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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

The effects of dietary inclusion of probiotic on the gut bacterial load of Japanese quails (*Coturnix japonica*)

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Abstract

This study was conducted to investigate the effect of different levels of Protexin on the bacterial load in the gut of Japanese quails. Forty eight Japanese quails (*Coturnix japonica*) were equally allocated to four treatments containing three pens (include 3 female and 1 male) in each treatment. Birds received diets supplemented with 0, 0.250, 0.500, and 1.000 gm Protexin/kg as treatments 1 (control), 2, 3 and 4 respectively. At the age of 14 weeks, four quails (two male and two female) were randomly selected from each treatment and slaughtered. The carcasses were immediately opened and the entire intestine was removed aseptically. Bacterial load of *Campylobacter*, *Closterdium perfringens* and *Lactobacillus* were measured using Real Time Polymerase Chain Reaction (PCR). *Lactobacillus* bacteria concentration as beneficial digestive bacteria was the highest in treatment 2 followed by treatments 4 and 3. Concentration of *Closterdium perfringens* in quails fed treatment 2 had the highest concentration compared to the control. Concentration of *Campylobacter* in quail's colon fed treatment 2 had the highest concentration compared to the control group. The results showed that the supplementation of 500 gm/kg Protexin has a beneficial effect on gastrointestinal flora of laying quails.

Keywords: *Coturnix japonica;* gut bacteria; probiotics

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Introduction

There are generally two different types of bacterial populations which can become established in the digestive tract of all warm blooded animals soon after birth. The first is that which exists in close association with the gut epithelium and the second is that which occurs free in the gut lumen. This would ensure that at all times the animal would have the proper microbial balance. This, of course, cannot be guaranteed under natural field conditions. However, if micro-organisms and/or substances which contribute to the proper microbial balance are added to the diet then the animal would continually receive a "boost" to establishing the proper microbial population. The term "probiotic" has been used to indicate substances or micro-organisms which contribute to an ideal microbial balance (Vali, 2009).

Probiotics are defined as microorganisms promoting the growth of other microorganisms. The use of probiotics in poultry was pioneered by Tortuero (1973), who reported an increase in growth rate in chicks given a *Lactobacillus acidophilus* culture in drinking water for 11 days from hatching. Similar results on the beneficial effects of *Lactobacillus* cultures on the growth of chickens were also reported by several researchers (Kalbane et al., 1992; Jin et al., 1997).

One of the probiotics used in poultry feed is Protexin. Protexin is a multi-strain probiotic containing live microbes to establish, enhance or re-establish essential microflora in the gut. Protexin is a highly concentrated pre-mix containing seven strains of bacteria and two yeasts. All the micro-organisms in the protexin are naturally occurring and have been isolated from a wide range of feed, plant, animal, bird and human sources (Ayasan et al., 2006). However, there is

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incomplete knowledge on the effect of probiotic supplementation on gut characteristics of poultry, especially in Japanese quail. Because of the importance of poultry as an economic and nutritious form of animal protein and the fast growing characteristics of this animal, this study was conducted to investigate the effect of different levels of Protexin on the bacterial load of Japanese quails gut.

Materials and Methods

Forty eight Japanese quails (*Coturnix japonica*), having six weeks of age were equally allocated to four treatments containing three pens in each. In each replicate, three female and one male bird were included. The ingredients and composition of basal diet in this study are shown in Table 1. Diets were formulated to meet the nutrient requirements for poultry (NRC, 1994). Birds received diets supplemented with 0, 0.250, 0.500, and 1.000 gm Protexin/kg diet. The experiment lasted for nine weeks. At the end of the experiment, four quails (two male and two female) were randomly selected from each treatment and were slaughtered. The carcasses were immediately opened and the entire intestines were removed aseptically. Samples of the small intestinal and ceca contents were collected in polymerase chain reaction (PCR) tubes. The substances in Table 2 were mixed in each PCR especial tube (Ott et al., 2004). Bacterial load of *Campylobacter*, *Closterdium perfringens* and *Lactobacillus* were measured using Real time (PCR) (Mackay, 2004). To recognize bacterial strains temperature program of real time PCR steps (Table 3), was used by method of Ott et al. (2004). Rotorgene 6000 software, version 1.7 was used for variables and PCR setting (Ott et al., 2004).

Results and Discussion

Lactobacillus

The figure 1 shows the *Lactobacillus* concentration in the colon of quails. *Lactobacillus* bacteria concentration as beneficial digestive bacteria was the highest in treatment 2 followed by treatments 3 and 4. This result was in agreement with Falaki et al. (2011), who reported that supplementation diet with different levels of proiotic (PRIMALAC) slightly increased the *Lactobacillus* population and total count of microrganisms in the ileum of chickens in early ages. In ileum the population of useful bacteria like L*actobacillus* and *Bifidobacteria* increases and the pH of the gut due to increase production of volatile fatty acids (VFAs) decreases. Therefore, the environment of gut becomes unsuitable for the activity and proliferation of pathogens like Salmonella.

Table 1: Composition of the basal diet fed to Japanese quails (*Coturnix japonica*)

feed ingredient	(%)
Corn	49.44
Soybean meal	18.29
Wheat	20
Fatty acid	2.34
Oyster shell	7.41
Bone meal	1.71
Common salt	0.28
Vitamin-Mineral premix[1]	0.5
DL-Methionine	0.12
Calculated values	
ME. Kcal/Kg	2900
Cp %	16.5
Calcium %	3.4
Available phosphorus %	0.32
Sodium %	0.15
Lysine %	0.69
Methionine %	0.34
Methionine-Cysteine. %	0.55
Tryptophan %	0.18

[1]Each kg of vitamin premix contains 9000000 IU Vitamin A; 2000000 IU Vitamin D3; 1800 mg Vitamin B1; 6600 mg Vitamin B2; 10000 mg Vitamin B3; 3000 mg Vitamin B6; 15 mg Vitamin B12; 18000 mg Vitamin E; 2000 mg Vitamin K3; 1000 mg Vitamin B9; 30000 mg Vitamin B5; 100 mg Vitamin H2; 21 mg Folic acid; 65 mg Niacin; 14 mg Biotin; 500000 mg Choline Chloride; 100000 mg Manganese; 85000 mg Zinc; 50000 mg Iron; 10000 mg Copper; 1000 mg iodine; 200 mg selenium.

Table 2: polymerase chain reaction (PCR) special tube contents

Substance	Volume (µl)
PCR Buffer 10X	2.5
$MgCl_2$ 50mM	1
dNTP Mix 10mM	0.5
Smar Taq DNA Polymerase(5U/µl)	0.25
Primer F	1
Primer R	1
CYBR Green	1
Template DNA	2
Sterile dH_2O	16.25
Total Volume	25

Table 3: Temperature program of real time polymerase chain reaction (PCR) steps[1]

Steps	Time	Temperature (˚C)	Replicate number
Hot state	5min	95	1
Denaturation	30 sec	95	45
Annealing	40 sec	58	45
Extension	40 sec	72	45

[1]This program is setting for recognizing bacterial strains (Ott et al., 2004)

Combylobacter

The result of PCR test showed that the concentration of *Campylobacter* in quail's colon had the highest concentration compared to the control and treatment 3 and 4 (Fig. 2).

Clostridium perfringens

Figure 3 shows that the concentration of *Closterdium perfringens* in quails fed treatment 2 was the highest compared to the control group.

Numerous reports indicated that addition of probiotics in feed, either solely or in combination with other feed additives like prebiotics, could regulate the intestinal microflora in order to increase the concentration of the beneficial bacteria such as Lactobacillus and Streptococcus and inhibit the reproduction of harmful bacteria in the gut (Line et al., 1998). Also other studies reported that probiotics beneficially affect the host by improving its intestinal balance (Fuller, 1989). They create conditions that suppress harmful microrganisms and favours beneficial ones (Line et al., 1998; Mead, 2000).

Siriken et al. (2003) investigated the effects of two commercially available probiotics, alone and in combination with an antibiotic, on the caecal flora of Japanese quail (*Coturnix japonica*) reared under unstressed conditions. The total count of aerobic bacteria, *Lactobacilli*, *Enterobacteriaceae*, *Coliforms*, *Enteroccoci*, *Salmonellae*, sulphite-reducing anaerobic bacteria (*clostridia*) and pH values in the caecal content of the birds had no significant difference among the four groups. These results are in disagreement with our results.

Strompfova and Marcinakova (2005) investigated the effect of *Lactobacillus fermentum* in Japanese quail (*Coturnix japonica*). The results demonstrated that the application of this strain significantly increased the population of lactic acid bacteria *Lactobacilli* and *Enterococci* in faeces and significantly decreased the count of *E. coli*. Jin et al. (1997) showed a decrease of the Coliform population in the caecum of broilers after the addition of *L. acidophilus*. A reduction of pathogenic *E. coli* was also observed in the gastrointestinal tract of gnotobiotic chickens dosed with *L. acidophilus* (Watkins and Miller, 1982). The study of Jin et al. (1997) showed that *Lactobacilli* added to the diet of broilers increased the concentrations of volatile fatty acids in the ileum and caecum and decreased the pH. In their study, the non-volatile fatty acids, lactic and succinic acids, in the cecum and ileum of broilers were not influenced (Strompfova and Marcinakova, 2005).

In conclusion, our results indicated that the supplementation of 500 g/kg Protexin has a beneficial effect on gastrointestinal flora of laying quails.

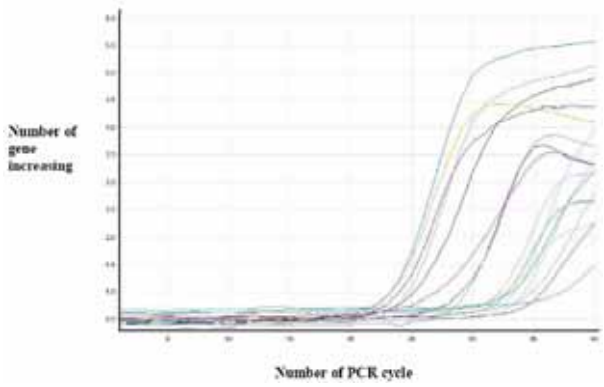

Fig. 1: Real time polymerase chain reaction (PCR) diagram analysis for *Lactobacillus*

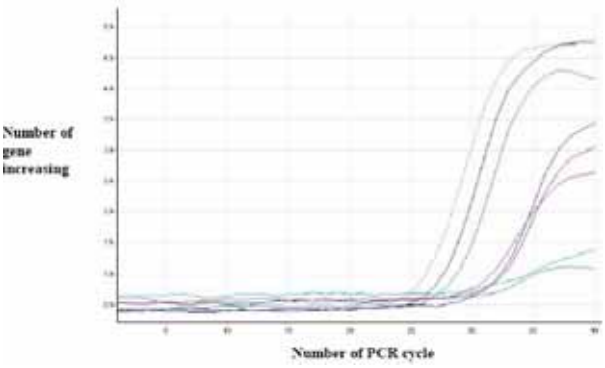

Fig. 2: Real time polymerase chain reaction (PCR) diagram analysis for *Campylobacter*

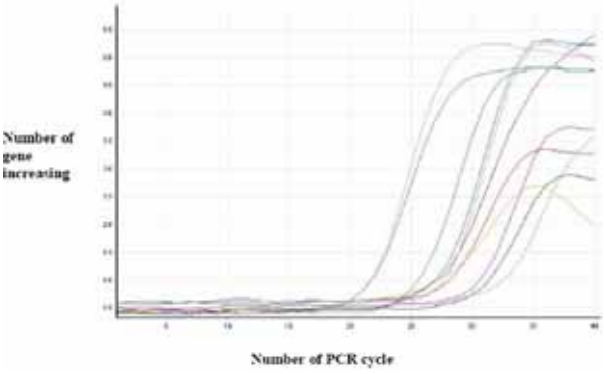

Fig. 3: Real time polymerase chain reaction (PCR) diagram analysis for *Clostridium perfringens*

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