Original Article



The Effects of Different Level of Synbiotic Supplementation in Diet of Broiler on Growth Performance, Intestinal Histology and Microbial Colony

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ABSTRACT

The objective of this study was to investigate the impact of varying levels of synbiotic supplementation on the growth performance and intestinal physiology of broiler chickens. A total of 360-day-old broiler chicks were randomly assigned to six different treatments, with four replicates per treatment and 15 birds per replicate. The control treatment was not supplemented, while the remaining treatments were supplemented with four different levels (0.25, 0.5, 0.75 and 1 g/kg) of synbiotic to the basal diets. The treatments were as follows: (1) control (not any supplement), (2) zinc bacitracin 0.04 g/kg, and (3) the remaining four treatments, which were supplemented with four different levels of synbiotic. On days 10, 24 and 35, the feed remaining and the birds were weighed in order to measure the body weight, weight gain, feed intake and feed conversion ratio. On day 10 and throughout the experimental period, there was a significant increase (P<0.05) in both body weight and weight gain, as well as a significant improvement in feed conversion ratio (FCR) with rising level of synbiotic. The control group exhibited a poorer feed conversion ratio than the other experimental groups (P<0.05). Up to 10 days, there was a significant increase in feed intake in birds on diets supplemented with 0.25 and 0.75 g/kg synbiotic. However, when the data from the 35-day experimental period were analyzed, it was found that the birds that had received 0.75 g/kg of synbiotic had significantly (P<0.05) decreased feed intake compared to the other experimental groups. The relative weight of the internal organs was not affected by the dietary treatments. The carcass yield and breast meat were found to increase significantly (P<0.05) with rising levels of dietary synbiotic. The length of the villi was found to be significantly affected by the treatment, with the villi in birds on diets supplemented with 0.5 g/kg of synbiotic being longer than those in the control group. Significantly, the shortest villi were observed in birds that received the highest supplement level (1 g/kg) of synbiotic. The number of Escherichia coli in the ileum was not affected by the dietary treatments. It can be concluded that synbiotic dietary supplementation exerts beneficial effects on growth output at an early age and during the broiler development cycle. In terms of performance, synbiotics supplementation resulted in an improvement in performance and a positive effect on carcass yield and breast meat production. The current research has demonstrated that the administration of synbiotics at a dosage of 0.75 g/kg exerts beneficial effects on the efficiency and subsequent physiological processes of broilers during the course of their

Keywords: Broiler, Growth performance, Intestinal histology, Microbial colony, Symbiotic.

1. Introduction

It is well documented that broiler chickens are particularly susceptible to a range of diseases, pathogens and general health issues as a result of the intensive rearing system employed. The utilisation of antibiotics represents the most efficacious methodology for the control of disease, the enhancement of health and the promotion of growth, a practice that has been extensively implemented within the poultry industry (8). However, following the emergence of undesirable side effects, including the development of antibiotic resistance in bacteria and the accumulation of residual antibiotics in poultry products, the role of antibiotics has become a matter of public concern (25). Consequently, the utilisation of antibiotics in animal diets for the purpose of disease control and growth promotion has been prohibited in numerous countries (4) and by the European Union in 2006, due to concerns regarding the emergence of antibiotic resistance in human pathogens (28). However, the restriction or complete banning of subtherapeutic doses of antibiotics in poultry production has resulted in significant economic challenges due to the rising incidence of enteric disease (15). Consequently, strategies of animal nutrition have been subject to significant modification, with the poultry industry pursuing the substitution of antibiotic growth promoters with alternative natural solutions (5). Among the proposed alternative feed additives, agents such as prebiotics, probiotics and synbiotics have recently emerged as a subject of considerable interest. Probiotics are defined as a mixture of live, non-pathogenic microorganisms that have been demonstrated to exert beneficial effects on the health of the host. These effects include the enhancement of gastrointestinal barrier function, which is achieved by regulating the homeostasis of the intestinal microbial load (10). However, prebiotics are defined as non-digestible food additives that selectively stimulate the growth and activity of one or a small number of bacteria in the colon, thereby affecting the host in a beneficial manner (12). The combination of a probiotic and a prebiotic, known as a synbiotic, has been proposed as a means of enhancing the metabolic activity of one or more beneficial microorganisms, or of selectively promoting their growth in a manner that benefits the host. The prebiotic component of the synbiotic mixture has been demonstrated to enhance the survival of the probiotic microorganisms within the intestinal tract, while simultaneously stimulating the endogenous bacteria of the host (29). It is thought that synbiotics can reduce the number of harmful bacteria and assist in the adherence of beneficial bacteria by reducing intestinal pH (30). Furthermore, synbiotic supplementation has demonstrated to preserve populations of unprofitable or potential pathogens, such as Escherichia coli, within the cecal digesta and small intestine at relatively low numerical levels (31). The administration of synbiotics

has been demonstrated to significantly enhance the average daily body weight gain, carcass ratio and feed conversion ratio (FCR) in broilers, as compared to the control group (2). The beneficial effects of synbiotics are more pronounced when administered soon post-hatch, as this allows for the improvement of intestinal barrier function and the fortification of the undeveloped intestinal microbial load in an early age. A significant barrier to the colonisation of chickens by potentially pathogenic bacteria is the gastrointestinal microflora of the chickens themselves (18). The beneficial effects of synbiotics on the growth efficiency and physiology of broiler chickens have been documented by (1) and (17). The primary objective of this study was to examine the performance and physiological responses of broilers fed with varying supplementary levels of synbiotic (Poultry Star ME) in comparison to a non-supplemented basal diet and an antibiotic-supplemented diet.

2. Materials and Methods

2.1. Experimental Design and Bird Management

In a feeding trial, 360-day-old Ross 308 broiler chicks were arranged in a completely randomised design and assigned to six different treatments, with each treatment comprising four replicates and 15 birds per replicate. The treatments were designed as a control (basal diet), a zinc bacitracin-supplemented (0.04 g/kg) treatment as an antibiotic source, and the remaining four treatments were supplemented with four different levels (0.25, 0.50, 0.75 and 1 g/kg) of synbiotic to the basal diets. The chicks were reared on floor pens (100 × 100 cm) bedded with wood shavings. The birds were fed three age-dependent diets: a starter diet until 10 days, a grower diet until 24 days, and a finisher diet until 35 days. The diets were formulated in accordance with the nutritional requirements for Ross 308 broiler chickens, as outlined in Table 1. The temperature of the room was reduced gradually from 35 to 24 degrees Celsius over the course of 35 days. The duration of illumination was maintained at eighteen hours per day throughout the experimental period, with the exception of the initial seven-day period, during which the duration was extended to twenty-three hours per day. The animals were provided with feed and water in quantities sufficient to meet their needs. On days 10 and 35, the remaining feed and the birds were weighed in order to ascertain the body weight, weight gain, feed intake and feed conversion ratio. Any mortalities were duly recorded as they occurred, and the mortality rate was subsequently corrected to reflect the feed-to-gain ratio.

Experiment design have been shown as:

T1 = Basal diet without addition any supplement.

T2 = Basal diet + 0.040 kg per ton of antibiotic (Zinc Bacitracin).

T3 = Basal diet + 0.250 kg per ton of synbiotic (Poultry star ME).

T4 = Basal diet + 0.500 kg per ton of synbiotic (Poultry star ME).

T5 = Basal diet + 0.750 kg per ton of synbiotic (Poultry star ME).

T6 = Basal diet + 1 kg per ton of synbiotic (Poultry star ME).

Poultry star ME is a mixture of probiotic and prebiotic. It contains fructo-oligosaccharides as a prebiotic, comprising four microbial strains selected from the different segments of the gastrointestinal tract. These are Lactobacillus reutter isolated from the crop; Enterococcus faecium from the jejunum; Bifidobacterium animals from the ileum; and Pediococcus acidilactici from the cecum. The probiotic component is produced by BIOMIN (America Inc., San Antonio, TX USA).

2.2. Intestinal Histology and Microbial Colony Analyses

Jejunal tissue samples were obtained, flushed with buffered saline and fixed with 10% neutral buffered formalin. Subsequently, the sectioned samples were subjected to staining with haematoxylin and eosin after incorporation into paraffin wax and separation. The prepared tissue sections were imaged magnification using a digital camera mounted on a microscope (Dino-Eye Microscope Eyepiece Camira, program version 23). The resulting images were then analyzed using a dedicated software program to determine the morphometric indices. The images were digitised, and the height of the villus (from the tip of the villus to the junction of the villi with the crypts) and the depth of the crypt (from the villus/crypt junction to the muscular junction) were measured for each well-oriented villus in the jejunal segment, with a minimum of seven and a maximum of ten villi measured per segment.

2.3. Statistical Analysis of Data

A one-way ANOVA was conducted using Minitab 2017 to statistically analyse the collected data in a completely randomised arrangement. The Duncan multiple range test was employed to ascertain the discrepancies between the mean values.

3. Results

3.1. Performance Data

The outcomes of the growth performance, as indicated by body weight (BW), body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR), have been presented for 10 days of age in Table 2 and for 35 days of age in Table 3. As demonstrated in Table 2, body weight exhibited a statistically significant increase (P<0.05) in birds fed a synbiotic-supplemented diet compared to those receiving the control and antibiotic-supplemented treatments. The addition of increasing levels of synbiotic to the diets resulted in a notable increase in body weight. The same trends were observed for body weight gain, with birds fed synbiotic-containing diets demonstrating a greater weight gain (P<0.05) than other groups. The body

weight gain (BWG) exhibited a significant increase (P<0.05) as the level of synbiotic in the diet increased. In general, the broilers in all supplemented groups exhibited greater body weight than those in the control group. A similar pattern was observed in weight gain, with broilers synbiotic-supplemented diets demonstrating a statistically significant (P<0.05) increase in weight compared to other experimental groups. In general, the weight gain of birds on supplemented diets was greater than that of birds on control diets. In general, feed intake was increased by dietary treatments over the 10-day period, with a particularly notable increase observed in birds fed a synbiotic diet containing 0.750 kg per ton of synbiotic, which exhibited an intake of 274.80. However, the observed effect was only significant (P < 0.05) in birds on diets containing synbiotic at levels of 0.25 and 0.75 g/kg and those that received antibiotic, in comparison to the control and other experimental groups (Table 2). The birds that were offered diets containing 0.75 g of synbiotic consumed a greater quantity of feed than the other experimental groups. A 35-day experimental period revealed that birds that received 0.75 g/kg of synbiotic consumed less feed than other experimental groups, with the exception of T4 (Table 3). This difference was statistically significant (P<0.05). The feed conversion ratio exhibited a statistically significant improvement (P<0.05) with the inclusion of increasing levels of synbiotic in the broiler diets. The FCR ratio of the control group was 1.71, which was the poorest performance among all experimental groups.

3.2. Carcass Characteristics

As demonstrated in Table 4, there was a statistically significant increase (P < 0.05) in carcass yield with elevated levels of dietary synbiotic supplementation. The birds that received 0.75 g/kg of synbiotic in their diets exhibited a higher dressing percentage. A similar trend was observed in the breast meat. However, no effect was observed on the weight of the thigh, drumsticks, wings, back and neck as a result of the dietary treatments.

3.3. Jejunum Histology

Among the measured micromorphological parameters (tip width, basal width, crypt depth, villous height/crypt depth, muscle thickness), the villi length was found to be significantly affected by the treatments (Table 5). Villus length was observed to be longer in birds that had been fed a diet supplemented with antibiotics and synbiotics (T2, T4). However, this difference was only found to be statistically significant (P<0.05) when comparing birds that had been fed a diet supplemented with 0.50 g/kg of synbiotic to the control and 1 g/kg of synbiotic (T6). The villi were observed to be shorter in birds that received the highest level of synbiotic supplementation compared to the other experimental treatments. The Escherichia coli in the ileum were not affected by the treatments, and thus the data are not presented here.

Table 1. The composition of basal diets of feed.

	Basal diet (g/kg)					
Ingredients	Starter	Grower	Finisher			
Corn	155	250	400			
Wheat	470	420	305.5			
Soyabean	320	270	240			
Vitamin and minerals premix ¹	15	15	15			
Oil	17	25	25			
L-Lysine	1.2	0	0			
L-Threonine	0.2	0	0			
Limestone	16.5	15.5	10.2			
Mono calcium phosphate	1.5	0.5	0.3			
Salt	2	2	2			
Anti-toxin	2	2	2			
Synbiotic	0	0	0			
Antibiotic	0	0	0			
Total	1000	1000	1000			
Chemical composition						
ME (Kcal/Kg)	3010	3160	3210			
Crude protein* %	24.1	22	20.2			
Crude ash* %	5.59	5.52	5.02			
Crude fiber* %	3.50	3.99	3.71			
Crude fat* %	2.78	2.97	2.93			

The content of vitamin and mineral **piremix**¹: Vitamin A 666.667 IU, vitamin D3 266.667 IU, vitamin E 5000 mg, vitamin K3 167 mg, vitamin B1 200 mg, vitamin B2 467 mg vitamin B6 2333 mg, vitamin B12 2567 mg, folic acid 67 mg and colin chloride 40000mg, : iron 3333mg, copper 667mg, manganese 5333mg and zinc 4000 mg.

Table 2. Body weight (BW), weight gain (WG), feed intake (FI), and feed conversion ratio (FCR) at 10 days of age of broiler chickens received different dietary levels of symbiotic.

Treatments	Performance parameters at 10 days of age						
	BW	WG	FI	FCR			
T1	230.45±1.37 °	185.91±0.85 d	254.17±1.5 °	1.367±0.003 ab			
T2	233.63±3.65 °	190.50±3.75 ^{cd}	266.77±3.5 ab	1.411±0.013 ^a			
Т3	T3 241.52±2.10 b		266.27±1.2 ab	1.321±0.008 bc			
T4	241.40±1.35 ^b	196.93±0.95 bc	260.93±1.24 bc	1.325±0.011 bc			
T5	256.27±1.25 a	211.50±1.34 a	274.80±4.25 a	1.299±0.002 ^{cd}			
Т6	250.70±4.21 a	208.48±2.85 a	263.37±7.2 bc	1.263±0.025 ^d			
F value	14.52	19.85	3.39	10.54			
P Value	P Value 0.001		0.025	0.001			

a,b.c:Mean values on the same column not sharing a superscript are significantly different (P<0.05).

^{*}Analysed

Table 3. Body weight (BW), weight gain (WG), feed intake (FI) and feed conversion ratio (FCR) at 35 days of age of broiler chickens received different dietary levels of symbiotic.

Treatments	Performance parameters at 35 days of age							
	BW	WG	FI	FCR				
T1	1890.8±6.25 ^d	1846.2±6.25 ^d	3151.9±12.3 ab	1.71±0.022 a				
T2	1927.0±5.15 ^{cd}	1883.8±4.87 ^{cd}	3128.1±18.6 ab	1.66±0.023 ab				
Т3	1928.3±9.65 ^{cd}	1884.8±9.72 bcd	3121.4±13.85 ab	1.66±0.026 ab				
T4	1949.3±12.05 bc	1904.8±11.95 bc	3066.3±12.5 bc	1.61±0.022 bc				
T5	1978.6±4.18 ab	1933.9±4.31 ab	3024.5±18.25 °	1.60±0.021 bc				
T6	2017.7±10.15 ^a	1972.0±10.07 a	3158.7±14.25 a	1.56±0.016 °				
F Values	6.99	6.87	2.97	5.60				
P Value	0.001	0.001	0.04	0.001				

a,b.c: Mean values on the same column not sharing a superscript are significantly different (P<0.05).

Table 4. Carcass yield and the weight of carcass cuts of broiler chickens at d 35 at various synbiotic supplementation levels (Means±SEM).

Treat ments	Carcass yield	Breast	Thigh	Drum	Wing	Back	Neck
T1	68.1±0.39 c	34.4±0.45bc	15.20±0.63	13.83±0.30	9.84±0.25	22.34±0.47	3.31±0.07
T2	68.6±0.48 bc	34.1±0.92 c	15.65±0.44	13.28±0.15	9.18±0.44	22.26±0.38	3.58±0.31
Т3	67.2±0.70 c	34.7±0.42 bc	15.82±0.56	14.22±0.25	9.72±0.34	21.45±0.67	3.58±0.32
T4	70.3±1.65 ab	36.5±0.91 ab	15.35±0.43	13.07±0.32	8.99±0.21	21.27±0.61	2.97±0.22
T5	71.1±0.24 a	37.4±0.21 a	15.34±0.15	13.45±0.22	8.45±0.11	20.78±0.58	3.20±0.23
T6	70.2±0.75 ab	36.2±1.08 abc	14.61±0.56	13.23±0.12	8.75±0.64	21.94±0.48	3.52±0.31
P Value	0.048	0.03	0.60	0.07	0.10	0.31	0.493
F Value	5.05	3.17	0.74	2.45	2.14	1.28	0.91

a,b.c:Mean values on the same column not sharing a superscript are significantly different (P<0.05).

Table 5. Jejunum micromorphological parameters of broiler chickens at d 35 at according to treatments (Means±SEM).

Treatments	Villi length mµ	Tip width mµ	Basal width mµ	Crypt depth mµ	¹V/C	Muscle thickness mµ	² VSA mm ²
T1	1398±10.0bc	21.6±18.3	30.9±18.3	386.0±53.5	4.59±0.82	216.9±19.4	3.65±0.50
T2	1612±19.8ab	20.6±16.1	24.1±16.1	315.4±36.5	5.47±1.21	254.0±9.90	3.65±0.46
Т3	1574±31.1ab	22.1±50.1	32.1±50.1	318.1±6.7	4.98±0.36	239.4±11.4	4.27±0.61
T4	1768±19.1a	21.5±9.5	25.3±9.9	423.6±36.1	4.30±0.86	272.1±15.1	4.10±0.25
T5	1528±17.5 abc	20.0±15.9	26.2±15.9	388.0±21.1	4.14±0.67	232.4±5.05	3.55±0.26
T6	1292±8.5°	16.7±7.4	23.9±7.2	380.7±24.7	4.46±0.35	240.0±6.45	2.62 ± 0.07
P Value	0.03	0.42	0.10	0.81	0.55	0.70	0.11
F value	1.30	1.04	2.14	0.45	0.82	0.59	2.09

a,b.c: Mean values on the same column not sharing a superscript are significantly different (p<0.05); $^{1}V/C=$ Villous height/crypt depth; $^{2}VSA=$ Villous surface area.

4. Discussion

4.1. Growth Performance

The administration of synbiotics in the diet of broiler chickens resulted in improved body weight and weight gain, as observed up to 10 or 35 days post-treatment. In the feed additive groups exhibiting the most pronounced impact, the synbiotic groups demonstrated the greatest improvement in feed efficiency. This finding was consistent with the results reported by (32), who demonstrated that synbiotic feeding improved the feed conversion ratio of 42day-old broilers. (17) reported that dietary synbiotic improved the growth performance of broiler chickens. The present study corroborates the hypothesis that dietary supplementation with synbiotics during the starter period of broilers enhances the benefits observed. It can be concluded that dietary supplementation of synbiotics enhances appetite and feed intake in early life to a similar extent as antibiotics. Similar outcomes have been documented by (24) when distinct concentrations of synbiotic (0.1%, 0.2%, 0.3% and 0.4%) were administered to Japanese quails. This may be attributed to the fact that dietary antibiotic and synbiotic supplementation enhanced the digestive processes of newly hatched chicks, given that they are in a developmental stage. Therefore, it is essential to enhance the absolute feed consumption and the availability of essential nutrients to facilitate the rapid growth and ensure the early development of the internal systems and body composition of birds. Moreover, (1) indicated that the supplementation of prebiotics, probiotics and synbiotics to broiler chicken diets resulted in increased body weight and feed conversion ratio. The positive effects of feeding synbiotics on the growth performance of broilers were also confirmed by (20). Additionally, (22) indicated that the incorporation of a synbiotic at a dosage of 0.9 g/kg into broiler diets did not elicit any discernible effects on body weight and body weight gain. The favourable growthpromoting effects of synbiotics may be attributed to the synergistic interaction between probiotics and prebiotics, which enhances the digestion and absorption of nutrients (13). The use of biological feed additives, such as synbiotics, has the potential to enhance the availability and utilisation of nutrients, improve the absorptive capacity of the intestine, and alter the intestinal pH and microbial community (9). This, in turn, can contribute to the maintenance of intestinal integrity and function (21).

4.2. The Relative Weight of Visceral Organs

The impact of the synbiotic supplementation on the relative weight of the organs under examination was found to be statistically insignificant. These findings are inconsistent with those of (1), who observed an increase in the relative weight of internal organs when a synbiotic at a dosage of (0.38, 0.63 and 1.13 g/kg) was incorporated into the broiler diets. (6) additionally indicated that the weight of the bursa fabricius was augmented by the dietary supplementation of a synbiotic (1.5 g/kg) in broiler diets. However, this outcome is not aligned with the findings of our experiment. It may be the case that the effect of the additive, which

includes antibiotics and a synbiotic, is functional rather than structural.

4.3. Carcass Characteristics

The primary objective of the poultry industry is to achieve a higher carcass yield and an increase in the edible portions. The current study demonstrated that birds fed synbiotic-supplemented diets exhibited increased body weight, carcass yield, and breast muscle weight, confirming the positive impact of dietary synbiotics on the performance of broiler chickens. These findings align with those of (2), who reported a significant positive effect of dietary synbiotics (1g/kg) on the carcass yield and breast meat of broilers. Additionally, (16) observed an increase in carcass percentage and breast muscle weight when two distinct commercial prebiotics were administered to broiler eggs. However, our results differed from those of (1), who used synbiotics in broiler diets at 0.38, 0.63 and 1.13 g/kg, despite our levels being slightly different from these.

4.4. Jejunum Histology

The current study revealed an improvement in the structural development of the morphology of the jejunal mucosa microscopic examination. The supplementation of a synbiotic resulted in a significant increase in the length of villi in the jejunum of the broiler. The lengthening of villi may be indicative of enhanced absorption and utilisation of available nutrients, thereby increasing the intestinal absorptive surface area. Similar findings have been reported by (3), who employed different levels of synbiotic supplementation (2.5 g/kg and 5 g/kg) in broiler diets. These findings are consistent with those of (11), who reported a significant increase in jejunal villous height following synbiotic supplementation in broiler diets. Furthermore, (27) observed an increase in villus length following the administration of a synbiotic supplement to broiler diets. Probiotic-friendly bacteria, such as Lactobacillus, which are present in the synbiotic colonization, facilitated these ultrastructural improvements by increasing the regulation of epithelial cell turnover (26). Furthermore, the combination of prebiotic and probiotic bacteria in the intestine may facilitate the implantation of other species of native bacteria with a protective effect (23). Prior research has demonstrated that probiotics, such as L. johnsonii BSNE (19), and synbiotics, comprising a combination of beneficial bacteria and a prebiotic (3), can facilitate intestinal development.

4.5. Escherichia Coli Number in the Broiler Ileum

The number of E. coli colonies in the ileum digesta was demonstrably reduced by the dietary supplementation of synbiotics to broiler diets. However, the dietary antibiotic resulted in an unexpected increase. These results were consistent with those reported by (33), who observed a significant reduction in E. coli numbers in the gastrointestinal tract of broilers following dietary synbiotic supplementation, and an increase following antibiotic administration. It was therefore concluded that the observed increase in bacterial density in the antibiotic group could be attributed to the development of immunity against these

bacteria. Furthermore, a reduction in the number of cecal E. coli has been documented in studies conducted by (7). This may be attributed to the ability of synbiotics to alter the bacterial population within the gastrointestinal tract of birds. Therefore, bacteria that are beneficial to health are selectively stimulated, while the number of harmful bacteria is decreased. The findings of the current study were encouraging and offered some proof of the beneficial effects of synbiotic dietary supplementation on growth output at an early age and during the broiler development cycle. Nevertheless, the optimal level of synbiotics to be included in broiler diets remains a topic of ongoing debate. With regard to performance, the administration of synbiotics was associated with an improvement in body weight (BW), body weight gain (BWG) and feed conversion ratio (FCR) in the birds. Furthermore, the beneficial effects of synbiotics were evident in both carcass yield and breast meat production. The administration of synbiotic supplementation was observed to induce favourable alterations in the histomorphology of the jejunum, particularly at the medium supplementary level (0.50 g/kg), which was associated with an increase in villus length. The relative weight of the visceral organs remained unaltered following the administration of the various treatments. The addition of synbiotics to the broiler diets did not result in any notable alterations in the relative weight of visceral organs, serum biochemistry, or the level of thyroid hormones. The findings of this study suggest that the administration of synbiotics during the early stages of chick development may be beneficial, as it has been demonstrated to enhance early growth and development. The present study has demonstrated that the administration of synbiotics at a dosage of 0.75g/kg exerts beneficial effects on the efficiency and subsequent physiological processes of broilers during the growth phase.

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Authors' Contribution

This article forms part of my master's thesis, which has been overseen by: Professor Dr. Filiz Karadaş, and Co-Supervisor: I am grateful to be able to assist. Professor Dr. Sleman Said Mohammed BESKI

Ethics

The procedure of this trial was approved by the Animal Ethics Committee of the College of Agricultural Engineering Sciences, University of Duhok (Approval No: AEC 120120203).

Conflict of Interest

The authors declare that they have no conflict of interest.

Data Availability

Data will be available upon request to the corresponding author.

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