



I See Inside semi quantitatively analysis of nutritional disturbances lesions in the liver and breast muscle of broiler during the starter period

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Abstract

Nutritional disorders are problems in the poultry industry. This experiment aimed to study the effect of high doses of soy oil and lysine (Lys) on the liver and Pectoralis muscle (PM) in broiler during the starter period and apply I See Inside (ISI) and semiquantitative methodology, determined the correlation between pathological lesions in both organs and regression assessment for the effects of myopathies on the liver. Forty broilers were divided into T1, receiving basal diet T2 and T3, high additive oil and lysine 4.56 and 1.66% respectively, and T4, which received both high additives; the trial continued for 12 days. The total ISI index for histopathological lesions in the liver and PM was 74 and 41, respectively, represented by vascular, degeneration alteration, and necrosis; ISI assessment revealed highly significant lesions in the liver of all groups in comparison with T1, significance endomias tissue formation in T4 at 4.00, splitting PM fiber in both T3 and T4 1.60, the intermittent muscle fiber of the broiler at same treatments was 1.00, furthermore semi-quantitative analysis investigated significant correlation between infiltration of inflammatory cells with highly significant congestion correlation with necrosis in the liver. In contrast, in the muscle, the necrosis is significantly correlated with hemorrhage in the liver; the regression plot analysis also determined the R2 value for microscopic lesions in the muscle, which affected hepatic tissue. This study concludes that ISI with semi-quantitatively analysis is a sound methodology for determining the significant deleterious effects of high doses of oil and lysine in broil ration.

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Introduction

Commercial broilers are among the animals that grow faster and have improved nutritional efficiency. However, even with these higher productivity thresholds, broiler production faces challenge (1). The balance of the poultry diet in terms of quantity and quality and the complement of its nutritional components such as protein, fats, carbohydrates, vitamins, minerals, and salts are essential in the growth and maintaining the immune and health status of the chicks (2). One or more of these dietary components lacking in rations or present in insufficient levels might cause low yield, disease, and even death (3,4). The nutrients for all

animals are classified into two classes: macronutrients, which are essential in large amounts, and their principal functions are to improve energy and body growth performance such as carbohydrates, protein, and lipids, while micronutrients are required in small quantities (milli to micro) grams (5) as well as the feed supplementation as Yeast, probiotic even the blue algae (6,7). The nutritional composition of the diet is recognized as the one environmental factor that primarily affects muscle growth and, consequently, meat quality. For instance, it has been shown that changes in the histology, initial energy, and metabolic properties of the muscle might affect meat quality due to dietary nutrient density (8). One way to modify the

growth trajectory without stimulating compensatory intakes is to vary the concentration of a single nutrient instead of the overall dietary density. Since Lysine (Lys) makes up the most significant amount of any critical amino acid (AA) in PM as a percentage of crude Protein, lysine is an ideal target for single nutrient deletion. The control of protein turnover at the cellular level in breast muscle demonstrates notable responsiveness to variations in dietary intake (9) in order to receive feedback at the processing facility (10). Moreover, feed-grade Lysine (Lys) is commonly in poultry diets, and as a result, it could be readily eliminated from one's diet; fats are essential because they are considered a concentrated source of energy more than proteins and carbohydrates; feed restriction programs have become more popular due to rising feed costs and their ability to decrease abdominal fat deposition and enhance feed efficiency. Despite the rapid growth rate of broiler chickens, it is accompanied by poor production efficiency, lameness, and ascites; therefore, limiting feeding was applied to reduce these health problems for birds (11,12). The liver is a primary organ for metabolic processes, which has vital physiological roles such as biosynthesis, lipid metabolism, detoxification, and host immunity (13). Liver injury is one of the primary diseases that occur as a result of many risk factors of xenobiotics (drug, heavy metals, etc.), chronic diseases, and malnutrition (14,15), Phillips *et al.* (16) suggesting the correlation between liver and muscular disorder.

Therefore, the significant goals of this study are to determine the statistical analysis ISI, histopathological alteration, and semi-quantitatively analysis in both muscle and liver in broiler fed with an unbalanced ratio during the starter period and determine the correlation between the architecture defects and study the hypothesis, which referred to that myopathies affected hepatic tissue.

Materials and methods

Ethical approve

All methods and procedures used in a recent study were completed according to the Scientific Ethical Committee on Animal Experimentation guidelines at the College of Veterinary Medicine, University of Mosul, UM.VET.2023.028.

Birds and house management

In this experiment, forty-one-day-old, unsexed broiler Ross 308 (17) was transported from a local hatchery to a pen 2x1.5 m² in the form of the house care animal unit, College of Veterinary Medicine- University of Mosul. The broiler house was kept disinfected BAYGENOX (BAYPS TECHNOLOGY - UK) at 4g/l and in suitable ventilation chambers. They were exposed to photoperiod at 23L:1D by using electric bulbs (200 watts) for the first five days; the temperature was set at 34°C and then gradually reduced to with progressing broiler age to keep comfort (18,19). Clean,

fresh wood shavings have been used as litter at deep four cm with a hanging feeder (1 kg capacity) and one nipple drinker water line for each pen. Moreover, the broilers were weighed immediately after they arrived on the farm and were vaccinated for Newcastle disease and Infectious bronchitis.

Dietary design

The basal ratio was formulated NRC (20), and birds were divided randomly into four groups. T1 chicks were fed on starter basal diet with Lys 1.25%, crude protein 22.31%, crude fat 2.433. T2 chicks were fed on a starter basal diet with a high percentage of oil 4.56%. T3 chicks were fed on a starter basal diet with a high percentage of Lys 1.66%. T4 chicks were fed on a starter basal diet with a high percentage of both oil and Lys 4.56 and 1.66%.

Sample collection

The specimen of liver and major pectoralis muscle was collected from the birds of each group at the end of the starter period (12 days) and kept in the 10% buffer formalin for at least 72 days, then continued kept in the ascending ethylene alcohol for dehydration then the samples should have kept them in the xylene for clearance and finally embedded in the wax for sectioning and staining with H&E (21).

I See Inside (ISI) technique

The ISI is a statistical method based on a numeric score of microscopic alteration. The term impact factors refer to macroscopic and histological alteration, and their value ranges from 1-3 depending on the extent of the damage and the organ's loss of its vital function, the severity of pathological lesions, and their frequency tissue compared with unaffected tissue is evaluated with the score as in table 1 (22,23).

Table 1: Score and frequency of histopathological alteration

Frequency of histopathological lesions (field)	Score
Absence of histological lesion	0
Histological lesion > 25%	1
Histological lesion 25 -50%	2
Histological lesion < 50%	3

The final rate of the ISI index for each liver and breast muscle is calculated according to the following formula: the impact factor of each histological lesion is multiplied by several scores (Table 2).

Statistical analysis

The experimental data for liver and muscle was analyzed by one-way ANOVA, and CRD design Duncken multiple range test (24) was utilized to determine differences in the lesions means ($P \leq 0.05$) and the Person correlation Coefficient used to detect the relation between liver and muscle (25).

Table 2: ISI histopathological lesions scores assessed in the liver and significant pectoralis muscle

Organs	Lesions	Impact factor		Score	Final score	Total ISI
Liver	Congestion	1	×	3	3	41
	Hemorrhage	3	×	2	6	
	Thrombus	3	×	3	9	
	Perivascular cuffing	2	×	2	4	
	Infiltration of inflammatory cells	2	×	2	4	
	Dilatation of sinusoid	2	×	1	2	
	Vacuolar degeneration	2	×	2	4	
	Necrosis	3	×	3	9	
Muscle	Congestion	1	×	2	2	74
	Hemorrhage	3	×	1	3	
	infiltration of inflammatory cells	2	×	2	4	
	Fibroblast proliferation	2	×	3	6	
	Muscle fiber intermittently	3	×	3	9	
	Muscle fiber atrophy	3	×	3	9	
	Splitting of muscle fiber	3	×	3	9	
	Loss straight	3	×	2	6	
	Endomysia tissue accumulation	3	×	3	9	
	Lipidosis	3	×	3	9	
Necrosis	3	×	3	9		

Results

Histological and ISI analysis of liver

The microscopic examination revealed pathological lesions presented by thrombus and perivascular cuffing in broilers, liver fed with high fat 4.56% in the ratio during the starter period, vacuolar degeneration and multifocal infiltration of inflammatory cells as in (Figure 1), the circulatory disturbances is more predominant histopathological lesions in the liver of broiler fed with diet lysin 1.66% it represented by perivascular cuffing and congestion with edema and dilatation of sinusoids. Furthermore, the histological examination revealed hyperplasia of the epithelial cells lining the bile duct and thickening of the wall of the central vein (Figure 2). Also, hepatic coagulative necrosis was observed in the broiler fed with both high fat and lysin 4.56 and 1.66% (Figure 3).

In this experiment, through the ISI method, it was detected that all treated groups presented significant histopathological lesions in all groups in contrast to control groups, except the hemorrhage, which declined significantly, and thrombus highly significantly occurrence in the liver of broiler in T2 in compared to histological results in T1, T3, and T4. Also, hepatic cell degeneration declined significantly in the T3 and T4 groups, and hepatic necrosis and tissue architecture declined significantly in the T3 groups (Table 3).

The histologic investigation detected zinckier necrosis as homogenous, eosinophilic amorphous fibers and loss of striation in the pectoralis muscle in broilers fed in ratio with high fat 4.56% (Figure 4). In contrast, in the microscopic examination of broilers, breast muscle fed with high Lys

1.66% in the ratio investigated as well as to the loss of striation, there was endomysia tissue formation, muscle fibers appear intermittently with myofiber necrosis, with hemorrhage, infiltration of inflammatory cells with muscle fibers (Figure 5). The lesions in the broilers, breast muscle fed with high fat and Lys 4.56 and 1.66% respectively in the ratio show atrophy degeneration muscle fiber, proliferation of fibroblast in the extracellular matrix with endomysia tissue formation with splitting of muscle fibers as well as there was accumulation of fibrous connective tissue between the heterogeneity in their diameters (skinny) and size of myofiber, edema and an arrangement muscle fiber with zinckier necrosis (Figure 6).

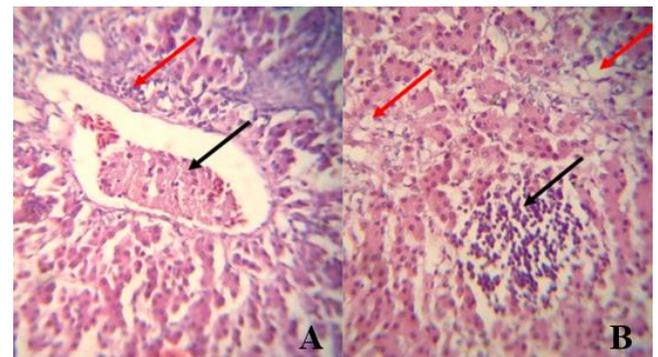


Figure 1: A- Photomicrography of broilers, liver with high fat 4.56% in the ration show recent thrombus black arrow and perivascular cuffing red arrow, B- vacuolar degeneration red arrow, focal infiltration of inflammatory cells black arrow H&E, 400X.

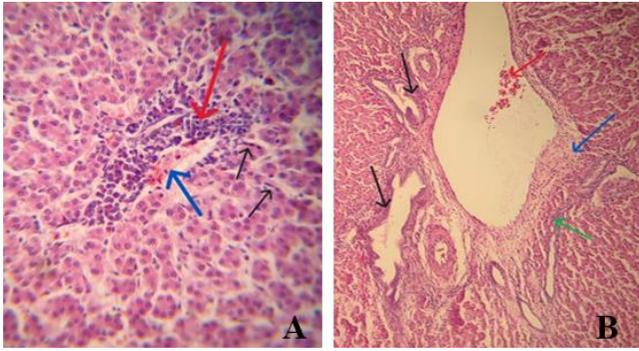


Figure 2: A- Photomicrography of broilers, liver with high Lys (1.66%) in the ration show perivascular cuffing red arrow, edema blue arrow and dilatation of sinusoids black arrow, B- congestion red arrow, thickening the wall of central vein blue arrow, hyperplasia of the epithelial cells lining bile duct black arrow and perivascular cuffing green arrow H&E, 400X.

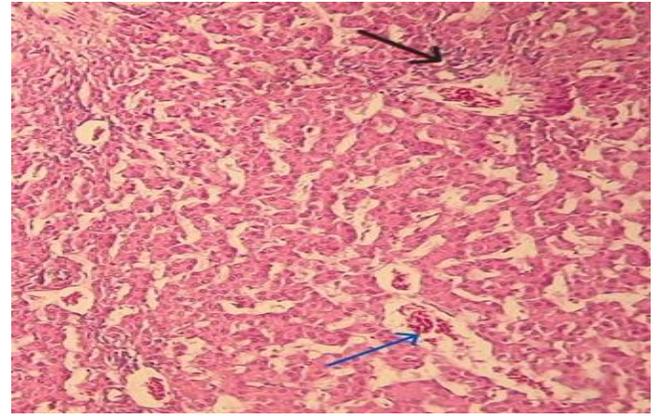


Figure 3: Photomicrography of broilers, liver with high fat and Lys 4.56 and 1.66% respectively in the ratio show congestion blue arrow, infiltration of inflammatory cells red arrow with coagulative necrosis, H&E, 100X.

Table 3: ISI analysis of histopathological lesions in the liver of broiler during the starter period

Lesions catalog	Mean±SD			
	Control	High oil ratio	High lysine ratio	High oil and lysin
Congestion	0.40±0.55 b	3.60±2.70 a	2.20±1.92 ab	4.20±1.64 a
Hemorrhage	0.20±0.45 b	0.60±0.55 b	2.60±1.67 a	2.60±1.52 a
Thrombus	0.00±0.00 b	4.60±2.88 a	1.00±1.22 b	0.20±0.45 b
Perivascular cuffing	0.20±0.45 a	1.40±1.52 a	2.60±2.70 a	1.00±0.82 a
Infiltration of inflammatory cells	0.40±0.55 b	2.20±2.49 ab	4.00±3.67 a	0.80±1.30 ab
Dilatation of Sinusoid	0.40±0.55 a	1.80±2.17 a	1.20±1.64 a	0.40±0.55 a
Degeneration	0.00±0.00 b	1.60±1.14 a	0.00±0.00 b	0.00±0.00 b
Necrosis	0.00±0.00 b	1.80±1.48 a	0.00±0.00 b	2.00±0.00 a

At the probability level ($P<0.05$), distinct letters signify the existence of noteworthy variations amongst the coefficients. Histological and ISI analysis of Pectoralis major muscle.

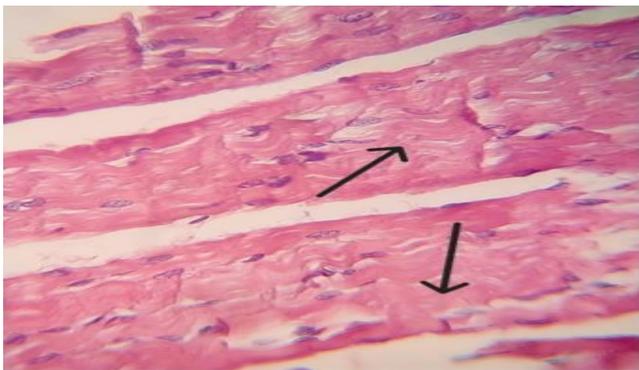


Figure 4: Photomicrography of broilers, breast muscle with high fat (4.56%) in the ration show zinckier necrosis, H&E, 400X.

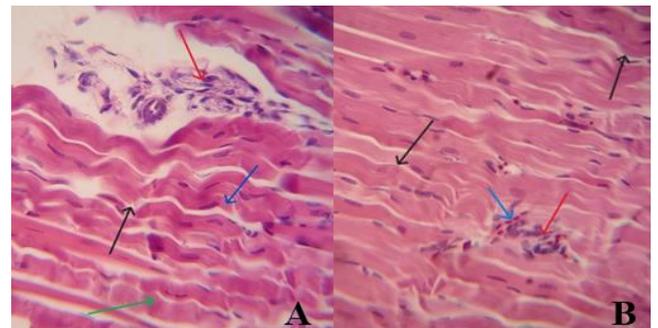


Figure 5: A- Photomicrography of broilers, breast muscle with high Lys (1.66%) in the ration show endomysia tissue red arrow, Muscle fibers appear intermittently blue arrow and loss straight black arrow with zinckier necrosis, B- hemorrhage blue arrow, infiltration of inflammatory cells red arrow with muscle fibers intermittently black arrow H&E, 400X.

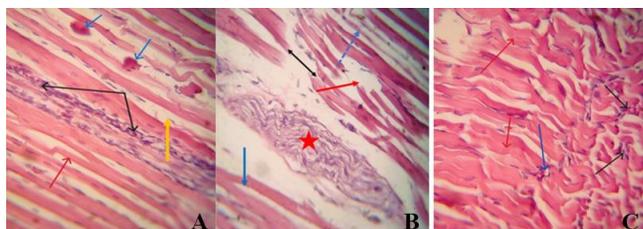


Figure 6: A- Photomicrography of broilers, breast broiler muscle with high fat and Lys (4.56 and 1.66)% respectively show atrophy degeneration muscle fiber in the ration of the blue arrow, proliferation of fibroblast black arrow and endomysia tissue yellow arrow with splitting of muscle fibers red arrow, B- in the ration show accumulation of fibrous connective tissue red star, small diameter of muscle fibers dotted-blue arrow, edema red arrow with splitting of muscle fibers blue arrow, C- un arrangement muscle fiber with zinckier necrosis red arrow, proliferation of fibroblast black arrow with hemorrhage blue arrow H&E, 400X.

By the ISI methodology, it was investigated that endomias and atrophy were highly significant in T4 and T3, respectively, in comparison to other groups. The mean atrophy between T4 and T2 did not differ significantly. The microscopic examination and ISI revealed that intermittently of muscle fibers and splitting of muscle fibers were highly significant in the broiler breast muscle in both groups T3 and T4, and dead tissue and necrosis were significantly increased in all groups for T2 1.80, T3 2.00, and T4 1.80 in contrast to control which was 0.00 (Table 4). In order to achieve the goal of this study and answer the question: Is there a correlation between pathological lesions in the liver and the broiler pectoralis muscle? A correlation test was conducted, and it was observed a significant correlation between congestion and infiltration of inflammatory cells and highly significant with necrosis in the liver of broiler, while in the muscle, the significant correlation investigated only in the necrosis and hemorrhage (Table 5).

Table 4: ISI analysis of histopathological lesions in the major pectoralis muscle of broiler during starter period

Lesions catalog	Mean±SD			
	Control	High oil ratio	High lysine ratio	High oil and lysin
Congestion	0.40±0.55 a	0.40±0.55 a	1.00±1.41 a	3.00±3.74 a
Hemorrhage	0.00±0.00 a	0.00±0.00 a	0.40±0.89 a	0.00±0.00 a
Thrombus	0.00±0.00 a	0.60±0.89 a	0.00±0.00 a	0.00±0.00 a
Perivascular cuffing	0.20±0.45 a	0.20±0.45 a	0.40±0.55 a	0.20±0.45 a
Infiltration of inflammatory cells	0.00±0.00 b	0.40±0.55 b	1.20±1.30 b	2.80±1.48 a
Dilatation of Sinusoid	0.00±0.00 a	0.60±0.55 a	0.40±0.55 a	0.20±0.45 a
Degeneration	0.00±0.00 c	2.00±0.71 b	1.60±0.89 b	4.00±1.58 a
Necrosis	0.00±0.00 c	0.60±0.55 ab	0.40±0.55 bc	1.00±0.00 a

At the probability level (P<0.05), distinct letters signify the existence of noteworthy variations among the coefficients.

Table 5: Correlation between histopathological lesions in broiler liver and major pectoralis muscle

Organs		Liver				Muscle			
		CON	HEM	INF	NEC	CON	HEM	INF	NEC
Liver	CON	1							
	HEM	0.059	1						
	INF	.445*	0.084	1					
	NEC	.709**	0.014	-0.020	1				
Muscle	CON	0.166	0.247	-0.136	0.269	1			
	HEM	-0.165	-0.075	-0.078	-0.188	-0.130	1		
	INF	0.104	-0.113	0.126	-0.075	0.109	-0.132	1	
	NEC	0.424	.493*	0.282	0.270	0.259	0.160	0.134	1

* Refer to significant correlation between traits at P<0.05. ** Refer to the high significant correlation between traits at P<0.01. Congestion (CON), Hemorrhage (HEM), infiltration of inflammatory cells (INF), Necrosis (NEC).

The regression plot for this study shows that the R² value between the congestion in both the breast muscle and the liver has reached 0.028, this means that the effect of congestion in the muscle can affect the liver by 2.8%, while the R² value of both hemorrhage and infiltration of

inflammatory cells in both organs was 0.006 and 0.016 respectively, this means this two pathological lesions in the breast muscle affected liver at 0.6 and 1.6%, necrosis is the other histopathological lesions has been investigated in the recent study, and it is R² value in muscle and liver was 0.073,

and the deleterious effects on muscle may affect the liver architecture at 7.3% (Figure 7).

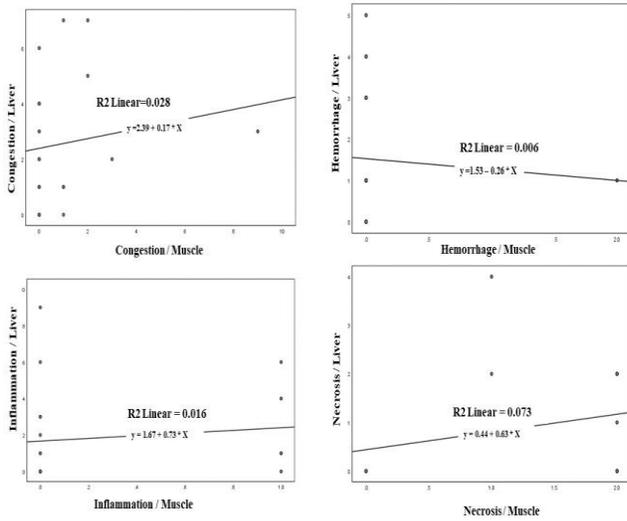


Figure 7: Regression chart of histopathological lesions in pectoralis muscle and liver.

Discussion

A metabolic disorder is one of the problems in poultry industries; it may result from an increase or inhibition of synthesis or the transport of a hormone or enzyme or may result from high nutritional intake or deficiency; this disorder mainly affects the cardiovascular and muscular skeletal systems (26). Plant Oils are considered a source of energy. It plays crucial roles in the absorption of minerals such as calcium and vitamins, mainly fat soluble, but the elevated concentration in the broiler ratio led to increased lipid peroxidation; aldehyde groups may react with amino groups of protein and reduce its activity and bio viability to amino acid (27,28). So, the increasing percentages of sunflower oil and lysin to 4.5 and 1.66%, respectively, in the experimental groups of this study cause deleterious effects in the liver architecture characterized by vascular disturbances, hepatic cell degeneration, and necrosis with dead tissue (29,30), also in the pectoralis muscle of broiler, which revealed significant pathological abnormalities of the breast muscle as circulatory disturbances, inflammatory infiltration, myodegeneration, split fibers, compromised fibrous connective tissue, endomysia tissue formation, and necrosis, whereas this result described abnormalities of breast muscle come with other results of previous studies (31-33).

Stringer *et al.* (34) reported that unsaturated oil mainly used as source of energy in diet of broiler and mainly the Sunflower oil and its rich in linoleic, linolenic and oleic acids which is mainly affected by lipid peroxidation and their byproduct as free radical which is the primary key for

functional anomalies, and pathologic alteration and cytotoxic in many organs and tissue, as well as although the lysin is one of the most limiting and essential amino acids that play a role in the formation of muscle fibers, an imbalance in adding it to the diet leads to muscle disorders, and this is what was recorded in histopathological examination in this stud, Jia *et al.* (35) reported that broiler exposed to overdose of lysin led to adverse effects on the tissue architecture as liver cause unbalance nucleic acid and protein metabolism and decline serum concentration of the valine, arginine, leucines and histidine which has essential roles in the cell and tissue physiological activity, the high dose of lysin cause depletion and atrophy of pectoralis muscle (36,37).

Changes in broilers' morphological and structural characteristics have been observed recently due to their high breast output. Increases in muscle fiber diameter have decreased the amount of space in the connective tissue in broilers chosen for high breast yield, which has affected muscle metabolism, decreased blood flow, and caused muscle lesions, such as myopathies (38). According to Kawasaki *et al.* (39), these pathological lesions may relate to an inadequate blood supply. In our study, circulatory disturbances have been significantly detected in the muscle of broiler in all treatments; this vascular alteration and accumulation of lactic acid, decline of space for connective tissue, and inhibition of the fiber regeneration by mediated satellite cells; all this pathway promote degenerative disorder and necrosis (38). According to Wang *et al.* (40), these abnormality indices are connected to the process of muscle development and expansion because they may restrict the circulatory system, making broilers more vulnerable to the oxidative stress linked to myopathy. Furthermore, this study's findings on necrotic fibers may indicate structural muscle fiber injury (41).

The ISI is a recent and significant methodology in histopathological practice (42). It investigates the ability of the tested high percentages of fat and lysin in the ratio of the broiler to affect variable histological areas of the liver and breast muscle. Therefore, it revealed that all histopathological lesions in the muscle are significant (43). The result of the recent experiment is one of the first previous studies to statistically analyze the deleterious effect of an unbalanced ratio on the histological architecture of the liver and muscle while investigating the extent of the correlation between pathological lesions. Furthermore, the result was determined through a regression chart analysis that metabolic disorders that lead to myopathies had an effect, according to R² values, on the occurrence of histological lesions in the liver.

Conclusion

It is concluded from this study that disturbance and imbalance in broiler diets, such as an increase in energy sources and amino acids, leads to the occurrence of

pathological changes such as myopathy and pathological lesions in the liver. Applying the ISI and semi-quantitative methodology for histopathological changes is one of the modern and innovative methods for determining the significance and correlation of histopathological criteria. In addition to finding a, it was concluded through regression analysis that myopathy is one of the systemic diseases that has an impact on the occurrence of histopathological changes in the liver.

Acknowledgments

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Conflict of interest

There was no conflict of interest.

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أرى داخل التحليل شبه الكمي لآفات الاضطرابات الغذائية في الكبد وعضلات الصدر في الفروج اللاحم خلال فترة البادئ

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الخلاصة

تعتبر الاضطرابات الغذائية من المشاكل التي تواجه صناعة الدواجن. هدفت هذه الدراسة إلى البحث في تأثير الجرعة العالية من زيت فول الصويا واللايسين على الكبد وعضلة الصدر في الفروج اللاحم خلال فترة البادئ وتطبيق منهجية أرى داخل التحليل شبه الكمي مع تحديد الارتباط بين الآفات المرضية في كلا العضوين وتقييم الانحدار لتأثير الاعتلال العضلي على الكبد. تم تقسيم أربعين فرخ إلى أربع مجاميع حصلوا على العليقة الأساسية المعاملة الأولى، وفي المعاملة الثانية والمعاملة الثالثة حصلوا على نسبة عالية من الزيت واللايسين ٤,٥٦ و ١,٦٦% على التوالي وفي المعاملة الرابعة حصلوا على كلتا النسبتين العاليتين استمرت التجربة لمدة ١٢ يوم. بينت النتائج أن مؤشر أرى بالداخل للآفات المرضية في الكبد والعضلة الصدرية كان ٤١ و ٧٤ على التوالي وتمثلت بالتغيرات الوعائية والتنكس والنخر في كلا العضوين، أظهرت نتائج أرى بالداخل فروق معنوية للآفات المرضية في الكبد ولكل المعاملات مقارنة مع المجموعة الأولى، فضلاً عن تكون النسيج الاندومياسي في المعاملة الرابعة ٤,٠٠، وانشطار الألياف العضلية في كلا من المجموعة الثالثة والرابعة والذي كان ١,٦٠ وعدم استمرارية الألياف العضلية في فروج نفس المعاملتين ١,٠٠ إضافة إلى التحليل شبه الكمي الذي أظهر علاقة الارتباط بين الخلايا الالتهابية والاحتقان والذي كان عالية المعنوية مع نخر الكبد، أما في العضلات فقد كان النخر معنوي الارتباط مع نرف الكبد وكذلك حدد تحليل الانحدار قيمة مربع التغيير للتغيرات المجهرية في العضلة والتي أثرت على نسيج الكبد. يستنتج من هذه الدراسة أن منهجية أرى داخل التحليل شبه الكمي جيدة في تحديد معنوية التأثير الضار للجرع المرتفعة من الزيت واللايسين في علائق الفروج اللاحم.