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EFFECT OF PREBIOTIC ON GROWTH PERFORMANCE AND IMMUNE RESPONSE OF JAPANESE QUAIL CHICKS

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ABSTRACT: This study was conducted to investigate the effect of prebiotic (Alphamune) supplementation on growth performance, immune responses, histopathological test and carcass characteristics of Japanese quail.

A total number of 117 unsexed Japanese quail chicks, day old, were weighed and distributed randomly into three experimental groups. The birds in each group were distributed to 3 replicates with 13 birds each and were housed in separate pens in battery cages with raised wire floors. Birds of the first experimental group served as control group (T_1). The birds of the second experimental group (T_2) were fed a ration containing prebiotic (Alphamune) at a level of 0.5 g / kg diet. The birds of the third experimental group (T_3) were fed a ration containing 1.0 g/ kg diet of the prebiotic. The conditions of housing and management of birds for all groups were similar during the experimental time.

The obtained results could be summarized as follows: Body weight gain was significantly ($P \leq 0.05$) higher for group fed 1000g prebiotic /ton feed compared to other groups but there was no significant difference among all groups in feed intake. Feed conversion ratio was significantly ($P \leq 0.05$) superior for groups fed levels of prebiotic compared with control groups. Regarding to carcass characteristics, there were no significant differences among all groups of study.

Results of humoral immune response indicated that in primary immune response (after a week of first injection), both groups 2 and 3 displayed slightly higher antibody titers compared to control group. While, in secondary immune response after a week of booster dose, both prebiotic treated groups demonstrated obvious higher antibodies titer than control group. Same trend was found-

with the percent of phagocytic and phagocytic index. On the other hand, lysozyme activity and nitric oxide values for quail treated with prebiotic show insignificant higher values than control.

Results of histopathological test revealed that group 2 shows the best values in villi, crypt and goblet cells compared with the other groups. In general, this study suggested that adding prebiotic to Japanese quail diet at level of 0.5 and 1g/ Kg diet improved body weight gain, feed conversion ratio, humoral immune response, phagocytic activities, lysozyme activity (immunoenhancing agent in poultry farms to protect against infection and increase vaccination efficiency) and enhanced the absorption compared to control group.

INTRODUCTION

Enteric diseases are an important concern to the poultry industry because of lost productivity, increased mortality and the associated contamination of poultry products for human consumption. With increasing concern about antibiotic resistancy, the ban on sub therapeutic antibiotic usage in Europe and other many countries, there is increasing interest in finding alternatives to antibiotics for poultry production (Patterson and Burkholder, 2003; and Chichlowski *et al.*, 2007).

Prebiotics is one of several approaches that have potential to reduce enteric diseases in poultry production. Prebiotics are defined as a non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/ or activity of endogenous bacteria such as Bifidobacteria and Lactobacilli, which benefit the host (Gibson and Roberfroid, 1995; Gibson, 1999). They include mannanoligosaccharide, White *et al.*, (2002), lactose, Szilagyi (2002), oligofructose and inulin, Teitelbaum and Walker (2002).

Several studies have shown that administering prebiotics can improve weight gain, feed intake and feed conversion in broiler (Waldroup *et al.*, 1993, Grimes *et al.*, 1997; *Wu et al.*, 1999; Xu *et al.*, 2006; Pelicano *et al.*, 2004 and Rodrigues *et al.*, 2005). Prebiotic supplementation also display favorable effects on intestinal microflora and improve immune response of poultry (Savag *et al.*, 1996; Sahin *et al.*, 2008 and Taherpour *et al.*, 2009). However, some reports indicate that prebiotics supplementation did not affect body weight gain and feed conversion (Stanczuk *et al.*, 2005).

The objective of the present experiment was to study the influence of prebiotic as an additive to growing Japanese quail diets, on growth

performance, immune responses, histopatholgical test and carcass characteristics of growing Japanese quail.

MATERIALS AND METHODS

This study was conducted at poultry experimental station belonging to Animal Production Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt.

Birds and Experimental Design

A total number of 117 unsexed Japanese quail chicks, day old, were weighed and distributed randomly into three experimental groups (39 birds each). Subsequently, the chicks in each group were distributed to 3 replicates with 13 birds each and were housed in separate pens in battery cages with raised wire floors. Birds of the first experimental group served as control group (T_1). The birds of the second experimental group (T_2) were fed a ration containing prebiotic (Alphamune) at a level of 0.5 g / kg diet (500 g/ Ton). The birds of the third experimental group (T_3) were fed a ration containing 1.0 g/ kg diet (1000 g/ Ton) of the prebiotic. The conditions of housing and management of birds for all groups were similar during the experimental time (five weeks).

All diets were formulated to meet all nutrient requirements of growing Japanese quails according to **NRC (1994).** Feed and water were offered to birds ***ad libitum*** throughout the experimental period. The composition and calculated analysis of experimental diets are presented in Table (1).

Data Collection

Body weight, feed consumption and feed conversion ratio were calculated weekly. Birds of each replicate were weighed individually every week. Unconsumed feed was measured at the end of each week, and then the average weekly bird consumption was calculated by dividing total feed consumed during the week by number of quails per pen. Feed conversion ratio was calculated weekly by dividing total feed consumed in a pen by the total gain in body weight of the birds in that pen.

Carcass Yield

At the end of experiment, 24 birds were slaughtered (3 males and 3 females from each group) for carcass test.

Table (1): The composition and calculated analysis of experimental diets used for growing Japanese quail

Ingredients	Control	Prebiotic levels (Alphammune)	
		500g/Ton	1000g/Ton
Ground, yellow corn.	54.93	54.93	54.89
Soybean meal (44%).	34.10	34.00	34.00
Corn gluten meal (60%).	8.06	8.11	8.10
Calcium carbonate.	1.427	1.427	1.427
Dicalcium phosphate.	0.9415	0.9415	0.932
Sodium chloride (Salt).	0.370	0.370	0.370
DL- Methionine.	0.031	0.031	0.031
L-Lysine HCL.	0.1405	0.1405	0.150
Prebiotic (Alphamune).	0.00	0.05	0.10
Total (Kg)	**100.00**	**100.00**	**100.00**
Calculated Analysis			
Crude protein (%).	23.63	23.63	23.62
Metabolizable energy (Kcal/Kg diet).	2900.42	2900.47	2898.33
Calcium (%).	0.85	085	0.85
Available phosphorus (%).	0.33	0.33	0.33
Methionine (%).	0.51	0.51	0.51
Lysine (%).	1.32	1.32	1.33
C/P ratio.	122.74	122.74	122.71
Analyzed.			
Crude protein %.	23.78	23.75	23.73

Histopathological Test

At the end of experiment, autopsy samples were taken from the small intestine (duodenum) of five birds of different groups and fixed in 10% formalin saline for twenty four hours for histopathological investigation. Washing was done in tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in Xylene embedded in paraffin at 56 degree in hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by slidge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by

Aldehyde Fuchsin for detection of Beta cells and examination was done through the light electric microscope (Banchroft *et al.*, 1996).

Blood Samples and Immune Response Test

After two weeks of experiment, twelve birds (6 males and 6 females) from each group were intramuscular injected with 0.5 ml of 10 %, sheep red blood cells (SRBCs.) (T-dependent antigen). Blood samples (serum) were collected after 48 hours to determine serum lyzosoyme. Also, blood samples (serum) were collected weekly after week from the first injection with SRBCs to determined antibodies against SRBCs. Same birds were injected again (second injection) with 0.5 ml of 10 % SRBCs, as booster dose after 4 weeks of experiment. Blood samples were collected after 48 hours to determine serum lyzosoyme. Also, blood samples were collected weekly after the second injection with SRBCs to evaluate second immune response.

Humoral immune response was evaluated by haemagglutination (HA) test, haemagglutinating antibodies to sheep red blood cells were estimated by HA assay according to Dohms and Jaeger (1988).

Serum was collected at 7 day later from first inoculation of SRBC to evaluate primary immune response and after week from booster dose of SRBC to evaluate secondary immune response as well as estimate lysozyme activity and nitric oxide value.

Measurement of Phagocytic Activity

Measurement of phagocytic activity of peripheral blood monocyte using candida albicans was adopted as described by Anthony *et al.*, (1985) and Chu and Dietert (1989). Separation of peripheral blood mononuclear cells using ficoll-hypaque density gradient was carried out as described by Boyum (1968). Mononuclear cell layer was collected, washed and resuspended in RPMI-1640 supplemented with 10% foetal calf serum and viability was done after Hanks and Wallace (1985). Phagocytic percentage and index was estimated as follows:

Phagocytic % = 100 X No. of macrophage ingesting candida albicans/ total No. of macrophages.

Phagocytic index = No of macrophages ingesting more than 3 blastopores / total No. of macrophages with ingested blastopores.

Lysozyme Activity

Lysozyme activity was determined according to Schltz (1987) using agarose gel lysis assay. Briefly; lysoplates were prepared by dissolving 1% agarose in 0.06% ml of PBS at pH 6.3, 500 mg of micrococcus lysodeikticus in 5 ml saline were added to 1 liter of agarose. Plates were poured, then 25 µl of serum sample and standard lysozyme were put in each well. After 18 hours, the cleared zones diameter were measured to both standard lysozyme and serum sample, then the concentration was estimated.

Measurement of Nitric Oxide Production

Nitric oxide production was determined according to Pertile *et al.*, (1995). Briefly; 100 µl of serum sample was transferred into flat-bottom 96-well ELISA plates and 100 µl of Greiss reagent were added to each well. The absorbance was read at 570 nm with ELISA plate reader, then absorbance was converted to (µm) of nitrite by comparison with absorbance values of sodium nitrite standard curve fit.

Statistical Analysis

The obtained data were statistically analyzed using analysis of variance and comparing between groups was performed using least significant difference (LSD) at $P \leq 0.05$ according to Petrie and Waston (1999) and computerized using SPSS (1999).

RESULTS

Results of body weight, feed intake and feed conversion ratio of Japanese quail fed diets supplemented with 0.5 or 1g Alphamune are presented in Table (2). Results showed that body weight gain was significantly ($P \leq 0.05$) higher for group fed 1g prebiotic /kg diet compared to the control group. Group fed 0.5g prebiotic/ kg diet had insignificant increase in body weight gain compared to control group. The results revealed also that dietary treatments improved significantly ($P \leq 0.05$) Feed/ Gain ratio compared with control group (Table 2). However, the differences among groups fed prebiotic (Alphamune) were insignificant.

The effect of experimental treatments on the composition of the bird carcasses (in gram) and relative to live body weight (%) are given in Table (3). Results of carcass variables showing no significant effect of prebiotic treatments on carcass characteristics among all groups.

Table (2): Effect of prebiotic supplementation (Alphamune) on Body weight gain, feed intake and feed conversion ratio during the experimental period (0 – 5 weeks of age).

Treatment	Body Weight Gain (g)	Feed Intake (g)	Feed Conversion Ratio (Feed/Gain)
Control (T_1)	180.01 **b** ± 4.14	844.80 ± 19.42	4.69 **a** ± 0.116
500 mg / ton feed (T_2)	187.25 **ab** ± 1.77	824.34 ± 7.79	4.40 **b** ± 0.032
1000 mg / ton feed (T_3)	193.24 **a** ± 1.91	801.60 ± 7.92	4.1 5 **b** ± 0.047

a, b..., means in the same column with different letters are significantly different at ($P < 0.05$).

Humoral Immune Response

Results of geometric antibody titers against sheep red blood cells referred to primary immune response after first injection of antigen that represented in 1st and 2nd week revealed slight increase in antibody titers of both group (2) and group (3) compared to control group. Secondary immune response represented at 1st week after booster dose of antigen (SRBCs), showed obvious higher antibodies titer of both prebiotic treated groups than control group (Table 4).

Data of phagocytic activity of macrophages in experimental groups of quail is presented in Table (5). Both prebiotic treated groups (2) and group (3) showed significant increase in phagocytic % and index in comparison to control group.

Results of serum lysozyme activity and nitric oxide did not show significant changes among experimental groups (Table 6). However, group 2 and 3 recorded higher values of serum lysozyme activity and nitric oxide than control group.

Histopathological Test

Concerning group of birds kept as control, The villi from duodenum of small intestine showed diffuse goblet cells formation in the lining mucosal epithelium and the average length was 7 microns while, the crypts had 6 microns length and 1.5 microns width associated with congestion in the serosal layer (Fig. 1 and 2).

Table (3): Effect of dietary prebiotic (Alphamune) fed to Japanese quail diets on the carcass characteristics.

Treatment / Traits	Control (group 1)	Group 2 (0.5 kg/ton)	Group 3 (1 kg/ton)
Live body weight.	217.11 ±8.74	220.00 ±1.66	224.73± 4.31
Empty body weight.	147.57 ± 7.10	153.37 ± 3.84	153.67 ± 3.01
Carcass %.	67.97	69.72	68.38
Liver weight.	3.39 ±0.17	2.90 ±0.02	3.74 ±0.40
Liver %.	1.56	1.32	1.66
Gizzard weight.	2.50 ± 0.11	2.47 ±0.19	2.78 ±0.15
Gizzard %.	1.15	1.12	1.24
Heart weight.	1.69± 0.14	1.83± 0.21	1.93± 0.08
Heart %.	0.78	0.83	0.86
Spleen weight.	0.08± 0.009	0.08± 0.01	0.09± 0.01
Spleen %	0.03	0.04	0.04
Neck weight.	8.12± 0.93	7.45± 0.64	7.00± 0.59
Wings weight.	11.04 ± 1.24	10.42 ± 0.55	10.43 ± 0.78
Legs weight	3.83	3.68	3.98
Head weight.	8.38	8.42	7.47
Intestinal weight.	8.27 ± 0.26	6.42 ± 0.28	7.88 ± 0.31
Intestinal %.	3.81	2.92	3.51
Abdominal fat weight.	4.03 ± 0.62	3.36 ± 0.55	3.94 ± 0.62
Abdominal fat %.	1.86	1.53	1.75

Differences between treatments were insignificant.

Fig. (1): Small intestine (duodenum) of Japanese quail from control group, showing the Villi (**V**), lining mucosal epithelium with goblet cells formation (**arrow**) and intestinal crypts (**C**). HSE X 40

Fig. (2): Small intestines (duodenum) of Japanese quail from control group, showing sever congestion in serosal layer (**bV**). HSE X 40

Table (4): Antibody titers against sheep red blood cells (SRBCs) expressed as Geometric mean titer in Japanese quail treated with prebiotic (primary immune response and secondary immune response).

Time / Group	1st week after injection of antigen (RBCs)	2nd week after injection of antigen (SRBCs)	1st week after booster dose of antigen injection
	21d of age	28d of age	35d of age
Control (group 1)	2.1	2.5	2.8
Group2(0.5 g/ton)	2.8	2.8	4.9
Group 3 (1 kg/ton)	3	3	6.1

Table (5): Phagocytic activity (percentage and index) of quail macrophages treated with prebiotic at the end of experiment.

Parameter / Group	Phagocytic %	Phagocytic index
Control (group 1)	^{b} 53 ± 1.8	^{b} 0.15 ± 0.02
Group 2 (0.5 kg/ton)	^{a} 68 ± 1.9	^{a} 0.26 ± 0.01
Group 3 (1 kg/ton)	^{a} 71 ± 2.3	^{a} 0.31 + 0.01

a, b..., means in the same column with different letters are significantly different at ($P < 0.05$).

Table (6): Serum lysozyme activity (µg/ml) and nitric oxide (µ mol/ml) of quail treated with prebiotic at 2 days post first antigen injection and at 2 days post the booster dose of antigen injection (SRBCs).

Time / Group	Serum lysozyme µg/ml		Nitric oxide µm/ml	
	2 days post first SRBCs injection (at 16 days of age)	2 days post the booster dose SRBCs injection (at 25 days of age)	2 days post first SRBCs injection (at 16 days of age)	2 days post the booster dose of SRBCs injection (at 25 days of age)
Control (group 1)	61 ± 7	37 ± 3.2	21 ± 1.3	19 ± 1.6
Group 2 (0.5 kg/ton)	68 ± 8	48 ± 5.5	21 ± 1.5	21 ± 1.8
Group 3 (1 kg/ton)	75 ± 7	48 ± 5.5	21 ± 1.3	21 ± 1.3

No significant different among groups at $P < 0.05$ using least significant difference (LSD).

Group of birds administrated 0.5g prebiotic/ kg diet show best results of histopathological test than control group and group supplemented with 1g prebiotic/kg diet. The length of the villi was 10 microns while, the crypts showed 8 microns length and 1.5 microns width with thick muscular layer and massive number of goblet cells formation in the mucosal lining epithelium (Fig.3, 4, 5 and 6). Regarding the group of birds administrated 1g prebiotic /kg diet, there was narrowing and adhesion between the villi with average length of 6 microns whereas, the crypts had 5 microns length and one micron width or might be absent associated with inflammatory cells infiltration in the lamina propria of the mucosal layer and sever congestion in the serosal layer. This finding may refer to some defaults happened during taking the samples and processing (Fig. 7, 8 and 9).

DISCUSSION

Mannanoligosaccharide is one from prebiotics which is used to replace antimicrobial growth promoters (AGP) as well as produced the positive effect in immunostimulation and disease prevention in fish (Li *et al.*, 2005 and Walker *et al.*, 2007).

When the results of body weight gain (BWG) were evaluated, it was seen that the highest body weight gain ($P<0.05$) occurred in the third treatment group. The BWG values calculated in this study are consistent with the previous findings in quail, turkey and broilers (Fritts and waldroup, 2003 ; Guclu, 2003 and Flemming *et al.*, 2004), respectively . Also, Guclu (2003) reported that addition of mannanoligosaccharide (MOS) to quail diets at a level of 0.75 and 1 g / kg feed enhance feed conversion ratio. The feed / gain ratio values calculated in this study are consistent with the previous finding (Flemming *et al.*, 2004). According to Savage and Zakrzewska (1997) and Waldroup *et al.*, (2003), mannan oligosaccharides improved feed conversion rate significantly in broilers and in turkey.

There were no significant differences among the groups on carcass yields in quail. This study also supports, the literature reported by Fritts and waldroup (2003) and Guclu (2003).

Fig. (3): Small intestines (duodenum) of Japanese quail from group two (500g prebiotic/ton diet), showing long villi (V) and wide crypts (C). HSE X 40

Fig. (4): Small intestines (duodenum) of Japanese quail from group two (500g prebiotic/ton diet), showing intact thick muscular layer (m). HSE X 64

Fig. (5): Small intestines (duodenum) of Japanese quail from group two (500g prebiotic/ton diet), showing wide crypts **(C).** HSE X 80

Fig. (6): Small intestines (duodenum) of Japanese quail from group two (500g prebiotic/ton diet), showing diffuse goblet cells formation allover the mucosal lining epithelium **(arow).** HSE X 80

Fig. (7): Small intestines (duodenum) of Japanese quail from group three (1000g prebiotic/ton diet), showing narrow crypts **(C).** HSE X 40

Fig. (8): Small intestines (duodenum) of Japanese quail from group three (1000g prebiotic/ton diet), showing inflammatory cells infiltration **(m)** in lamina propria of the mucosal layer. HSE X 64

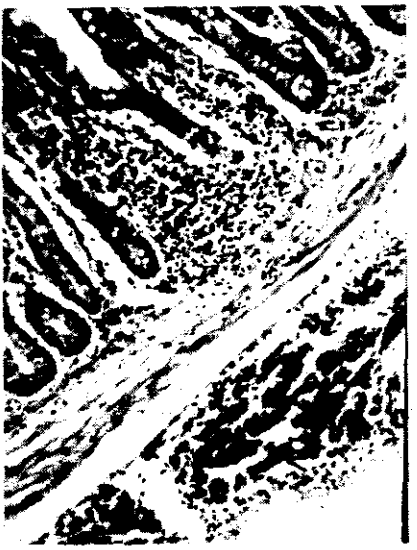

Fig. (9): intestines (duodenum) of Japanese quail from group three (1000g prebiotic/ton diet), showing sever congestion in serosal layer (bV). HSE X 64

Immunoglobulines play an important protective role by acting as opsonins to enhance phagocytosis, stimulating the complement pathway or blocking pathogens from adhering to mucosal surface (Singh *et al.*, 2008), therefore, the humoral immune response of prebiotic against (T-dependent antigen) sheep red blood cells, had been evaluated in the current study which indicated that prebiotic treated groups exhibited high-level of antibody titers. The greatest value of antibody titers was prominent at 35 day of age post secondary immunization in group (3). This finding agrees with Haschke *et al.*, (2001) who recorded that fructooligosaccharide mix. with cereals, significantly improved antibody response to measles vaccine in infants. Also, White *et al.*, (2002) and Hosono *et al.*, (2003) recorded that addition of prebiotics to animal diets improve mucolal immune system function, particularly increase levels of immunoglobulin in serum of pig and in the intestinal lumen of mice respectively. Roller *et al.*, (2005 and 2007) found that rats fed prebiotic inulin enriched with oligofructose for 4 weeks, increased secretary immunoglobulin A (IgA) in the cecum as well as enhanced production of interleukin-10 in peyer's patches. Moreover, Benyacoub *et al.*, (2008) studied the effect of prebiotic on murine response to salmonella vaccine, results indicated significantly increase in specific immunoglobulin G (IgG) and fecal IgA, the authors reported that prebiotic in diet acts as mucosal adjuvant. Furthermore, Gou *et al.*, (2008) suggested that mannan functionalized nanoparticles might enhance the humoral immune response against target antigen and it may has a great potential application in vaccine delivery system.

In contrast, Vos *et al.*, (2006) reported that dietary supplementation of prebiotic oligosaccharides in influenza vaccination using mice, resulting in non significant changes in specific antibody production and splenocyte proliferation.

Regarding to macrophage activity (phagocytic % and index), results obtained herein agree with Li *et al.*, (2004) who found that supplementation of prebiotics (Grobiotic A and brewers yeast) to fish enhanced respiratory burst of head kidney leucocytes as well as increased resistance against streptococcus iniae infection. Trushina *et al.*, (2005) reported that rats fed diet containing prebiotic (inulin and oligofructose) resulting in increased activity of peritoneal macrophages that confirmed by enhance phagocytosis and superoxide anion, enlargement of a number of T-cells and increasing of major histocompatability complex (MHC-II)

molecule on the surface of an antigen-presenting cells as well as increase interleukin-2-and 4. Besides, Benyacoub *et al.*, (2008) noticed significant increase in peritoneal macrophage phagocytic activity in mice fed diet supplemented with prebiotic as well as increase in production of cytokines, γ interferon, interleukin 12 and tumor necrosis factor alpha. Although mechanism of action by which prebiotic impacts immunity still not fully understood, Nevertheless, Brandtzaeg *et al.*,(2001) and Rescigno *et al.*, (2001) speculated one possible direct interaction of prebiotic with macrophage and/or dendretic cells underlying the gut mucosa either via transfer through epithelium or direct sampling in the lumen by dendretic cells, that can penetrate the gut epithelial monolayer. Dietary supplementation of manonoligosaccharide to marron, improves the health status and immunity of marron under bacterial infection (Sang *et al.*, 2009).

In respect to lysozyme activity, there was no significant change among groups. Lysozymes are proteins of low molecular weight found in polymorphnuclear cells and synthesized also in mononuclear cells. Lysozymes are considered as a number of innate humoral factors that elaborated from the body and showed a dramatic increase in their concentration (Weir, 1983). Similar result was obtained by Li *et al.*, (2005) who found that dietary supplementation of prebiotic to fish, did not show significant alteration in serum lysozyme activity.

Concerning serum nitric oxide, results did not reveal significant difference in prebiotic treated groups compared to control. These results may be due to that prebiotic had no notable effect in these parameters.

Overall, administration of prebiotic to Japanese quail diet improved growth performance, had potent immunostimulating effect that confirmed by increase antibody response, macrophage phagocytic activity while lysozyme activity and nitric oxide did not affected. These results suggest that prebiotic can be used as growth promoter and immunoenhancing agent in poultry production to protect against infection and increase vaccination efficiency.

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تأثير البريبيوتك علي اداء النمو والاستجابة المناعية في كتاكيت السمان الياباني

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الملخص العربي

اجريت هذه الدراسة بهدف معرفة تأثير اضافة البريبيوتك علي النمو؛ الاستجابة المناعية، إختبار الهستوباثولوجي، وصفات الذبيحة في السمان الياباني.

استخدم في هذه الدراسة ١١٧ كتكوت سمان ياباني عمر يوم وتم توزيع الطيور عشوائياً في البطاريات إلي ثلاثة مجموعات متساوية العدد (٣٩ طائر) وكل مجموعة قسمت الي ثلاثة مكررات كل مكررة احتوت علي ١٣ كتكوت. تغذت المجموعة الاولي (كنترول) علي عليقة الكنترول حسب ال NRC 1994 والمجموعة الثانية علي عليقة الكنترول مضافاً اليها نصف جرام الفاميون/كجم علف. والمجموعة الثالثة علي عليقة الكنترول مضافاً اليها واحد جرام الفاميون/كجم علف. ربيت جميع الطيور تحت ظروف واحدة وقدم لها العلف والماء بحرية طول فترة التجربة التي استمرت لمدة ٥ اسابيع.

ويمكن تلخيص النتائج المتحصل عليها في التالي:

- الزيادة في وزن الجسم كانت اعلي معنوياً للمجموعة الثالثة التي تغذت علي عليقة احتوت علي ١٠٠٠ جم الفاميون/طن علف تلتها المجموعة التي تغذت علي ٥٠٠ جم الفاميون/طن علف مقارنة بمجموعة الكنترول. علما بأنه لم يوجد هناك اي اختلافات معنوية بين المجموعات في كمية الغذاء المأكول.
- معدل تحويل الغذاء كان افضل معنويا لصالح المجموعات المعاملة بالبريبيوتك مقارنة بمجموعة الكنترول. ايضا لم يكن هناك تأثير للمعاملة بالبريبيوتك علي صفات الذبيحة مقارنة بمجموعة الكنترول.
- بالنسبة للاستجابة المناعية كان هناك زيادة معنوية في الاجسام المضادة للمجموعات التي تغذت علي البريبيوتك خاصة بعد حقن الانتجين في الجرعة التنشيطية. ايضا اظهرت نتائج Phagocytic % and phagocytic index للمجموعات المغذاه علي البريبيوتك زيادة معنوية مقارنة بمجموعة الكنترول بينما لم يكن هناك اختلافات معنوية في Lysozyme activity and nitric oxide وإن كانت قيم المجموعات التي تغذت علي البريبيوتك اعلي من مجموعة الكنترول.
- اظهرت نتائج الهستوباثولوجي ان المجموعة التي تغذت علي ٥٠٠ جم الفاميون/طن علف كانت الافضل في ارتفاع الخملات وعرض المنخفضات (Crypts) وعدد الخلايا الكأسية مقارنة بمجموعة الكنترول.

اوضحت الدراسة ان اضافة الألفاميون بمعدل ٥٠٠ او ١٠٠٠ جم/طن علف ادي الي تحسن وزن الجسم، كفاءة تحويل الغذاء وتحسين الاستجابة المناعية فضلا عن دوره فى الحماية ضد العدوى وزيادة فعالية التحصينات وتحسين الامتصاص من خلال الامعاء مقارنة بمجموعة الكنترول.