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Effects of Dried Whey (Prebiotics) and Probiotics in Laying Hen's Performance and Intestinal Flora

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Abstract: This study was conducted to evaluate the effects of adding levels of dried whey powder and probiotic to practical-type diets on the performance and Intestinal micro organism of laying hen's. Dried whey was used in isocaloric and isonitrogenous diets at levels of 0, 2.5, 5 and 7.5% with two Level of probiotic (0 and 1000 g^{-1} Bioplus 2B ton^{-1} feed providing 0 and 3.2×10^{6} cfu g^{-1} Feed concentration). About 72 white leghorn Hy-Line, w-36 strain used in experiment. Adding dried whey powder and probiotic to the diets caused highly significant increase ($p<0.05$) in egg production and lactobacillus in ileum but significant decrease ($p<0.05$) coliform in ileum. Using dried whey and probiobic improve egg weight, egg mass, shell thickness, shell hardness and Haugh unit but not significant ($p>0.05$). Level of 5% dried whey had significant decrease feed conversion ($p<0.05$). Other traits did not show significant different by using dried whey and probiotic.

Key words: Dried whey, probiotics, laying hen, performance, intestinal flora, Iran

INTRODUCTION

Prebiotics and Probiotics are two of several approaches that have potential to reduce enteric disease in poultry and subsequent contamination of poultry products. Probiotic, witch means For life in Greek (Gibson and Fuller, 2000) has been defined as live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance (Fuller, 1989). Prebiotics are defined as a non digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Gibson and Roberfroid, 1995), Combinations of prebiotics and prebiotics are known as synbiotics.

Whey or a liquid remaining from cheese or casein production is one of the most valuable protein sources in human food chain. In spite of its balanced nutrients liquid whey is disposed as a waste product. Environmental pollution is also a concern in many counties (Thivend, 1977). Dried whey that is produced from its liquid from can be used in poultry diets (Brunner, 1981). Recently uses on antibiotics are growth promoter is banned so use of probiotic and prebiotics or fermentable sugars instead of antibiotics is going to be popular in birds in order to improve the useful microbial population of gastrointestinal tract. Lactose that is a major component of dried whey (Zadow, 1984) is a prebiotic but since poultry are lacking Lactase, Lactose can not be digested or absorbed efficiently and almost reaches to final intestine tract. In final tract the population of useful bacteria like Lactobacillus and bifid bacteria (Harms *et al*., 1977) increases and the ph of the GIT due to increasing production of volatile fatty acid, decrease.

Therefore the environment of GIT becomes unsuitable for the activity and proliferation of pathogens (Nurmi and Rantala, 1973). Feeding viable lactobacillus at 1100 mg kg^{-1} (4.4×10^{7} cfu mg^{-1}) increased daily feed consumption, egg size, nitrogen and calcium retentions and decreased intestinal length from 7-59 weeks of age (Nahashon *et al*., 1996). Haddadin *et al*. (1996) reported that egg production, egg size and quality were improved by the addition of a liquid culture of lactobacillus acidophilus to the basal diet.

Goodling *et al*. (1987) observed no improvement in hen day egg production, egg efficiency and egg size when laying pullets were fed a drived non-viable lactobacillus product. Mahdavi *et al*. (2005) observed significant decrease in plasma Cholesterol, plasma triglyceride and egg cholesterol when laying hen's were fed a probiotic. It is shown that dietary supplementation of whey powder linearly increases body weight gain and nitrogen retention in Turkey poults and in broiler chickens (Kermanshahi and Rostami, 2006). Dietary

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supplementation of dried whey to monogastric significantly improved digestible protein and fat, feed to gain ratio and increases the absorption of minerals like Ca, P, Cu, Fe and Mg (Gleaves and Salim, 1982). The purpose of this study was to investigate the effect of dried whey (prebiotic) and probiotic on Laying hen's performance, egg quality and some microbial populations of the gastrointestinal tract.

MATERIALS AND METHODS

Birds and experimental diets: About 72 leghorn hens Hy-Line, w-6 strain were allocated in a factorial completely randomized design considering 8 treatments with 3 replicates and 3 samples in each. Supposed treatments including four level of dried whey (0, 2.5, 5 and 7.5%) were included to a corn-soybean based diet (Table 1) that met the laying hen's requirement and two level of probiotic concentration (0 and 1000 g ton^{-1} feed providing 0 and 3.2×10^{6} cfu g^{-1} feed concentration). All diets of experiment were isocaloric and isonitrogenous. Bioplus 2B, a commercial probiotic preparation was used in this study. The product contained 2 strains of bacilli. *Bacillus subtilis* (CH201) and *Bacillus iicheniformis* (CH200) with a minimum of 3.2×10^{9} cfu g^{-1} of the product.

Data and sample collection: During the 8 weeks of the experiment (41-49 weeks old) hen's had free access to feed and water. The photoperiod was 14 h light day^{-1}. Feed consumption was recorded at the end of 4 weeks of the experimental period. Egg weight, shell thick ness, shell hardness, yolk quality and albumen quality were measured for 3 consecutive day at the end of 4 weeks period and egg production were recorded daily.

Table 1: Composition of experimental diets

		Dried Whey	Levels (%)	
Ingredients (%)	0	2.5	5	7.5
Corn	57.10	54.00	50.60	43.30
Soybean meal	25.30	25.80	26.60	27.00
Fish meal	2.50	2.00	1.50	1.00
Dried whey	0.00	2.50	5.00	7.50
Soybean oil	2.85	3.50	4.20	5.00
Oyster shell	5.50	5.50	5.50	5.50
Limestone	3.80	3.77	3.77	3.85
Dicalcium phosphate	1.30	1.25	1.20	1.15
Salt	0.17	0.17	0.17	0.17
Bicarbonat sodium	0.15	0.15	0.15	0.15
DL-methionine[1]	0.15	0.15	0.15	0.15
Mineral complex[2]	0.50	0.50	0.50	0.50
Vitamin D	0.10	0.10	0.10	0.10
Vitamin E	0.10	0.10	0.10	0.10
Calculated analysis				
ME (kcal kg^{-1})	2800.00	2800.00	2800.00	2800.00
Crud protein (%)	17.50	17.50	17.50	17.50
Calcium (%)	4.03	4.03	4.03	4.03
Available phosphate (%)	0.42	0.42	0.42	0.42
Methionine (%)	0.42	0.42	0.42	0.42
Methionine+cystein (%)	0.70	0.70	0.70	0.70
Lysine (%)	0.94	0.94	0.96	0.96

[1]Vitamin complex provided per kilogram of diet: vitamin A, 10000 IU; vitamin D_3, 2500 IU; vitamin E, 10 IU; vitamin B_1, 2.2 mg; vitamin B_2 4 mg; pantothenic acid, 8 mg; vitamin B_6, 2 mg; niacin , 30 mg; vitamin B_{12}, 0.15; Folic acid, 0.5 mg; biotin, 0.15 mg; Colin chloride, 200 mg; [2]Mineral premix provided per kilogram of diet: manganese, 80 mg; Copper, 10 mg; iodine, 0.8 mg; cobalt, 0.25 mg; selenium, 0.3 mg; zinc, 80 mg; Iron, 80 mg

Microbial count: On end of experiment 2 birds was slaughtered by the cervical dislocation method. After slaughtered by the abdominal surface of the carcass and areas around it, the internal organs was removed. Then about 7 cm from the length of the ileum were sampled, To determine the microbial population, 1 g of ileum content was used to make 10 fold dilution using buffered peptone water and then 0.1 mL of the appropriate ileum dilution were spread on MRSA plates (to detect lactic acid bacteria) and VRBA (to detect Coli forms) (Izat *et al*., 1990). The culture of lactic acid and Coli form bacteria was made anaerobically form. The plates were incubated at 37.5°C for 48 h. After counting the number of colonies in each plate, the number so obtained was multiplied by inverse of the dilution on the result was stated as the number of colony forming unit (cfu) in l g of the sample (Downes and Lto, 2001).

Data analysis: Data were analyzed using General linear Module (GLM) procedure of Statistical Analysis System (SAS, 1999) and when the means were significant ($p<0.05$), Duncan multiple range test was used to determine treatments difference.

RESULTS AND DISCUSSION

Production characteristics: Analysis of the egg production, egg weight, Feed consumption, feed conversion ratio and egg mass data are shown in Table 2. Inclusion of dried whey and probiotic no significant affects ($p>0.05$) in feed Consumption, egg weight and egg mass but use dried whey and probiotic improve these factors. Dried whey and probiotic had significant effect $p<0.05$) at egg production.

Levels 5 and 7.5% of dried whey and probiotic increase egg production. Used dried whey showed significant effect on feed conversion ratio ($p<0.05$) and level of 5% dried whey had least feed conversion but add probiotic did not effect ($p>0.05$). Researchers attributed unidentified factor dried whey to its balanced amino acids, high protein efficiency ratio (Susmel *et al*., 1995) and rich source of water soluble vitamins (Modler, 1982) have characteristics of dried whey that can cause increase egg production and improve other factor production. Probiotic increase egg production, this results are in agreement

Table 2: Effect of dried whey and probiotic on production characteristics in laying hen's

Source variation	Egg weight (g)	Egg production (%)	Feed consumption (g/hen/day)	Feed conversion ratio(g/g)	Egg mass (g/hen/day)
Dried whey (%)					
0	58.380	78.660^{b}	99.200	2.164^{a}	45.980
2.5	57.770	79.190^{b}	96.400	2.092^{ab}	46.320
5	61.570	81.700^{a}	100.200	1.997^{b}	50.370
7.5	59.280	82.620^{a}	100.100	2.049^{ab}	48.970
Probiotic levels (cfu g^{-1} feed)					
0	58.890	79.310^{b}	97.600	2.099	46.760
302×10^{6}	59.620	81.780^{a}	100.200	2.052	49.000
SE	0.798	0.662	1.087	0.027	0.861

Means followed by the same superscript letters in each column are not significant (p>0.05)

Table 3: Effects of dried whey and probiotics on egg quality traits

Source of variation	Shell thikness (mm)	Shell hard-ness kg cm^{-1}	Haugh unit	Yolk index
Dried whey (%)				
0	0.360	3.281	60.52	0.413
2.5	0.368	3.23	61.94	0.401
5	0.376	3.29	63.45	0.408
7.5	0.375	3.28	68.58	0.416
Probiotics levels (cfu g^{-1} feed)				
0	0.360^{b}	3.17	61.28	0.408
3.2×10^{6}	0.380^{a}	3.36	65.98	0.411
SE	0.004	0.121	1.77	0.004

Means followed by the same superscript letters in each column are not significant (p>0.05)

with Yoruk *et al.* (2004) and Panda *et al.* (2003) who reported statistically significant increase of product egg in leghorn lying hens during the whole laying period. Result Feed consumption, egg weight, egg mass and feed conversion ratio agreement with Mohan *et al.* (1995) and Haddain *et al.* (1996) but Nahashon *et al.* (1996) and showed that using vital Biomass of probiotic supplements affects the egg weight significantly (p<0.05).

Egg quality traits: Analysis of the shell thickness, sell hard, Haugh unit and yolk index are shown in Table 3. Addition of probiotic had significant effect (p<0.05) on shell thickness and dried whey improve shell thickness but not significant (p>0.05). Effect probiotic and dried whey on shell hardness, index yolk and Haugh unit not significant (p>0.05). Adding whey to diet contributes to digestibility and absorption of nutrient in diet, this is because of the fact that whey produces an acidic condition that suitable for growth lactobacillus and cause to increase of digestibility and absorption of nutrient (Bilgili and Moran, 1995).

There are some reports showing whey powder increase absorption of some minerals like Ca, P, Cu, Fe and Mg (Gleaves and salim, 1982), these factors can improve the egg quality traits. Addition of probiotic had no significant effect on shell hardness and thickness (Haddadin *et al.*, 1996; Mohan *et al.*, 1995) that results hardness this experiment agreement with them. Damron and Jan Found significant Improvements in interior egg quality as measured by Hough United in hens fed distillers feeds and corn fermentation soluble. Subsequent studies indicated that trace elements my have been involved. But described that the variations in plasma mineral concentration were not Sufficient to implicate supporting the hypothesis that trace elements improve albumen quality with microbial Supplementation.

Table 4: Effect of dietary of dried whey and probiotic on Ileum microbial population of laying hen's

Source variation	Coli form	Lactobacillus
Dried whey (%)		
0	5.46^{a}	6.84^{c}
2.5	5.29^{ab}	7.16^{b}
5	4.94^{bc}	7.52^{a}
7.5	4.70^{c}	7.68^{a}
Probiobic levels(cfu g^{-1} feed)		
0	5.30^{a}	7.12^{b}
3.2×10^{6}	4.90^{b}	7.48^{a}
SE	0.098	0.087

Means followed by the same superscript letters in each column are not significant (p>0.05)

Microbial culture: The results of the effect of the mentioned additives on the population composition of the microbe's ileum are shown in Table 4. Results coli form indicate that the birds under dried whey and probiotic treatments had decrease significant (p<0.05) relative to the control treatment and additive of dried whey up to 5% and probiotic significant increase (p<0.05) lactobacillus in the ileum.

Fairchild *et al.* (2001) and Spring *et al.* (2000) reported that the use of probiotics in the ration reduces the total population of coliforms in the intestinal lumen. Rada *et al.* (1995) found that the use of lactobacillus salivary in the chickens ration can reduce the coliform bacteria population significantly as camped with the control treatment by reducing the intestinal pH level. Despise of this, Yang *et al.* (2007) and Ceylan *et al.* (2003) observed no change in the gastrointestinal microbial flora by adding probiotic.

The probiotic microorganisms, prevent the growth of pathogenic bacteria in the intestinal environment by production of acids (such as acetic acid and lactic acid) and other components (Fuller, 1989).

Lactose in dried whey can not digested or absorbed so fermented by lactic acid bacteria, production of lactic acid and reduction in the Ph-value, multiplication of pathogenic bacteria is reduced (Chung and Day, 2004).

CONCLUSION

It is concluded that the use of dried whey and probiotic can reduce the intestinal pH and provide a good environment for lactobacillus growth and limited multiplication coliform bacteria.

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