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RESEARCH ARTICLE

The Study of Different Growth Promoters on Growth Performance, Intestinal Bacteriology and Haematological Chracteristics

Raed Hussein Salih Rabee[1] and Yahya Sabah Abdulameer[2]

[1]Department of avian pathology, Faculty of Veterinary Medicine, Al Qasim Green University, Babylon, Iraq.
[2]Department of Animal and Poultry Public Health, Faculty of Veterinary Medicine, Al Qasim Green University, Babylon, Iraq.

Abstract

Probiotics are rich sources of viable microorganisms (bacteria or yeasts) and these have a beneficial effect on host health after ingestion. This trial was conducted to assess the effects of growth performance of broilers fed on diets containing different growth promoters. A total of 200 male chicks was allotted into four dietary treatments in which they were fed none, antibiotic (Virginiamycin), probiotic (*Lactobacillus plantarum* K KKP 529/p, 1.0×10^6 (cfu/g) or Thymus vulgaris. The growth performances and Lymphocyte cells were the highest in probiotic and Thymus vulgaris ($p<0.05$) compared with control. The probiotic and herbal plant caused significant decrease ($p<0.05$) of hetrophile and reduced of H/L Ratio ($P<0.05$). The *Eschertia coli* and total bacteria count were lowered with growth promoters when compared to control ($p<0.05$). These findings confirm the valuable impact of growth promoters on growth performance, haematogical characteristics and on reducing intestinal bacteriology.

Keywords: Bacteriology, growth promoter, performance, haematological.

***Correspondence:** yha_sabir@yahoo.com; 0064-7811160174

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INTRODUCTION

Now a day, there are limitations on the use of antibiotic because of its resistance development after prolong consumption. So there is constant search and development for the new other alternatives such as prebiotics, probiotics and phytobiotics[1-4]. The principle site for digestion, nutrient assimilation and energy harvest is small intestine. These absorbed nutrients are directly contributing to the body weight gain. While, in large intestine water is absorbed and finally, indigestible food is dried out. Therefore, the microbiota from the small and large intestine are varied significantly. Probiotics are rich sources of viable microorganisms (bacteria or yeasts) and these have a beneficial effect on host health after ingestion. Several studies have reported that the addition of probiotics to the diets of broilers leads to improved their weight and performance[5,6]. Probiotics is defined as a nondigestible food ingredient which has beneficial activities on the host. Probiotic has a selective activity for stimulating and activating the growth and metabolism of a number of health promoting bacteria in the intestinal tract, which in turn improves hosts microbial balance.

Growth promoters may be required for treating intestinal problems after banning the use of antibiotics as growth promoters. The intestinal tract of chicks provides a suitable ecological niche for pathogens[7]**.** In this regard, the use of pre/pro-biotic and photobiotic increase the rapid colonization of beneficial bacteria in the intestine[8,9]. The role of probiotics and phytobiotics has been well documented in regards to the development of the intestinal mucosa as a result of the early colonization of normal micro-flora in the intestine[10-11]. Although growth promoters are widely used in the production of poultry, it is not yet known whether this use under Iraqi field conditions has an impact on productivity or haetological characteristics. This study was conducted to evaluate the effectiveness of growth promoters under Iraqi farm conditions on growth performance, intestinal bacteriology and haetological characteristics in broiler chickens.

MATERIALS AND METHODS

Animals and housing

The experiment was carried out in the Poultry Section of the Research Institute of the University of AL Qassim green. A total of 200 newly hatched male Ross 308 broiler chicks were purchased from a local hatchery and the birds were weighed and randomly divided into four treatments according to a completely randomized design. Each dietary treatment had five repetitions with 10 birds, which were kept for 40 days in litter pens with a 1.2 × 3 meter dimension under standard management practices and environmental conditions. The temperature was regulated at 32 ± 1°C in the first week and reduced by 3°C per week to arrive at 21°C in the third week. Each pen was equipped with an aspirate feeding trough and water was supplied via nipple drinkers. A continuous lighting schedule was used throughout the experimental period.

Experimental diets

The broilers were fed according to the following feeding programme: starter 1–21 days, grower 21–35 days, and finisher 36–40 days. Feed and water were provided *ad libitum*. Feed for all groups were made in mashed shape from the same ingredient materials (Table 1, Calculated on an as-fed basis). Nutrients in the diets were formulated using the User-friendly Feed Formulation, Done Again, programmed by J. Hargrave, University of Georgia, Athens, GA, USA) and were balanced to be iso-caloric and iso-nitrogenous in order to meet the nutritional requirements of the birds in accordance with the Ross 308 recommendations for all nutrients[12]. The dietary treatments were as follows:

1. Basal diet (as controls)
2. Basal diet + 20 mg/ kg Virginamcin (Ab)[13]
3. Basal diet + 1.0 g/kg *Lactobacillus plantarum* K KKP 529/p® (a commercial probiotic including *Lactobacillus plantarum,* 1.0×10^6 cfu/g) (Pro)
4. Basal diet +10 g/kg *Thymus vulgaris* 10 % (a commercial herbal plant).

Measurements

Body weight and feed were recorded at the time of placement and then biweekly for approximately 40 days per pen. The body weight

Table 1. Ingredient profile (g/kg) and calculated nutritional composition (g/kg unless otherwise stated) of experimental Basal diets (on an as-fed basis)

Ingredients (g/Kg on as fed basis)	Starter (1-14 d)	Grower (15-28d)	Finisher (29-40d)
Corn	537.2	572.1	640.0
Soybean Meal	393.0	360.0	290.0
Vegetable Oil	25.0	28.0	30.4
Calcium Di-phosphate	19.0	16.5	15.5
Calcium Carbonate	12.5	11.0	11.5
Salt	3.9	3.9	3.7
DL-methionine	2.4	1.9	2.0
Lysin HCl	2.0	1.6	1.9
Vit. + Min. premix*	5.0	5.0	5.0
Calculated Chemical Composition (g /Kg)			
Metabolisable energy (MJ /Kg)	12.13	12.55	12.76
Crude protein	219	207	182
Total arginine	15.02	14.18	12.30
Total lysine	13.35	12.25	10.75
Total methionine	5.86	5.24	5.03
Total methionine + cystine	9.25	8.48	7.87
Total threonine	8.85	8.43	7.90
Calcium	10.3	9.1	8.9
Available phosphorus	5.0	4.5	4.2
Sodium	1.7	1.7	1.6

*Each kg of the vitamin and mineral supplied: Vitamin A (retinyl acetate, 13000 IU); Vitamin D3 (cholecalciferol, 4000 IU); Vitamin E (DL-α-tocopheryl acetate, 80 IU); Vitamin K (menadione sodium bisulfite, 3 mg); Riboflavin (6.0 mg); Pantothenic acid (6.0 mg); Niacin, (20 mg); Pyridoxine (2 mg); Folic acid (0.5 mg); Biotin (0.10 mg); Thiamine (2.5 mg); Vitamin B_{12} (20 μg); Mn (120 mg); Zn (90 mg); Fe(30 mg); Cu (10 mg); I (1.5 mg); Se (0.2 mg); ethoxyquin (100 mg).

gain (BWG), Feed Conversion Ratio (FCR), feed intake (FI) were calculated at 14, 28 and 40 days. Mortality was recorded as it occurred.

Blood haematological and microbiological analysis

On day 35 of rearing, 8 broilers (males) were taken from each treatment for slaughter. Blood was sampled from the wing vein for haematology. Blood samples (3 mL) were put into anticoagulant tubes for blood pictures. Leukocytes (WBC) were counted with a cell-counter (Nihon Cohden Cenltax–MEK-6450K) Japan (without division into fraction) WBC was assessed by calculating their numbers using the chamber method. A fecal samples of ileo cecal junction was collected for microbial analysis. The fecal samples were weighed and diluted 10 times with normal saline, and then again diluted from 10^{-1} to 10^{-5}. From the last three tubes 0.1 mL was put onto the suitable agar media for counting intestinal microbiota (in triplicate). Isolation and enumeration microbiological slides consists of the following indicators:

Total number of *Enterobacteriaceae* – VRBG medium (BioMérieux, France), deep culture, incubation at 37°C; *Eschertia coli* – TBX Agar medium (Bio-Rad, France), 42°C, deep culture.

Statistical Analysis

The statistical differences in mean values were determined using the ANOVA test and LSD test. The significance level was α =0.05 (Analysis of variance, ANOVA), SPSS/17 statistical package[14].

RESULTS

Growth performance

The results presented in Table 2 show that the effect of growth promoters (GPs) on BW was significant (P< 0.001) throughout the

experimental period; for example, adding the diet with antibiotic caused a significant increase in body weight when compared to the control diet. The difference between GPs (antibiotic with probiotic and herbal plant) and control group was significant at both 28 and 40 days of age. At the end of the experiment, the lowest BW belonged to the birds that were fed a PRO diet. The effect of GPs on FI was significant during days 29-40 (P< 0.01) and days 1-40 (P<0.01). The diet contained antibiotic increased FI in comparison to the control and probiotic groups after 28 days. Meaning that the birds that were fed a diet with antibiotic consumed the highest amount of feed, while the lowest FI was related to the birds that were fed a diet supplemented with probiotic. Adding the diet with antibiotic reduced FCR when compared to probiotic and herbal during days 15-28 (P<0.05) and days 1-40 (P<0.001) (Table 3). At the end of the experiment, the highest FCR belonged to the birds that were fed a diet including antibiotic and the lowest FCR belonged to the birds that received the herbal plant.

Haematology findings

Effect of growth promoters on WBC number and differential count and Heterophil to Lymphocyte Ratio (H/L) in broiler chickens reared for 40 days revealed in Table 4.

Table 2. Effects of different growth promoters on body weight and feed intake of Broilers[1] (1-40 days of age)

Treatments	Body weight (g/bird)			Feed Intake (g/bird)			
Growth Promoters	14 d	28 d	40 d	1-14 d	15-28 d	29-40 d	1-40 d
-	471	1594^{b}	2899^{bc}	509	1672	2416^{bc}	4467
Antibiotic	506	1697^{a}	3063^{a}	544	1716	2528^{a}	4669
Probiotic	492	1611^{ab}	2869^{c}	552	1690	2400^{c}	4516
Herbal plant	492	1623^{ab}	2888^{bc}	549	1714	2441^{abc}	4602
Pooled SEM	9.6	38.2	37.3	13.4	19.3	28.6	59.0
Main effects				Significance			
Growth Promoters	*	**	***	NS	NS	**	NS

[1]Values represent the means of 5 repetitions of 10 birds per treatment,[a-c] means that a column with the same superscript is not significantly different ($P < 0.05$), Pooled SEM = Pooled Standard error of means, N.S, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 3. Effects of different growth promoters on feed conversion ratio (FCR) and productivity efficiency index (PEI) of Broilers[1] (1-40 days of age)

Treatments	FCR (g/g)			
Growth promoters	1-14 d	15-28 d	29-40 d	1-40 d
-	1.190	1.505	2.123	1.614^{bcde}
Antibiotic	1.174	1.461	2.037	1.580^{e}
Probiotic	1.226	1.512	2.193	1.637^{abc}
Herbal plant	1.223	1.528	2.169	1.653^{ab}
Pooled SEM	0.020	0.028	0.049	0.014
Main Effect			Significance	
Growth Promoters	*	*	NS	***

[1]Values represent the means of 5 repetitions of 30 birds per treatment,[a-e] means that a column with the same superscript is not significantly different ($P < 0.05$), Pooled SEM = Pooled Standard error of means, N.S, *$P > 0.05$, **$P < 0.01$, ***$P < 0.001$.

The effect of different Gps on all the types of WBC count was not noticed with Feed additives groups (Virgianmycin, probiotic and herbal plant (*Thymus vulgaris*) at (P < 0.05) on compared with control group (non-treated group). While Lymphocyte was the highest in probiotic and herbal plant (p<0.05) compared with control. The probiotic and herbal plant caused significant decrease (p<0.05) of Hetrophile and reduced of H/L Ratio (P<0.01) especially with plant plant.

Intestinal bacteria enumeration

Growth promoters reduced (p<0.05) the total bacteria count, in addition the pathogenic bacteria was lower (p<0.01) with Growth promoters compared to control. Beneficial bacteria significantly reduced (p<0.05) with Virginiamycin (Table 5).

Table 4. Effect of different growth promoters on hematological variables in Ross 308 Broilers

Treatments	White blood cell ($\times 10^4$)	Lymphocytes (%)	Heterophil (%)	Heterophil/(%) Lymphocyte
-	1.414	58.92^{b}	35.69^{ab}	0.61^{ab}
Antibiotic	1.400	62.15^{ab}	33.23^{ab}	0.57^{ab}
Probiotic	1.453	63.07^{ab}	32.33^{ab}	0.52^{ab}
Herbal plant	1.600	64.71^{a}	30.00^{b}	0.48^{b}
Pooled SEM	0.124	1.72	1.88	0.04
		Significance		
Main effect	Growth Promoters	NS	**	* **

$^{A-C, a-b}$ means within the same columns with no common superscripts have significant differences. SEM = Standard error of means, NS = not statistically significant,*P ≤ 0.05, **P ≤ 0.01

Table 5. Effects of different growth promoters on o microbial analysis of intestinal content after 40 days of broiler (CFU g^{-1})

Treatments Main Effects	Total Count	*E. coli*
-	12.60^{a}	14.1^{a}
Antibiotic	7.97^{ab}	4.87^{b}
Probiotic	3.23^{b}	2.06^{b}
Herbal plant	1.57^{b}	2.98^{b}
Pooled SEM	2.91	2.64
Significance		
GPs	*	**

[1]Values represent the means of 10 birds per treatments, ileoceacal digesta was taken from 2 birds per pen on day 25 under hygienic condition. GPs: Growth promoters, Antibiotic (Virginamycin), Herbal plant (*Thymus vugaris),* SEM= Standard error of means, NS: Not-statistically significant. $^{a-b,}$ means within a column that do not share common superscripts have significant differences. *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001.

DISCUSSION

Growth performance

The mode of action of virginamycin as growth promoter (GP) may be attributed to the reduced thickness of the intestinal wall and thus increase in the absorption of nutrient[15]. These results were in agreement with previous reports. Ao et al.[16] found that feed additives with prebiotic (Oligossaccharide), antibiotic resulted in improved BW and FCR. Many studies have shown that use of antibiotic as growth promoters have a positive impact on the growth of broiler chickens[11,17-19]. In addition, Riyaza et al.[20] showed that of antibiotic (Avilamycin 150 ppm), probiotic (150 ppm) and herbal plant had no positively affected the FCR; BW; BWG; FI and mortality. The response to probiotic or herbal plant was not observed on growth performance and may be attributed to hygienic condition. The efficacy of dietary herbal plant can be affected by intrinsic and extrinsic factors such as nutritional status of animals, infection diet composition and environment[21]. This result is similar to Ashayerizaden et al.[22] who pointed out that growth parameters were not effected by probiotic and prebiotic compared with control. This finding may be correlated with practice processes and environmental condition of experiment. In broilers that are well nourished, healthy status may not respond significantly to natural GP[23]. The obtained data supported those reported previously by Shams et al.[24] stated that there was no significant effect of probiotic and phytobiotic on BWG and FI in the starter, grower, finisher and entire period (0-40 days) compared with control. Sadeghi et al.[25] reported that herbal plant mixture which contained thyme, turmeric and cinnamon did not effect on growth performance of male broiler chicken. Diarra et al.[26] explained that high fibre content of some herbal plants may be a reason for no significant difference in growth performance when compared with basal diet. The results showed that the growth promoters, particularly antibiotic, were able to improve growth parameters. These results corroborate those of a previous study which pointed out that some of the GPs may improve the performance of birds[11,27]. In contrast, the natural growth promoters results (probiotic and Herbal plant) were inconsistent with Mokhtar et al,[18]

studied the effect of natural growth promoters on growth performance and ileal mucosal development, they showed that GP increased BW and decreased FCR.

Haematology parameters

The role of growth promoters in alleviation of stress factors which were caused by unhealth condition that measuring by WBC and H /L ratio are documented[28,29]. Concerning the results of GP on WBC count and lymphocyte, heterophils and H/L ratio revealed that Lymphocte count of chicks fed diet containing probiotic and herbal plant had recorded significant increase ($p<0.01$) and reduced hetrophils and L/H ratio, but the WBC count was not altered with GPs. Data of this experiment was in agreement with that of Ali[30] who concluded that herbal plant (*Digestarom*) did not alter WBC count in broiler chicken. Rahimi et al.[31] evaluated the effect of dietary supplementation of antibiotic and probiotic on bird under heat stress condition. It revealed that probiotic supplementation at 0.1 % decreases heterophil to lymphocytes compared to control. A low ratio of H/L with natural growth promoters (probiotic, herbal plant) in this study was an indicator of low level of stress. However, some author reported that the herbal plant extract had no significant effect on proportion of H and L, as well as H/L ratio[32].

Intestinal bacteriology

The current study findings suggest that pathogenic bacteria are decreased by the dietary addition of growth promoters. This result is noted by Guo et al.[33,34] concluded that herbal plants reduced the number of *Bacteroides* spp and *E. coli* in ceca. Rahimi et al.[31] reported that herbal plant (*Thyme*) reduced number of *E.coli* in ilea cecal section. Furthermore, growth promoters (antibiotic, probiotic and herbal plant) have all documented to minimize the colonization of the intestine by pathogens and subsequently reduction the incidence of some diseases with varying levels of success[25,35-37]. One other reasons to reduce the number of pathogens, they could attached with the probiotics or fibre in herbal plant instead of attaching to intestinal epithelial cells and, therefore, move through the intestine without colonization[11].

CONCLUSION

The Avalmycin or herbal plant (*Thymus vulgaris*) can be used in Iraqi field condition for increasing BW and enhancing the feed conversion of broiler chickens. Also, using feed additives such as probiotic and herbal plants especially after banned of antibiotic can use for reducing the gut bacteria.

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