Effect of Adding Different Levels of Dried Yellow Mealworm as a Protein Supplement to Broiler Diets on Some Microbial and Histological Characteristics of the Small Intestine

Hadeel Nawfal Majeed AL-Saadi1*, Ammar Talib Dhiab AL-Tememy2
1student at Diyala University - College of Agriculture – Department of Animal Production 2prof.
Dept. Animal production – Agriculture of college-University of Diyala – Iraq
*corresponding author's email: hadeelnawfal@gmail.com
Email addresses of coauthors: Ammaraltememy@uodiyala.edu.iq

Abstract

This study was conducted at the poultry farm Department of Animal Production - College of Agriculture – University of Diyala for the period from 24/9/2024 until 28/10/2024 with the target of finding out the effect of adding the dried yellow mealworm to broiler diets (Ross308) on some microbial and histological characteristics of the intestine. 225 unsexed one day broiler chick (Ross308) with an average weight 38 g were used , randomly distributed into five treatments by three replicates for each treatment (15 bird/replicate). The treatments were as follows : T1= (control) standard diet without any additives, T2= 15 g/kg of dried yellow mealworm added to the standard diet , T3= 30 g/kg of dried yellow mealworm added to the standard diet, T4= 45 g/kg of dried yellow mealworm added to the standard diet, T5= 60 g/kg of dried yellow mealworm added to the standard diet, The results showed that significant superiority ($P \leq 0.05$) in the microbial balance and the environment of the small intestine which decrease the numbers of colonic bacteria significantly, and the total bacteria and lactic acid bacteria were increased in the broiler that has been fed with dried yellow mealworms compared to control diet. Whoever, the histological characteristics observed big superiority ($P \leq 0.05$) in the treatments contained dried yellow mealworms compared to the control treatment in the length and thickness of villi and the depth of crypts. Therefore, it concluded that adding dried yellow mealworms has improved the number of the good bacteria and the histological characteristics in the jejunum of the small intestine.

Keywords: Yellow mealworm, Protein supplements, Broiler, Microbial and histological characteristics.

Introduction

Last years the poultry industry has witnessed rapid and considerable development, it became dependent on modern scientific basis, due to the significant advancement in all sides of animal production, and the value of poultry is highlighted as a rich source of a high quality nutrients, such as the animal protein which is founded in the meat and egg, which are considered two fundamental elements in human feeding , for this reason the poultry projects receives a distinctive attention according to the rising request for its products . The poultry diet is considered a significant agent in improving their performance, as the diet formulations significantly effects on their growth, health and the quality of their products. Thus, the focus has moved to find out diet additives that acquire superior performance , with lower cost and ensuring

animal welfare and product integrity ,priority in this industry [8] in Iraq the private sector production capacity was measured in broiler meat about 156.5 thousand tons in 2020, which recorded an increase amount of 8.38 thousand ton compared to 148.2 thousand ton in 2019, by achieving a 5.6% increase [9]. Protein requirements composes about 45% of total poultry production [2]. Decrease protein and amino acids in the diet negatively affects the growth and development of the chicken in the optimal way [4], whereas the protein plays an essential role in building and protection of body tissues, it's also important for enzymatic and tissues functions [18]. For greater productivity for broiler, can be adding supplements contains a highly protein components and adding new protein ingredient to the broiler's diet might help decreasing protein deficiency and decrease the feeding cost, And for this reason poultry scientists and feed producers looking for economic protein sources and supplements to produce high quality and low cost diets [12]. With the increasing of population and growing demand for animal products, the organic waste was increased significantly. According to Makkar [16], the world produces 1.3 billion tones from the organic wastes every year. Accumulation of these wastes poses an environmental and health threat if not transact with it appropriately [25]. The insects plays an important role in processing these wastes , which it helps with reducing the environmental impurity and converting the wastes to a vital block rich with protein [14]. Recycling of the organic waste products depends on the collecting , grinding and mixing of these wastes to produce organic mixture, then put it in special containers and add insects larvae on it, which starts with engorging this mixture , and growing and multiplying during one week, which leading to conversion 75% from this mixture to larvae and 25% to larvae's wastes [21]. In the end, the larvae are collected to extract protein concentrates with high nutritional value, where the protein percentage reaches between 42%-47% on a dry matter basis, this protein is rich with essential amino acids, vitamins and minerals [11]. As for the remains of larvae are dried and converted to plants fertilizers like to peat moss [19]. Many types of insects are used as a protein sources such as yellow or mealworms (Tenebrio molitor), black soldier fly (Hermetia illucens), house fly (Musca domestica), Beetle (Tenebrio molito), (house crickets) and (silkworm) [17]. Our study focused on yellow mealworm which contains a high average of protein between 45%-60% and amount of fats reaches to 15%-40% . This insects protein highlighted specifically for its high content of essential amino acids , where the yellow mealworm amino acids profile exceeds that of soybeans flour, and equal to or sometimes exceed fish meal [21] as well as it considered a rich source of unsaturated fatty acids including oleic and linolenic acids [10]. It is also contains a group of various minerals and vitamins such as Zinc, copper, iron, magnesium, potassium and phosphorus besides to vitamins E,B12,B3,B2,B5 and B7 [13]. That is why this study aimed to know the effect of adding different levels of dried yellow mealworm to the diet of broiler and study its impact on some microbial and histological characteristics of the intestine.

Material and Methods

This experiment was conducted in the poultry field of the Animal Production Department - College of Agriculture - University of Diyala for a period of 35 days, from September 24, 2024, to October 28, 2024. The study aimed to investigate the effect of adding yellow

ISSN 2072-3857

mealworms to the diet of broiler chickens (Ross 308) on some microbial and histological characteristics of the intestine. A total of 225 unsexed one-day-old hybrid broiler (Ross 308), with an average weight of 38±1 g, were used in the experiment. The chicks were randomly distributed into five treatments, with three replicates per treatment (15 birds per replicate), as illustrated in the experimental design diagram. The treatments were as follows:

.1T1: (control) standard diet with out any additives.

.2T2: standard diet added to it 15 g/kg of dried yellow mealworm.

.3T3: standard diet added to it 30 g/kg of dried yellow mealworm.

.4T4: standard diet added to it 45 g/kg of dried yellow mealworm.

.5T5: standard diet added to it 60 g/kg of dried yellow mealworm.

The chicks were obtained from Baz Al-Jazeera hatchery a privately owned facility in Karbala, with an intermediate weight of 38 g at one day old and were unsexed. They were raised in a semi-closed housing system, with the floor of the designated cages covered with a 5 cm layer of wood shavings and bedding paper until the end of the first week. The chicks were housed in floor pens with an area of 1.5 m² per pen, with each pen containing 15 birds. During the first week, plastic dish feeders and inverted plastic drinkers were used. Afterward, the feeders were replaced with suspended circular feeders (45 cm in diameter), and the drinkers were replaced with automatic hanging plastic drinkers until the birds reached market age (five weeks). Regarding the lighting program, 23 hours of light with 1 hour of darkness was applied during the first three days, followed by 20 hours of light and 4 hours of darkness until the marketing age (five weeks), based on Aviagen's guidelines [3]. The chicks were initially housed on the first at a heat of 33°C on the first day, and the heat was gradually reduced by 0.5°C per day till reaching 21°C by the finish of the third week, where it remained stable until market age. At night, brooders with electric heaters were used to provide the appropriate temperature from the start of the rearing period until the end of the experiment.

The dried yellow mealworms were obtained from a local bird feed store, imported from Belgium in the form of whole dried worms, and subjected to chemical analysis to determine their nutritional composition, as shown in table 1. The birds were fed a ready-made Evan feed, produced by Evan Feed Company and Factory / Erbil. The starter feed contained 22-23% crude protein and 2,900-2,950 calories/kg of energy, while the grower feed contained 20-21% crude protein and 3,030-3,050 calories /kg of energy. The birds were fed the starter feed from day 1 to day 14, followed by the grower feed from day 15 to day 35. The dried yellow mealworms were added to the diets according to the specified amounts for each treatment. The worms were manually mixed with a small portion of feed, gradually increasing the amount while ensuring thorough mixing until achieving the desired uniformity for the weekly allocated feed of each treatment. After mixing, the feed was packed into sealed, gg bags for each treatment to maintain the effectiveness of the added materials.

ISSN 2072-3857

Table 1. Chemical analysis, amino acids, fatty acids and minerals in dried yellow mealworm.

Ingredients (%)		**Amino acid (%)**	
Protein	52.01	Aspartic	4.20
Fat	33.80	Serine	2.31
Fibers	5.80	Glutamic	6.60
Ash	3.40	Threonine	2.05
Minerals (%)		Glycine	2.81
Sodium(Na)	36.40	Alanine	3.70
Calcium(Ca)	3.80	Cysteine	0.71
Potassium(K)	8.50	Valine	3.10
Phosphorus(P)	7.00	Methionine	0.83
Magnesium(Mg)	2.00	Praline	3.22
Zinc(Zn)	1.00	Isoleucine	2.19
Fatty acids g/100g		Leucine	3.72
Myristic acid	2.15	Tyrosine	3.79
Palmitic acid	16.69	Phenylalanine	2.21
Palmitoleic acid	1.56	Lysine	2.95
Stearic acid	3.46	Histidine	1.68
Oleic acid	31.56	Arginine	2.77
Vaccenic acid	0.56		
Linoleic acid	36.42		
Linolenic acid	2.43		
Eicosenoic acid	0.39		

Results and Discussion

Microbial characteristics

Table 2 showed the presence of moral superiority (P≤0.05) in the total numbers of bacteria for T5 compared to T1,T2,T3 and T4, while T4 is significantly exceeded compared with T1,T2 and T3, and its found that there is significant superiority in T3 compared with T1 and T2 also T2 has significant superiority compared with T1 . As for the numbers of coliform bacteria are significantly increased (P≤0.05) in T1 compared to T2,T3,T4 and T5 and was found that there is significant increase in T2 and T3 over T4 and T5. Also, it was found that T4 siignificantly was higher than T5, while no significant difference was found between T2 and T3 . In the numbers of lactic acid bacteria it was noticed significant superiority (P≤0.05) in T5 compared to T1,T2,T3 and T4 while T4 significantly exceeded T1,T2 and T3 , and same thing T3 was significantly higher T1 and T2 and T2 significantly exceeds T1 .

ISSN 2072-3857

Table 2. effect of adding dried yellow mealworm to broiler diet (Ross 308) on total numbers of bacteria and Coliform bacteria and lactic bacteria inside the jejunum of the intestine (means ± SE.(

Treatment	Total bacteria (cfu/gm10^5)	Coliform bacteria (cfu/gm10^5)	Lactic acid bacteria (cfu/gm10^5)
T1	8886.66 ± 25.23 e	380.00 ± 18.70 a	490.00 ± 46.36 e
T2	1056 ± 38.94 d	329.16 ± 24.57 b	727.50 ± 57.77 d
T3	1163.33 ± 28.04 c	303.33 ± 10.80 b	860.00 ± 22.58 c
T4	1268.33 ± 38.94 b	268.33 ± 26.77 c	1000.00 ± 36.24 b
T5	1350.00 ± 52.44 a	237.50 ± 3061 d	1112.50 ± 81.77 a
p-value	1.234E-16	1.1068E-9	2.7394E-15

T1: Control (without additives), T2: Addingdried yellow mealworm 15 g/kg feed. T3: Adding dried yellow mealworm 30 g/kg diet . T4: Adding dried yellow mealworm 45 g/ kg diet. T5: Adding dried yellow mealworm 60 g /kg diet.

The presence of different letters in a single column indicates statistically significant differences between the means of the coefficients at the P≤0.05 level, as determined by Duncan's multiple range test.

The study was indicated that adding yellow mealworm in the diet positively affect on the good bacteria to the diet has positive effect on the good bacteria of the small intestine [1]. [22] found that there was a decreasing in the numbers of Escherichia coli and other colonic bacteria due to antibacterial agents which is produced by yellow mealworm such as chitosan which has anti-microbial action against many typed of harmful bacteria. Also the microbial composition of the insects which includes lactobacilli (lactic acid bacteria) enhances useful bacterial balance in the intestine , which is contributes with improving the health of digestive system and enhances the good microbial environment [7.[

Our study results did not align with those of [24], which evaluated the effect of adding yellow mealworms on microbial traits. Their study observed no significant differences in the total bacterial count, coliform bacteria, and lactic acid bacteria between the experimental groups and the control group.

Histological characteristics

Table 3 showed significant increase (P≤0.05) in villi length in T5 compared with T1,T2,T3 and T4, while the treatments T2,T3 and T4 significant higher than T1, while no differences between T2 and T3 and T4. As for the villi thickness has significant superiority (P≤0.05) in T5 compared with T1,T2,T3 and T4, while T2,T3,T4 were significantly higher than T1, and no differences were found between T2,T3 and T4. While a significant superiority (P≤0.05) was found in the crypt depth in T2,T3,T4 and T5 compared with T1, while no significant superiorities found between T2 and T3 and T4 and T5.

ISSN 2072-3857

Table 3 affect of adding dried yellow mealworm to the broiler diet (Ross 308) on the length and depth of the villi and crypt depth inside the jejunum of the intestine (means ± SE

Treatment	Length of villi	Thickness of villi	Depth of the crypts
T1	647.40 ± 129.59 c	137.20 ± 32.790 c	79.40 ± 12.99 b
T2	723.20 ± 78.84 b	145.13 ± 32.85 b	1117.73 ± 30.20 a
T3	780.60 ± 193.87 b	154.87 ± 31.26 b	113.47 ± 33.53 a
T4	780.40 ± 130.39 b	158.78 ±23.70 b	106.69 ± 12.96 a
T5	923.40 ± 137.40 a	184.74 ±30.98 a	108.78 ± 14.27 a
p-value	0.000003	0.000014	0.000038

T1: Control (without additives), T2: Addingdried yellow mealworm 15 g/kg feed. T3: Adding dried yellow mealworm 30 g/kg diet . T4: Adding dried yellow mealworm 45 g/ kg diet. T5: Adding dried yellow mealworm 60 g /kg diet.The presence of different letters in a single column indicates statistically significant differences between the means of the coefficients at the P≤0.05 level, as determined by Duncan's multiple range test.

The morphology of the small intestine plays an important role in the efficacy of digestion and absorption of the nutrients , which is directly affecting the health and production of the broiler, among the essential factors that affect the efficiency of the digestive system are the long and depth of the villi and the crypt depth in the intestine. The studies are indicate that these factors are closely related to the ability of the bird to benefit from nutrients, which is reflects on the growing rates and efficiency of feed conversion [6.[

The length of the villi is considered one of the most important factors that affect the absorption, where the increase in its length mean the expansion of the surface area that is available to absorb the nutrients and this is contributed with enhancing the efficiency of absorption and utilization of feed spent, also the length of villi enhances the activity of the digestive enzymes which is leading to improve the digestion and achieving higher benefit from the nutrients and for this reason enhancement in the growth rate and increase weight were noticed which makes birds more productive and best economic performance[15 . [

The depth of villi is consider an indicator to the intestinal health and epithelial strength, the thick villi can provide extra protection to the intestinal cells which enhancing the intestine to resist against the environmental and nutritional pressure . As well as it decreases the possibility of damage that caused by the external factors such as fungal poisons or pathogens , which is contributing with improving the health of the digestive systems in general . This matter is positively reflects on the efficiency of digestion and the absorption of the nutrients which lead to enhancing the bird's performance for improving its immunity against the illnesses [5.[

our study did not align with the findings of [6], which assessed the impact of yellow mealworm supplementation in broiler diets on intestinal histological traits. They reported no

significant improvement in villus height and crypt depth between the supplemented groups and the control group. Additionally, our results did not agree with [7], who evaluated the effect of yellow mealworm supplementation in broiler diets on intestinal histological characteristics. Their study found that the control group outperformed the supplemented groups in terms of crypt depth. Furthermore, our findings did not align with those of [20], which investigated the impact of yellow mealworm supplementation in broiler diets on intestinal histological traits. Their study found no significant effect between the control group and the supplemented groups regarding villus height and crypt depth

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Conclusion

The adding of 60g from dried yellow mealworm to each 1 kilogram of diet led to improvement of the microbial and histological characteristics for the broiler's small intestine during the production period.

Acknowledgment: I can only extend my sincere thanks and gratitude to everyone who contributed on the completion of this thesis. Firstly, I would like to express my deep gratitude to my honorable supervisor for his continuous support and guidance, which was the main reason for the completion of this work. I do not forget to thank my friends and colleagues who provided me with support and assistance throughout the study period. would also like to thank the professors who provide us with valuable information and guidance that paved the way for the completion of this study. Many thanks and appreciation to all of you.

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ISSN 2072-3857