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IMPACT OF DIETARY PROBIOTIC ON GROWTH PERFORMANCE, CARCASS TRAITS, SERUM BIOCHEMICAL CONSTITUENTS AND IMMUNE RESPONSIVENESS IN BROILER RABBITS

Al-Homidan, I. H.[1], S. S. Alharbi[1], M. F. Aljtayli[1], M. M. Fathi[1,2], T. A. Ebeid[1,3], and O. K. Abou-Emera[1,4]

[1]Anim. Poult. Prod. Dept., Coll. Agric. Food, Qassim Univ., Al-Qassim 51452, Saudi Arabia
[2]Poult. Prod. Dept., Fac. Agric., Ain Shams Univ., Hadayek Shoubra, 11241 Cairo, Egypt
[3]Poult. Prod. Dept., Fac. Agric., Kafrelsheikh Univ., Kafr El-Sheikh 33516, Egypt
[4]Poult. Breed. Dept., Anim. Prod. Res. Inst., Agric. Res. Center, Dokki, Giza, 12618, Egypt

Corresponding author: M. F. Aljtayli Email: mfjutil@yahoo.com

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ABSTRACT: The objective of the present study is to investigate the impact of dietary probiotic administration on growth parameters, carcass parameters, serum biochemical constituents and immune responsiveness in growing rabbits. A total number of 54 rabbits aged 4 weeks were allocated randomly into three experimental treatments with levels of dietary probiotic (0, 1 and 2%). The probiotic containing 2.67 x 10^{11} CFU/g of *Bacillus licheniformis*. There were 9 replicates of each dietary treatment with 2 rabbits each. Dietary probiotic had no significant effect on body weight gain, feed conversion ratio, carcass % and carcass parts percentages including front%, mid%, hind %, skin %, and head%. Also, dietary probiotic supplementation had no significant impact on internal organs including heart, testes, lunges, kidney and thymus, while liver % was reduced due to dietary 2% probiotic in growing rabbits. Probiotic supplementation had insignificant impacts on all blood biochemical traits including total protein, albumin, globulin, cholesterol, triglycerides and urea in fattening rabbits. Dietary probiotic supplementation had a beneficial impact on cell-mediated immunity at 24 h post-injection of phytohemagglutinin P (PHA-P) into the left ear in growing rabbits. It could be concluded that dietary probiotic had a positive impact on cell-mediated immunity, while growth parameters, carcass traits, and serum biochemical constituents were not affected.

Keywords: Probiotics, growth performance, cellular immunity.

INTRODUCTION

Antibiotics were utilized as growth promotors in poultry and animal farming for a long time. However, overuse of antibiotics has resulted in pathogenic bacteria developing resistance and antibiotic residues in poultry products, both of which are harmful to individual health (Gadde et al. 2018). Thus, since 2006, it has been illegal to use antibiotics to promote growth; instead, their usage is limited to vital medicinal purposes. Natural growth promoters, such as probiotics, prebiotics, organic acids, phytobiotics, and enzymes, have gained attention as antibiotic substitutes. However, stopping the use of antibiotics has serious consequences, including poor animal performance, increased susceptibility to enteric illnesses, and nutrient malabsorption (Ebeid et al., 2021). Consequently, there is an increasing need to find effective substitutes to stop the spread of resistant germs and fight infectious diseases.

In order to promote growth and stability against digestive issues, the weaning and post-weaning phases are crucial for rabbits (Fortun-Lamothe and Boullier, 2007). According to Pogány Simonová et al. (2020), probiotics are live microbes which are introduced to the diets of rabbits to modify the gut microbiome and improve health and performance. Probiotic microorganisms typically administered to rabbits include bacteria (such Lactobacillus, Lactococcus, Bacillus, Bifidobacterium, and Enterococcus fecium), yeasts (like Saccharomyces cerevisiae), and fungi (like Aspergillus awamori). Supplementing with probiotics improved gut health and growth performance (Abdel-Wareth et al., 2021; Nwachukwu et al., 2021). Additionally, probiotics enhanced immunological responsiveness (Guo et al., 2017) and antioxidative qualities (El-Deep et al., 2021). Abdel-Azeem et al. (2018) observed that oral administration of 0.25 or 0.5 ml probiotic (ZAD®)/rabbit improved final live body weight, body weight gain (BWG), and feed conversion ratio (FCR), nutrients digestibility (crude protein, ether extract, and crude fiber), carcass parameters (dressing percentage, giblets percentage, and total edible parts percentage), and cecum volatile fatty acids concentration as well as elevated the abundance of *Lactobacillus* spp. and minimized total coliforms and total anaerobic bacteria in cecum of growing rabbits. The aim of the current study was to examine the influence of dietary probiotic administration on growth performance, carcass characteristics, blood biochemical constituents and immune response in growing rabbits.

MATERIALS AND METHODS

Rabbit Husbandry:

This study was carried out on one fifty four (54) Growing rabbits aged 4 weeks, which fed two levels of probiotic (1% and 2%) compared to the control group. Each treatment has (9) replicates; the number of rabbits within each replicate is 2 rabbits. The probiotic containing 2.67 x 10^{11} CFU/g of *Bacillus licheniformis*. The rabbits were placed in individual wire cages under lighting schedule of 16 h/d light cycle. All rabbits were reared under identical environmental, biosecurity and nutritional conditions. Feed for all rabbits were weighed throughout the experiment. The diet was formulated to contain approximately 18 % crude protein and 2050 kcal/kg ME in a standard rabbit diet and was mixed every week. The composition and calculated chemical analysis of diets are presented in Table (1). The care and handling of the rabbits were in accordance with regulations of animal care committee of Qassim University.

Probiotic strains:

Description GalliPro® Tect WS contains a source of live (viable), naturally occurring microorganisms.

Guaranteed Analysis:

Bacillus licheniformis, minimum: 2.67 x 10^{11} CFU*/gram.

*Colony Forming Units

Ingredients: Maltodextrins, Dried *Bacillus Licheniformis* Fermentation Product.

Measurements and Observations

Growth performance measurements and feed conversion ratio

Body weight in gram was weekly determined until the end of the experiment on individual basis using a second decimal scale. To determine the feed efficiency, body weight in gram, feed intake (FI), weight gain and FCR were estimated on a weekly basis. The FCR (kilograms feed intake per kilograms BW gain) was calculated for each replicate within each treatment. Feed consumption and FCR were adjusted for mortalities when appropriate.

FCR = Total feed intake / Total body weight gain

BWG = Body weight – Initial weight

Carcass dissection

At the end of 8 week of age, 20 rabbits from each treatment group were randomly assigned, individually weighed and slaughtered after 8h of fasting, then carcass washed, drained and eviscerated for carcass yield measurements. Live body weight, dressed eviscerated carcass (in which intestine, inner organs were removed), carcass, skin, head, front, mid, hind, liver, testes, heart, lungs, kidney, thymus were dissected, weighed and calculated as a percentage of live body weight.

Blood plasma constituent's analysis and antioxidant profile:

At slaughtering, 2 blood samples were taken from each slaughtered rabbit containing 2.0 ml each, with a total number of 120 blood samples. Blood serum was separated (centrifuged at 3000 rpm for 15 min), and plasma obtained was stored at -20C° until later analysis. The frozen plasma was thaw prior to analysis. Plasma total protein, albumen, total cholesterol, triglyceride and urea were determined using spectrophotometer with commercial kits (Biodiagnostic, diagnostic and research reagents, Dokki, Giza, Egypt). The globulin was calculated as the difference between the total protein and albumen.

Evaluation immune response profile:

Cell-mediated immunity assay- PHAP

The cutaneous basophil hypersensitivity test, a measure of T-cell mediated response, was evaluated in vivo by injection of the mitogen phytohemagglutinin-P (*PHA*-P) into the ear. 10 rabbits aged 6 weeks were randomly assigned from the considered groups (3 groups/ 20 each). Each rabbit was intradermally injected in the left ear with 100 µg phytohemagglutinin-P (PHA-P) (Sigma Chemical Co., St. Louis, MO 63178) in 0.1 ml sterile saline. The thickness of the ear was measured with a constant tension caliper preinjection and at 24, 48 and 72 h postinjection. The ear swelling was calculated as the difference between the thickness of the web before and after injection.

Statistical Analysis

Data were subjected to a one-way ANOVA with probiotic in feed supplementation (P) using SAS Ver. 8.2 (SAS, 2002). The model applied is as follows:

$Y_{ijk} = \mu + P_i + e_{ij}$

Where:

Y_{ij} = Trait measured,

μ = Overall mean,

P_i = Probiotic In feed level,

e_{ij} = Random error assumed to be independent normally distributed with mean = 0 and variance = σ^2.

All results are presented as mean and the pooled SEM. The significance of difference among the groups was assessed using Duncan's multiple-range test. Significance was set as P<0.05.

SAS institute (2000). JMP statistics and graphics guide. Ver. 8.2, SAS Institute Inc, Cary, NC.

RESULTS AND DISCUSSION

Results shown in Table (2) illustrated the influence of probiotic on FI, BWG, and FCR in growing rabbits. No significant impacts on FI at 5,7, and 8 weeks old, while overall FI was reduced due to using the 1% probiotic. Dietary probiotic supplementation had no significant effect on BWG and FCR. As shown in Table (3) inclusion of probiotic had no significant effect on BW during the whole fatting period. These results agree with other trails which indicated that probiotics did not have a positive impact in rabbit productivity (Fathi et al., 2017; Tag El Din, 2019; Emmanuel et al., 2019). Fathi et al. (2017) also elucidated that dietary *Bacillus subtilis* (4.0×10^9 CFU/g) had no significant influence on final BW and BWG in fattening rabbits. Likewise, Emmanuel et al. (2019) reported that addition of *Saccharomyces cerevisiae* (0.12g/kg) did not alter final BW, BWG and FCR in broiler rabbits. However, these results are contrarily with several studies, which elucidated that, dietary supplementation of probiotics increased BWG, feed intake, FCR, and production efficiency factor in broiler rabbits (Kadja et al., 2021; Abdel-Wareth et al., 2021; Nwachukwu et al., 2021). Indeed, probiotics do not always have a positive effect on rabbit and poultry productivity. It might be indicated that different types of probiotics work differently and give varying results. The different results can be linked to differences in animal species, size, age, sex, environmental conditions, feed components, crude protein and energy levels (Ebeid et al., 2021).

The impacts of dietary probiotic addition on carcass traits of growing rabbits are summarized in Table (4). Dietary probiotic administration had no impact on carcass % and carcass parts percentages including front%, mid%, hind %, skin %, and head%. Also, dietary probiotic administration had no influence on internal organs including heart, testes, lunges, kidney and thymus, while liver % was reduced due to dietary 2% probiotic in growing rabbits. Also, numerous previous studies indicated that probiotics had no influence on carcass traits in broiler rabbits (Bhatt et al., 2017; Ayyat et al., 2018; Beshara et al., 2018; Abdel-Wareth et al., 2021). Bhatt et al. (2017) elucidated that dietary supplement of *Lactobacillus acidophilus* and *Lactococcus lactis* (1.0×10^7 CFU/g) on dressed weight, head weight, skin weight, kidney weight, heart weight, and lung weight in growing rabbis. Also, Abdel-Wareth et al. (2021) observed that supplying broiler rabbits' diets with 450 mg probiotics/ kg diet had no significant effects on carcass characteristics in growing rabbits. On the other hand, numerous studies elucidated that probiotics supplementation enhanced carcass traits and edible parts in developing rabbits (Mohamed et al., 2017; Fathi et al., 2017). Fathi et al. (2017) demonstrated that rabbits fed *Bacillus subtilis* (400 g/ton) improved carcass traits (carcass weight, dressing %, and cuts of mid part and hind part as a percentage of live BW) in growing rabbits.

Results of blood biochemical measurements as influenced by probiotic administration are presented in Table (5). Probiotic supplementation had insignificant impacts on all blood biochemical traits including total protein, albumin, globulin, cholesterol, triglycerides and urea in fattening rabbits. These findings are in agreement with Fathi et al. (2017) who demonstrated that rabbits fed *Bacillus subtilis* (400 g/ton) did not affect plasma values of total protein, albumin, globulin, and triglycerides, while plasma cholesterol concentration was decreased in growing rabbits. However, Alderey et al. (2024) demonstrated that inclusion of 0.5 gm/kg dry yeast elevated serum contents of total protein, albumin, globulin, and albumin/ globulin ratio in growing rabbits.

Results tabulated in Table (6) demonstrated that dietary probiotic addition at the highest level (2%) had a helpful impact on cell-mediated immunity at 24 h post-injection of PHA-P into the left earcompared with low level (1%) of addition and control diet in growing rabbits. However, at 48 and 72 h post-injection of PHA-P no significant effects were observed. Likewise, Fathi et al. (2017) elucidated that addition of *Bacillus subtilis* (4.0×10^9 CFU/g) enhanced cell-mediated immunity at 48 h post-injection of PHA-P into the left ear in broiler rabbits. Also, Guo et al. (2017) observed that rabbits supplemented with *Bacillus subtilis* (1.0×10^6 CFU/g) had higher serum concentrations of immunoglobulins (IgG and IgA), greater index of immune organs (thymus % and spleen %), and higher expression of chief innate immunity genes participated in starting and regulating immune responsiveness including *IFN-γ*, *IL-1β*, *IL-4*, *IL-8*, α-defensin, and β-defensin in rabbits. It could be concluded that dietary probiotic had a positive effect on immune responsiveness, while growth parameters, carcass traits, and serum biochemical constituents were not affected.

Table (1): The composition and calculated chemical analysis of the diets.

Calculated chemical analysis	Content/ kg feed
Ash, %	7
ME, Kcal/kg	2050
Fats, %	3
Fiber, %	12
Moisture, %	8
Crude protein, %	18
Starch, %	19
Calcium, %	1
Available phosphorus, %	0.6
Sodium, %	1
Potassium, %	0.9
Manganese, ppm	60
Zinc, ppm	30
Copper, ppm	15
Iodine, ppm	1
Iron, ppm	25
Cobalt, ppm	0.3
Selenium, ppm	0.15
Vitamin A, IU	5000
Vitamin D, IU	500
Vitamin E, IU	10

Table (2) : Feed intake, body weight gain and feed conversion ratio means of rabbit fed different levels of probiotics.

Parameter	Probiotic Level (%)			SEM	Prob.
	0	1	2		
Feed intake at 4 week	550.58[a]	403.75[b]	528.57[a]	14.8	≤0.01
Feed intake at 5 week	599.94	582.50	604.52	13.1	NS
Feed intake at 6 week	570.29[a]	449.06[b]	543.31[a]	13.1	≤0.01
Feed intake at 7 week	550.31	551.25	563.33	12.7	NS
Feed intake at 8 week	531.25	571.56	547.22	13.1	NS
Overall feed intake	2811.19[a]	2548.00[b]	2795.67[a]	33.9	≤0.01
Body weight gain	757.31	723.13	784.76	24.6	NS
Feed conversion ratio	3.65	3.65	3.63	0.08	NS

Table (3): Weekly body weight means of rabbit fed different levels of probiotics

Parameter	Probiotic Level (%)			SEM	Prob.
	0	1	2		
Initial body weight	1392.00	1392.75	1399.11	26.7	NS
Body weight at 4 week	1549.88	1504.19	1494.53	25.6	NS
Body weight at 5 week	1733.12	1696.31	1692.21	24.8	NS
Body weight at 6 week	1873.76	1805.19	1832.74	22.8	NS
Body weight at 7 week	2044.94	1968.00	1984.89	23.1	NS
Body weight at 8 week	2150.75	2115.67	2144.5	25.4	NS

Table (4): Carcass parts % of rabbit fed different levels of probiotics.

Parameter	Probiotic Level (%)			SEM	Prob.
	0	1	2		
Carcass, %	53.40	55.07	54.88	0.59	NS
Skin, %	13.94	13.87	13.48	0.21	NS
Head, %	4.92	4.64	5.32	0.15	NS
Front, %	19.35	18.79	19.97	0.24	NS
Mid, %	13.01	14.47	13.74	0.33	NS
Hind, %	21.04	21.77	21.18	0.31	NS
Liver, %	3.30^{a}	3.01^{ab}	2.68^{b}	0.08	0.003
Testes, %	0.21	0.19	0.21	0.01	NS
Heart, %	0.28	0.31	0.28	0.00	NS
Lungs, %	0.67	0.64	0.63	0.02	NS
Kidney, %	0.58	0.60	0.60	0.01	NS
Thymus, %	0.06	0.07	0.07	0.00	NS

Table (5): Some blood parameters means of rabbit fed different levels of probiotics.

Parameter	Probiotic Level (%)			SEM	Prob.
	0	1	2		
Protein, g/dL	5.81	6.01	6.21	0.13	NS
Albumin, g/dL	4.10	3.98	4.08	0.08	NS
Globulin, mg/dL	1.82	2.03	2.13	0.13	NS
Cholesterol, mg/dL	63.26	75.33	63.20	3.25	NS
Triglycerides, mg/dL	102.47	106.05	81.77	5.97	NS
Urea, mg/dL	49.92	49.00	53.38	1.34	NS

Table (6): Cell mediated Immune response of rabbit fed different levels of probiotics.

Parameter	Probiotic Level (%)			SEM	Prob.
	0	1	2		
Ear swelling After 24 h, mm	53.80^{ab}	43.77^{b}	62.23^{a}	3.61	0.01
Ear swelling After 48 h, mm	31.40	30.89	41.26	2.94	NS
Ear swelling After 72 h, mm	19.97	13.01	18.70	1.90	NS

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الملخص العربى

تأثيرعلائق البروبيوتيك على أداء النمو وخصائص الذبيحة والمكونات الكيميائية الحيوية للدم والاستجابة المناعية في الأرانب النامية

إبراهيم حمدالحميدان[1]، سلطان سعيد الحربي[1]، محمد فهدالجطيلي[1]، معتز محمد فتحي[1،2]،طارق امين عبيد[1،3]، أسامة قرنه أبوعميرة[1،4]

[1]قسم إنتاج الدواجن، كلية الزراعة، جامعة القصيم، القصيم 51452، المملكة العربية السعودية

[2]قسم إنتاج الدواجن، كلية الزراعة، جامعة عين شمس، حدائق شبرا، 11241 القاهرة، مصر

[3]قسم إنتاج الدواجن، كلية الزراعة، جامعة كفر الشيخ، كفر الشيخ 33516، مصر

[4]قسم سلالات الدواجن، تربية الحيوانات معهد بحوث الإنتاج، مركز بحوث الزراعة، الدقي، الجيزة، 12618، مصر

الهدف من الدراسة الحالية هو دراسة تأثير المكملات الغذائية من البروبيوتيك على أداء النمو وخصائص الذبيحة ومكونات الدم الكيميائية الحيوية والاستجابة المناعية لدى الأرانب النامية. تم توزيع إجمالي 54 أرنبًا يبلغ عمرهم 4 أسابيع بشكل عشوائي على ثلاث معاملات تجريبية بتركيزات من البروبيوتيك الغذائي (0 و1 و2%) احتوت البروبيوتيك على 2.67×10^{11} وحدة تشكيل مستعمرة/جم من Bacillus licheniformis. كان هناك 9 مكررات لكل معاملة بكل مكررة ٢ ارنب لم يكن للمكملات الغذائية من البروبيوتيك تأثير كبير على زيادة وزن الجسم ونسبة تحويل العلف. لم يكن للمكملات الغذائية من البروبيوتيك تأثير كبير على نسبة الذبيحة ونسب أجزاء الذبيحة بما في ذلك النسبة الأمامية والوسطى والخلفية والجلد والرأس. كما أن المكملات الغذائية للبروبيوتيك لم يكن لها تأثير كبير على الأعضاء الداخلية بما في ذلك القلب والخصيتين والرئتين والكلى والغدة الزعترية، في حين انخفضت نسبة الكبد بسبب البروبيوتيك الغذائي بنسبة 2٪ في الأرانب النامية. كان للمكملات الغذائية للبروبيوتيك تأثيرات ضئيلة على جميع المعايير الكيميائية الحيوية للدم بما في ذلك البروتين الكلي والألبومين والغلوبيولين والكوليسترول والدهون الثلاثية واليوريا في الأرانب المسمنة. ومن المثير للاهتمام أن المكملات الغذائية للبروبيوتيك كان لها تأثير إيجابي على المناعة الخلوية بعد 24 ساعة من حقن فيتوهيماجلوتينين (PHA-P) في الأذن اليسرى في الأرانب النامية. يمكن أن نستنتج أن البروبيوتيك الغذائي كان له تأثير إيجابي على الاستجابة المناعية، في حين لم يتأثر أداء النمو وخصائص الذبيحة ومكونات الدم الكيميائية الحيوية.