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RESEARCH ARTICLE

Effect of Dietary Probiotic Supplementation on Performance, Gut Microflora, and Hematology of Local Quails

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ABSTRACT

This research was conducted to evaluate the effect of probiotic supplementation on growth performance, gut microbiota, and hematology of local quails. Ninety-one-week-old quails were randomly distributed into three dietary treatments with three replicate per each for 42 days. The dietary treatments were: Treatment 1 which was control (no additive), standard diet added with *Lactobacillus animalis* bacteria 1 g/kg 1.35×10^9 colony forming units (CFU)/kg (PRO1) and commercial multi-strain probiotic (*Lactobacillus acidophilus* 2.75×10^{10} CFU, *Streptococcus faecium* 8.25×10^{10} CFU, and *Bacillus subtilis* 1.1×10^{10} CFU) (PRO2). Results showed that PRO1, isolated from chicken caeca, had probiotic properties to improve growth performance of local quail compared to the control group. In cecum digesta, addition of both types of probiotics significantly increased the number of *Lactobacillus* spp. and reduced the number of coliform bacteria at the end of the experiment. Furthermore, supplementation of both types of probiotics significantly increased the number of lymphocyte and reduced H/L ratio compared to the control group at the end of the experiment. While, no significant differences were observed between both types of probiotic. This research has established that *L. animalis,* which isolated in cecum of chicken origin, had the same data recorded when compared to commercial multi-strain bacteria probiotic by improved growth performance, gut microbiota, and hematology parameters and could be a convenient probiotic additive in dietary local quail.

Keywords: Gut microbiota; Hematology; *Lactobacillus*; Probiotic; Quails

INTRODUCTION

The fastest-growing project of agriculture and animal husbandry sector is poultry productions. Otherwise, diet is one of the expensive items in poultry production, accounting for 70% of total poultry production. The constant increase in the cost of poultry feed ingredients and compounded feed is making less profit to poultry farmers (Kapil et al., 2015).

Today probiotics have a large and significant role in promoting the growth of broilers and increase resistance to the diseases by provide the different types of beneficial microorganisms to the diets. Therefore, one option that has the prospective to decrease the opportunity of infection of the poultry farm is the use of probiotics. There is a different definition of probiotics such as FAO/WHO defined probiotics as an alone or multi-strain bacteria added in an appropriate amount that makes a wealthy interest to the body. However, the mechanisms of action of probiotics are not completely understood. Probiotic feed supplements have been used to modulate the composition of the gut microflora by successfully competing with pathogens through a competitive exclusion process.

Several studies resulted that probiotics in broiler feed ameliorate the performance compared to non-addition probiotics into the feed to be as efficacious such antibiotic evolution provider (Kalavathy et al., 2003; Mountzouris et al., 2010; Shim et al., 2010). Some researchers resulted the influence of administration of adding a monocular strain of probiotics in broiler feed (Khosravi et al., 2010; Mountzouris et al., 2007; Zakeri and Kashefi, 2011), while others have tested two strain (Anjum et al., 2005; Mehr et al., 2007; Nayebpor et al., 2005) or multi-strain of bacteria (Mountzouris et al., 2010; Apata, 2008; Li et al., 2008; Wang and Gu, 2010). Probiotics have been used and developed for poultry is based on the information on the microbiota in the gastrointestinal tract (GIT) which is participating in the impedance to enter the harmful bacteria and stopping the growth of harmful bacteria, where it has been illustrated to be participating in safeguarding against

a different kind of harmful bacteria including *Salmonella* spp., *Campylobacter* spp., *Clostridium* spp., and *Escherichia coli* (Jin et al., 1997; Murry et al., 2006; Ragione et al., 2004).

Most of the reported research on probiotics focuses on the use of different strains of *Lactobacillus* spp. While, there is no evidence that exert effect of *Lactobacillus animalis* on the quails. Thus, the present study was conducted to investigate the effects of *L. animalis* on performance, gut microbiota, and hematology of local quail. On the other hand, compare the *L. animalis* which was isolated from the cecum of chicken and commercial probiotic.

MATERIALS AND METHODS

Management and Design of Experiment

Ninety-one-day local quails were taken from a commercial farm (Erbil, Kurdistan, Iraq) and quails reared in the same place during the 1st week before starting the experiment. Then, after 1-week quails were randomly distributed into three treatments (30 quails per treatment) and reared in the same building. The quails were scaled and kept in nine cages (60 × 60 × 40 cm). The quails also located in a way to have full access to drinking water and feed. Each treatment had three replicates (10 quails/cage). The duration of the trial was 42 days. The dietary treatments include control diet (standard broiler diet), probiotic 1 = control diet administrated with 1 g *L. animalis*/kg of feed, comprises 1 g/kg 1.35×10^9 colony forming units (CFU)/kg (Plymouth University, United Kingdom), and probiotic 2 = control diet with 1 g/kg commercial multi-bacteria probiotic (*L. acidophilus* 2.75×10^{10} CFU, *Streptococcus faecium* 8.25×10^{10} CFU, and *Bacillus subtilis* 1.1×10^{10} CFU) with a commercial name (BIOZYME, India). Skim milk was used as a protective carrier for improving the survival ratio of *L. animalis*.

Growth Performance

All quails were weighed at the first (initial weight) and on day 42 individually after their arrival from the hatchery to the experimental farm. Final weight gain for each dietary treatment was calculated. Feed intake (FI) was recorded in the course of the whole experiment for each replicate, and the feed conversion rates (FCR) and European production efficacy factor (EPEF) were calculated subsequently.

Gut Microbiota Analysis

At the end of the experiment period, six quails were taken from treatments and their cecal digesta were fully aseptically separated to investigate the intestinal microorganisms (*Lactobacillus* spp. and total coliform bacteria). One hundred mg of each caecum digesta was mixed with 0.9 ml of sterile PBS (pH 7.0) and vortexed for 1 min to homogenize. The homogenate was diluted serially from an initial 10^{-1} dilution to 10^{-7}. For each dilution, 0.1 ml from the dilution was plated onto sterile selective medium agar to count targeted bacteria groups as following; MacConkey agar (Sigma-Aldrich, UK) for total coliform and MRS agar for *Lactobacillus* spp. The colonies number of microbial was then counted to determine the CFU. CFU/g for fresh cecal digesta was calculated and expressed as logarithms.

Hematology Parameters

At the end of the experiment period, three quails from each treatment randomly were selected and killed by cervical dislocation. The blood samples were collected in test tubes with anticoagulant di-potassium ethylene diamine tetraacetic acid. All parameters of blood (hemoglobin, WBC, lymphocyte, heterophil, and H/L ratio) were examined by a full-auto hematology analyzer (MCL 3800, China) (Pelicano et al., 2005; Baurhoo et al., 2007).

Statistical Analysis

The data obtained in the experiments were statistically analyzed using a one-way ANOVA test, SPSS program (Statistical Package for Social Science) (SPSS 22, 2005). Descriptive statistics aided for the analysis of the data. Therefore, means and standard error were calculated. Duncan test utilized and aided to calculate significant differences at 0.05 levels among the various parameters (Duncan, 1995).

RESULTS

The influences of probiotic administration on quality performance parameters are shown in Table 1. Insignificant

Table 1: Influence of probiotics administration on growth performance of local quails at 6 weeks of age (mean±standard error)

Growth performance	Treatment			*P* value
	CON	PRO1	PRO2	
Initial weight (g)	24.43±0.88[a]	23.26±0.68[a]	23.18±1.08[a]	0.577
Final weight (g)	193.79±3.46[b]	211.91±4.28[a]	206.35±3.53[a,b]	0.037
Weight gain (g/bird)	169.35±4.31[b]	188.64±3.68[a]	183.14±4.10[a,b]	0.037
Feed intake (g/bird)	508.62±12.73[a]	511.44±7.23[a]	508.56±8.51[a]	0.972
Feed convention ratio	3.00±0.12[a]	2.71±0.05[a]	2.78±0.10[a]	0.190
EPEF[1]	138.64±3.95[a]	136.77±1.77[a]	136.47±3.14[a]	0.869

[a,b]Data in the same row direction with different letters are differ significantly ($P<0.05$). [1]EPEF=Liveability (%) × live weight (kg) × 100/age (d) × FCR

differences ($P > 0.05$) were observed between treatments on final FCR, total FI, and EPEF. While, the quails with *L. animalis* (PRO1) showed a significant ($P < 0.05$) improvement in body weight and weight gain compared to other groups. While, both parameters, insignificant ($P > 0.05$) differences were observed between both types of probiotics and commercial multi probiotic (PRO2) with the control group. The best results recorded with PRO1 compared to other probiotic supplementation.

Table 2 showed the effects of *L. animalis* (PRO1) and commercial multi probiotic (PRO2) supplementation in diet on the microbiota composition in the cecum digesta of local quails at 6 weeks of age. Both types of probiotic supplementation increased significantly ($P < 0.05$), the counting of *Lactobacillus* spp. and reduced the number of coliform bacteria compared to control treatment. While, insignificant ($P > 0.05$) differences were observed between both types of probiotic supplementations in *Lactobacillus* spp. and coliform bacteria in the caeca of local quails compared to the control group.

Table 3 showed the influence of *L. animalis* (PRO1) and commercial multi probiotic (PRO2) on hematological parameters of local quails at 6 weeks of age. Both kinds of probiotics supplementation increased significantly ($P < 0.05$), the counting of WBCs compared to control treatment. Lymphocyte increased significantly ($P < 0.05$) in *L. animalis* (PRO1) compared to control treatment, while no significant ($P > 0.05$) differences were observed on lymphocyte between both kinds of probiotic supplementations and PRO2 with control treatment. Furthermore, heterophil and H/L ratio parameters reduced significantly ($P < 0.05$) using both kinds of probiotic supplementation compared to control treatment. The best result was recorded with *L. animalis* (PRO1) compared to the commercial multi probiotic (PRO2). While, no significant ($P > 0.05$) differences were observed among treatments on hemoglobin traits.

Table 2: Influence of probiotics administration on bacterial counts (Log_{10} CFU/mL) of microbiota in cecum digesta of quails at 6 weeks of age (mean±SE)

Microbes	Treatment			*P* value
	CON	PRO1	PRO2	
Lactobacillus ssp.	8.65±0.07[b]	9.39±0.06[a]	9.25±0.13[a]	0.005
Total coliform	7.28±0.10[a]	6.85±0.06[b]	6.85±0.11[b]	0.030

[a,b]Data in the same row direction with different letters are differ significantly (*P*<0.05)

Table 3: Influence of probiotics administration on the hematological parameters of quails at 6 weeks of age (Mean±SE)

Parameters	Treatment			*P* value
	CON	PRO1	PRO2	
Hemoglobin	153.17±6.02[a]	160.36±5.54[a]	153.45±3.60[a]	0.568
WBC	3.81±0.15[b]	6.79±.0.20[a]	6.14±0.31[a]	<0.001
Lymphocyte	68.66±2.02[b]	78.33±.2.90[a]	75.95±1.51[a,b]	0.050
Heterophil	24.11±1.49[a]	13.00±0.57[c]	18.22±0.78[b]	0.001
H/L ratio	0.35±0.03[a]	0.16±.0.08[c]	0.24±0.01[b]	0.002

[a,b]Data in the same row direction with different letters are differ significantly (*P*<0.05)

DISCUSSION

Nowadays, there are various production systems to improve poultry quality performance and health benefits one of them using probiotics that increase the products due to the recent ban of antibiotics. This study proved that the significant efficacy of dietary supplemented with probiotic *L. animalis* and commercial multi-bacteria probiotic on growth performance, cecal microbiota, and hematology of local quails. There are a different of beneficial microorganism species used as probiotics in poultry feed (Akoy, 2015; Mountzouris et al., 2010; Patterson and Burkholder, 2003; Khaksefidi and Rahimi, 2005). In general, beneficial bacteria that used in poultry feed belonging to the *Bifidobacterium*, *Streptococcus*, *Bacillus*, *Lactobacillus*, *Enterococcus*, *Aspergillus*, *Candida*, and *Saccharomyces* have a significant influence on broiler quality performance (Kalavathy et al., 2003; Zulkifli et al., 2000; Kabir et al., 2004; Gil De Los Santos et al., 2005), modification of small intestine microbiota, and suppress the harmful bacteria (Pascual et al., 1999).

The data of this research showed in Table 1 indicated that both types of probiotic administration were significantly ($P < 0.05$) improved body weight gain, feed conversion ratio and EPEF, this significant improvement in feed conversion ratio was showed by (Akoy, 2015; Zeweil, 1997; Chumpawadee et al., 2009; Devarestti, 2016; Mohammadreza et al., 2016). Probiotics are considered to encourage poultry performance and increase immune system, on the other hand, suppresses pathogen bacteria. The advantage of probiotic administration most important in poultry feed, not like antibiotics that kill all kind of bacteria and there is remain in the meats and eggs production that influence on the health of consumers. Today, probiotics are used strain-specific, live bacteria as probiotic cultures that produce a beneficial influence on the host. These microorganisms may be used such a single strain of bacteria or a group of many bacteria together, such us multi-strain beneficial bacteria all together, may have more influence on the health of poultry. These bacteria used as a probiotic generally isolated from the GIT of a healthy animal, then as probiotics will be applied to the diet of specific animals. Subsequently, these bacteria could become a portion of common microbiota in the small intestine, could be remained through GIT passage, and

ability of bacteria as probiotic to adhere and colonize the small intestinal tract. The study by Harimurti and Kurniasih (2010) showed that indigenous lactic acid bacteria isolated from the GIT of healthy Indonesian native adult chickens (*Ayam kampung*), including *Streptococcus thermophilus* Kd2, *Pediococcus acidilactici* Kp6, and *Lactobacillus murinus* Ar3 showed significant effect as a feed additive to get better live growth performance of chickens.

This research showed the effect of dietary probiotic administration on the microbiota profile of the digesta of cecum as revealed by culture method in local quails. Counting of *Lactobacillus* spp. was increased by adding either *L. animalis* which was isolated from cecum of chicken or multi-strain commercial probiotics. The improvement of the numbering of *Lactobacillus* spp. and lower coliform bacteria detected in quails with both types of probiotics could be due to decreasing pH value in the small intestine and raise the production of SCFA (Fuller, 2001). Lactic acid bacteria strains are capable of converting carbohydrate substrates into organic acids (mainly lactic acid) and producing a wide range of metabolites that effect on some microorganisms in GIT. The homolactic and heterolactic species are produced lactic acid that decreases the pH value of the intestine content and increases the short-chain fatty acid, which is harmful to some kind of harmful bacteria. Furthermore, lactic acid bacteria are produced acetic acid and hydrogen peroxide that restrained against coliforms bacteria, *Salmonella* spp., and *Clostridia* spp. Largely, *Lactobacillus* spp. remedy is claimed to improve the growth performance of layer hens and broilers by killing the harmful bacteria and influence of *E. coli* in the GIT (Mudalgi et al., 1993; Kumprecht et al., 1994; Kapil et al., 2015).

The present study showed that no significant differences were observed between single strain and multi-strain probiotic supplementation as commercial probiotics. This result is in agreement with Mountzouris et al. (2010), who showed that the addition of probiotic (PoultryStar ME, Biomin GmbH, Herzogenburg Austria) in the feed of broilers increased the number of *Lactobacillus* spp. and *Bifidobacterium* spp. compared to control treatment. The research conducted by Smirnov et al. (2005) resulted that the use of probiotic (2 g/kg of diet), including the live bacteria *Bifidobacterium bifidum*, *L. acidophilus*, *Lactobacillus casei*, and *Enterococcus faecium* (minimum 1.0×10^8 CFU/g) increased positively the number of *Lactobacillus* spp. in the ileum part of small intestine by 147% compared to control treatment. Probiotic supplementation of the intestinal microbiota in poultry, particularly deal with *Lactobacillus* species, showed a beneficial influence on resistance to infection by some kind of harmful bacteria such as *E. coli* (Jin et al., 1996), *Salmonella* sp. (Pascual et al., 1999; Wali, 2012; Akoy, 2015), *Campylobacter* sp. (Stern et al., 2001), and, more recently, *Eimeria acervulina* (Dalloul et al., 2003).

CONCLUSIONS

The present study indicates that the *L. animalis* strain used as a probiotic had important influences on growth performance, gut microbiota, and hematology parameters of local quails. Furthermore, the commercial bacteria (multi-strain bacteria) showed significant improvement compared to the control treatment. Furthermore, there were no significant differences observed between both types of probiotics.

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