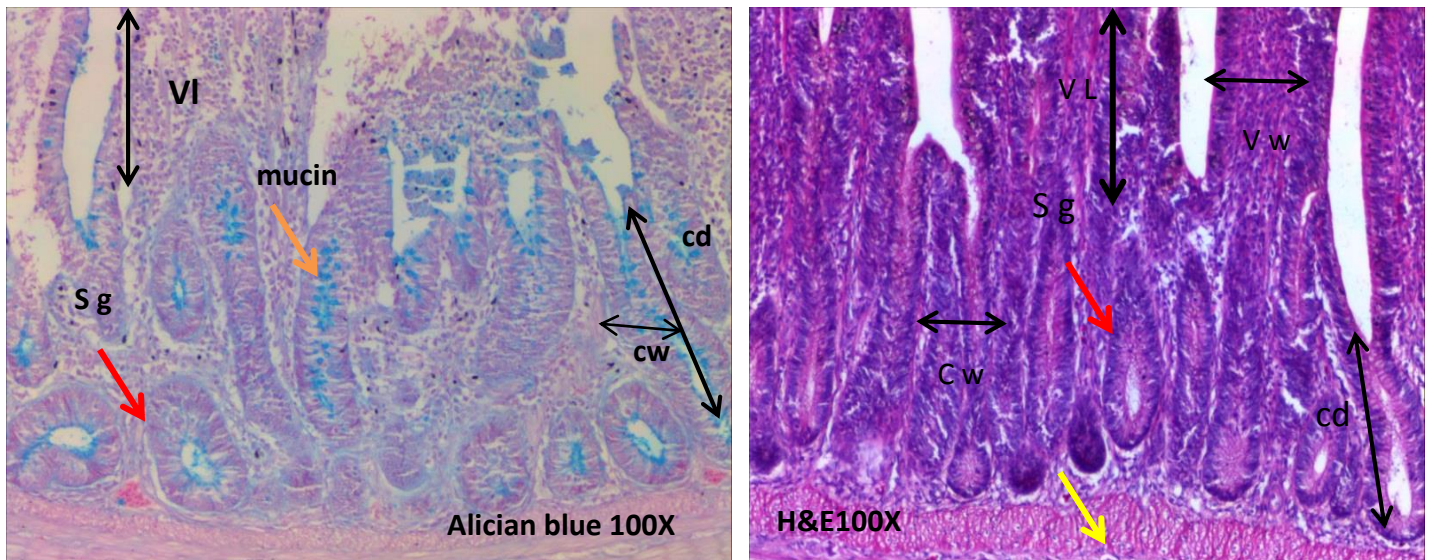
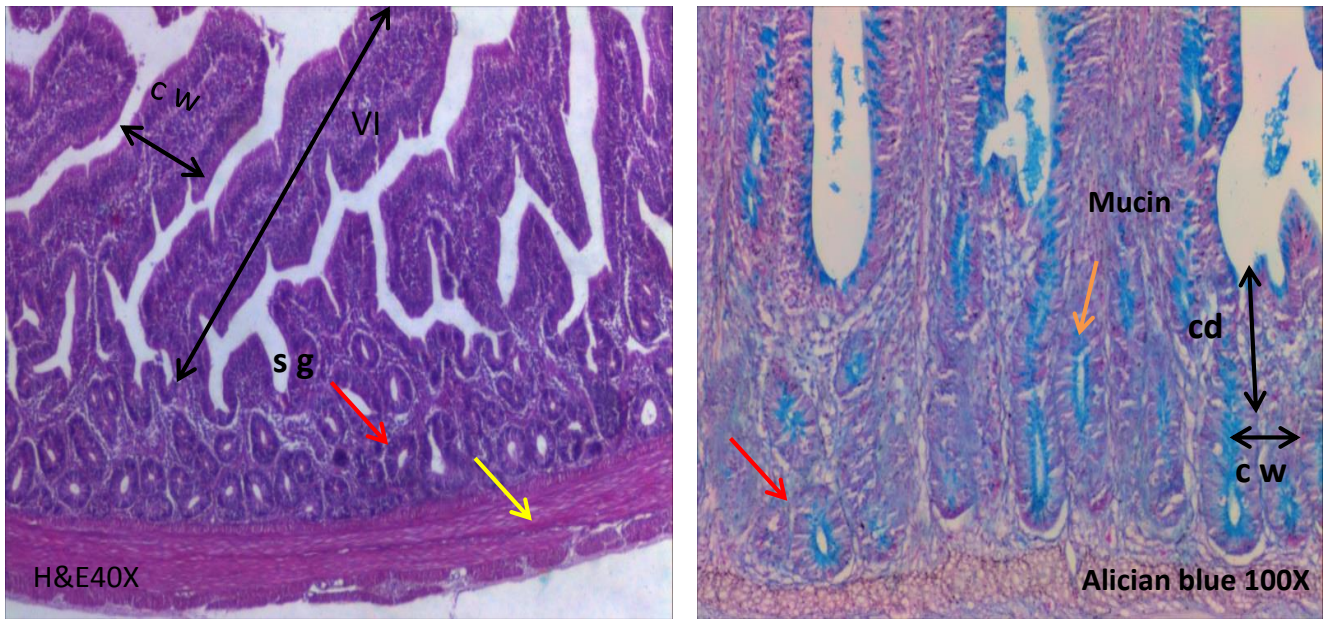


Figure (4.2) : T1: Photomicrograph of intestine section (jejunum), from a control birds group showing, histological architecture of the tubular wall which consists, of tunica mucosa, significant regular of sub mucosa connective tissue represented of cord entire villi (length villi VI black arrow ; width villi V w)with (orang arrow)mucin was observed in cytoplasm of secretory epithelial cells of crypt of Lieberkuhn (orang arrow)and villus epithelial cells glands (red arrow),also show up (crept depth cd ; crept width c w), and tunica muscles layer (yellow arrow)



Figure(4.3) : T1 :Photomicrograph of intestine section (ileum), from a control birds group showing, histological architecture of the tubular wall which consists, of tunica mucosa, significant regular of sub mucosa connective tissue represented of cord entire villi (length villi VI black arrow; width villi V w)with mucin was observed in cytoplasm of secretory epithelial cells of crypt of Lieberkuhn (orang arrow) and villus epithelial cells glands (red arrow), like that (crept depth cd ; crept width c w), and tunica muscles layer (yellow arrow)

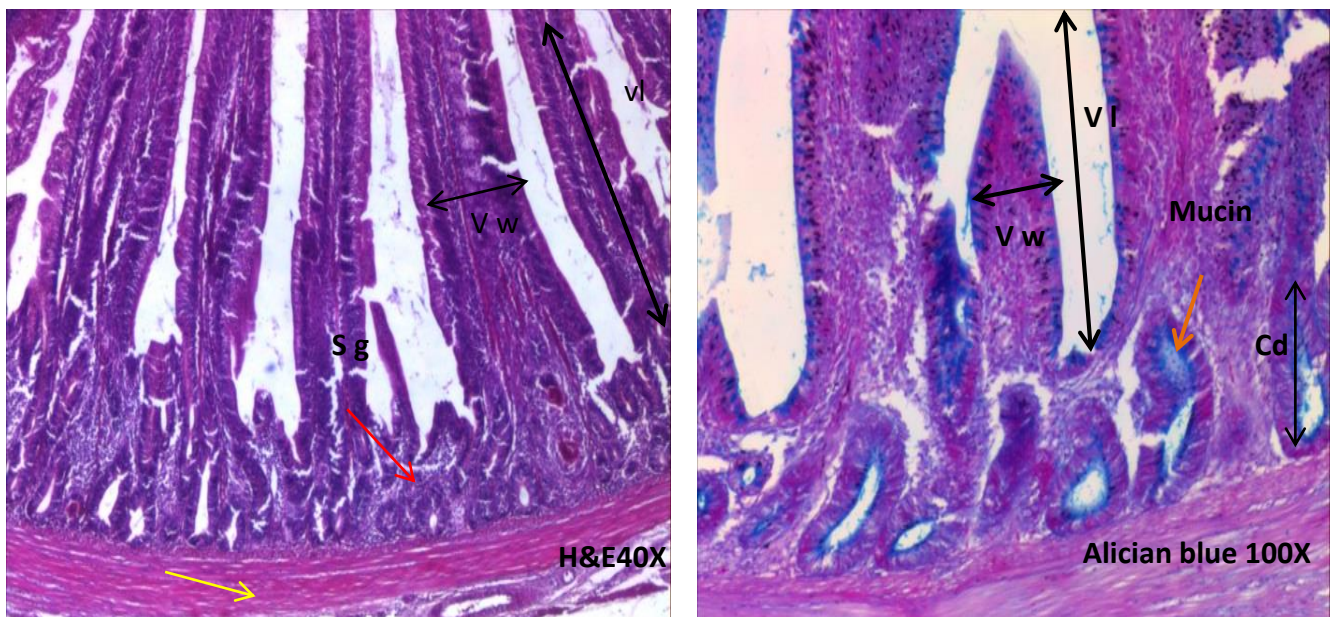


Figure (4.4) : T2 :Photomicrograph of intestine section (duodenum), from a control birds group showing, histological architecture of the tubular wall which consists, of tunica mucosa, significant regular of sub mucosa connective tissue represented of cord entire villi (length villi VI black arrow; width villi V w)with mucin was observed in cytoplasm of secretory epithelial cells of crypt of Lieberkuhn (orang arrow) and villus epithelial cells glands (red arrow), so show up (crept depth cd), and tunica muscles layer (yellow arrow)

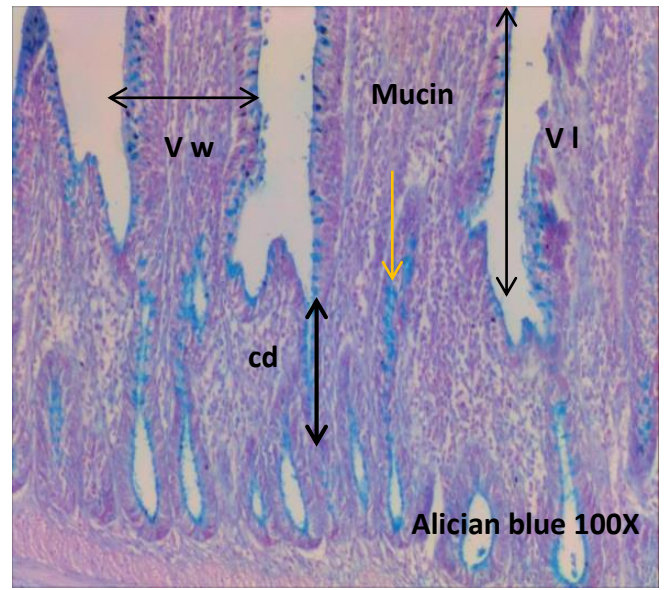
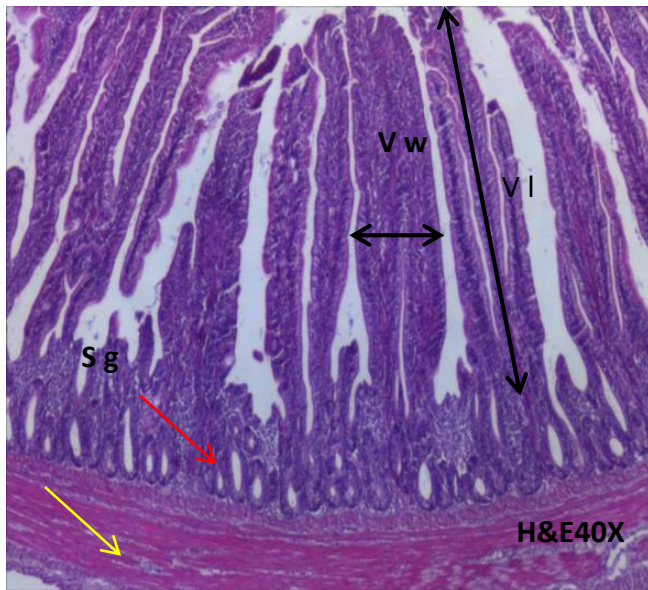


Figure (4.5) : T2e :Photomicrograph of intestine section (jejunum), from a control birds group showing, histological architecture of the tubular wall which consists, of tunica mucosa, significant regular of sub mucosa connective tissue represented of cord entire villi (length villi VI black arrow; width villi V w)with mucin was observed in cytoplasm of secretory epithelial cells of crypt of Lieberkuhn (orang arrow) and villus epithelial cells glands (red arrow), (crept depth cd), and tunica muscles layer (yellow arrow)

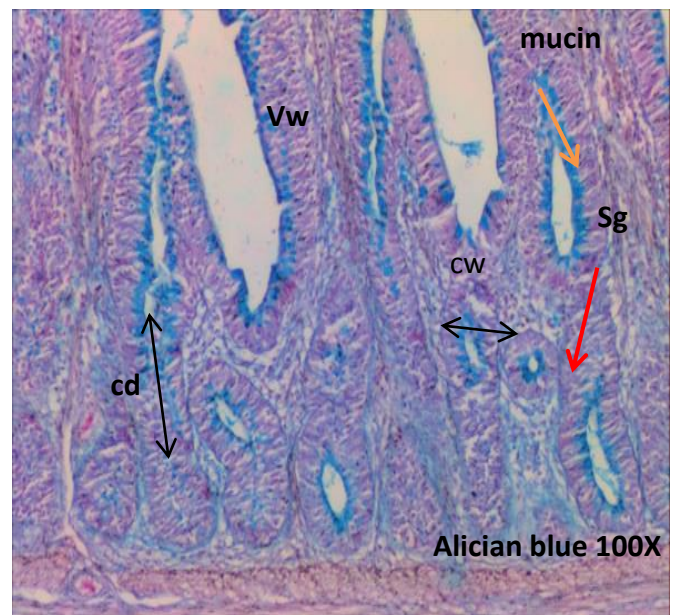
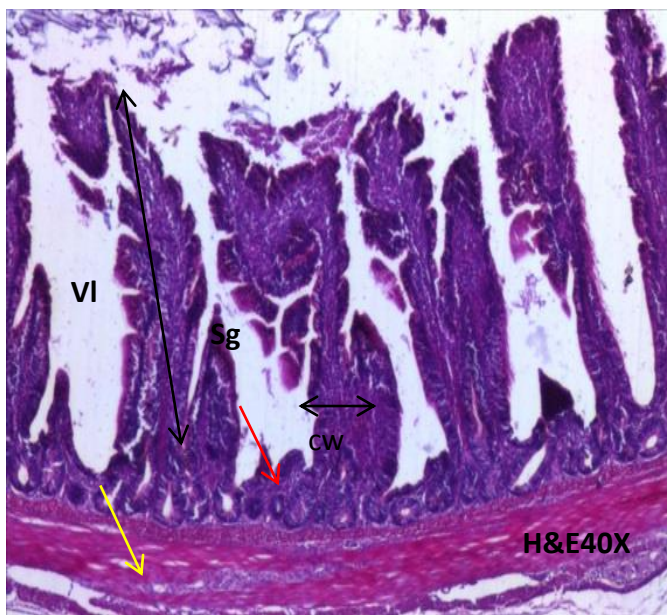
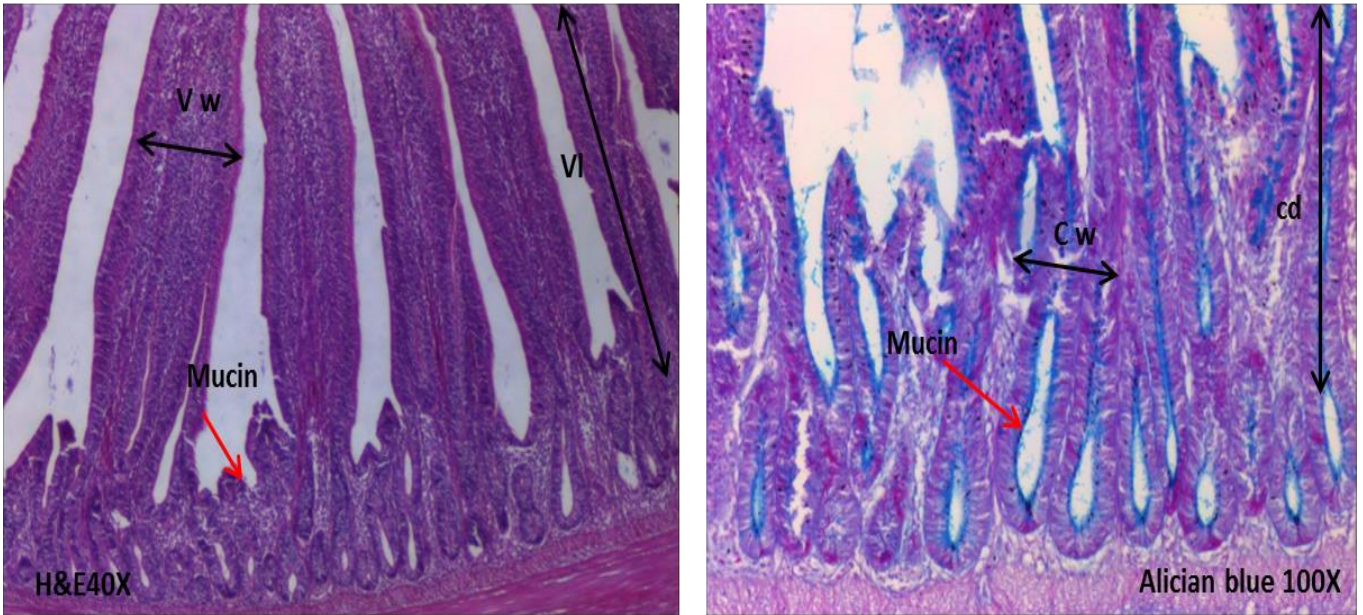


Figure (4.6) : T2:Photomicrograph of intestine section (ileum), from a control birds group showing, histological architecture of the tubular wall which consists, of tunica mucosa, significant regular of sub mucosa connective tissue represented of cord entire villi (length villi VI black arrow; width villi V w)with mucin was observed in cytoplasm of secretory epithelial cells of crypt of Lieberkuhn (orang arrow) and villus epithelial cells glands (red arrow), (crept depth cd), and tunica muscles layer (yellow arrow)



Figure(4.7) T3 :Photomicrograph of intestine section (duodenal), from a treated birds group showing, histological architecture of the tubular wall which consists, of tunica mucosa, significant regular of sub mucosa connective tissue represented of cord entire villi it become higher in number and larger area (length villi VI black arrow; width villi V w), also, we can notice more wider (c w) and most deepest crypts of the Lieberkuhn glands (cd), with significant an increase in the secretion of mucin in cytoplasm of the secretory epithelial cells (orang arrow) so, bigger size and raise number of intestinal glands (red arrows) when compared with the control and treated groups.

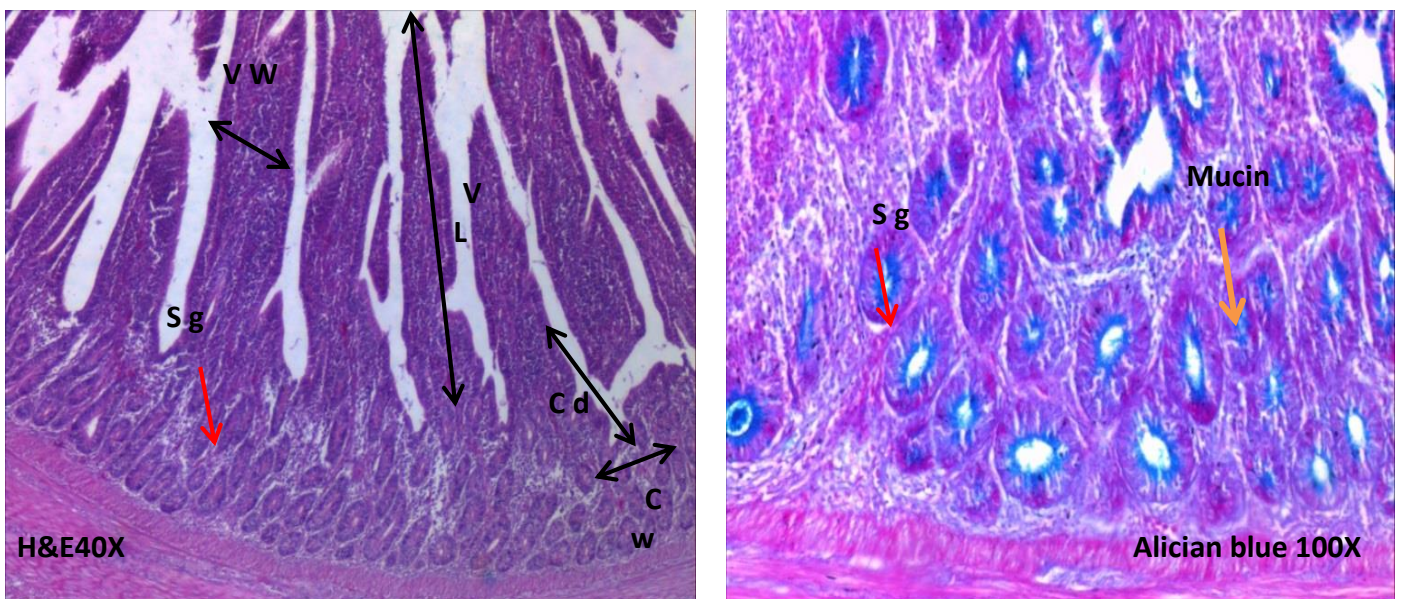


Figure (4.8) T3:Photomicrograph of intestine section (jejunal), from a treated birds group showing, histological architecture of the tubular wall which consists, of tunica mucosa, significant regular of sub mucosa connective tissue represented of cord entire villi it become higher in number and larger area (length villi VI black arrow; width villi V w), also, we can notice more wider (c w) and most deepest crypts of the Lieberkuhn glands (cd), with significant an increase in the secretion of mucin in cytoplasm of the secretory epithelial cells (orang arrow) and the number of intestinal glands appear the sheets (red arrows) when compared with the all research groups control and treated .

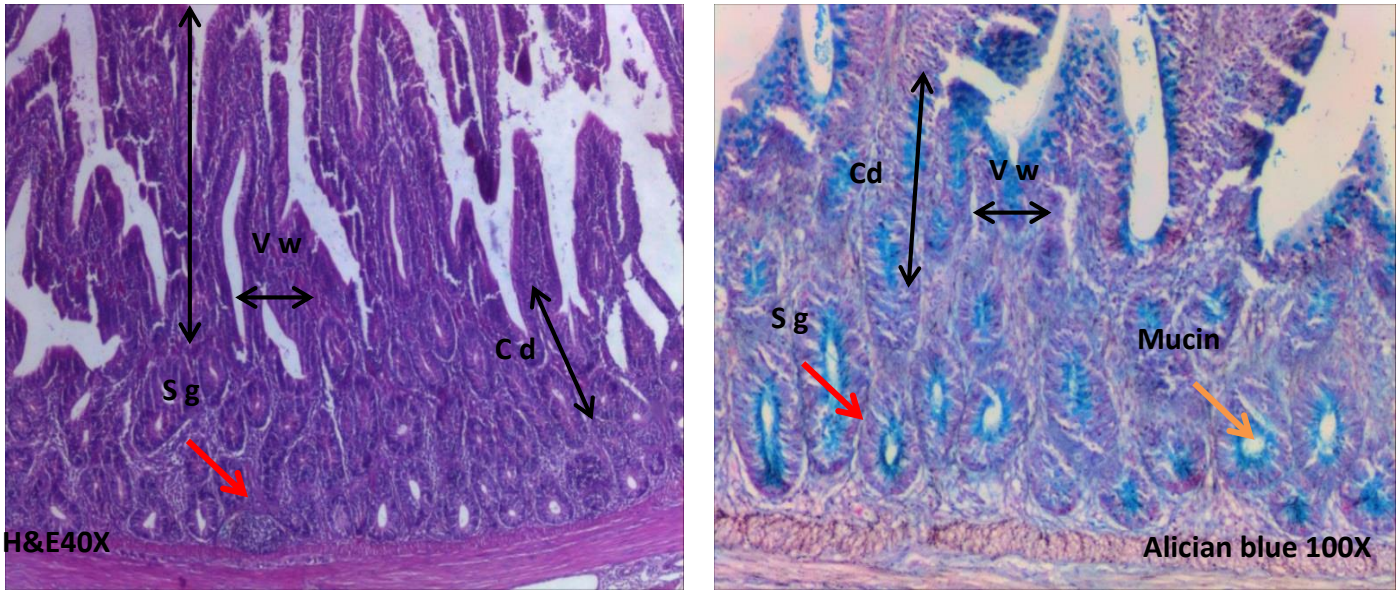


Figure (4.9) T3 :Photomicrograph of intestine section (ileum), from a treated birds group showing, histological architecture of the tubular wall which consists, of tunica mucosa, significant regular of sub mucosa connective tissue represented of cord entire villi it become higher in number and larger area (length villi VI black arrow; width villi V w), also, we can notice more wider (c w) and most deepest crypts of the Lieberkuhn glands (cd), with significant an increase in the secretion of mucin in cytoplasm of the secretory epithelial cells (orang arrow) and the number of intestinal glands formed numbering layers (red arrows) when compared with the control

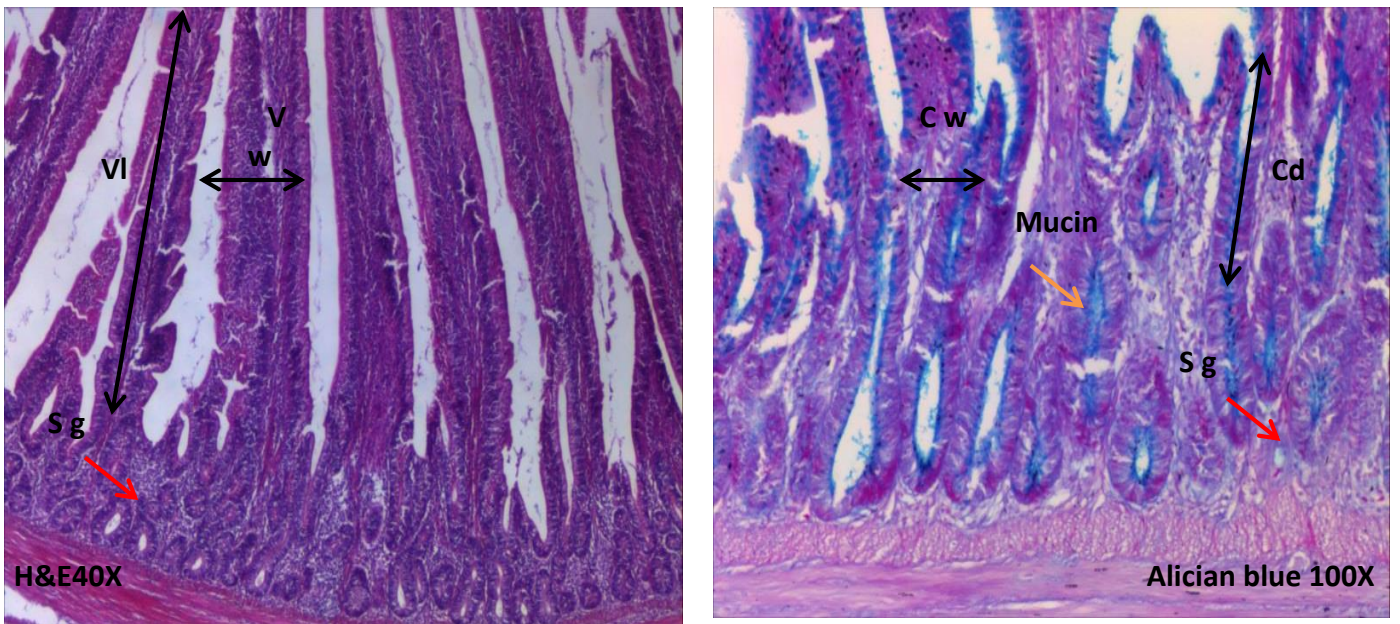


Figure (4.10) T4 :Photomicrograph of intestine section (duodenal), from a treated birds group showing, histological architecture of the tubular wall which consists, of tunica mucosa, significant regular of sub mucosa connective tissue represented of cord entire villi it become higher in number and larger area (length villi VI black arrow; width villi V w), also, we can notice wider (c w) and rather deepest crypts of the Lieberkuhn glands (cd), with an increase in the secretion of mucin in cytoplasm of the secretory epithelial cells (orang arrow) so, rather a raise a number of intestinal glands (red arrows) when compared with the control negative ,positive and ER, but, show up lower in villi in a number, area , crept depth , crept width and number with secretion mucin of cytoplasm epithelial cell glands when compression with treated group (FX).

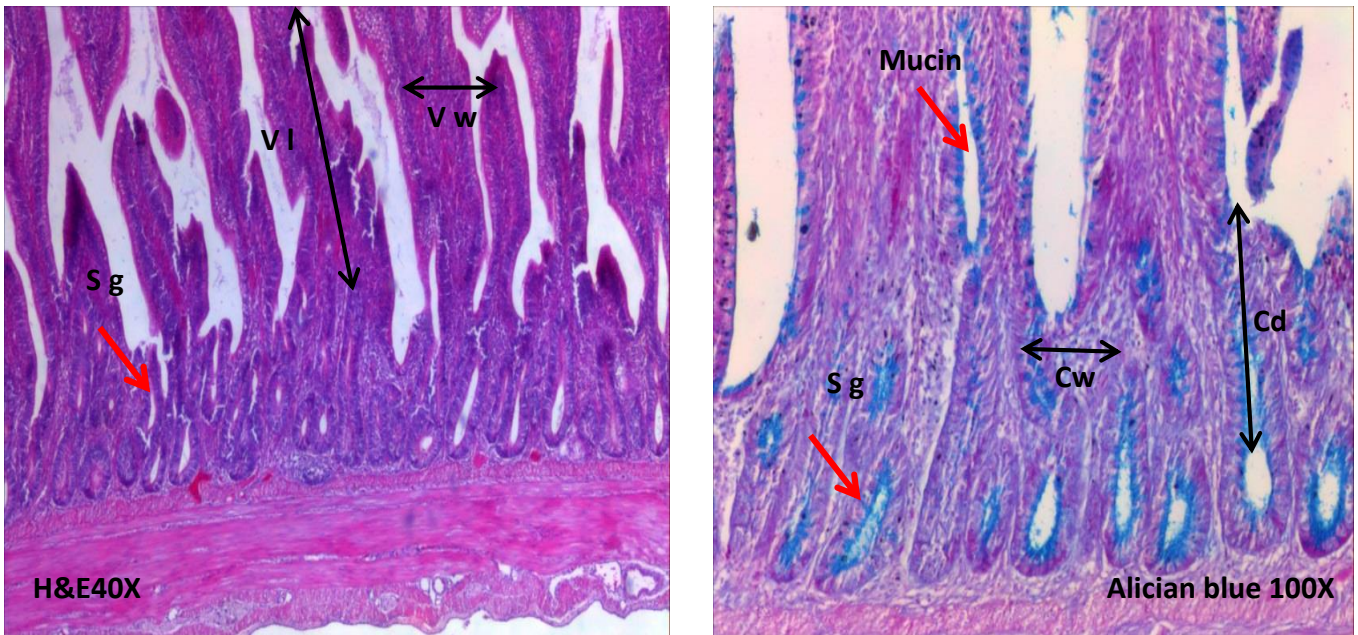


Figure (4.11) T4 :Photomicrograph of intestine section (jejunum), from a treated birds group showing, histological architecture of the tubular wall which consists, of tunica mucosa, significant regular of sub mucosa connective tissue represented of cord entire villi it become higher in number and larger area (length villi VI black arrow; width villi V w), also, we can notice wider (c w) and rather deepest crypts of the Lieberkuhn glands (cd), with an increase in the secretion of mucin in cytoplasm of the secretory epithelial cells (orang arrow) so, sort of a raise a number of intestinal glands (red arrows) when compared with the control negative ,positive and ER but, show up lower in villi in a number, area , crept depth , crept width and number with secretion mucin of cytoplasm epithelial cell glands when compression with treated group (FX)

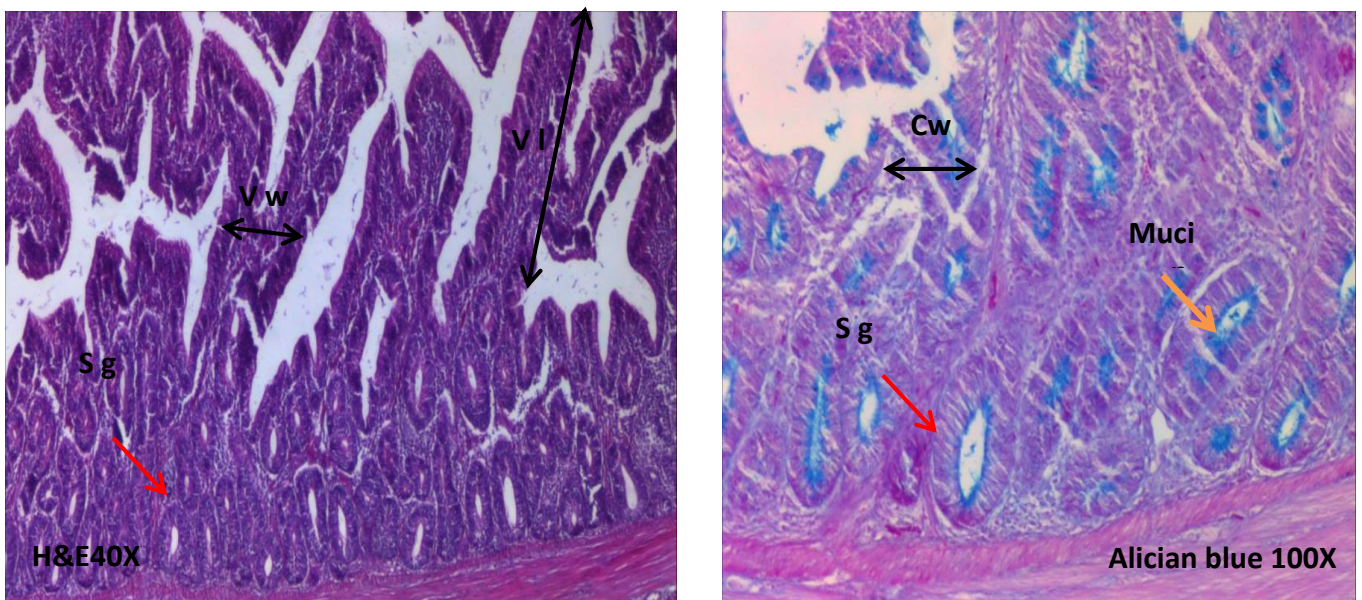


Figure (4.12) T4 :Photomicrograph of intestine section (ileum), from a treated birds group showing, histological architecture of the tubular wall which consists, of tunica mucosa, significant regular of sub mucosa connective tissue represented of cord entire villi it become higher in number and larger area (length villi VI black arrow; width villi V w), also, we can notice wider (c w) and rather deepest crypts of the Lieberkuhn glands (cd), with an increase in the secretion of mucin in cytoplasm of the secretory epithelial cells (orang arrow) so, somewhat a raise a number of intestinal glands (red arrows) . when compared with the control negative ,positive and ER but, show up lower in villi in a number, area , crept depth , crept width and number with secretion mucin of cytoplasm epithelial cell glands when compression with treated group (FX) .

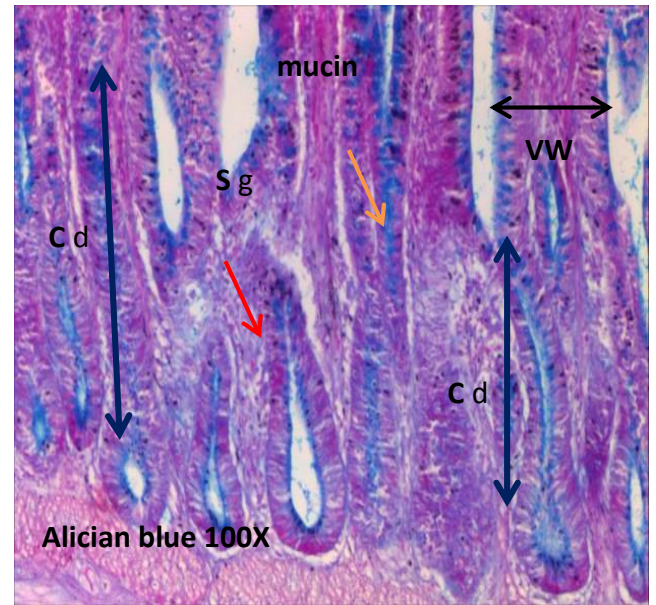
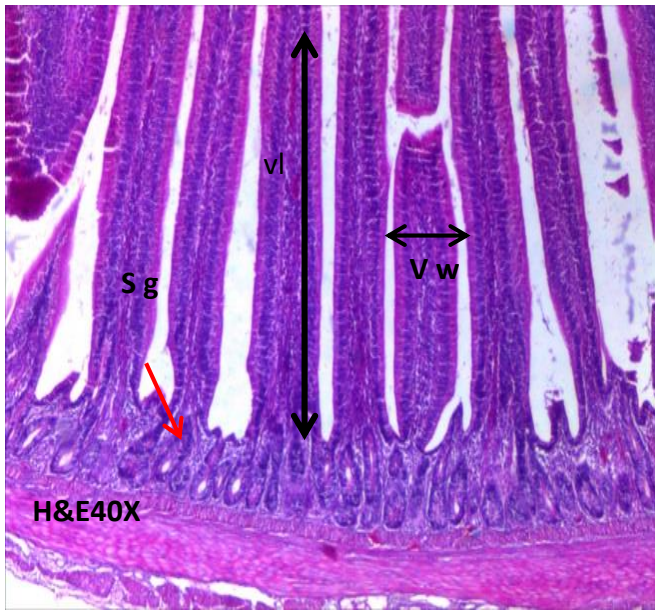
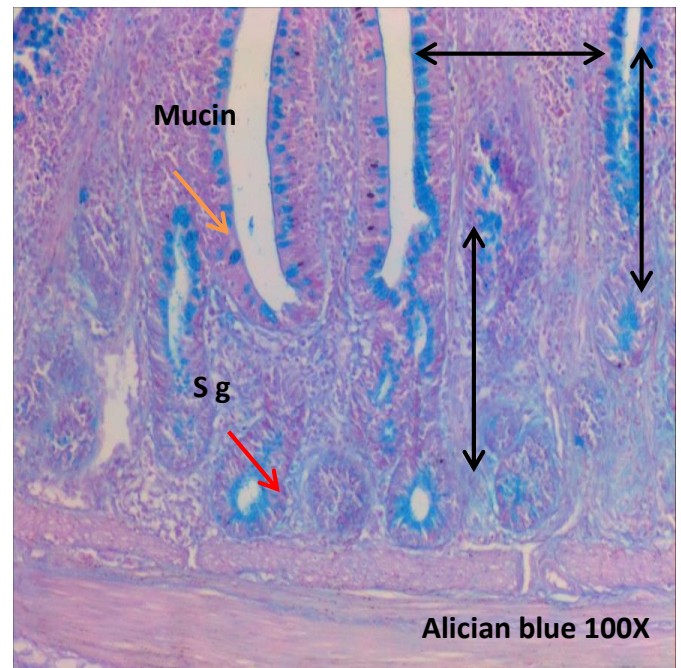
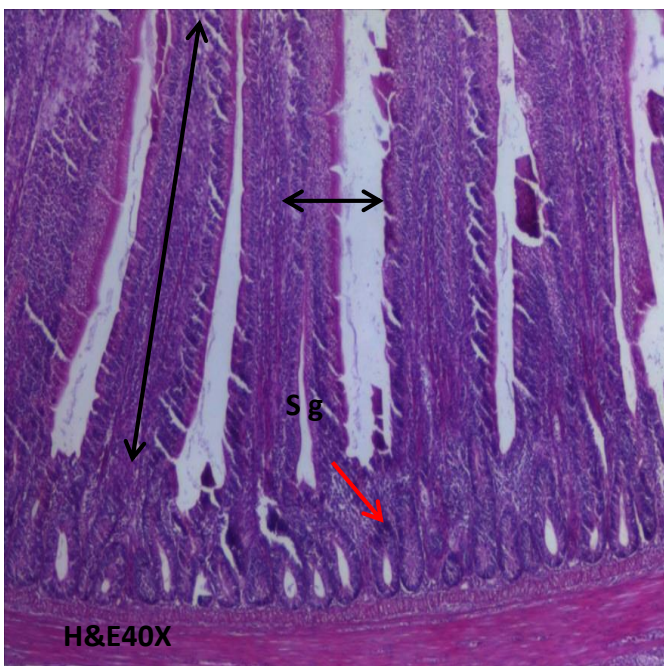


Figure (4.13) : T5 :Photomicrograph of intestine section (duodenum), from a treated birds group showing, histological architecture of the tubular wall which consists, of tunica mucosa, significant regular of sub mucosa connective tissue represented of cord entire villi it become higher in number and larger area (length villi VI black arrow; width villi V w), also, we can notice wider (c w) and deepest crypts of the Lieberkuhn glands (cd), with an increase in the secretion of mucin in cytoplasm of the secretory epithelial cells (orang arrow) and the number of intestinal glands (red arrows) when compared with the control groups



Figure(4.14) : T5 :Photomicrograph of intestine section (jejunum), from a treated birds group showing, histological architecture of the tubular wall which consists, of tunica mucosa, significant regular of sub mucosa connective tissue represented of cord entire villi it become higher in number and larger area (length villi VI black arrow; width villi V w), also, we can notice wider (c w) and deepest crypts of the Lieberkuhn glands (cd), with an increase in the secretion of mucin in cytoplasm of the secretory epithelial cells (orang arrow) and the number of intestinal glands (red arrows) when compared with the control groups

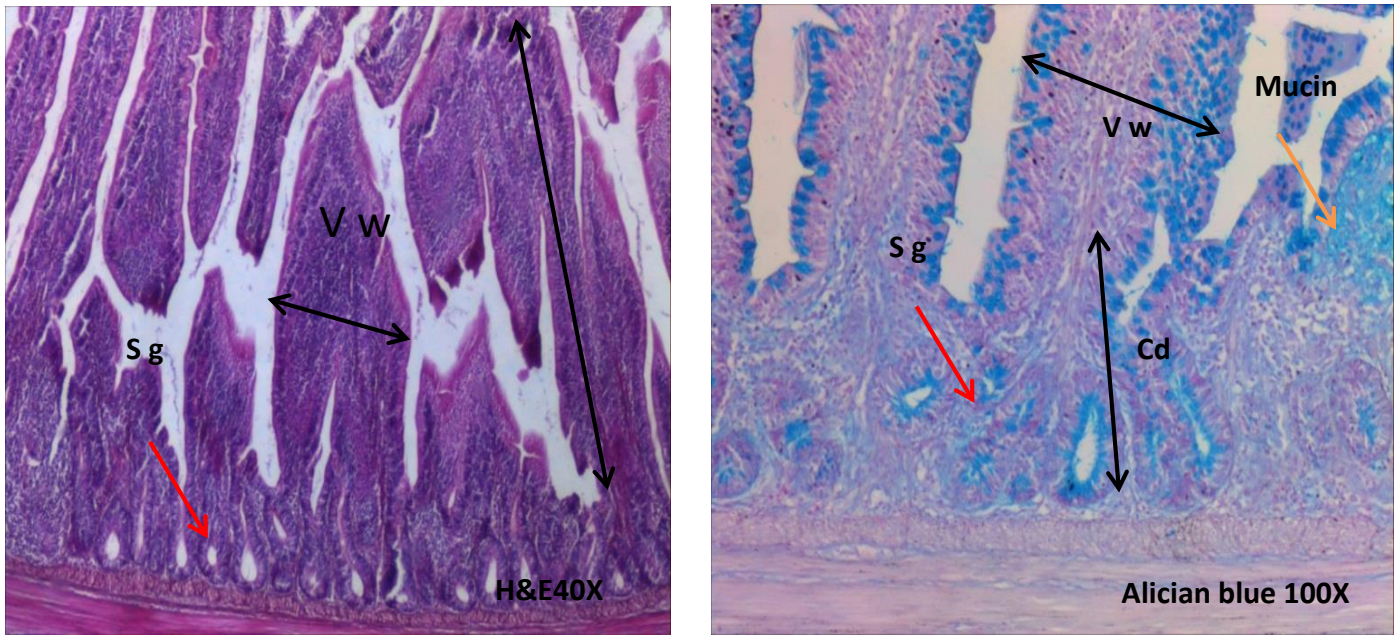


Figure : (4.15) :T5 Photomicrograph of intestine section (ileum), from a treated birds group showing, histological architecture of the tubular wall which consists, of tunica mucosa, significant regular of sub mucosa connective tissue represented of cord entire villi it become higher in number and larger area (length villi VI black arrow; width villi V w), also, we can notice wider (c w) and deepest crypts of the Lieberkuhn glands (cd), with an increase in the secretion of mucin in cytoplasm of the secretory epithelial cells (orang arrow) and the number of intestinal glands (red arrows) when compared with the control groups

The increase in the villi area and crypt depth in duodenum, jejunum and ileum was probably due to the enhancement MUC-2 gene responsible for producing the mucin layer that acts as a defensive factor against pathogens in the intestine and improving intestinal health and thus improving digestion absorption and weight. On the other hand, the improvement may have been due to the increase in the length of the villi, the surface area of the villi and the depth of the crypts. This was in agreement with (Obaid, 2023), which indicated that giving a feed from one-day old to 35 days' old containing 10% flaxseed led to an increase in the improvement of the MUC2 gene and intestinal health.

The decrease distance between adjacent villi may be due to n-3 fatty acids supplementation diet. Decreased distance between villi represents improved intestinal morphology, which could be more effective for nutrient absorption owing to a shorter distance of nutrients traveling and diffusion of the nutrients. This is in agreement with results (Wang *et al.*, 2021) indicating that feeding broiler chickens supplementation of n-3 fatty acids showed a more beneficial effect on improving intestinal morphology during the grower phase than the starter phase.

The enhancement expression of the MUC2 gene may be due to by omega-3 fatty acids. The main gel-forming mucus that covers the ileal epithelium to protect it from antigens is MUC2. The mucus layer of the intestinal epithelium mostly comprises mucus produced by goblet cells; a deficiency in mucin has been shown to hinder absorption and immune function. This is in agreement with results (Wang *et al.*, 2021) indicating that feeding broiler chickens supplementation of n-3 fatty acids Intestinal morphologic results were consistent with changes in gene expression implying a positive effect of n-3 fatty acids supplementation.

The improving gut health may be due to, which an omega-3 fatty acids decrease inflammatory responses and improve immune function in broilers. The n-3 fatty acids, including α -linolenic acid (18:3n-3), eicosapentaenoic acid (20:5n-3, and docosahexaenoic acid (22:6n-3), are polyunsaturated fatty acids (PUFA). The principal link between long-chain PUFA and immune function is primarily mediated by the synthesis of oxylipins from PUFA called eicosanoids (C20-derived) or docosanoids (C22-derived). This is in agreement with results (Calder, 2010) indicating that feeding broiler chickens supplementation of n-3 fatty acids intestinal morphologic lead to were inhance with improve immune function and decrease inflammatory responses a positive effect of n-3 fatty acids supplementation.

The increased dietary n-3 PUFA can modulate production of n-6 eicosanoids by increasing n-3 derived eicosanoids and eicosanoids because the precursor PUFA (n-6 or n-3) use the same enzymes for synthesis. The n-3 derived eicosanoids are less biologically potent than eicosanoids synthesized from arachidonic acid and thus dampen the inflammatory effects. In this way, n-3 fatty acids can improve gut health through reducing inflammation in the gastrointestinal tract. This is in agreement with results (Calder, 2010) indicating that feeding broiler chickens supplementation of n-3 fatty acids intestinal morphologic lead to were reducing inflammation in the gastrointestinal tract a positive effect of n-3 fatty acids supplementation.

We our here that whole flaxseed had beneficial effects in the duodenum, jejunum and ileum morphology, characterised by a significant increase in villus height and crypt depth probably leading to increased nutrient adsorption. The crypt depth and intestinal villus length are indicative of the intestinal absorptive capacity. High intestinal villus length triggers an increase in the mucosal surface area, which, in our study, may be due to the PUFA composition of flaxseed. This is in agreement with results (Kiela *et*

al., 2016) indicating that feeding broiler chickens supplementation of whole flaxseed intestinal morphologic lead to were had beneficial effects in the duodenum and the jejunum morphology, characterized by a significant increase in villus height and crypt depth.

That polyunsaturated fatty acid diets can reduce the severity of intestinal lesions. Omega-3 polyunsaturated fatty acids can be incorporated into cell membranes, affecting antioxidant signaling and helping to regulate levels of oxidative stress resulting from heat stress. This is in agreement with results (Agboola *et al.*, 2021) indicating that feeding broiler chickens supplementation of Omega-3 polyunsaturated fatty acids diets can reduce the severity of intestinal lesions caused by *Eimeria* infection, especially in the jejunum and regulate levels of oxidative stress resulting from heat stress.

In addition, n-3 PUFAs enhanced may be due to the improve resistance to free radical attacks, reduce lipid peroxidation, especially in cases of stress or infection and expression of tight junction genes (E-cadherin and ZO-1), and tissue repair was significantly enhanced with goblet cell abundance. This is in agreement with results (Liu *et al.* 2012) (Oppedisano *et al.*, 2020) indicating that feeding broiler chickens supplementation of Omega-3 polyunsaturated fatty acids diets can intestinal barrier functions and attenuation of inflammatory mediators by omega-3 supplementation suggest their protective role associated intestinal injury.

The omega-3 fatty acids (EPA and DHA) increased IgA and IgM levels in the cecum. SIgA antibodies are considered the main influencers in mucosal immunity as they play the key role in intestinal barrier defending against invading pathogen through binding with invading pathogen at intestinal mucosal surfaces and neutralizing their endotoxin within epithelial cells without causing tissue damage. This is in agreement with results (Yang *et al.*, 2006), who reported that omega-3 fatty acids increased IgA and IgM

levels in the cecum after taking fish oil supplements containing 18% EPA and 12% DHA.

Chronic heat stress in broiler chicks diminishes antioxidant capacity over time. The gut mucosa serves as the primary internal barrier, directly interacting with both nutritional and non-nutritional substances. This results in the generation of surplus reactive oxygen and reactive nitrogen species, hindering nutrient digestion and absorption (Mishra and Jha, 2019). The gut mucosa is susceptible to oxidation due to its significant workload, although it is safeguarded by an antioxidant defense system comprising T-AOC, T-SOD, and GSH-Px. It was discovered that a reduced calorie diet diminished T-AOC and T-SOD activity (Attia *et al.*, 2022).

The intestinal epithelial barrier is a key factor that orchestrates gut balance via creating communication between the microbiota and underlying immune lining cells (Okumura and Takeda, 2017). This barrier is guarded by tight junction proteins (TJPs), unique proteins, comprising occludin, claudins-1 and JAM which are crucial for providing integral physical barrier between intestinal mucosal cells and strictly governs its permeability (Nasu *et al.*, 2013).

The improvement in mucin activity may be due to the improvement in the gene expression of occludin and JAM-2 genes because of the effect of n-3, which indicates its effective role in strengthening the mucosal barrier and enhancing intestinal integrity. Our result agreements with (Verwoolde *et al.*, 2021) who reported that gene expression enhanced by adding n-3.

The effective role of n-3 PUFAs in restoring intestinal barrier integrity through antagonizing inflammation by replacing n-6 arachidonic acid, pro-inflammatory eicosanoids described to modify the gut microbiota and interrupts the intestinal barrier functions, and yield 100-fold weak pro-

inflammatory eicosanoids that accounted for inflammation un-recovery (Calder, 2012).

Additionally, n-3 PUFA enhanced the expression of tight junction genes (E-cadherin and ZO-1), and tissue restoration were significantly enhanced with the abundance of goblet cells. Our result agreements with (Liu *et al.*, 2012) who reported that Restoring intestinal barrier functions and subsiding of inflammatory mediators by supplementing Omega-3 indicating their protective role against gut injury associated with *Eimeria* infection.

Inflammation acts as a physiological innate immune reaction to a challenge, especially at the first week of chick's life when they take the first experiences toward their around environment, nutrition and development changes. Conversely severe or uncontrolled inflammations can handle the up taken nutrients to the response to the acute inflammations that cause decreased feed consumption, muscle protein deposition and muscle building, impair GIT development and weak intestinal barriers and tight junctions with increase the incidence of leaky gut syndrome, increase the metabolic rate, finally resulting in high incidence of diseases and poor production (Kong and Jha, 2022).

The increase in mucin activity was probably due to the enhancement MUC-2 gene responsible for producing the mucin layer that acts as a defensive factor against pathogens in the intestine and improving intestinal health and thus improving digestion, absorption and weight. Our result agreements with (Obaid, 2023), which indicated that giving a feed from one-day old to 35 days' old containing 10% flaxseed led to an increase in the improvement of the MUC2 gene and intestinal health.

Therefore, decreased distance between villi and increased gene expression levels of SLC15A1 that supplementation of n-3 fatty acids during

the grower phase showed a more beneficial effect on improving gut health t. The intestinal mucus barrier and nutrient transport would be enhanced by n-3 fatty acids supplementation in the grower diet. Our result agreements with (Wang *et al.*, 2021) who reported that gene expression enhanced by supplementing omega-3 decreased distance between villi and increased gene expression levels SLC15A1.

4.5 Impact of whole flaxseed and a reduce energy diet on IGF-1 gene expression in broiler chickens.

This is the first study to demonstrate IGF-1 gene expression in liver tissue of influence by whole flaxseed and whole flaxseed in reduced energy diet of broiler chickens. IGF_1 gene expression was significantly enhancing (Over regulated) (18.51 ± 0.113 -fold increase) in T3 group and T4 (14.09 ± 0.112 -fold increase) group compared with control group as show in table (4-9).

Table (4-10) Impact of whole flaxseed in reduced energy diet on IGF_1 gene expression in Broiler Chickens (Mean±SD):

Parameters	T1		T2		T3		T4		T5	
	Mean	±S. D	Mean	±S. D	Mean	±S. D	Mean	±S. D	Mean	±S. D
IGF_1	1	0.000	1.839	0.12	18.51	0.19	14.09	0.19	7.936	0.46

Tukey's multiple comparisons test	Significant?	Summary	Adjusted P Value
Control Negative vs. Heat stress	Yes	*	0.0139
Control Negative vs. Flax seed	Yes	****	<0.0001
Control Negative vs. Flax seed + Reduced energy	Yes	****	<0.0001
Control Negative vs. Reduced energy	Yes	****	<0.0001

Insulin is a crucial component regulating metabolism and growth, promoting anabolic processes, and facilitating the appropriate turnover of carbohydrates, lipids, and proteins. Insulin enhances glucose clearance and lipid metabolism. The insulin status in broiler chickens is influenced by age, diet, and genetics (Newman *et al.*, 2005). Insulin is strongly associated with insulin-like growth factors IGF-1 and IGF-2, which are polypeptide hormones possessing notable anabolic characteristics. These hormones enhance development and facilitate the function of insulin (Gao *et al.*, 2007).

The gene encoding insulin-like growth factor I (IGF-I) was utilized to examine the correlation among growth, carcass yield, and meat quality characteristics. IGF-I (accession numbers: M 74176) is situated on chromosome 1 (Molee *et al.*, 2018). IGF-I stimulates the proliferation, differentiation, and metabolism of myogenic cell lines across many species (Florini *et al.*, 1996). IGF-1 regulates bodily and muscular development in hens (Duclos *et al.*, 1999).

Omega-3 fatty acids may increase the synthesis of transcription factors and be responsible for the increased expression of the IGF_1 gene in the liver tissue of broiler chickens. (Nematbakhsh *et al.*, 2021). By changing the signal transduction pathways that link cell surface receptors to the activation of transcription factors in the nucleus, omega-3 polyunsaturated fatty acids may have an impact on gene expression. On the other hand, nuclear factor may be directly contacted by omega-3 polyunsaturated fatty acids, changing its activity. (Adkins and Kelley, 2010).

The complex relationship between omega-3 FAs and transcription factors and gene expression is probably, influenced by a number of factors, such as the amount of omega-3 fatty acids taken, the particular transcription factors at play, the type of cell being studied, and the physiological setting. (Gutiérrez *et al.*, 2019). 2. (Nematbakhsh *et al.*, 2021). Insulin is closely

linked to insulin-like growth factor IGF-1, a polypeptide hormone with significant anabolic properties. These hormones promote growth and augment insulin efficacy (Gao *et al.*, 2007).

Key hormones, such as thyroid hormone, growth hormone and IGF-1 (Fuentes *et al.*, 2013), predominantly regulate skeletal muscle hypertrophy. Skeletal muscle functions as an autocrine and paracrine organ by secreting IGF-1, which promotes the proliferation and differentiation of satellite cells, augments protein synthesis, and ultimately increases muscle mass. Additionally, PUFA enhanced mRNA expression of IGF-1, correlating with raised IGF-1 levels in the liver.

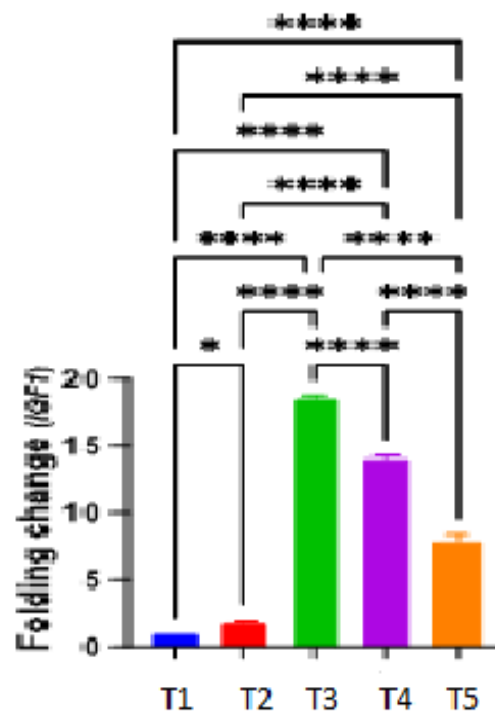


Figure (4.16): Folding change comparison among the groups expressed IGF-1 gene. This shows significant upregulation of the (T3) and (t4) groups compared with control groups.

4.6 Impact of whole flaxseed and reduced energy diet on carcass characterization in Chickens.

The results of this study show an increase significant ($p \leq 0.05$) in the live body weight, carcass without feather and carcass without visra. We notice an increase in the size of the heart, liver and gizzard in the groups (T3) and (T4) when compare with other groups, this examination did at age of broiler chickens 35 days as show in table (4-10).

Table (4-11) Effects of whole flaxseed and reduced energy diet on carcass characteristics/ mean \pm SD.

Groups Parameter g.	T1	T2	T3	T4	T5
Live body Wight	1980.52 \pm 20.21 BC	1805.38 \pm 39.1 D	2138.70 \pm 62.55 A	2025.62 \pm 46.06 B	1938.58 \pm 40.13 C
Carcass Without feather	1825.52 \pm 20.21 BC	1645.10 \pm 43.12 D	2006.50 \pm 47.84 A	1872.62 \pm 46.06 B	1784.32 \pm 40.04 C
Carcass Without visra	1426.32 \pm 20.21 C	1246.70 \pm 40.86 D	1609.70 \pm 47.74 A	1474.52 \pm 46.12 B	1385.32 \pm 40.04 C
Carcass with edible	1522.06 \pm 22.08 B	1334.66 \pm 43.87 D	1690.18 \pm 36.57 A	1564.62 \pm 46.06 B	1475.32 \pm 40.04 C
Gizzard	43.48 \pm 1.04 C	41..08 \pm 1.15 D	46.52 \pm 1.67 A	45.50 \pm 1.04 AB	44.46 \pm .56 BC
Liver	37.10 \pm .81 C	35.18 \pm 1.14 D	39.14 \pm 1.32 A	38.56 \pm 1.40 AB	37.66 \pm .38 BC
Heart	12.04 \pm .37 C	11.04 \pm .24 D	13.46 \pm .41 A	13.12 \pm .81 AB	12.74 \pm .33 B

Different Different letters among treatment show significant difference ($p \leq 0.05$). The control treatment (T1) received a basal diet without additives. (T2) fed basal diet without additives under heat stress; (T3) included whole flaxseed (7.5 % in starting feed and 10% in grower feed) during heat stress; (T4) incorporated whole flaxseed in reduced energy diet (7.5 % in starting feed and 10% in grower feed) during heat stress; (T5) consisted of reduced energy diet during heat stress.

The increase in live body weight, carcass characteristics and edible organs weight at 35 days of age, as a result of using 7.5% in starter feed and 10% in grower feed whole flaxseed, as shown in Table (4-10), was probably due to the use of whole flaxseed in the poultry diet. Which is rich in long-

chain polyunsaturated fatty acids that have many benefits, including improving metabolism and enhancing the breakdown and absorption of lipoproteins and their fatty acid components. Our result agreements with (Appersonl, 2015) who reported that final body weight were highest in flaxseed fed birds and effect relative organ weights was observed relative.

The increase in live body weight, carcass characteristics and edible organs weight was probably due to the enhancement MUC-2 gene responsible for producing the mucin layer that acts as a defensive factor against pathogens in the intestine and improving intestinal health and thus improving digestion, absorption and weight. On the other hand, the improvement may have been due to the increase in the length of the villi, the surface area of the villi and the depth of the crypts. This was in agreement with (Obaid & Jameel, 2023) which indicated that giving a feed from one day old to 35 days old containing 10% flaxseed led to an increase in the improvement of the MUC2 gene and intestinal health.

The improvement in live body weight, carcass characteristics and edible organs weight was may be due to, adding flaxseed oil to broiler feed, slowing the rate at which food passes through the gastrointestinal tract, improves palatability, energy efficiency, and vitamin absorption. It also increases the assimilation of all nutrient. Unsaturated vegetable oils have more metabolizable energy and less fecal energy loss than animal fats. Our result agreements with (Kishawy *et al.*, 2019) who reported that use flaxseed oil to broiler feed increase of carcass characteristics.

On the other hand, the increase in carcass characteristics under heat stress may be have been may be due to the antioxidant activity resulting from the use of a source of flaxseed oil that has a beneficial effect on birds. This is in agreement with (Ansari, 2024). The increase in carcass characteristics under heat stress conditions is may be due to the effect of polyunsaturated

fatty acids on some metabolic mechanisms, as it has a significant effect on the metabolism of protein, fat, carbohydrates and minerals in birds suffering from heat stress. This is consistent with (Elbaz *et al.*, 2023)) where it was concluded that polyunsaturated fatty acids work to reduce the metabolism of fats and carbohydrates while increasing the metabolism of protein.

The enhancement in live body weight, carcass characteristics and edible organs weight to flaxseed oil treated broilers may be due to, which that calories from unsaturated fats are primarily used in a variety of metabolic processes, calories from saturated fats are stored as adipose tissue. Those omega-3 fatty acids augment bile secretion and promote intestinal lipid degradation. Those omega-3 fatty acids might increase the release of dopamine and serotonin, reducing stress and encouraging calm in animals, which would improve their well-being and output. This is consistent with (Huo *et al.*, 2019) where it was concluded that flaxseed oil work to increases production performance, reduces fat in the carcass, and is useful in cases where the bird is exposed to external stress.

The increase in live body weight, carcass characteristics and edible organs weight to omega-3 polyunsaturated fatty acids treated broilers may be due to, facilitate breast muscle hypertrophy, particularly when accompanied by a diet abundant in the plant-derived precursor ALA, which influences the muscular development process. That the ingestion of omega-3 LC-PUFA enhances muscular anabolism in farm animals. One potential mechanism is linked to the heightened activation of the insulin cascade, as this hormone regulates the equilibrium between protein synthesis and proteolysis. The ALA-enriched diet seemingly resulted in increased activation of the insulin-dependent S6K1/S6 pathway in the breast muscle. This is consistent with (Tesseraud *et al.*, 2014) where it was concluded that

modulation of the insulin anabolic signalling cascade in growing chickens by omega-3 PUFA.

The improvement in live body weight, carcass characteristics and edible organs weights may be due to enhanced expression of IGF-1 gene, which is consistent with (Rodríguez-García *et al.*, 2022), where it was concluded that of using n-3 PUFA in broiler chicken diets. Where IGF-1 gene stimulates proliferation, differentiation and metabolism of muscle cell lines of various types.

Chapter Five

Conclusion and Recommendation

5.1 Conclusion.

According to the results of present study, the conclusion can be reported as follows:

1. An increase in live body weight, weight gain and decrease feed intake and feed conversion ratio of chicks fed flaxseed and flaxseed reduced energy diet (7,5% in starting feed and 10% in growing feed) in the treatments since 1st week until end of this study, also the treatment groups showed an improvement of to the end of the experiment.
2. Increase omega-3 in meat of broiler in the treatment groups (T3) and (T4) compared with control group.
3. Reduction in cholesterol, triglyceride, LDL and vLDL-cholesterol, and increase significantly in HDL-cholesterol concentrations especially of chicks fed flaxseed and flaxseed reduced energy in the treatment group.
4. The liver enzymes concentrations AST and ALT were decreased in the treatment groups (T3) (T4) compared with control group.
5. Increase IGF-1 gene expression in the treatment groups (T3) and (T4) compared with control group.
6. Improve of intestine histology by increase villi length in jejunum, villi area in duodenum and crypt depth in jejunum and ileum in the treatment groups (T4) and (T3) as compared to control group.
7. Using of whole flaxseed in reduced energy diet led to enhance IGF-1 gene expression, performance, and intestinal morphology and mucin activity.
8. Using of reduced energy diet will minimize the cost of feed and it's perfect in HS condition.

5.2 Recommendation

From the results of the present study, it can be recommended the following:

1. Recommended planting flaxseed in Iraq as a good alternative as source of protein and energy in the diet of chickens.
2. Whole flaxseeds improve gene expression for production IGF-1, which improves body and muscle growth. So use flaxseed in feed broiler chickens
3. Using a low energy feed of 200 kcal/kg of feed reduces feed costs on the one hand and reduces the effect of heat stress on the other hand.
4. Using whole flaxseeds as a source of omega-3 in broiler feed to produce a healthy meat enriched with Omega-3.
5. Recommended using flaxseed at a rate of 7.5% in the starter feed and 10% in the growing feed to broiler chicken.
6. Recommended studying the sensory properties of meat.

Chapter Six

Reverences

- Abd El-Hack, M. E., Abdelnour, S. A., Taha, A. E., Khafaga, A. F., Arif, M., Ayasan, T., ... & Abdel-Daim, M. M. (2020).** Herbs as thermoregulatory agents in poultry: An overview. *Science of the Total Environment*, 703, 134399.
- Abdelnour, S. A., Abd El-Hack, M. E., Khafaga, A. F., Arif, M., Taha, A. E., & Noreldin, A. E. (2019).** Stress biomarkers and proteomics alteration to thermal stress in ruminants: A review. *Journal of thermal biology*, 79, 120-134.
- Abdulla, N. R., Loh, T. C., Akit, H., Sazili, A. Q., Foo, H. L., Kareem, K. Y., ... & Abdul Rahim, R. (2017).** Effects of dietary oil sources, calcium and phosphorus levels on growth performance, carcass characteristics and bone quality of broiler chickens. *Journal of Applied Animal Research*, 45(1), 423-429.
- Abioja, M. O., & Abiona, J. A. (2021).** Impacts of climate change to poultry production in Africa: Adaptation options for broiler chickens. In *African handbook of climate change adaptation* (pp. 275-296). Cham: Springer International Publishing.
- Adkins, Y., & Kelley, D. S. (2010).** Mechanisms underlying the cardioprotective effects of omega-3 polyunsaturated fatty acids. *The Journal of nutritional biochemistry*, 21(9), 781-792.
- Abu-Zeid, E. H., El-Hady, E. W., Ahmed, G. A., Abd-Elhakim, Y. M., Ibrahim, D., Abd-Allah, N. A., ... & Abo-Elmaaty, A. (2024).** Nicotine exacerbates liver damage in a mice model of Ehrlich ascites carcinoma through shifting SOD/NF- κ B/caspase-3 pathways: ameliorating role of *Chlorella vulgaris*. *Naunyn-Schmiedeberg's Archives of Pharmacology*, 1-17
- Agboola, A. F., Omidwura, B. R. O., Okiwelu, E. I., & Agbuza, S. (2021).** Effects of dietary fish oil supplementation on performance,

gut morphology, protozoan load and histopathological indices of broiler chickens. *Egyptian Poultry Science Journal*, 41(2), 263-249.

Ahmed, E. M., Attia, A. I., Ibrahim, Z. A., Alshehry, G., Algarni, E. H., Aldekhail, N. M., & Abd El-Hack, M. E. (2024). The impacts of dietary inclusion of soybean oil and linseed oil on growth performance, carcass yield, and health status of growing Japanese quail. *Poultry Science*, 103(7), 103746.

Alan, H. B. (2006). Wu: Tietz Clinical Guide to Laboratory Tests, 4th ed. WB Saunders, 564.

Al-Fayadh, H., and S. A. Naji, (1989). Poultry Technology. 1st Edition. Administration of higher education press. Baghdad. Iraq. (In Arabic)

Al-kalo, O. A., & Al-Aalim, A. M. (2024). The Effect of Oils Additive on Some Immunological Cytokines Newcastle Disease-Vaccinated Broiler Chickens. *Egyptian Journal of Veterinary Sciences*, 1-7.

Al-Khalaifah, H., & Al-Nasser, A. (2021). Dietary source of polyunsaturated fatty acids influences cell cytotoxicity in broiler chickens. *Scientific Reports*, 11(1), 10113.

Al-Khalifa, H., Givens, D. I., Rymer, C., & Yaqoob, P. (2012). Effect of omega-3 fatty acids on immune function in broiler chickens. *Poultry Science*, 91(1), 74-88.

Al-Murrani, W. K., Al-Rawi, A. J., Al-Hadithi, M. F., & Al-Tikriti, B. (2006). Association between heterophil/lymphocyte ratio, a marker of 'resistance' to stress, and some production and fitness traits in chickens. *British Poultry Science*, 47(4), 443-448.

Altan, Ö. Z. G. E., Pabuçcuoğlu, A., Altan, A., Konyalioglu, S., & Bayraktar, H. (2003). Effect of heat stress on oxidative stress, lipid

peroxidation and some stress parameters in broilers. *British poultry science*, 44(4), 545-550.

Ansari, M. (2024). Recent strategies to mitigate reproductive aging in male broiler breeders: a review. *Animal Reproduction Science*, 107570.

Apperson, K. D. (2015). Enzyme Supplementation of Broiler Chicken Diets Containing Whole Flax Seed as a Means to Increase n-3 Fatty Acids in Human Diets.

Aviagen, (2016). Ross broiler handbook USA. pp: 14-110.

Aviagen, (2022). Ross broiler handbook USA. pp: 14-110.

Awad, E. A., Najaa, M., Zulaikha, Z. A., Zulkifli, I., & Soleimani, A. F. (2020). Effects of heat stress on growth performance, selected physiological and immunological parameters, caecal microflora, and meat quality in two broiler strains. *Asian-Australasian journal of animal sciences*, 33(5), 778.

Bamias, G., Arseneau, K. O., & Cominelli, F. (2014). Cytokines and mucosal immunity. *Current opinion in gastroenterology*, 30(6), 547-552.

Bartram, T. (2013). Bartram's encyclopedia of herbal medicine. Hachette UK.

Bechlin, T. R., Granella, S. J., Christ, D., Coelho, S. R. M., & Viecelli, C. A. (2019). Evaluation of grain and oil quality of packaged and ozonized flaxseed. *Journal of Stored Products Research*, 83, 311-316.

Bilal, R. M., Hassan, F. U., Farag, M. R., Nasir, T. A., Ragni, M., Mahgoub, H. A., & Alagawany, M. (2021). Thermal stress and high stocking densities in poultry farms: Potential effects and mitigation strategies. *Journal of Thermal Biology*, 99, 102944.

- Bostami, A. B. M. R., Mun, H., & Yang, C. (2017).** Breast and thigh meat chemical composition and fatty acid profile in broilers fed diet with dietary fat sources. *J. Food Process. Technol*, 8(5), 1000672.
- Calder, P. C. (2010).** Polyunsaturated fatty acids, inflammation, and inflammatory diseases.
- Calder, P. C. (2012).** Mechanisms of action of (omega-3) fatty acids. *The Journal of nutrition*, 142(3), 592S-599S.
- Calik, A., Emami, N. K., Schyns, G., White, M. B., Walsh, M. C., Romero, L. F., & Dalloul, R. A. (2022).** Influence of dietary vitamin E and selenium supplementation on broilers subjected to heat stress, Part II: oxidative stress, immune response, gut integrity, and intestinal microbiota. *Poultry Science*, 101(6), 101858.
- Campos, J. R., Severino, P., Ferreira, C. S., Zielinska, A., Santini, A., Souto, S. B., & Souto, E. B. (2019).** Linseed essential oil—source of lipids as active ingredients for pharmaceuticals and nutraceuticals. *Current Medicinal Chemistry*, 26(24), 4537-4558.
- Cassuce, D. C., Tinôco, I. D. F., Baêta, F. C., Zolnier, S., Cecon, P. R., & Vieira, M. D. F. (2013).** Thermal comfort temperature update for broiler chickens up to 21 days of age. *Engenharia Agrícola*, 33, 28-36.
- Chang, Q., Lu, Y., & Lan, R. (2020).** Chitosan oligosaccharide as an effective feed additive to maintain growth performance, meat quality, muscle glycolytic metabolism, and oxidative status in yellow-feather broilers under heat stress. *Poultry science*, 99(10), 4824-4831.
- Chang, V. C., Cotterchio, M., Boucher, B. A., Jenkins, D. J., Mirea, L., McCann, S. E., & Thompson, L. U. (2019).** Effect of dietary flaxseed intake on circulating sex hormone levels among postmenopausal women:

a randomized controlled intervention trial. *Nutrition and cancer*, 71(3), 385-398.

Changxing, L., Dongfang, D., Lixue, Z., Saeed, M., Alagawany, M., Farag, M. R., ... & Jianhua, L. (2019). *Heracleum persicum*: Chemical composition, biological activities and potential uses in poultry nutrition. *World's poultry science journal*, 75(2), 207-218.

Coşkuner, Y., & Karababa, E. (2007). Some physical properties of flaxseed (*Linum usitatissimum* L.). *Journal of food Engineering*, 78(3), 1067-1073.

Cozannet, P., Davin, R., Jlali, M., Jachacz, J., Preynat, A., & Molist, F. (2021). Dietary metabolizable energy, digestible lysine, available phosphorus levels and exogenous enzymes affect broiler chicken performance. *Animal*, 15(5), 100206.

Cui, X., Gou, Z., Fan, Q., Li, L., Lin, X., Wang, Y & Jiang, Z. (2019). Effects of dietary perilla seed oil supplementation on lipid metabolism, meat quality, and fatty acid profiles in Yellow-feathered chickens. *Poultry Science*, 98(11), 5714-5723.

Oybak Dönmez, E., & Hüryılmaz, H. (2024). Flax, Bupleurum and Other Plants at Early Bronze Age Yenibademli Höyük (Gökçeada), NW Turkey. *Environmental Archaeology*, 29(4), 333-351.

Daun, J. K., Barthet, V. J., Chornick, T. L., & Duguid, S. (2003). Structure, composition, and variety development of flaxseed. *Flaxseed in human nutrition*, (Ed. 2), 1-40.

De Silva, S. F., & Alcorn, J. (2019). Flaxseed lignans as important dietary polyphenols for cancer prevention and treatment: Chemistry, pharmacokinetics, and molecular targets. *Pharmaceuticals*, 12(2), 68.

- Deng, C., Zheng, J., Zhou, H., You, J., & Li, G. (2023).** Dietary glycine supplementation prevents heat stress-induced impairment of antioxidant status and intestinal barrier function in broilers. *Poultry Science*, 102(3), 102408.
- Dribnenki, J. C. P., McEachern, S. F., Chen, Y., Green, A. G., & Rashid, K. Y. (2007).** 2149 Solin (low linolenic flax). *Canadian journal of plant science*, 87(2), 297-299.
- Duclos, M. J., Beccavin, C., & Simon, J. (1999).** Genetic models for the study of insulin-like growth factors (IGF) and muscle development in birds compared to mammals. *Domestic Animal Endocrinology*, 17(2-3), 231-243.
- Elbaz, A. M., Zaki, E. F., Salama, A. A., Badri, F. B., & Thabet, H. A. (2023).** Assessing different oil sources efficacy in reducing environmental heat-stress effects via improving performance, digestive enzymes, antioxidant status, and meat quality. *Scientific Reports*, 13(1), 20179.
- Faintuch, J., Bortolotto, L. A., Marques, P. C., Faintuch, J. J., Franca, J. I., & Ceconello, I. (2011).** Systemic inflammation and carotid diameter in obese patients: pilot comparative study with flaxseed powder and cassava powder. *Nutricion hospitalaria*, 26(1), 208-213.
- Fébel, H., Mezes, M., Pálffy, T., Herman, A., Gundel, J., Lugasi, A., ... & Blazovics, A. (2008).** Effect of dietary fatty acid pattern on growth, body fat composition and antioxidant parameters in broilers. *Journal of Animal Physiology and Animal Nutrition*, 92(3), 369-376.
- Florini, J. R., Ewton, D. Z., & Coolican, S. A. (1996).** Growth hormone and the insulin-like growth factor system in myogenesis. *Endocrine reviews*, 17(5), 481-517.

- Fraz, A., Parker, N. B., Löhr, C. V., & Cherian, G. (2023).** Evaluating the impact of organic chromium with flax seed in broiler diets: effects on production performance, breast muscle pathology, and meat quality aspects. *Poultry Science*, 102(2), 102331.
- Fuentes, E. N., Valdés, J. A., Molina, A., & Björnsson, B. T. (2013).** Regulation of skeletal muscle growth in fish by the growth hormone–insulin-like growth factor system. *General and Comparative Endocrinology*, 192, 136-148.
- Galobart, J., Barroeta, A. C., Baucells, M. D., & Guardiola, F. (2001).** Lipid oxidation in fresh and spray-dried eggs enriched with ω 3 and ω 6 polyunsaturated fatty acids during storage as affected by dietary vitamin E and canthaxanthin supplementation. *Poultry Science*, 80(3), 327-337.
- Gao, F., Jiang, Y., Zhou, G. H., & Han, Z. K. (2007).** The effects of xylanase supplementation on growth, digestion, circulating hormone and metabolite levels, immunity and gut microflora in cockerels fed on wheat-based diets. *British poultry science*, 48(4), 480-488.
- Georganas, A., Giamouri, E., Pappas, A. C., Zoidis, E., Goliomytis, M., & Simitzis, P. (2023).** Utilization of agro-industrial by-products for sustainable poultry production. *Sustainability*, 15(4), 3679.
- Ghanbari, M., Mousavi, S. N., & Chamani, M. (2024).** Effects of different lipid sources on performance, blood lipid parameters, immune system activity, and expression of TNF α and TLR4 genes in broiler chickens. *Prostaglandins & Other Lipid Mediators*, 174, 106873.
- Gheorghe, A., Lefter, N. A., Idriceanu, L., Ropotă, M., & Hăbeanu, M. (2020).** Effects of dietary extruded linseed and *Lactobacillus acidophilus* on growth performance, carcass traits, plasma lipoprotein response, and caecal bacterial populations in broiler chicks. *Italian Journal of Animal Science*, 19(1), 822-832.

- Gieryńska, M., Szulc-Dąbrowska, L., Struzik, J., Mielcarska, M. B., & Gregorczyk-Zboroch, K. P. (2022).** Integrity of the intestinal barrier: the involvement of epithelial cells and microbiota—a mutual relationship. *Animals*, 12(2), 145.
- Hamosh, M. (2007).** Fatty acids, growth, and development. In *Fatty acids in foods and their health implications* (pp. 913-948). CRC Press.
- Haščík, P., Čech, M., Čuboň, J., Bobko, M., Kačániová, M., & Arpášová, H. (2022).** Effect of supplemental flax and pumpkin pomace on meat performance and quality of Ross 308 broiler chickens meat.
- Hashem, N. M., Abdelnour, S. A., Alhimaidi, A. R., & Swelum, A. A. (2021).** Potential impacts of COVID-19 on reproductive health: Scientific findings and social dimension. *Saudi Journal of Biological Sciences*, 28(3), 1702-1712.
- Heimbach, J. (2009).** Determination of the GRAS status of the Addition of Whole and Milled Flaxseed to Conventional Foods and Meat and Poultry Products. Virginia: Port Royal VA, 53.
- Hidayat, D. F., Mahendra, M. Y. N., Kamaludeen, J., & Pertiwi, H. (2023).** Lycopene in Feed as Antioxidant and Immuno-Modulator Improves Broiler Chicken's Performance under Heat-Stress Conditions. *Veterinary medicine international*, 2023(1), 5418081.
- Hirdyani, H., & Charak, B. (2015).** Nutritional, organoleptic evaluation and storage study of peanut chikki supplemented with flaxseeds. *International Journal of Home Science*, 1(3), 40-43.
- Hoek, J. B., & Pastorino, J. G. (2002).** Ethanol, oxidative stress, and cytokine-induced liver cell injury. *Alcohol*, 27(1), 63-68.
- Huerta, A., Trocino, A., Birolo, M., Pascual, A., Bordignon, F., Radaelli, G., ... & Xiccato, G. (2022).** Growth performance and gut response of

broiler chickens fed diets supplemented with grape (*Vitis vinifera* L.) seed extract. *Italian Journal of Animal Science*, 21(1), 990-999.

Huo, W., Li, M., Wang, J., Wang, Z., Huang, Y., & Chen, W. (2019). On growth performance, nutrient digestibility, blood T lymphocyte subsets, and cardiac antioxidant status of broilers. *Animal nutrition*, 5(1), 68.

Jameel, Y. J. (2018). Effect of prebiotic plus xylanase enzymes in reduced-energy high wheat Midd diets on broilers subjected to bursa vaccine and *Clostridium perfringens* challenge under heat stress. *Journal of Global Pharmacy Technology*, 10(01), 53-59.

Kajla, P., Sharma, A., & Sood, D. R. (2015). Flaxseed—a potential functional food source. *Journal of food science and technology*, 52, 1857-1871.

Kauser, S., Hussain, A., Ashraf, S., Fatima, G., Javaria, S., Abideen, Z. U., & Korma, S. A. (2024). Flaxseed (*Linum usitatissimum*); phytochemistry, pharmacological characteristics and functional food applications. *Food Chemistry Advances*, 4, 100573.

Kiela, P. R., & Ghishan, F. K. (2016). Physiology of intestinal absorption and secretion. *Best practice & research Clinical gastroenterology*, 30(2), 145-159.

Kim, H. W., Cramer, T., Ogbeifun, O. O., Seo, J. K., Yan, F., Cheng, H. W., & Kim, Y. H. B. (2017). Breast meat quality and protein functionality of broilers with different probiotic levels and cyclic heat challenge exposure. *Meat and Muscle Biology*, 1(1.(

Kishawy, A. T., Amer, S. A., Abd El-Hack, M. E., Saadeldin, I. M., & Swelum, A. A. (2019). The impact of dietary linseed oil and pomegranate peel extract on broiler growth, carcass traits, serum lipid profile, and meat fatty acid, phenol, and flavonoid contents. *Asian-Australasian journal of animal sciences*, 32(8), 1161.

- Kong, X., & Jha, R. (2022).** parent-offspring integration: gut health and physiological functions of animals. *Frontiers in Veterinary Science*, 8, 808074
- Kpomasse, C. C., Oke, O. E., Houndonougbo, F. M., & Tona, K. (2021).** Broiler production challenges in the tropics: A review. *Veterinary Medicine and Science*, 7(3), 831-842.
- Kumari, S., Yong Meng, G., & Ebrahimi, M. (2017).** Conjugated linoleic acid as functional food in poultry products: A review. *International Journal of Food Properties*, 20(3), 491-506.
- Lampros, M., Vlachos, N., Voulgaris, S., & Alexiou, G. A. (2022).** The role of Hsp27 in chemotherapy resistance. *Biomedicines*, 10(4), 897.
- Lara, L. J., & Rostagno, M. H. (2013).** Impact of heat stress on poultry production. *Animals*, 3(2), 356-369.
- Leeson, S., & Summers, J. D. (2009).** Commercial poultry nutrition. Nottingham university press.
- Li, L., Cui, Z., Wang, H., Huang, B., & Ma, H. (2023).** Dietary supplementation of dimethyl itaconate protects against chronic heat stress-induced growth performance impairment and lipid metabolism disorder in broiler chickens. *Journal of Animal Science*, 101, skad120.
- Li, Z., Liu, A., Xu, J., & Zhang, C. (2023).** Resveratrol attenuates heat-stress-impaired immune and inflammatory responses of broilers by modulating toll-like receptor-4 signaling pathway. *Brazilian Journal of Poultry Science*, 25, eRBCA-2022.
- Liu, L., Ren, M., Ren, K., Jin, Y., & Yan, M. (2020).** Heat stress impacts on broiler performance: a systematic review and meta-analysis. *Poultry Science*, 99(11), 6205-6211.

- Liu, X., Hong, H., Wang, J., Huang, J., Li, J., Tao, Y., ... & Bo, R. (2024).** Mucosal immune responses and protective efficacy elicited by oral administration AMP-ZnONPs-adjuvanted inactivated H9N2 virus in chickens. *Poultry Science*, 103(4), 103496.
- Liu, Y., Chen, F., Odle, J., Lin, X., Jacobi, S. K., Zhu, H., ... & Hou, Y. (2012).** Fish Oil Enhances Intestinal Integrity and Inhibits TLR4 and NOD2 Signaling Pathways in Weaned Pigs after LPS Challenge, 3. *The Journal of nutrition*, 142(11), 2017-2024.
- Lowcock, E. C., Cotterchio, M., & Boucher, B. A. (2013).** Consumption of flaxseed, a rich source of lignans, is associated with reduced breast cancer risk. *Cancer Causes & Control*, 24, 813-816.
- Lu, M. H., Ding, K. N., Liang, S. S., Guo, Y. N., He, Y. M., & Tang, L. P. (2023).** Resveratrol inhibits oxidative damage in lungs of heat-stressed broilers by activating Nrf2 signaling pathway and autophagy. *Ecotoxicology and Environmental Safety*, 258, 114949.
- Lu, M., Tan, L., Zhou, X. G., Yang, Z. L., Zhu, Q., Chen, J. N., ... & Wu, G. S. (2020).** Secoisolariciresinol diglucoside delays the progression of aging-related diseases and extends the lifespan of *Caenorhabditis elegans* via DAF-16 and HSF-1. *Oxidative medicine and cellular longevity*, 2020.
- Mahasneh, Z. M., Abuajamieh, M., Abedal-Majed, M. A., Al-Qaisi, M., Abdelqader, A., & Al-Fataftah, A. R. A. (2024).** Effects of medical plants on alleviating the effects of heat stress on chickens. *Poultry Science*, 103391.
- Maki, K. C., & Dicklin, M. R. (2019).** Strategies to improve bioavailability of omega-3 fatty acids from ethyl ester concentrates. *Current Opinion in Clinical Nutrition & Metabolic Care*, 22(2), 116-123.

- Mashaly, M. M., Hendricks 3rd, G. L., Kalama, M. A., Gehad, A. E., Abbas, A. O., & Patterson, P. H. (2004).** Effect of heat stress on production parameters and immune responses of commercial laying hens. *Poultry science*, 83(6), 889-894.
- Mirzaei, M., Bouyeh, M., Zahedi, A., Seidavi, A., Khan, R. U., Tufarelli, V., ... & Swelum, A. A. (2022).** Influence of dietary L-carnitine and lysine–methionine levels on reproductive performance and blood metabolic constituents of breeder ducks. *Reproduction in Domestic Animals*, 57(3), 253-261.
- Mishra, B., & Jha, R. (2019).** Oxidative stress in the poultry gut: Potential challenges and interventions. *Frontiers in veterinary science*, 6, 60.
- Moghadam, M. B., & Cherian, G. (2017).** Use of flaxseed in poultry feeds to meet the human need for omega-3 fatty acids. *World's Poultry Science Journal*, 73(4), 803-812.
- Mohsen, S., A El-Sayed, S., El-Adl, M., Fouda, M., & El-Sherbini, E. S. (2019).** Ameliorative effects of flaxseed and soybean oils on male rats fertility. *Mansoura Veterinary Medical Journal*, 20(1), 67-74.
- Molee, A., Kuadsantia, P., & Kaewnakian, P. (2018).** Gene effects on body weight, carcass yield, and meat quality of Thai indigenous chicken. *The Journal of Poultry Science*, 55(2), 94-102.
- Mridula, D., Kaur, D., Nagra, S. S., Barnwal, P., Gurumayum, S., & Singh, K. K. (2015).** Growth performance and quality characteristics of flaxseed-fed broiler chicks. *Journal of Applied Animal Research*, 43(3), 345-351.
- Nawab, A., Ibtisham, F., Li, G., Kieser, B., Wu, J., Liu, W., ... & An, L. (2018).** Heat stress in poultry production: Mitigation strategies to overcome the future challenges facing the global poultry industry. *Journal of thermal biology*, 78, 131-139.

- Nematbakhsh, S., Pei Pei, C., Selamat, J., Nordin, N., Idris, L. H., & Abdull Razis, A. F. (2021).** Molecular regulation of lipogenesis, adipogenesis and fat deposition in chicken. *Genes*, 12(3), 414.
- Newman, R. E., Bryden, W. L., Kirby, A. C., Storlien, L. H., & Downing, J. A. (2005).** Dietary omega-3 and n-6 fatty acids alter avian glucose metabolism. *British poultry science*, 46(1), 104-113.
- Nofal, M. E., Magda, A. G., Mousa, S. M. M., Doaa, M. M., & Bealsh, A. M. A. (2015).** Effect of dietary betaine supplementation on productive, physiological and immunological performance and carcass characteristic of growing developed chicks under the condition of heat stress. *Egyptian Poultry Science Journal*, 35(1).
- Obaid, M. N. (2023).** Used flaxseed as a source of omega-3 in feed of broiler and study the effects on productive performance, histological change and gene expression (Master's Thesis) University of Kebala College of Veterinary Medicine.
- Obaid, M. N., & Jameel, Y. J. (2023).** Effects of Whole Flaxseed on Productive Performance and Immune Response of Broiler Chickens. *Academic International Journal of Veterinary Medicine*, 1(1), 52-57.
- Oladeinde, A., Awosile, B., Woyda, R., Abdo, Z., Endale, D., Strickland, T., ... & Cook, K. (2023).** Management and environmental factors influence the prevalence and abundance of food-borne pathogens and commensal bacteria in peanut hull-based broiler litter. *Poultry science*, 102(2), 102313.
- Oppedisano, F., Macrì, R., Gliozzi, M., Musolino, V., Carresi, C., Maiuolo, J., ... & Mollace, V. (2020).** The anti-inflammatory and antioxidant properties of omega-3 PUFAs: Their role in cardiovascular protection. *Biomedicines*, 8(9), 306.

- Pawar, S. S., Sajjanar, B., Lonkar, V. D., Kurade, N. P., Kadam, A. S., Nirmal, A. V., ... & Bal, S. K. (2016).** Assessing and mitigating the impact of heat stress in poultry. *Adv. Anim. Vet. Sci*, 4(6), 332-341.
- Popescu, R. G., Voicu, S. N., Gradisteanu Pircalabioru, G., Gharbia, S., Hermenean, A., Georgescu, S. E., ... & Dinischiotu, A. (2021).** Impact of dietary supplementation of flaxseed meal on intestinal morphology, specific enzymatic activity, and cecal microbiome in broiler chickens. *Applied Sciences*, 11(15), 6714.
- Rahman, M., & Hoque, A. (2023).** Flax Breeding. In *The Flax Genome* (pp. 55-68). Cham: Springer International Publishing
- Raya-Sandino, A., Luissint, A. C., Kusters, D. H., Narayanan, V., Flemming, S., Garcia-Hernandez, V., ... & Nusrat, A. (2021).** Regulation of intestinal epithelial intercellular adhesion and barrier function by desmosomal cadherin desmocollin-2. *Molecular biology of the cell*, 32(8), 753-768.
- Reda, F. M., El-Kholy, M. S., Abd El-Hack, M. E., Taha, A. E., Othman, S. I., Allam, A. A., & Alagawany, M. (2020).** Does the use of different oil sources in quail diets impact their productive and reproductive performance, egg quality, and blood constituents?. *Poultry science*, 99(7), 3511-3518.
- Ringseis, R., & Eder, K. (2022).** Heat stress in pigs and broilers: role of gut dysbiosis in the impairment of the gut-liver axis and restoration of these effects by probiotics, prebiotics and synbiotics. *Journal of Animal Science and Biotechnology*, 13(1), 126.
- Rodríguez-García, C., Sánchez-Quesada, C., Martínez-Ramírez, M. J., & Gaforio, J. J. (2022).** PPAR γ Gene as a Possible Link between Acquired

and Congenital Lipodystrophy and its Modulation by Dietary Fatty Acids. *Nutrients*, 14(22), 4742.

Rostagno, M. H. (2020). Effects of heat stress on the gut health of poultry. *Journal of animal science*, 98(4), skaa090.

Rubilar, M., Gutiérrez, C., Verdugo, M., Shene, C., & Sineiro, J. (2010). Flaxseed as a source of functional ingredients. *Journal of soil science and plant nutrition*, 10(3), 373-377.

Saber, S. N., & Kutlu, H. R. (2020). Effect of including omega-3/n-6 fatty acid feed sources in diet on fertility and hatchability of broiler breeders and post-hatch performance and carcass parameters of progeny. *Asian-Australasian journal of animal sciences*, 33(2), 305.

Scanes, C. G. (2016). Biology of stress in poultry with emphasis on glucocorticoids and the heterophil to lymphocyte ratio. *Poultry science*, 95(9), 2208-2215.

Shahid, M. S., Wu, Y., Xiao, Z., Raza, T., Dong, X., & Yuan, J. (2019). Duration of the flaxseed diet promotes deposition of n-3 fatty acids in the meat and skin of Peking ducks. *Food & nutrition research*, 63.

Shakeri, M., & Le, H. H. (2022). Deleterious effects of heat stress on poultry production: Unveiling the benefits of betaine and polyphenols. *Poultry*, 1, 147–156

Shim, Y. Y., Gui, B., Wang, Y., & Reaney, M. J. (2015). Flaxseed (*Linum usitatissimum* L.) oil processing and selected products. *Trends in Food Science & Technology*, 43(2), 162-177.

Sierzant, K., Korzeniowska, M., Pólbrat, T., Rybarczyk, A., & Smoliński, J. (2022). The use of an optimised concentration of quercetin limits peroxidation of lipids in the meat of broiler chickens fed a diet containing flaxseed oil rich in omega-3. *animal*, 16(8), 100603.

- Simopoulos, A. P. (2011).** Evolutionary aspects of diet: the omega-6/omega-3 ratio and the brain. *Molecular neurobiology*, 44(2), 203-215.
- Sokoła-Wysoczańska, E., Czyż, K., & Wyrostek, A. (2024).** Different sources of omega-3 fatty acid supplementation vs. blood lipid profiles—a study on a rat model. *Foods*, 13(3), 385.
- Stillhart, C., Vučićević, K., Augustijns, P., Basit, A. W., Batchelor, H., Flanagan, T. R & Müllertz, A. (2020).** Impact of gastrointestinal physiology on drug absorption in special populations—An UNGAP review. *European Journal of Pharmaceutical Sciences*, 147, 105280.
- Sugiharto, S. (2020).** Alleviation of heat stress in broiler chicken using turmeric (*Curcuma longa*)-a short review. *Journal of Animal Behaviour and Biometeorology*, 8(3), 215-222.
- Sun, S., Li, B., Wu, M., Deng, Y., Li, J., Xiong, Y., & He, S. (2023).** Effect of dietary supplemental vitamin C and betaine on the growth performance, humoral immunity, immune organ index, and antioxidant status of broilers under heat stress. *Tropical Animal Health and Production*, 55(2), 96.
- Surai, P. F., Kochish, I. I., Fisinin, V. I., & Kidd, M. T. (2019).** Antioxidant defence systems and oxidative stress in poultry biology: An update. *Antioxidants*, 8(7), 235.
- Szalai, K., Tempfli, K., Zsédely, E., Lakatos, E., Gáspárdy, A., & Papp, Á. B. (2021).** Linseed oil supplementation affects fatty acid desaturase 2, peroxisome proliferator activated receptor gamma, and insulin-like growth factor 1 gene expression in turkeys (*Meleagris gallopavo*). *Animal bioscience*, 34(4), 662.
- Tesseraud, S., Chartrin, P., Métayer-Coustard, S., Hermier, D., Simon, N., Peyronnet, C., ... & Baéza, E. (2014).** Modulation of the insulin anabolic signalling cascade in growing chickens by omega-3 PUFA. *British journal of nutrition*, 111(5), 761-772.

- Tietz, N.W. (1995).** ed. Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia, PA: W.B. Saunders, 76.
- Tóth, R., Tokodyné Szabadi, N., Lázár, B., Buda, K., Végi, B., Barna, J., ... & Gócza, E. (2021).** Effect of Post-Hatch Heat-Treatment in Heat-Stressed Transylvanian Naked Neck Chicken. *Animals*, 11(6), 1575.
- Uyanga, V. A., Musa, T. H., Oke, O. E., Zhao, J., Wang, X., Jiao, H., ... & Lin, H. (2023).** Global trends and research frontiers on heat stress in poultry from 2000 to 2021: A bibliometric analysis. *Frontiers in Physiology*, 14, 1123582.
- Velmurugan, N., Kalpana, D., Cho, J. Y., & Lee, Y. S. (2018).** Chemical composition and antioxidant capacity of the aqueous extract of *Phellodendron amurense*. *Journal of forestry research*, 29, 1041-1048.
- Verwoolde, M. B., van den Biggelaar, R. H., de Vries Reilingh, G., Arts, J. A., van Baal, J., Lammers, A., & Jansen, C. A. (2021).** Innate immune training and metabolic reprogramming in primary monocytes of broiler and laying hens. *Developmental & Comparative Immunology*, 114, 103811.
- Waheed, R., El Asely, A. M., Bakery, H., El-Shawarby, R., Abuo-Salem, M., Abdel-Aleem, N., & Abdeen, A. (2020).** Thermal stress accelerates mercury chloride toxicity in *Oreochromis niloticus* via up-regulation of mercury bioaccumulation and HSP70 mRNA expression. *Science of the total environment*, 718, 137326.
- Wang, J., Clark, D. L., Jacobi, S. K., & Velleman, S. G. (2021).** Supplementation of vitamin E and omega-3 fatty acids during the early posthatch period on intestinal morphology and gene expression differentiation in broilers. *Poultry science*, 100(3), 100954.
- Wang, Y. W., Sunwoo, H., Cherian, G., & Sim, J. S. (2004).** Maternal dietary ratio of linoleic acid to alpha-linolenic acid affects the passive immunity of hatching chicks. *Poultry Science*, 83(12), 2039-2043.

- Wang, Y., Yang, X., Li, S., Wu, Q., Guo, H., Wang, H & Wang, J. (2023).** Research Note: Heat stress affects immune and oxidative stress indices of the immune organs of broilers by changing the expressions of adenosine triphosphate-binding cassette subfamily G member 2, sodium-dependent vitamin C transporter-2, and mitochondrial calcium uniporter. *Poultry Science*, 102(8), 102814.
- Wasti, S., Sah, N., Singh, A. K., Lee, C. N., Jha, R., & Mishra, B. (2021).** Dietary supplementation of dried plum: a novel strategy to mitigate heat stress in broiler chickens. *Journal of Animal Science and Biotechnology*, 12, 1-17.
- Whittle, R. H., Kiarie, E. G., & Widowski, T. M. (2024).** The effect of feeding flaxseed as a source of omega-3 fatty acids to broiler and layer breeders during rearing and lay on body weight, reproductive performance, and hatchability performance indices. *Canadian Journal of Animal Science*.
- Yang, C., Luo, P., Chen, S. J., Deng, Z. C., Fu, X. L., Xu, D. N., ... & Liu, W. J. (2021).** Resveratrol sustains intestinal barrier integrity, improves antioxidant capacity, and alleviates inflammation in the jejunum of ducks exposed to acute heat stress. *Poultry Science*, 100(11), 101459.
- Yang, X., Guo, Y., Wang, Z., & Nie, W. (2006).** Fatty acids and coccidiosis: effects of dietary supplementation with different oils on coccidiosis in chickens. *Avian Pathology*, 35(5), 373-378.
- Zaboli, G., Huang, X., Feng, X., & Ahn, D. U. (2019).** How can heat stress affect chicken meat quality?—a review. *Poultry science*, 98(3), 1551-1556.
- Zampiga, M., Laghi, L., Zhu, C., Mancinelli, A. C., Mattioli, S., & Sirri, F. (2021).** Breast muscle and plasma metabolomics profile of broiler

chickens exposed to chronic heat stress conditions. *Animal*, 15(7), 100275.

Zhang, P., Yan, T., Wang, X., Kuang, S., Xiao, Y., Lu, W., & Bi, D. (2017). Probiotic mixture ameliorates heat stress of laying hens by enhancing intestinal barrier function and improving gut microbiota. *Italian journal of animal science*, 16(2), 292-300.

Zhang, X., Akhtar, M., Chen, Y., Ma, Z., Liang, Y., Shi, D & Liu, H. (2022). Chicken jejunal microbiota improves growth performance by mitigating intestinal inflammation. *Microbiome*, 10(1), 107.

Zhang, Y., Cao, Z., Wang, L., Dong, B., Qi, S., Xu, X & Chen, G. (2023). Effects of linseed oil supplementation duration on fatty acid profile and fatty acid metabolism-related genes in the muscles of Chinese crested white ducks. *Poultry Science*, 102(10), 102896.

Zollitsch, W., Knaus, W., Aichinger, F., & Lettner, F. (1997). Effects of different dietary fat sources on performance and carcass characteristics of broilers. *Animal Feed Science and Technology*, 66(1-4), 63-73.

Appendices

Appendices

Appendix I

Estimation of serum cholesterol concentration (mg/dl):

Principle:

Ester of cholesterol + H₂O $\xrightarrow{\text{Chol. esterase}}$ Cholesterol + Fatty acids

Cholesterol + O₂ $\xrightarrow{\text{Chol. oxidase}}$ Cholest-4-en-one + H₂O₂

H₂O₂ + 4-Aminophenazone + phenol $\xrightarrow{\text{peroxidase}}$ Quinonimine

Reagent:

Reagent (1) Buffer solution: pipes PH 6.9 mmol/L, phenol 26 mmol/L

Reagent (2) vial of enzyme: cholesterol oxidase 300 U/L, peroxidase 1250

U/L, cholesterol esterase 300 U/L, 4-aminophenazone 0.4 mmol/L

Reagent (3): cholesterol standard 200 mg/dl

1. Manual procedure: Cholesterol concentration in serum samples was measured according to the following
 - a. Reagent and serum samples were brought to room temperature
 - b. Serum sample, blank and standard were treated as follow:
 - c. Tube contents were mixed and left to stand for 5 minutes at 37° C before reading.
 - d. the absorbance of the standard was measured and sample was read via spectrophotometer at wavelength 505 nm against the blank.

Tubes	Blank	Standard	Sample
Cholesterol standard (s)	-	10 ml	-
Sample	-	-	10 ml
Working Reagent	1 ml	1 ml	1 ml

Calculation:

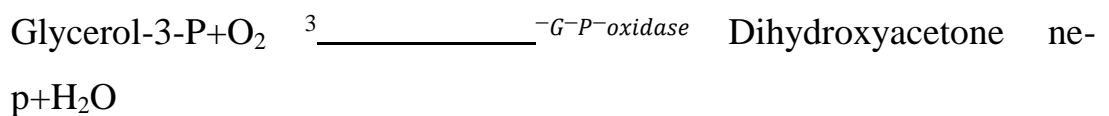
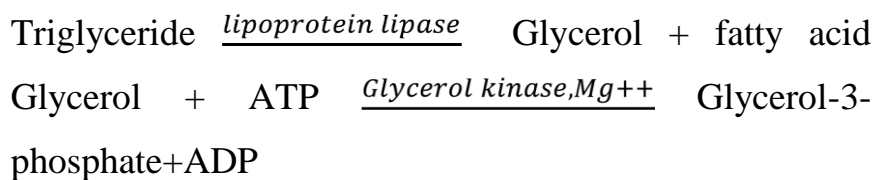
Result were calculated according to the following equation:

$$\text{Total Cholesterol concentration} = (\text{O.D sample}) / (\text{O.D/ standard}) \times \text{nn} = 200 \text{ mg/dl}$$

Appendix II

Estimation of serum triglyceride concentration (mg/dl):

Principle:



Reagent:

Reagent (1) buffer solution: pipes buffer PH 7.2, 50 mmol/L, p-chlorophenol 2 mmol/L

Reagent (2) Enzyme: lipoprotein lipase 150 000 U/I, glycerol kinase 800 U/I, glycerol-3-phosphate oxidase 4000 U/I, peroxidase 440 U/I, 4aminophenazone 0.7 mmol/L, ATP 0.3 mmol/L.

Reagent (3) triglyceride Standard (S): Glycerol 200mg/dl.

Procedure:

Triglyceride concentration in serum samples was measured according to the following:

- a. Wave length/filter. 505nm (Hg546nm)/green
- b. Temperature 37°C/R. T
- c. Light path 1 cm

Pipette into clean dry test tubes labelled as Blank (B), standard (S), and Test (T).

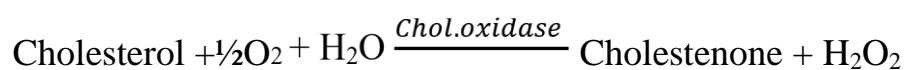
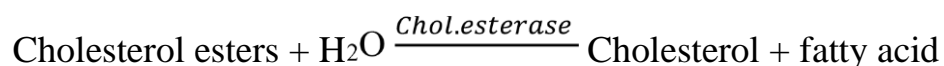
Mix well and incubated at 37°C for 5 min or at R. T (25°C) for 15min.

measure the absorbance of the standard **Calculation:**

Results were calculated according to the following equation:

$$\text{Triglyceride concentration mg/dl} = (\text{O.D sample}) / (\text{O.D standard}) \times n = 200 \text{ mg/dl}$$

Additive sequence	Blank	Standard	Test
Working reagent	1.0	1.0	1.0
Distilled water	0.01	-	-
Triglyceride standard	-	0.01	-
Sample	-	-	0.01

Appendix III**Estimation of serum HDL-Cholesterol concentration (mg/dl):****Principle:**



Reagent:

Reagent (1) Good's buffer (pH 6.6) 100 mmol/l, cholesterol esterase 1400 U/l, cholesterol oxidase 800 U/l, catalase 600 kU/l, N-(2-hydroxy-3-sulfoethyl)3,5-dimethoxyaniline (HDAOS) 0.6 mmol/l

Reagent (2) Good's buffer (pH 7.0) 100 mmol/l peroxidase 3 kU/l 4-aminoantipyrine (4-AA) 4 mmol/L

Tubes	Blank	Standard	Sample
Distilled water	50 ml	-	-
Cholesterol standard (S)	-	50 ml	-
Sample supernatant	-	-	50 ml
Reagent (B)	1.0 ml	0.5 ml	1 ml

Procedure:

HDL-Cholesterol concentration in serum sample was measured according to the following steps: serum sample 40 – 60 mg/dl 1.04 1.55mmol/l, wavelength 600 nm, temperature 37°C CORMAY HDL DIRECT is intended for automated analysers.

- Reagent (A, B) and serum sample were brought to room temperature.
- Serum sample, blank and standard were treated as followed:
- 0.2 ml of sample was mixed with 0.5 ml of reagent (A) in centrifuge tube and let stand for 10 minute at room temperature.
- Centrifuged at a minimum of 4000 r.p.m. for 10 minutes.
- The temperature was collected carefully.
- Sample supernatant, blank, standard and reagent (B) were treated as follows:

g. Tubes contents were mixed thoroughly and incubated for 10 minute at 37°C.

h. the absorbance (A) of the standard was measured and sample was read via spectrophotometer at wave length 500 nm against the blank.

Calculation: results were calculated according to the following equation: HDL-cholesterol concentration in the sample (mg/dl) = (Absorbance of the sample/Absorbance of standard) × concentration of standard × sample dilution factor (1.7).

Appendix IV

Estimation of serum LDL-Cholesterol concentration (mg/dl):

Principle:

Cholesterol ester $\xrightarrow{\text{chol.esterase}}$ chol. + fatty acid

Cholesterol + O₂ $\xrightarrow{\text{chol.oxidase}}$ chol. H₂O₂

2H₂O₂ $\xrightarrow{\text{catalase}}$ H₂O +

O₂ Reagent:

Reagent (1) Good's buffer (pH 7,0) 50 mmol/l, cholesterol esterase 600 U/l, cholesterol oxidase 500 U/l, catalase 1200 kU/l, ascorbate oxidase 3 kU/l, TOOS [N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline] 2.0 mmol/l

Reagent (2) Good's buffer (pH 7,0) 50 mmol/l, peroxidase 5 kU/l, 4-aminoantipyrine (4-AA) 4 mmol/l.

Procedure: wavelength 600 nm, temperature 37°C, CORMAY LDL DIRECT is intended for automated analysers. serum/plasma <

100 mg/dl < 2.59 mmol/l. As LDL cholesterol is affected by a number of factors such as smoking, exercise, hormones, age and sex, each laboratory should establish its own reference ranges for local population.

Calculation:

A comparison between LDL cholesterol values determined at Biolis 24i Premium (y) and at COBAS INTEGRA 400 (x) using 52 samples gave following results:

$$y = 0.9642 x - 0.8968 \text{ mg/dl};$$

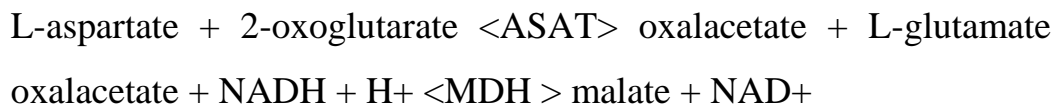
$$R = 0.9762 \text{ (R – correlation coefficient).}$$

Appendix V

Serum Aspartate aminotransferase (AST) activity determination:

Aspartate aminotransferase activity is (ASAT, AST, GOT) Measured by cormay GOT kit produced by PZ CORMAY S.A. company (Tietz, 1995).

Principle:



The rate of absorbance changing at $\lambda=340$ nm is directly proportional to aspartate aminotransferase activity.

	(24-TRAY)	(36-TRAY)
1-Reagent	6 x 40 ml	8 x 23 ml
2- -Reagent	6 x 12.5 ml	8 x 7.5 ml

Reagent:

Tris (pH 7.8) 80 mmol/l, L-aspartate 240 mmol/l, MDH > 10 μ kat/l, LDH >

20 μ kat/l, 2-oxoglutarate 15 mmol/l, NADH 0.18 mmol/l

ROCEDURE

These reagents may be used in automatic analysers Prestige 24i, Biolis 24i and Sapphire 400.

1-REAGENT and 2-REAGENT are ready to use.

Parameter	Liquick Cor-ALAT	Liquick Cor-ALAT “bulk”
1-ALAT	3 x 400 ml	--
2-ALAT	1 x 300 ml	--

1-Reagent put on basic position in reagent tray.

2-Reagent put on start position in reagent tray. For reagent blank deionized water is recommended. Reagent blank is required each day

Calculation:

A comparison between ASAT values for samples obtained on Prestige 24i (y) and obtained on COBAS INTEGRA 400 (x) using 100 samples gave following results: $y = 1.1501 x - 2.8845$ U/l;

$R = 0.9972$ (R - correlation coefficient)

Appendix VI

Serum Alanine aminotransferase (ALT) activity determination:

Principle:

L-alanine + 2-oxoglutarate \xrightarrow{ALAT} pyruvate + L-glutamate

pyruvate + NADH + H⁺ \xrightarrow{LDH} lactate + NAD⁺

The rate of absorbance changing at $\lambda=340$ nm is directly proportional to alanine aminotransferase activity.

Reagent:

Tris (pH 7.5) 100 mmol/l, L-alanine 500 mmol/l, LDH > 36.7 μ kat/l
2oxoglutarate 15 mmol/l, NADH 0.18 mmol/l

PROCEDURE

These reagents may be used both for manual assay (Sample Start and Reagent Start method) and in several automatic analysers.

Applications for them are available on request.

Simple start method:

Pipette into the cuvette. working reagent 1000 μ l, bring up to the temperature of determination. Then add sample 100 μ l, Mix and incubate at adequate temperature. After about 1 min. read the absorbance against air or water. Repeat the reading after exactly 1, 2 and 3 minutes. Calculate the mean absorbance change per minute (DA/min.).

Calculation:

ALAT activity [U/l] = DA/min. x F

Appendix VII

Principle:

Fat was determined based on the method (AOAC 1995) using a fat extractor (Soxholet). The sample was prepared according to the method approved by (AOAC 1995), which is based on the esterification of fats, by its interaction with methanolic potassium hydroxide, prepared by dissolving 11.2 g of potassium hydroxide and dissolving it in 100 ml of methanol, then taking 1 gm of fat and adding 8 ml of methanolic potassium hydroxide to it. With 5 ml of hexane, shake quickly for 30 seconds, then leave to separate into two layers, taken from the upper layer (the hexane layer) that contains the esterified fat and injected into the device.

Appendix VIII

Estimation of gene expression :

Insulin-like growth factors I (IGF-1) Gene expression

Total RNA extraction using Easy-spin™ (DNA free) total RNA extraction Kit

1. Preparation of 50-100 mg of fresh tissue.
2. Add 1ml of Lysis Buffer (easy-BLUETM reagent) and homogenize tissue sample using a homogenizer or equivalent.
3. Vigorously vortex in room temperature for 10sec.
4. Add 200µl of Chloroform and apply vortex.
5. After centrifuging the solution at 13,000 rpm (4°C) for 10 min, transfer 400µl of the upper fluid to an empty 1.5ml tube.

6. Add 400 μ l of Binding Buffer and mix it well by pipetting or gently inverting the 2-3 times. Do not centrifuge and leave it for 1min at room temperature.
7. Load the upper solution to the column, but do not load the whole upper solution because the maximum volume of the column reservoirs is 800 μ l. After loading the optimum of the upper solution to the column, and centrifuge at 13,000rpm for 30sec. Discard the flow-through after centrifuging and place the spin column back in the same 2ml collection tube. And then repeat this step.
8. Add 700 μ l of Washing Buffer A to the column. Close the tubes gently, and centrifuge for 30 sec. at 13,000rpm to wash the column. Discard the flow-through and place the spin column back in the same 2ml collection tube.
9. Wash by adding 700 μ l of Washing Buffer B to the column and centrifuge for 30 sec. at 13,000rpm. Discard the filtrates and place the spin column back in the same 2ml collection tube.
10. Centrifuge for 1-2 min at 13,000rpm to dry the column membrane
11. Place the column in a clean 1.5ml microcentrifuge tube (not provided), and add 50 μ l of Elution Buffer directly onto the membrane. Incubate at RT for 1min, and centrifuge for 1min at 13,000rpm to elute.

Preparation of primers

Gene	Accession	Sequence	Size (pb)
------	-----------	----------	-----------

IGF1	M74176.1	F: 5'-CAGAGCAGATAGAGCCTGCG-3'	655
		R: 5'-TCTGCAGATGGCACATTCAT-3	

According to instruction of the primer synthesiser company, the primers (originally lyophilized), were dissolved in the free ddH₂O to obtain a final concentration of 100 pM/μl which served as a stock solution that stored at -20 °C. A concentration of 10 pM/μl was prepared from the stock primers to be used as a work primer.

Primers used in this study

Protocol of GoTaq® 1-Step RT-qPCR System for Real-Time qPCR (Gene expression assay):

1. Program a real-time instrument for standard or fast mode one-step RT-qPCR (Table 2).
2. Thaw the components of the GoTaq® 1-Step RT-qPCR System, the RNA templates and the primer pair on ice, at room temperature or at 37°C. Immediately mix each thawed component thoroughly. If using a vortex mixer, mix at low speed to minimize aeration. Keep thawed reagents on ice.
3. Prepare the RNA samples (mRNA [500fg–100ng]) in water or another qPCR compatible diluent.
4. Combine reaction components (Table 1) in a non-stick, sterile tube on ice. Mix gently after each addition. Carefully pipet reaction volumes to plate on ice.
5. Transfer plate from ice into the pre-programmed instrument. Start the run immediately.

6. When the run is complete, collect the data and analyse the results.

Table 1: Preparation of Real-Time PCR solutions

Components	Concentration	Volume (20µl)
GoTaq™qPCR master mix, 2X	1X	10 µl
Forward primer	10 µM/µl	2µl
Reverse primer	10 µM/µl	2 µl
GoScript™ RT mix for 1-step RT-qPCR	1X	0.4 µl
ddH ₂ O	-	3.6 µl
RNA template	250 ng	2µl

Table 2: Real-Time PCR conditions (According to the instruction of GoTaq® 1-Step RT-qPCR System)

Stage	Ta (°C)	Time	Cycles
Reverse transcription	42	15 min	1
RT inactivation/Hot-start activation	95	10 min	1X
Denaturation	95	10 sec.	40X
Annealing/data collection	60	30 sec.	
Extension	72	30 sec.	
Dissociation	72	2 min	1X

Appendix IX

Histological examination:

Histological sampling was collected from each replicate at 35 days. Section from the Meckel's diverticulum of duodenum, jejunum and ileum (about 0.6 cm in length) were excised longitudinally at the ant mesenteric attachment and gently flushed with NaCl (9 g. L1). These samples were fixed in a solution of formalin buffer (90 mL.L-1) for 12 to 24 24 h at 4 oC, then rinsed and stored in 70% ethanol at 4 oC until analysis. Villi and crypts were carefully individualized under a dissecting microscope. The preparation was then mounted between slides and coverslips, with addition of an aqueous

agent for microscopy (Aqua mount improved gun, VWR, West Chester, PA). Ten villi and 10 crypts of Lieberkühn from each segment of each bird were measured using an optical microscope. The sample of duodenum, jejunum and ileum of 2 birds from each line, representative of the population on the basis of BW, were rehydrated with PBS and stored at 4 °C until analysis. Each sample was then embedded in medium in liquid nitrogen, cut at – 20 °C into 5–10 µm-thick cross-section using a cryostat, and placed on gelatine – treated glass slides. Three cross-sections were obtained from each sample for further and observation. A routine procedure was carried out using Meyer hemalun and eosin (Sigma Chemical Company). The preparation were then mounted between slides and coverslips with the addition of an aqueous agent for microscopy. The slides were examined using an optical microscope. The thickness of the muscularis layer was measured on all section (Boroojeni *et al.*,2019)

After the staining was completed the slide was examined by using a light microscope and a graduated lens was used to measure the villi height and crypt depth under magnified to X 10.

Appendix X

Procedure of determination immunity of ND and IBD by ELISA test
ELISA Kit (Synbiotics–USA)

The procedure used in this test was performed according to the manufacturer instructions listed in the ProFLOK® (ND and IBD) ELISA Kit (Synbiotics–USA), which is a rapid serologic test for the detection of IBV antibody in chicken serum samples. It was developed primarily to aid in the detection of pre and postvaccination (ND and IBD) antibody levels in chickens.

ELISA was performed as the following:- Each test sample 1:500 was diluted by adding 1 μ l to 0.5 ml of sample diluents. 1. Fifty μ l of diluents was added to each plate that contained coated antigen. 2. Fifty μ l of positive control was added in wells (H11, A3, and A1) for the plate.

3. Fifty μ l of negative controls was added in wells (A2, A10, and H12) for the plate. 4. Fifty μ l of diluted serum was added to the suitable wells for the presided plate. 5. Plate was incubated for 30 minutes at room temperature. 6. Plate holes were washed by using washing solution. 7. Hundred μ l of diluted antiserum was added at ratio of 1:100 and conjugated with (horseradish peroxides) for each hole. 8. Plate was incubated for 30 minutes at room temperature.

9. All the plate holes were washed by using washing solution in amount of 300 μ l twice with good drying. 10. Hundred μ l of substrate was added to expose the color, and then the plates were left at room temperature in a dark place. 11. Hundred μ l of stop solution (H_2SO_4) was added. 12. Results were read through recording the optical density absorbance of the control and the samples antibody titers were calculated automatically, by using ELISA reader using profile flock software.

ملخص:

إن التكيف مع ارتفاع درجات الحرارة العالمية مع الحفاظ على كفاءة الإنتاج يشكل تحديًا مهمًا يواجه صناعة الدواجن. ففي ظل درجات الحرارة المرتفعة خاصة في العراق، تقلل الطيور من تناولها للعلف، وهذا هو العامل الرئيسي الذي يفسر تدهور أداء الطيور. أجريت هذه التجربة لتحديد تأثيرات بذور الكتان الكاملة في نظام غذائي منخفض الطاقة على معايير أداء فروج اللحم مثل وزن الجسم (BW)، الزيادة الوزنية (WG)، العلف المستهلك (FI)، وكفاءة التحويل الغذائي (FCR)، والصورة الدهنية، وجودة اللحوم، ووظائف الكبد، الحالة المناعية، وتم تحديد كمية التعبير عن جين IGF_1 في أنسجة الكبد باستخدام تفاعل البوليميراز المتسلسل الكمي في الوقت الفعلي، وشكل الامعاء لفروج اللحم وصفات الذبيحة تحت ظروف الإجهاد الحراري.

تم تجهيز 250 فرخ من سلالة (Ross 30) بعمر يوم واحد قسمت إلى خمس مجاميع عشوائية كل مجموعة تضم (50) طير توزعت على مكررين. تم تقسيم كل مكرر إلى 25 طائرًا/حظيرة. تم تغذية معاملة السيطرة (T1) على عليقة غذائية من الذرة وفول الصويا. المعاملة الثانية (T2) غدت على عليقة من الذرة وفول الصويا تحت الإجهاد الحراري. المعاملة الثالثة (T3) غدت على عليقة من بذور الكتان والذرة وفول الصويا باستخدام بذور الكتان 7.5 غم / كغم في البادئ والنامي 10 غم / كغم تحت الإجهاد الحراري. المعاملة الرابعة (T4) غدت على عليقة من بذور الكتان _ منخفضة الطاقة باستخدام بذور الكتان 7.5 غم / كغم في البادئ والنامي 10 غم / كغم تحت الإجهاد الحراري. المعاملة الخامسة (T5) غدت على عليقة منخفضة الطاقة (KCL 200) تحت الإجهاد الحراري، كانت فترة التجربة خمسة أسابيع. اجريت هذه الدراسة في حقل دواجن من 27 كانون الأول 2023 لغاية 31 كانون الثاني 2024. تم جمع عينات الدم في نهاية التجربة (بعمر 35 يوم) من التجربة. لوحظ وجود فروق معنوية ($P \leq 0.05$) بين المعاملات في وزن الجسم الحي الأسبوعي (BW) والزيادة الوزنية (WG) والعلف المستهلك (FI) وكفاءة التحويل الغذائي (F.C.R). وقد أظهرت زيادة معنوية ($P \leq 0.05$) في الصفات الإنتاجية (وزن الجسم، والزيادة الوزنية) في مجموعتي (T3) و(T4) اللتين تغذتا على بذور الكتان. أما نسبة الدهون في الدم ونسبة تحويل الغذاء فقد انخفضت معنويًا ($P \leq 0.05$). كما تحسنت جودة اللحوم في (T3 وT4) بزيادة محتوى اللحوم من أحماض أوميغا 3 [حمض ألفا لينولينيك وحمض الإيكوسابنتاينويك وحمض الدوكوساهيكسانويك] معنويًا ($P \leq 0.05$) باستخدام تقنية كتلة الكروماتوجرافيا الغازية في مجموعتي (T3) و(T4) مقارنة بالمجموعة السيطرة التي تغذت على الذرة وفول الصويا. كما تحسنت نسبة الدهون في مجموعتي (T3) و(T4) مقارنة بالمجموعة السيطرة. وانخفضت نسبة الكوليسترول والدهون الثلاثية

والكوليسترول الضار والكوليسترول الضار جداً بشكل معنوي ($P \leq 0.05$) في مجموعتي (T3) و(T4) مقارنة بمجموعة السيطرة. ومع ذلك، فقد زاد HDL بشكل ملحوظ ($P \leq 0.05$). وانخفض نشاط انزيمات الكبد (AST) و (ALT) بشكل ملحوظ ($P \leq 0.05$) في (T3) و (T4) مقارنة بالمجموعة الضابطة. تحسنت الحالة المناعية ضد فيروس ND و IBD بشكل ملحوظ ($P \leq 0.05$) في المجموعتين (T3) و (T4) مقارنة مع مجموعة السيطرة. زادت المجموعتان (T3) و (T4) من التعبير الجيني IGF1 في الكبد مقارنة بمعاملة السيطرة ($P \leq 0.05$). تم الحصول على زيادات ملحوظة ($P \leq 0.05$) في ارتفاع الزغابات وعمق التكتل وعرض الزغابات ومساحة الزغابات في (T3) و (T4) مقارنة بالمجموعة الضابطة. من خلال هذه الدراسة نستطيع إضافة بذور الكتان الكاملة وبذور الكتان الى علف منخفض الطاقة بمعدل 7.5% من الكتان في البادئ والنامي 10% في علف دجاج اللحم أدى إلى تحسين أداء النمو، واللحوم الصحية المخصبة بأوميغا 3 (ALA، EPA، DHA) باستخدام تقنية كتلة GC. بالإضافة إلى ذلك، فإن تقليل استخدام الزيت والذرة في العليقة.

ان إضافة بذور الكتان الكاملة كمصدر لأحماض أوميغا 3 عزز أداء دجاج التسمين وأدى الى زيادة تركيز نسبة أوميغا 3- في اللحم. النظام الغذائي المكون من بذور الكتان الكاملة ادى إلى تعزيز نشاط التعبير الجيني للكبد IGF-1 المسؤول عن التمايز واستقلاب سلالات الخلايا العضلية من أنواع مختلفة. IGFs تنظم نمو الجسم والعضلات في دجاج التسمين. كما وان استخدام بذور الكتان الكاملة في نظام غذائي منخفض الطاقة عزز اداء التعبير الجيني لـ IGF-1 ومورفولوجيا الأمعاء ونشاط المخاط. ومع ذلك، فإن استخدام نظام غذائي منخفض الطاقة سيقبل من تكلفة العلف وهو مثالي في حالة الاجهاد الحراري. نتائجا تتفق مع توصية Aviagen، مواصفات التغذية، (2022).



جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة كربلاء / كلية الطب البيطري

فرع الصحة العامة البيطرية

تأثير احلال بذور الكتان الكاملة في النظام الغذائي منخفض الطاقة على أداء الدجاج اللحم والتعبير الجيني المعرض للإجهاد الحراري

الرسالة مقدمة الى

مجلس كلية الطب البيطري في جامعة كربلاء كجزء من متطلبات نيل درجة الماجستير في الطب البيطري / فرع الصحة العامة

بواسطة

حارث مضر جواد

بإشراف

أ.د. ياسر جمال جميل