Effect of dietary probiotic on performance and humoral immune response in layer hens

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SUMMARY

In the present study, the effects of dietary supplementation of commercial probiotic (Protexin™) on daily feed consumption, egg yield, egg weight, food conversion ratio and humoral immune response in layer hens were investigated. In 7 replicates, a total of 280 40-week-old *Hysex Brown* layers were fed diets containing either 0, 250, 500 or 750 parts per million (ppm) for 90 days.

When compared with the controls, the food consumption, food conversion ratio and the damaged egg ratios were found lower in the group consuming 500 ppm probiotic ($P<0.05$).

There was no significant difference between the controls and the groups receiving 250 and 750 ppm probiotic on food consumption, food conversion ratio and damaged egg ratio. Similarly, the egg yield, egg weight, specific gravity, and peripheral immune response showed no statistically significant differences between the groups.

Keywords: layers, probiotic, performance, immune response

INTRODUCTION

The US National Food Ingredient Association defined probiotic (direct fed microbial) as a source of live naturally occurring microorganisms, and this includes bacteria, fungi, and yeast (Miles and Bootwalla, 1991). The addition of probiotic to the diet has been found to improve egg production, food conversion ratio (Abdulrahim et al., 1996; Nahashon et al., 1994b; 1996a,b; Mohan et al., 1995; Tortuero and Fernandez, 1995), food consumption (Nahashon et al., 1994a), egg weight (Nahashon et al., 1992, 1993, 1996a; Jin et al., 1997; Tortuero and Fernandez, 1995), specific gravity (Nahashon et al., 1994c; Mohan et al., 1995) in layers. During the laying phase (20-59 weeks of age), layers fed a 15.3 % CP diet plus *Lactobacillus* produced significantly larger eggs ($P<0.05$) than those fed a similar diet without *Lactobacillus* (Nahashon et al., 1996a). Similar results have also been reported by others (Tortuero and Fernandez, 1995; Mohan et al., 1995; Abdulrahim et al., 1996). In contrast, some conflicting results have been reported on the effects of probiotic on the

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egg production (Nahashon et al., 1994a) and food conversion ratio (Goodling et al., 1987) in laying hens.

Nearly all of the probiotics currently on the market contain lactobacilli and/or streptococci. Of these lactobacilli are the most studied group. The results obtained with lactobacilli are, therefore, of relevance to our understanding of the probiotic effect. Lactobacilli given orally have been reported to be able to migrate from the gut to the systemic circulation. They could translocate and could survive for many days in the spleen, liver and lungs. Cell wall products might have a co-stimulatory role on the induction of the systemic immune response (Fuller, 1988; Erickson and Hubbard, 2000). Oral administration of *Lactobacillus casei* has been reported to enhance activity of splenic NK cells and to stimulate phagocytic activity (Saito et al., 1981; Matsuzaki et al., 1998). Erickson and Hubbard (2000) have reported that lactobacilli also have the effect of increasing serum anti-*E.coli* IgM levels in rodents and the production of autocoids by probiotic bacteria might have a pronounced influence on the induction of immunity, although the underlying mechanisms by which that occurs are largely unclear.

The purpose of this study was to investigate the effect of a commercial probiotic direct fed microbial, including nine species of bacteria on performance of layers and systemic antibody synthesis.

MATERIAL AND METHODS

Animals

A total of 280 40-week-old *Hysex-Brown* layer hybrids, which were obtained from University of Selçuk, Faculty of Veterinary Medicine, Animal Husbandry and Research Unit, were used in this study.

Experimental design

The animals were randomly divided into four groups and then, each of the four groups (seven replicates of 10 layers making a subgroup) were housed in seven different cages. The subgroups were distributed randomly among the different compartments of the cage system. Each subgroup consisted of 2 cages, each of which was 55x45x40 cm in dimensions. 5 hens/cage (55×45×40 cm) were placed. The distribution resulted in 56 cages and 280 laying hens. Experimental period was 90 days.

Diet and probiotic

The composition of the basal diet is shown in table 1. The basal diet was supplemented with a commercial probiotic *(Protexin^{TM}; Novartis Probiotics International, UK),* at the levels of 0, 250, 500 and 750 ppm and prepared using a food mixer. Analysis of diet is presented in table 2. The probiotic and the microbial content of the probiotic are shown in table 3.

Table 1. Composition of the diets

Ingredients	%
Corn	20.00
Wheat	48.90
Soybean meal	17.00
Fish meal	1.50
Oil	2.50
Limestone	9.00
DCP	0.50
Salt	0.25
Vitamin-premix[1]	0.25
Mineral-premix[2]	0.10

[1] Per 2.5 kg of vitamin premix contains 3.6 mg vitamin A, 0.05 mg vitamin D_3, 30 mg vitamin E, 3 mg vitamin K_3, 3 mg vitamin B_1, 6 mg vitamin B_2, 5 mg vitamin B_6, 0.015 mg vitamin B_{12}, 25 mg niacin, 0.04 mg biotin, 8 mg karotenoid, 1 mg folic acid, 300 mg choline chloride, 50 mg vitamin C.

[2] Per kg of mineral premix contains 80 mg Mn, 35 mg Fe, 50 mg Zn, 5 mg Cu, 2 mg I, 0.4 mg Co, 0.15 mg Se.

Table 2. Analysis of diets

ME, kcal/kg *	2788
Crude protein, %	16.12
Dry matter, %	91.63
Ash, %	8.85
Crude fibre, %	5.35
Ether extract, %	3.45
Ca, %	3.42
P, %	0.65
Methionine + Cysteine,* %	0.55
Lysine,* %	0.90

*Obtained by calculation.

Table 3. Probiotic and its microbiological contents

Microorganism	Microorganism CFU* / kg probiotic
Lactobacillus plantarun	1.89×10^{10}
Lactobacillus delbruecki subsp. bulgaricus	3.09×10^{10}
Lactobacillus acidophilus	3.09×10^{10}
Lactobacillus rhamnosus	3.09×10^{10}
Bifidobacterium bifidum	3.00×10^{10}
Streptococcus salivarus subsp. thermophilus	6.15×10^{10}
Enterococcus faecium	8.85×10^{10}
Aspergillus oryza	7.98×10^{9}
Candida pintolopesii	7.98×10^{9}

* Colony forming units

Hen-day egg production

The hen-day egg production was recorded during 7-days and ensured similar pre-production values of treatments. The hen-day egg production were then recorded daily at the same time and calculated as follows: total number of eggs collected divided by total number of live hens per day in each group. The collected eggs were classified as "*normal*" or "*damaged*; the latter included the following: broken eggs (an egg with broken shell and destroyed membrane), cracked eggs (an egg with broken shell but intact membrane), the eggs without shell (an egg without shell but with intact membrane).

Food consumption and food conversion ratio

Feed and water were supplied for *ad libitum* consumption throughout the 90-day experimental period. Food consumption (FC) and food conversion ratio (FCR) were determined at 14 day intervals.

Egg weight and specific gravity

Egg weight and specific gravity were determined monthly using the methods described by Hamilton (1982) and Hempe et al. (1988).

Nutrient composition of experimental diets

Crude protein, dry matter, ash, crude fibre, lipid content, Ca and P values of the experimental diet were determined by chemical analysis (AOAC, 1980).

Test of humoral immune response

A La Sota vaccine (Delwax ND) was applied to the animals by drinking water on day 1 of the experiment. Antibodies to Newcastle Disease (ND) antigen in blood sera from 32 hens/treatment group were measured on days 1, 45, and 90 by haemagglutination inhibition (HI) test as described by Brown et al. (1990). ND antigen was supplied from the Institute of Poultry Diseases and Vaccine Production, Manisa, Turkey.

Statistical analysis

One-way analyses of variance of egg yields, egg weights, FC, FCR and damaged egg ratios were conducted. Any significant differences were further analysed by Duncan's multiple range test (SPSS, 1993).

RESULTS AND DISCUSSION

This study was carried out to evaluate the effect of a commercial probiotic supplementation in layer diets on egg production, egg weight, FC, FCR, specific gravity, mortality and humoral immune response. Chemical composition of the diet is shown in table 2. The results presented in tables 4 and 5 show the effects of dietary probiotic on performance and immune response of layer hens.

Table 4. Data on the performance of egg production and shell quality at different experimental periods

Experimental period,	G R O U P S			
Days	*Control*	250 ppm probiotic	500 ppm probiotic	750 ppm probiotic
Egg production, % eggs/hen/day				
1-30	76.33±1.73	77.38±1.44	78.00±0.78	77.62±0.72
30-60	74.52±0.74	73.33±1.15	75.91±0.86	74.91±0.58
60-90	72.72±0.89	71.81±0.91	73.10±0.83	72.67±0.58
1-90	74.52±0.71	74.18±0.76	75.67±0.56	75.06±0.48
Food consumption, g/hen/day				
1-30	112.98±1.02	113.38±0.60	110.93±1.00	112.60±1.02
30-60	113.71±0.79	111.42±1.22	111.07±1.04	112.80±0.96
60-90	113.00±1.33[a]	111.92±0.84[ab]	109.62±0.94 [b]	113.24±0.94[a]
1-90	113.23±0.60 [a]	112.24±0.53 [a]	110.54±0.57 [b]	112.88±0.55[a]
Food conversion ratio, kg food/kg egg				
1-30	2.51±0.06	2.47±0.05	2.41±0.03	2.47±0.03
30-60	2.60±0.03 [a]	2.62±0.06 [a]	2.48±0.04 [b]	2.55±0.03 [a b]
60-90	2.68±0.05	2.68±0.04	2.58±0.04	2.62±0.04
1-90	2.59±0.03 [a]	2.59±0.03 [a]	2.49±0.02 [b]	2.55±0.02 [a b]
Damaged eggs, %				
1-30	1.16±0.22	1.32±0.29	0.49±0.19	1.22±0.37
30-60	1.60±0.25	1.82±0.51	1.01±0.28	1.97±0.38
60-90	1.51±0.41	1.77±0.35	0.78±0.23	1.77±0.37
1-90	1.42±0.17 [a]	1.64±0.23 [a]	0.76±0.14[b]	1.65±0.22 [a]
Egg weight, g				
1st	58.12±0.55	58.42±0.26	58.22±0.31	58.59±0.73
45th	58.33±0.67	58.37±0.35	58.54±0.37	59.41±0.60
90th	58.14±0.86	58.19±0.40	58.04±0.52	59.36±0.63
Specific gravity, g/cm^3				
1st	1.081±0.002	1.079±0.002	1.084±0.002	1.083±0.001
45th	1.083±0.001	1.080±0.001	1.082±0.001	1.079±0.002
90th	1.083±0.001	1.082±0.001	1.083±0.002	1.081±0.003
Mortality, %				
0-90	1.42	0.00	1.42	1.42

a, b: Means within columns with no common superscripts differ significantly (P<0.05).
Values represent the mean ±SEM of 4 groups of 70 layers hens (7 replicates of 10 layers in each) per treatment.

As seen in table 4, there was no statistically significant difference in hen-day egg production between the groups with respect to the whole period of the experiment (P>0.05). It was determined that hen-day egg production values from the groups control, 250, 500, and 750 ppm were 74.52, 74.18, 75.67 and 75.06 %, respectively. The lowest value for the egg yield was from the group 250 ppm (74.18 %) while the highest was from that fed 500 ppm (75.67 %). In a

study by Nahashon et al. (1994a) 28 weeks old layers have been fed diets supplemented 0, 1100, and 2200 ppm *Lactobacillus* for 24 weeks. It has been revealed in that study that the hen-day egg production values for these groups were 88.9, 90.4, and 89.5 %, respectively and the egg production value for 1100 was statistically different from that of the control ($P<0.05$). In another report, Mohan et al. (1995) have shown that layers fed 100 ppm probiotic have 5 % higher production value than the control. Svetic et al. (1994) have shown that values for egg production were slightly (1%) higher in the group fed 700 ppm than that of control. The difference between the present study and previous works may be related to the differences of ages of layer hens.

Table 5. Anti-ND haemagglutination-inhibition titres of the hens fed on diets with different levels of probiotic supplementation

Probiotic supplementation (ppm)	Titres		
	1 day	45 day	90 day
- (Control)	5.50±2.07	10.33±3.21	8.08±2.84
250	6.33±3.34	9.08±3.37	7.67±3.20
500	6.50±3.10	10.00±2.45	8.80±1.50
750	5.92±3.53	10.25±2.30	8.33±1.90

No differences were observed between the groups ($P>0.05$).
Values represent the mean ± Sd of 4 groups of 35 layer hens per treatment.

The lowest value (109.62 g) for the food consumption obtained from the days 30-60 was found in the group that consumed 500 ppm probiotic while the highest (113.24 g) was from the group receiving 750 ppm. Figures for the food conversion from the control and 250 ppm groups were found close to each other (113.0 and 111.92 g, respectively). In addition, the food conversion values from the control and 750 ppm groups were not significantly different but they differed significantly from the group 500 ppm ($P<0.05$). In a study by Nahashon et al. (1994a), food conversion figures from the hens fed with a diet having no probiotic supplementation have been reported to be significantly lower than those from the groups supplemented with probiotic at various levels. However, controversial results arose from another study (Nahashon et al., 1994c) in which no significant difference was observed between the hens that consumed diets with or without probiotic. From the present experiment it was also obvious that the highest value for daily food consumption for days 1-90 day group was obtained from the control group (113.23 g). The food consumption values for the same period from the groups 250, 500 and 750 ppm were determined as 112.24, 110.54, and 112.88 g. In one of the similarly conducted studies, it has been reported that the highest significant values for the food consumption obtained from groups either fed on probiotic diets, or control groups (Nahashon et al., 1994a). Nahashon et al. (1994b,c) have also reported that no differences were seen between any of the groups. Our findings are consistent with those from Nahashon et al., (1994a).

Food conversion values for all the periods of the study are shown in table 4. The highest food conversion ratio (2.62) from the days 30-60 was obtained in the 250 ppm group while the lowest (2.48) was in the 500 ppm group. Food conversion ratios obtained from the both control and 250 ppm groups were found to differ significantly from that of 500 ppm ($P<0.05$) while no difference was found between the ratios from the group receiving 500 ppm and those from 750 ppm groups ($P>0.05$). Food conversion ratios for whole period of the study were 2.59, 2.59, 2.49 and 2.55, respectively ($P<0.05$). The probiotic supplementation at 500 ppm has had a positive effect on FCR and such effect was significantly different from those of both control and 250 ppm groups ($P<0.05$) (Table 4). However, the value from the group receiving 500 ppm probiotic was similar to that from 750 ppm probiotic. This result agrees with those reported by others (Nahashon et al., 1994a,b; Svetic et al., 1994) and suggests that the type of the strain plays an important role in the conversion of the food *in vivo*.

No significant effect of probiotic supplementation on damaged egg ratios was seen when the periods 1-30 days, 30-60 days and 60-90 days were taken into account ($P>0.05$) (Table 4). Of these values, the lowest was from the 500 ppm group ($P<0.05$). In the total period, the damaged egg ratios for the groups were 1.42, 1.64, 0.76 and 1.65 %, respectively ($P<0.05$). Feeding with 500 ppm probiotic diet caused statistically significant reduction on damaged egg ratio ($P<0.05$). Our results corroborate a study by Svetic et al. (1994) who indicated that a similar probiotic supplementation has beneficial effects on the damaged egg ratio. Nahashon et al. (1992, 1993, 1994a, 1996b) have found that supplementation of *Lactobacillus* cultures in corn/soybean diets increased calcium retention in layers. We suggest that the decreased damaged eggs ratio observed in this study could be caused by such retention.

In the 90^{th} day of the experiment, the average values for egg weights in the four groups (control, 250, 500, and 750 ppm) showed no statistical differences (Table 4); the highest value was obtained from the group 750 ppm ($P>0.05$). The effect of supplementation of diet with probiotic could be said to be controversial since Nahashon et al. (1994a,b) found supplementation significantly increased the egg weight whilst Nahashon et al. (1994c) reported no significant effects.

In terms of specific gravity, there were no statistical differences between the groups. At the and of the experiment, the mortality figures for the groups (control, 250, 500 and 750 ppm) were found to be 1.42, 0, 1.42 and 1.42 %, (Table 4).

The probiotic supplementation did not affect specific antibody synthesis to ND vaccine antigen administered via drinking water (Table 5). The best antibody response among the groups after 90 days was in the 500 ppm probiotic-consumed group. In fact, not much spread was observed within the group titres. However, to conclude that obtaining such homogenous antibody titres can be caused by probiotic supplementation (500 ppm) is an

oversimplification since this might also be caused by the size of population from which sampling was made. Thus, no stimulatory effect by probiotic supplementation could be caused for several reasons; firstly, competitive exclusion, which is one of the important mechanisms for the control of pathogens in the intestinal flora that has been introduced in chicks (Jin et al., 1997). Since localisation of bacteria is mainly governed by this mechanism which solely depends on age, our results on the immune ineffectiveness of probiotic could be explained by the age of the hosts. Secondly, Goodling et al. (1987) pointed out that host specificity of the probiotic used was important factor in the effectiveness of the culture in any terms. Again, no stimulation of humoral immunity by probiotic administered in this study could be attributed to the non-host specific strains, species or even genera of the microorganisms. On the other hand, Maassen et al. (1998) reported that strain dependent cytokine production profiles were induced after oral administration of *Lactobacillus*, concluding that such a cytokine profile seemed to determine the direction and efficiency of the humoral response. The findings of the present study could imply that proper selection of the microorganisms did not occur. Perdigon et al. (1990) showed that the layer treated with *Lactobacillus* supplementation increased cellularity of Peyer's Patches and this indicated a stimulation of the mucosal immune system that responded to antigenic stimuli by secreting immunoglobulin (IgA). In our study, the immunity measured was basically related to the systemic immunity.

CONCLUSIONS

In conclusion, evidence from this study suggests that supplementation with a mixed culture of microorganisms improved hen-day egg production and food conversion ratio in 40 week-old layers but no effect was seen on egg weight. Serological data from the present study showed the ineffectiveness of probiotic supplementation only on systemic immunity. However, further work might be considered to focus on planning more comprehensive experimental designs examining mucosal cellular and humoral immunities, effects of age and/or strain-host interactions.

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