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المجلة العراقية لبحوث السوق
وحماية المستهلك

USE OF SHRIMP WASTE POWDER (*METAPENAEUS AFFINIS*) AND PROTEASE ENZYME IN BROILERS FEED AND ITS EFFECT ON PHYSIOLOGICAL AND MICROBIAL CHARACTERISTICS AND INDICATORS OF FAT OXIDATION IN MEAT

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ABSTRACT

This study was conducted in the poultry field of the Department of Animal Production/ College of Agricultural Engineering Sciences / University of Baghdad for the period from 42 days. Aiming to know the effect of using shrimp waste powder (Metapenaeus Affinis) and enzyme in broilers diet on physiological and microbial performance and indicators of fat oxidation in meat. 250 one-day-old ROSS308 chicks were used. The chicks were fed on diets containing shrimp waste treated with enzyme and not treated with protease enzyme by 0,4,6 %. The experiment included five treatments, with 5 replicates for each treatment, and each replicate contained 10 birds. The results showed a significant decrease (P≤0.05) in the concentration of ALT and AST for all treatments of the experiment compared to the control treatment, and a significant increase (P≤0.05) in the concentration of calcium element for the T5 treatment compared to all treatments and the control treatment. In addition, there was a significant increase (P≤0.05) in the concentration of phosphorous element for treatment T2 compared to the rest of the treatments. The results showed that there was a high significant decrease (P≤0.01) in the indicators of fat oxidation, MDA, PV and FFA, in the treatments of the experiment compared to the control treatment for the two periods of 1 day and 30 days of storage. Both T2 and T3 recorded a significant increase (P≤0.05) for the logarithmic number of Lactobacillus, and a significant decrease (P≤0.05) for the logarithmic number of coliform bacteria (E.coil) in treatment T2. We conclude from this study the possibility of using shrimp waste in broilers diets at rates of 4 and 6% to obtain a positive improvement in the physiological and microbial characteristics and indicators of fat oxidation in the meat and to increase the storage period of chicken meat for 30 days without deteriorating its quality.

Key words: Broilers, Shrimp waste, Physiological characteristics, Microbial characteristics, Indicators of oxidation in meat.

[*] **The article is taken from the master's thesis of the first researcher .**

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استعمال مسحوق مخلفات الروبيان *Metapenaeus affinis* وانزيم االبروتياز في عليفة فروج اللحم وتأثيره في الصفات الفسلجية والميكروبية و مؤشرات اكسدة الدهن في اللحم

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الخلاصة

اجريت هذه الدراسة في حقل الدواجن التابع لقسم الانتاج الحيواني/ كلية علوم الهندسة الزراعية/ جامعة بغداد لمدة 42 يوماً. بهدف معرفة تأثير استعمال مسحوق مخلفات الروبيان والانزيم في عليقة فروج اللحم في الاداءالفسلجي و الميكروبي و مؤشرات اكسدة الدهن في اللحم. استخدم 250 فرخ من نوع ROSS308 بعمر يوم واحد وغذيت الافراخ على علائق استعمل فيها مخلفات الروبيان المعاملة بالانزيم وغير معاملة بانزيم البروتييز وبنسب 0,4,6 %. وتضمنت التجربة خمس معاملات وبواقع 5 مكررات لكل معاملة وكل مكرر احتوى على 10طيور. واظهرت النتائج حصول انخفاض معنوي ($P\leq0.05$) في تركيز كلاً من ALT و AST لجميع المعاملات التجريبية مقارنة بمعاملة السيطرة و حصول ارتفاع معنوي ($P\leq0.05$) في تركيزعنصر الكالسيوم للمعاملة T5 مقارنة بجميع المعاملات ومعاملة السيطرة. فضلاً عن ذلك حصل ارتفاع معنوي ($P\leq0.05$) في تركيز عنصر الفسفور للمعاملة T2 مقارنة بباقي المعاملات. واظهرت النتائج حصول انخفاض معنوي عالي ($P\leq0.01$) في مؤشرات اكسدة الدهون MDA و PV و FFA معاملات التجريبية مقارنة بمعاملة السيطرة للمدتين 1 يوم و 30 يوم من الخزن. سجلت كلاً من T2و T3 ارتفاعاً معنوياً ($P\leq0.05$) للاعداد اللوغارتمية لبكتريا حامض اللينيك وانخفاضاً معنوياً ($P\leq0.05$) للاعداد اللوغارتمية لبكتريا العصيات القولونية (*E. coil*) في المعاملة T2 . نستنتج من هذه الدراسة امكانية استعمال مخلفات الروبيان *Metapenaeus affinis* في علائق فروج اللحموبالنسب4 و 6% للحصول على تحسن ايجابي في الصفات الفسلجية والميكروبية ومؤشرات اكسدة الدهون في اللحم وزيادة فترة خزن لحوم الدجاج لمدة 30 يوماً من دون تدهور نوعيتها.

الكلمات المفتاحية: فروج اللحم، مخلفات الروبيان، الصفات الفسلجية، الصفات الميكروبية، مؤشرات اكسدة في اللحم..

INTRODUCTION

Poultry meat is one of the most nutritious sources of animal protein **(Schiavone *et al.*, 2019)**. Researchers have made efforts to contribute to its development by increasing productivity and improving physiological condition**.** And since broiler chickens consume fodder to supply their energy and protein needs **(Qassim *et al.*, 2022)**, therefore, it is necessary to provide high-quality fodder to obtain the best productive and physiological performance of broilers chickens. This is done by improving or raising the quality of protein by using valuable feed alternatives of protein and high biological value **(Schiavone *et al.*, 2019; Qassim & Bandar, 2019; Awad** & **Zanganeh, 2020)**, including shrimp waste in diets, as it is a good protein source **(Yin *et al.*, 2019)**. In terms of nutritions, shrimp waste has high protein because it is easily digestible (85% digestibility). Shrimp waste is characterized by its containment of minerals such as copper, magnesium, calcium, zinc and phosphorus **(Singh *et al.*, 2018)** and vitamins such as D, A, E, B6 & B12 **(Ali *et al.*, 2015)** It is considered one of the natural sources of effective carotenoids that have biodegradability and have distinctive antioxidant and anti-inflammatory characteristics and are used in the medical and immunological fields **(Al-Kanaan &Al-Ali, 2017)**. In order to improve the nutritional value of shrimp waste and fully benefit to the birds, some enzymes may be added to the broilers diet. (**Alrubaee *et al.*, 2017; Al-Saidi & Al-Asadi, 2018; Hussain *et al.*, 2019)**.

The aim of this study is to use shrimp waste in the broilers diet in different proportions with and without the addition of the protease enzyme, it is to use shrimp waste in the diet in addition to other components and its containing of protein and active substances an effect on

the physiological performance and health status of the bird by measuring some physiological characteristics and the number of bacteria in intestines and its effect as an antioxidant by storing broilers' meat for two periods (1 day &30 days) and measuring indicators of fat oxidation in the meat.

MATERIALS AND METHODS

This experiment was conducted in the poultry field of the Department of Animal Production/ College of Agricultural Engineering Sciences/ University of Baghdad (Abu Ghraib) during the period from 42 days old 250 Ross 308 chicks were used in the experiment, with an average weight of 41.8 grams. The chicks were housed in a closed form consisting of 25 pen. The chicks were distributed randomly on the pen, at the rate of 10 birds per pen. The birds were fed on a three-stage diet, according to table (1, 2, 3). Local shrimp waste purchased from local markets in Basra Governorate, dried for 3 days under the sun, sterilized in an autoclave, and ground into powder. was used and according to the chemical analysis table for it, Table (4), by 0 %, 4%, 6% of the diet for each of the treatments T1, T2, T3, and shrimp waste was also used by 4.6%, With the addition of 0.05% of pure protease enzyme (EC 3.4.21.) purchased from Amman/Jordan, according to the recommendations of the elected company for each of the treatments T4, T5.Blood samples were collected at the end of the experiment at the age of 42 days, by taking 5 random birds from each treatment, and collecting blood samples through the pterygoid vein and placing them in test tubes containing an anticoagulant (EDTA). The concentration of calcium and phosphorus were measured according to the method of **Yonng *et al.* (1975)** using a kit produced by the Italian company Giesse Diagnostic. At the age of 42 days after the birds were slaughtered and dissected, the small intestine was extracted and its contents were collected from the jejunum in a test tube 10 mm/ 1 gram of the contents of the small intestine for two of each replicate, and after the microbial atoms were made in the dishes in order to estimate the logarithmic numbers of bacterial colonies for both linic acid bacteria and bacillus coli according to the method of **Harrigan & Mccance (1976).** In addition, meat samples were collected after slaughtering the birds at the age of 42 days, by taking 2 random birds from each replicate, and they were stored at a temperature of -20 °C for two periods, 1 day and 30 days, with an estimated amount of malonaldehyde according to the method of **Witte *et al.* (1970)** and the value of peroxide and free fatty acids according to the method of **Pearson & Dustson (1985).** The data of the experiment were analyzed using a completely randomized design (CRD) using the ready-made statistical program **(SPSS, 2017),** and significant differences between the averages were compared using Duncan's polynomial test **(Duncan, 1955)**.

Table (1): The percentages of the components of the diets used in the study and their chemical composition in the starter diets.

Ingredient (%)	Treatment		
	Control	T_2+T_4	T_3+T_5
Yellow Corn	34	34	34
Wheat	24	21	20
Soybean meal [1]	31.5	29.6	28.6
Protein concentrate [2]	5	5	5
Sunflower oil	3	4	4
Dicalcium phosphate (D.C.P)	0.8	0.7	0.7
Salt	0.1	0.1	0.1
Lime stone	1.2	1.2	1.2
Methionine	0.25	0.25	0.25
Lysine	0.15	0.15	0.15
[3] shrimp waste powder	-	4	6
Total	100	100	100
chemical composition [4]			
M.E(kcal/kg)	3031.75	3047.87	3025.31
C.P %	23.01	22.76	22.67
C.F %	2.8	3.2	3.4
Lysine %	1.4	1.34	1.31
Meth +Cys %	1.11	1.08	1.06
Ca %	1	1.15	1.23
Av. P	0.51	0.54	0.57

1. The used soybean meal is from an Argentine source, the percentage of crude protein in it is 48%, and 2440 kilocalories / kg a metabolized energy.
2. The protein concentrate used is animal, produced by a Dutch company Brocon (imported). It contains 40% crude protein, 2107 kilocalories / kg protein a metabolized energy, 5% crude fat, 2.20% crude fiber, 5% calcium, 2.65% phosphorus, 3.85% lysine, 3.70% methionine, 4.12% methionine + cysteine, 0.42% tryptophan, 1.70% threonine.
3. Shrimp waste powder, a metabolized energy 1652 kilocalories / kg, crude protein 25.9%, ether extract 2.4%, crude fiber 13.2%, methionine + cysteine 0.0408%, lysine 0.025%.form the local markets in basra governorate.
4. According to the chemical composition of the diets based on (NRC 1994)
5. T1 control diet did not use shrimp waste powder, T2, T4 used shrimp waste powder by 4 and 6%, T3 and T5 used shrimp waste powder by 4 and 6% with 0.05% protease enzyme.
6. protease enzyme (EC 3.4.21.-).

Table (2): The percentages of the components of the diets used in the study and their chemical composition in the growth diets.

Ingredient (%)	Treatment		
	Control	**T_2+T_4**	**T_3+T_5**
Yellow Corn	39.5	38	39
Wheat	22	21	17.74
Soybean meal [1]	28	25.5	26
Protein concentrate [2]	5	5	5
Sunflower oil	3.5	4.5	4.5
Dicalcium phosphate (D.C.P)	0.5	0.5	0.4
Salt	0.1	0.1	0.1
Lime stone	1.14	1.14	1
Methionine	0.13	0.13	0.13
Lysine	0.13	0.13	0.13
shrimp waste powder [3]	-	4	6
Total	100	100	100
chemical composition [4]			
M.E(kcal/kg)	3113.20	3126.83	3103.86
C.P %	21.55	21.13	21.57
C.F %	2.7	3.1	3.3
Lysine %	1.29	1.21	1.22
Meth +Cys %	0.96	0.96	0.91
Ca %	0.90	1.07	1.08
Av. P	0.45	0.50	0.51

1. The used soybean meal is from an Argentine source, the percentage of crude protein in it is 48%, and 2440 kilocalories / kg a metabolized energy.
2. The protein concentrate used is animal, produced by a Dutch company Brocon (imported). It contains 40% crude protein, 2107 kilocalories / kg protein a metabolized energy, 5% crude fat, 2.20% crude fiber, 5% calcium, 2.65% phosphorus, 3.85% lysine, 3.70% methionine, 4.12% methionine + cysteine, 0.42% tryptophan, 1.70% threonine.
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4. According to the chemical composition of the diets based on (NRC 1994)
5. T1 control diet did not use shrimp waste powder, T2, T4 used shrimp waste powder by 4 and 6%, T3 and T5 used shrimp waste powder by 4 and 6% with 0.05% protease enzyme.
6. protease enzyme (EC 3.4.21.-).

Table (3): The percentages of the components of the diets used in the study and their chemical composition in the final diets.

Ingredient (%)	Treatment		
	Control	T2+T4	T3+T5
Yellow Corn	40.5	48	52
Wheat	25	13.6	10
Soybean meal [1]	23.04	22.59	20.24
Protein concentrate [2]	5	5	5
Sunflower oil	4.5	5	5
Dicalcium phosphate (D.C.P)	0.5	0.4	0.4
Salt	0.1	0.1	0.1
Lime stone	1.1	1.05	1
Methionine	0.13	0.13	0.13
Lysine	0.13	0.13	0.13
shrimp waste powder [3]	-	4	6
Total	100		100
chemical composition [4]			
M.E(kcal/kg)	3209.28	3204.95	3202.33
C.P %	19.63	19.66	18.94
C.F %	2.7	3	3.1
Lysine %	1.16	1.13	1.06
Meth +Cys %	0.90	0.88	0.85
Ca %	0.87	1	1.06
Av. P	0.44	0.48	0.50

1. The used soybean meal is from an Argentine source, the percentage of crude protein in it is 48%, and 2440 kilocalories / kg a metabolized energy.
2. The protein concentrate used is animal, produced by a Dutch company Brocon (imported). It contains 40% crude protein, 2107 kilocalories / kg protein a metabolized energy, 5% crude fat, 2.20% crude fiber, 5% calcium, 2.65% phosphorus, 3.85% lysine, 3.70% methionine, 4.12% methionine + cysteine, 0.42% tryptophan, 1.70% threonine.
3. Shrimp waste powder, a metabolized energy 1652 kilocalories / kg, crude protein 25.9%, ether extract 2.4%, crude fiber 13.2%, methionine + cysteine 0.0408%, lysine 0.025%. form the local markets in basra governorate.
4. According to the chemical composition of the diets based on (NRC 1994)
5. T1 control diet did not use shrimp waste powder, T2, T4 used shrimp waste powder by 4 and 6%, T3 and T5 used shrimp waste powder by 4 and 6% with 0.05% protease enzyme.
6. protease enzyme (EC 3.4.21.-).

Table (4): Chemical composition of shrimp waste.

Chemical composition of shrimp waste							
E.E	C.F	Ash	Mois	CHO	M.E	B-carotein	Astaxanthen
(%)	(%)	(%)	(%)	(%)	Kcal/kg	µg/ mg	µg/ mg
2.4	13.2	15.8	32.5	10.1	1652	46.98	42.11

C.P: Crud Protein
E.E: either Extract
C.F: Crude Fibre
Mois: moisture
CHO: carbohydrates
M.E: metabolizable energy

RESULTS AND DISCUSSIONS

Table (5) shows the effect of using shrimp waste powder in different proportions with and without protease enzyme in broiler diets on the levels of AST and ALT enzymes, calcium (Ca) and phosphorous (P). We note that there was a significant increase ($P\leq0.01$) for the AST enzyme in T2 compared to T4 and there was no significant difference in T2 and T4 treatment compared with control treatment, T3 and T5 treatment, and in ALT enzyme there was a high significant decrease ($P\leq0.01$) in T3 treatment compared with control treatment, and there was no significant difference in control treatment, T2 and T3 treatment Compared with T4 and T5 treatment.

We note that there is a significant difference ($P\leq0.05$) calcium Ca in treatment T5 compared with the rest of the treatments of the experiment, and a significant increase ($P\leq0.05$) in treatment T2, T3 and T4 compared with the control treatment. And there was a significant increase ($P\leq0.05$) in T2 compared with the rest of the treatments phosphorous P, and a significant increase ($P\leq0.05$) in the treatment T3 compared with the control treatment, T4 and T5, and a significant increase ($P\leq0.05$) in the treatment T4 compared with the control treatment and T5 treatment was not significantly different from the control treatment.

Table (5): Effect of using shrimp waste in broiler diets on liver enzymes, calcium and phosphorus concentration**.**

Traits	Treatment					Sg
	T1	T2	T3	T4	T5	
AST	104.00±2.05ab	119.00±2.17a	93.00±2.55ab	88.40±2.64b	92.60±1.33ab	**P≤0.01**
ALT	22.00±2.43a	21.20±1.02a	13.80±1.16b	18.40±.51ab	18.00±.55ab	**P≤0.01**
Ca	9.10±0.28c	13.80±2.03b	16.14±1.31b	14.48±1.36b	24.42±1.53a	**P≤0.05**
P	3.57±0.44d	8.13±0.38a	6.84±0.28b	5.38±0.40c	4.76±0.50cd	**P≤0.05**

- The different letters between the averages in one line indicate that there are significant differences at the level of P≤0.01. There are no significant differences in AST and ALT. The different letters between the averages in one line indicate that there are significant differences at the level of P≤0.05. There are no significant differences in Ca and P.T1 control diet did not use shrimp waste powder, T2 and T4 used shrimp waste powder by 4 and 6%, T3 and T5 used shrimp waste powder by 4 and 6% with 0.05% protease enzyme.

Chart (1) shows a significant increase (P≤0.05) in the logarithmic numbers of Lactobacillus in treatments T3 and T4 compared to the control treatment and treatments T4 and T5, in addition to that, treatments T3 and T4 recorded increases (P≤0.05) in the same number of bacteria compared with the control treatment.

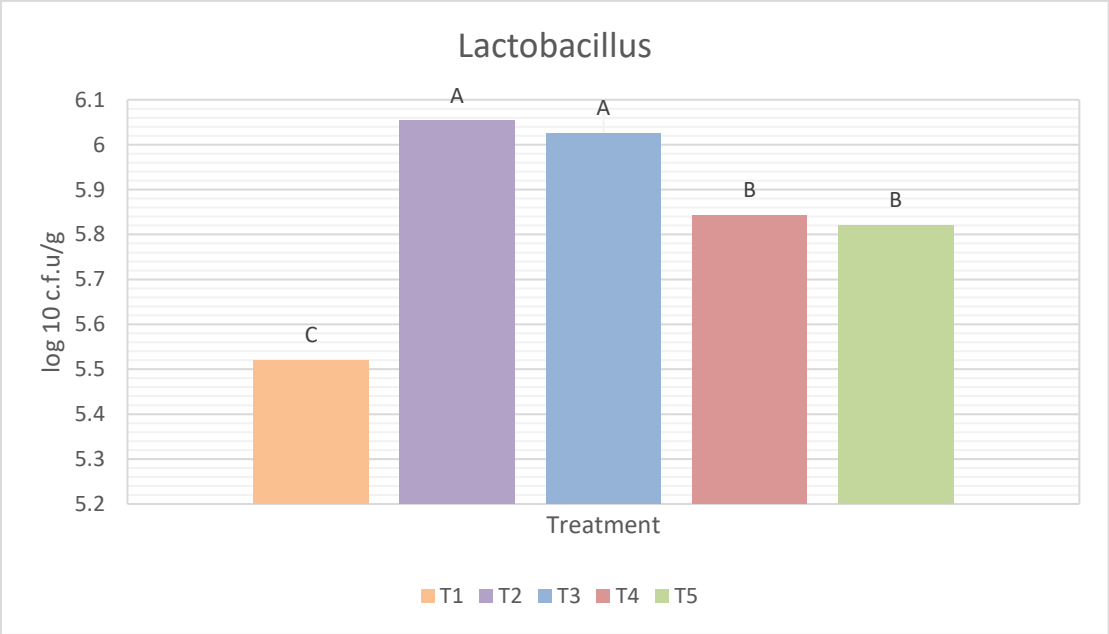

Chart (1): The effect of using shrimp waste in broilers diets on the logarithmic numbers of Lactobacillus bacteria.

Chart (2) shows a significant decrease (P≤0.05) in the logarithmic number of bacteria (E. coli) for all treatments compared to the control treatment. In addition, treatment T2 recorded a decrease (P≤0.05) in the number of E. coli compared to treatment T3, T4 and T5.

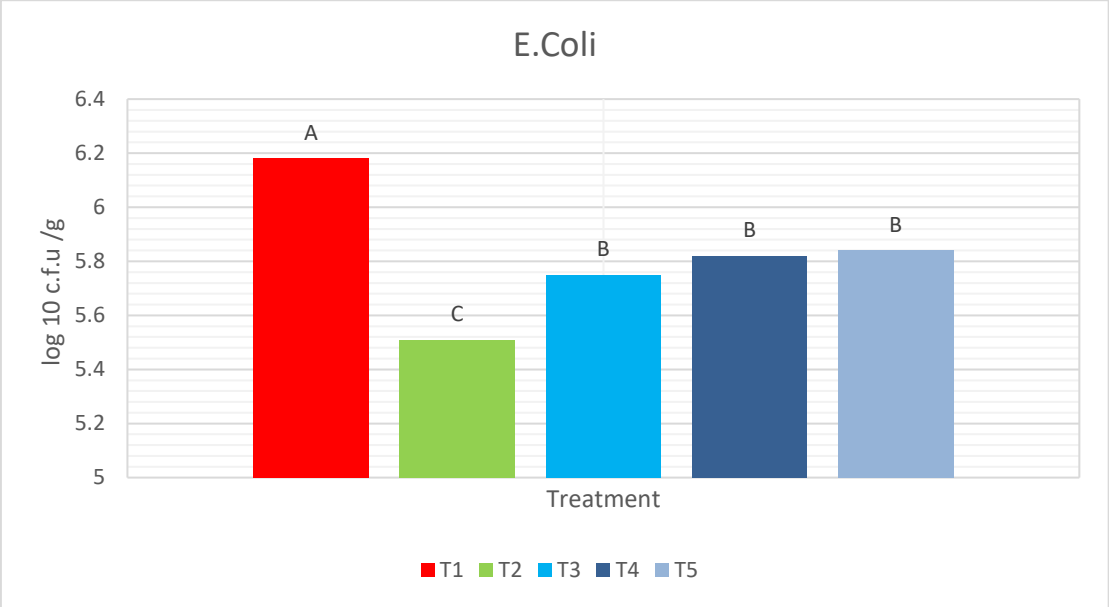

Chart (2): The effect of using shrimp waste in broilers diets on logarithmic numbers of E. coli bacteria.

Table (6) showed a high significant decrease (P≤0.01) in the value of peroxide (PV) and malondialdehyde (MDA) for all treatments compared to the control treatment in the first period of storage 1 day. The treatment T5 and T3 recorded a high significant decrease (P≤0.01) in the value of PV and MDA compared to treatment T2 and it did not differ significantly (P≤0.01) from T4 and did not differ significantly (P≤0.01) from T2 for the first period of storage 1 day. Table (6) showed a significant decrease (P≤0.01) in the concentration of free fatty acids FFA of 1 day storage for all treatments of the experiment compared to the control treatment, as the T5 treatment recorded a high significant decrease (P≤0.01) in the concentration of FFA compared to the T2 treatments and did not differ significantly with T3 and T4, these two treatments did not differ significantly from T2 after 1 day of storage. The results showed in Table (6) that there was a high significant (P≤0.01) decrease in the value of PV in all treatments of the study compared to the control treatment at the end of the second period of storage (30 days), and in the same period a high significant decrease (P≤0.01) was observed in the value of peroxide for T3, T4 and T5 treatment compared to T2 treatment. All treatments in the second storage period recorded a high significant decrease (P≤0.01) in the concentration of MDA compared to the control treatment, as T5 recorded a lower value of MDA and decreased significantly (P≤0.01) than T2, but it did not differ significantly from T3 and T4, and T2 did not differ significantly (P≤0.01) from T3 and T4, and the results showed a high significant decrease (P≤0.01) in the concentration of FFA for the second period of storage in all treatments compared to the control treatment, as T2 recorded the lowest value of FFA compared to T2 and did not differ

significantly (P≤0.01) from T4 And T5 and these two treatments did not differ significantly (P≤0.01) from T2 at the end of the 30 days period.

Table (6): The effect of using shrimp waste in broilers diets on indicators of fat oxidation in meat.

Traits	Treatment					Sg
	T1	T2	T3	T4	T5	
storage period 1 day						
PV	3.075±0.014a	2.620±0.023b	2.200±0.012c	2.285±0.009bc	2.123±0.007c	**P≤0.01**
MDA	0.043±0.001a	0.029±0.001b	0.023±0.001c	0.027±0.001b	0.021±0.001c	**P≤0.01**
FFA	0.530±0.006a	0.430±0.006b	0.305±0.003bc	0.345±0.003bc	0.250±0.006c	**P≤0.01**
storage period 30 day						
PV	5.622±0.306a	3.915±0.014b	2.823±0.015c	3.033±0.028c	2.470±0.017c	**P≤0.01**
MDA	0.075±0.002a	0.043±0.001b	0.030±0.001bc	0.036±0.001bc	0.027±0.001c	**P≤0.01**
FFA	0.733±0.012a	0.523±0.015b	0.333±0.009c	0.413±0.012bc	0.407±0.007bc	**P≤0.01**

- The different letters between the averages in one line indicate that there are significant differences at the level of P≤0.01, NS there are significant differences. T1 control diet did not use shrimp waste powder, T2 and T4 used shrimp waste powder by 4 and 6%, T3 and T5 used shrimp waste powder by 4 and 6% with 0.05% protease enzyme.

The clear improvement in the levels of liver enzymes (AST and ALT) may be due to the fact that shrimp waste contains many biologically active and functional substances such as chitosan, which works to improve the physiological state of the liver, as this is reflected in the decrease in the concentration of enzymes (AST and ALT) in the treatments that contain shrimp waste or no significant changes in some treatments **(AbedAllaw *et al.*, 2016)**. Also, shrimp waste contains both astaxanthin and chitosan pigments, which play an important role in influencing the liver's production of antioxidant enzymes such as glutathione peroxidase and catalase, restraining and preventing the formation of free radicals **(Ulaiwi & Al-Khafaji, 2020; Awadh & Zangana, 2021)**. Or the effect of astaxanthin pigment on reducing free radicals by binding to improve the state of fat oxidation in meat, evident in the decrease in the concentrations of the oxidation indicators MDA, PV and FFA in the treatments in which shrimp waste was used in it and with an increase in the use rate **(Yamashita, 2015; Abed Allaw et al., 2016; Awad, 2020)**. It was observed through the results that there was a significant decrease in the numbers of E.coli bacteria in the treatments in which shrimp waste was used in it because it contains many active substances, including chitosan, which acts as an antibacterial through its association with the main phosphate group in the microbial cell membrane, which leads to an increase in the permeability of the cell membrane of the microbial cell **(Zheng & Zhu, 2003; Elserafy *et al.*, 2021)**, and the creation of appropriate conditions to improve the histological composition of the intestine and increase the absorption of nutrients, which reflects positively on the health of the bird and its physiological state **(Chen *et al.*, 2000).**

CONCLUSIONS

1. Modification in the levels of liver enzymes (AST and ALT) with an increase in the levels of shrimp waste as it is considered an antioxidant, 6% of shrimp waste with protease enzyme is the best percentage of calcium, and 4% of shrimp waste is the best percentage of phosphorus in broilers at the age of 42 days.
2. Using 4% shrimp waste in broiler diets increases the beneficial bacteria Lactobacillus and reduces the harmful bacteria E.coil in the jejunum, thus affecting production performance.
3. Increasing levels of shrimp waste in diets has a positive effect on food oxidation indicators in stores for 30 days.

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