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Influence of Supplemental Dried Whey on Broiler Performance and Cecal Flora

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Abstract: A study was conducted to evaluate the effects of adding levels of dried whey powder (DW) to practical-type diets on the performance and cecal micro organism of broilers. DW was used in isocaloric and isonitrogenous diets at levels of 0, 2, 4 and 8 percent and fed from 1-49 days of age. Samples of cecal contents at 7, 14 and 35th days of age were collected for counting total micro organism, coliforms and salmonella in suspension of cecal contents. Body weight gain of chickens fed 2 to 8 percent DW was improved at 49 days of age. Adding DW to the diets from 2 to 8 percent, improved feed to gain ratio at 21-42 and 42-49 days of age. Carcass weight in chickens received 2 and 4 percent DW was maximum ($p < 0.05$) at 49 days of age. Gastrointestinal tract weight of chickens fed DW was significantly increased ($p < 0.05$) when compared to control group. Total counts and coliforms of cecal contents significantly increased in broilers received DW during 0–14 days of age ($p < 0.05$). No salmonella colony was isolated from the samples. Findings of the present study indicate that 2-4 percent DW in the diet of broilers had beneficial effects on performance. Changes in cecal micro flora indicated an environment more hostile to salmonella establishment from 0-21 days of age.

Key words: Dried whey, cecal micro flora, performance, nutrition, chickens

Introduction

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. Liquid whey has a high biological oxygen demand so its disposal in rivers kills living organisms. Environmental pollution is also a concern in many countries (Thivend, 1977). In theory protein content of whey includes alpha and beta-lactoglobulins and can be used a valuable source of protein to animals (Brunner, 1981).

Dried whey (DW) that is produced from its liquid form can be used in chickens (Susmel *et al.*, 1995). For years, DW is used in monogastric nutrition (Balloun and Khajarearn, 1974; Damron *et al.*, 1971). It is shown that dietary supplementation of whey powder linearly increases body weight gain and nitrogen retention in turkey poults (Balloun and Khajarearn, 1974) and in broiler chickens (Al-Ubaidi and Bird, 1964). Dietary supplementation of DW to monogastrics significantly improved digestible protein and fat, feed to gain ratio (Balloun and Khajarearn, 1974) and increases the absorption of minerals like Ca, P, Cu, Fe and Mg (Earl and Salim, 1982).

Recently use of antibiotics as growth promoter is banned (Ziggers, 2000). Use of 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 (GIT). Lactose that is a major component of DW (Zadow and Csiro, 1984), is a prebiotic but since poultry are lacking lactase (Harms

et al., 1977), lactose can not be digested or absorbed efficiently and almost reaches to ceca and large intestine intact (Langhout, 1989; Ziggers, 2000). In ceca the population of useful bacteria like lactobacillus and bifidobacteria (Ziggers, 2000) increases and the pH of the GIT, due to increasing production of volatile fatty acids (VFAs), decreases. Therefore the environment of GIT becomes unsuitable for the activity and proliferation of pathogens like Salmonella. Based on Nurmi concept of competitive exclusion (Nurmi and Rantala, 1973), pathogens will be expelled out of the gut by useful bacteria if it already occupied the gut sites. Colonization of useful micro flora in the gut of young birds that is related to gut conditions, can inhibit further colonization of pathogens (Nurmi and Rantala, 1973). Lactose as a prebiotic can alleviate the conditions of the gut in favour of colonization of useful bacteria (Langhout, 1998; Ziggers, 2000). Therefore, the objective of this study was to evaluate the effect of dietary levels of DW on performance of broiler chickens and its effect on some microbial populations of the ceca and the possible excretion of salmonella from feed origin during rearing period.

Materials and Methods

Birds and experimental diets: 240 day-old mixed Ross broiler chickens were randomly divided to 20 groups of 12 birds each and reared for 49 days. Four levels of DW (0, 2, 4 and 8 %) were included to a corn-soybean based diets (Table 1) that met the broiler requirements (NRC, 1994). Feed and water were provided *ad libitum*. All

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Table 1: Composition of experimental diets

starter (0-21 days)				
Ingredients (%)	Dried whey levels (%)			
	0	2	4	8
Com	47.50	49.07	50.65	45.49
Soybean meal	30.13	30.34	30.55	30.00
Fish meal	5.45	4.69	3.93	3.00
Dicalcium phosphate	0.89	0.89	0.90	0.85
Limestone	1.08	1.10	1.11	1.14
Premix ¹	0.50	0.50	0.50	0.50
Iodized salt	0.34	0.28	0.23	0.11
Soybean oil	6.00	5.00	4.00	1.81
DL-Methionine	0.11	0.12	0.12	0.11
Sand	8.00	6.00	4.00	0.00
Dried whey powder	0.00	2.00	4.00	8.00
Calculated chemical composition:				
Crude protein (%)	20.86	20.86	20.86	20.86
ME (Kcal/Kg)	2900	2900	2900	2900
grower (21-42 days)				
Ingredients (%)	Dried whey levels (%)			
	0	2	4	8
Com	68.56	67.50	66.62	64.34
Soybean meal	23.54	23.15	23.88	21.99
Fish meal	3.00	3.00	3.00	3.00
Dicalcium phosphate	2.40	2.04	0.85	0.97
Limestone	1.72	1.58	1.04	1.17
Premix ¹	0.50	0.50	0.50	0.50
Iodized salt	0.26	0.20	0.00	0.00
Soybean oil	0.00	0.00	2.00	0.00
DL-Methionine	0.03	0.03	0.03	0.03
Sand	8.00	6.00	4.00	0.00
Dried whey powder	0.00	2.00	4.00	8.00
Calculated chemical composition:				
Crude protein (%)	18.12	18.12	18.12	18.12
ME (Kcal/Kg)	2900	2900	2900	2900
finisher (42 to end of experiment)				
Ingredients (%)	Dried whey levels (%)			
	0	2	4	8
Com	59.91	62.17	64.47	66.80
Soybean meal	25.41	24.42	23.40	21.78
Dicalcium phosphate	1.18	1.10	1.02	1.53
Limestone	1.00	1.00	1.00	1.00
Premix ¹	0.50	0.50	0.50	0.50
Iodized salt	0.00	0.00	0.00	0.07
Soybean oil	3.96	2.77	1.58	0.00
DL-Methionine	0.03	0.02	0.02	0.02
Sand	8.00	6.00	4.00	0.00
Dried whey powder	0.00	2.00	4.00	8.00
Calculated chemical composition:				
Crude protein (%)	16.31	16.31	16.31	16.31
ME (Kcal/Kg)	2900	2900	2900	2900

¹Supplied per kilogram of diet: vitamin A, 10000 IU; vitamin D₃, 9790 IU; vitamin E, 121 IU; B₁₂, 20 µg; riboflavin, 4.4 mg; calcium pantothenate, 40 mg; niacin, 22 mg; choline, 840 mg; biotin, 30 µg; thiamin, 4 mg; zinc sulfate, 60 mg; manganese oxide, 60 mg.

Table 2: Effect of dietary supplementation of dried whey on broiler performance at starter, grower and finisher periods

DW ¹ (%)	0-21 days			21-42 days			42-49 days		
	BWG (g)	FI (g)	F/G	BWG (g)	FI (g)	F/G	BWG (g)	FI (g)	F/G
0	528.3	875.8 ^{b2}	1.658 ^b	1183.3 ^{bc}	3067.8 ^{ab}	2.59 ^a	358.2 ^b	1310.4	3.66 ^a
2	528.9	904.5 ^{ab}	1.710 ^a	1234.1 ^{ab}	3134.7 ^a	2.54 ^a	494.6 ^a	1458.9	2.95 ^b
4	509.7	877.3 ^b	1.721 ^a	1278.2 ^a	2905.0 ^{ab}	2.27 ^b	450.3 ^a	1308.7	2.91 ^b
8	516.6	922.8 ^a	1.786 ^a	1145.3 ^c	2857.5 ^b	2.50 ^{ab}	486.3 ^a	1313.4	2.70 ^b
±MSE ³	11.78	14.24	0.025	22.37	86.35	0.075	26.28	58.63	0.233

¹DW, dried whey; BWG, body weight gain; FI, feed intake; F/G, feed to gain ratio. ²Means in each column with different superscripts are significantly different (P<0.05). ³Mean standard error.

Table 3: Effect of dietary supplementation of dried whey on the overall broiler performance from 0-49 days of age

DW ¹ (%)	BWG (g)	FI (g)	F/G
0	2069.8 ^{b2}	5254.1	2.54 ^a
2	2257.7 ^a	5498.1	2.44 ^{ab}
4	2238.2 ^a	5090.9	2.28 ^b
8	2148.2 ^b	5093.7	2.37 ^b
±MSE ³	31.29	126.05	0.081

¹ DW, dried whey; BWG, body weight gain; FI, feed intake; F/G, feed to gain ratio. ² Means in each column with different superscripts are significantly different (P<0.05). ³ Mean standard error.

diets in 3 periods of 0-21, 21-42, and 42-49 days of experiment were isocaloric and isonitrogenous. Starter, grower and finisher diets contained 2900 kcal/kg Men and 20.86, 18.12 and 16.31 percent respectively. Sweet DW that prepared from local sources, contained 4 percent moisture, 14.5 percent crude protein, 0.2 percent fat and 67.7 percent lactose (factory analysis) and had no coliforms contamination. Its colour, texture and taste were whitish cream, uniform and natural respectively.

Data and sample collection: Body weight gain (BWG) and feed intake (FI) recorded weekly for 7 weeks. At 7, 14, and 35 days of age, one bird from each replicate of treatments was killed by cervical dislocation and the cecal samples were thawed, diluted with sterile water and froze for further analysis. At 49 days of age one bird from each replicate of treatments was also killed and the fat pad, GIT weight, and dressed carcass weight were measured.

Microbial counts: Cecal samples were defrost, diluted by ceilin and a serial dilutions were prepared. By using McKagi agar (MCA) and eosin blue agar (EBA) cultures, the possible presence of salmonella was tested by standard plate counts. In order to count the total microbial growth, plate count agar (PCA) was used (APHA, 1993). Prepared cultures incubated for 24-48 hours at 29-31°C. Colonies were counted and evaluated (APHA, 1993; Neiman *et al.*, 1987) by aerobic plate count (APC) and surface plate count (SPC). To make sure of the presence of salmonella, remained cecal contents were mixed and transferred to sterile selenite broth and incubated for 18-24 hours at 37°C. The enriched samples were then transferred to MCA and brilliant green agar (BGA) cultures.

Data analysis: Data were analyzed using general linear model (GLM) of SAS (SAS institute, 1985) and when the means were significant (P<0.05), Duncan multiple range test was used (Duncan, 1955).

Results

The performance of chickens including body BWG, FI and feed to gain ratio (F/G) at 0-21, 21-42 and 42-49

days of age are presented in Table 2. Levels of DW had no effect on BWG at 0-21 days of age but was significant (P<0.05) at 21-42 days of age. By increasing the levels of DW up to 4 percent, BWG improved. BWG was also increased at 42-49 days of age (P<0.05). 8 percent DW had no effect on BWG when compared to control (no added DW). At 42-49 days of age, DW had a significant effect on BWG but this effect for 2, 4, and 8 percent DW was not significant. The FI at 0-21 days of age and 8 percent DW was significantly higher than those of the other treatments (Table 2) but at 21-42 days of age, this level of DW decreased FI significantly (P<0.05). At this period, the highest FI was for 2 percent level of DW (3134.7 g). FI was not affected by dietary treatments at 42-49 days of age, although the same trend as 21-42 days of age was observed. At 0-21 days of age, DW had a negative effect on F/G (P<0.05) but this effect reversed and the best F/G was for 4 percent DW (Table 2). However, this effect between 4 and 8 percent DW was not significant. The overall BWG (0-49 days) was significantly improved (P<0.05) by addition of 2 and 4 percent DW (Table 3). The overall FI among treatments was not affected by dietary treatments. However, addition of DW improved the overall F/G of broilers significantly (P<0.05). Addition of DW up to 4 percent level significantly improved the overall F/G although between 4 and 8 percent DW, this effect was not significant (2.28 and 2.37 vs 2.54 and 2.44). Carcass weight at 49 days of age was significantly increased by addition of 2 and 4 percent DW (P<0.05). However at this stage, 8 percent DW had no significant effect on carcass weight when compared with control group (Table 4). The amount or the ratio of fat pad to live body weight in 4 percent DW treatment was significantly higher than those of the others (60.04 g and 2.81 percent respectively). Dietary supplementation of DW significantly increased GIT weight (gut fill) but as the percentage of live body weight, it was not significant.

The appearance of ceca in chickens received DW were expanded and foamy when compared with control group (no added DW) group (Fig. 1). Samples of cecal contents at different ages in MCA and EMB cultures didn't show any salmonella colony but the number of E. coli colonies and total counts were significantly higher (P<0.05) in 4 and 8 percent DW (table 5) at 7 and 14 days of age. At 35 days of age treatments had no significant effect on the number of coliforms. No salmonella contamination was also observed in cultures that are usually detectable by the formation of colourless and red to pink colonies.

Discussion

For the years whey powder has been known as a source of unidentified factor (UGF) and used in poultry diets (Susmel *et al.*, 1995). BWG at 49 days of age in chickens fed DW was higher than that of control group. This trend



Figure 1: Effect of dietary levels of dried whey (D W) on ceca appearance at 49 days of age

Table 4: Effect of dietary supplementation of dried whey on carcass characteristics of broiler chickens at 49 days of age

DW ¹ (%) / Traits	CW (g)	FPW (g)	(FPW/LBW)*100	GITW (g)	(GITW/LBW)*100
0	2040.0 ²	49.62 ^b	2.24 ^b	198.96 ^b	8.97
2	2228.0 ^a	48.44 ^b	2.13 ^b	233.76 ^a	9.09
4	2208.0 ^{ab}	60.04 ^a	2.81 ^a	209.22 ^{ab}	9.19
8	2112.0 ^{bc}	46.78 ^b	2.16 ^b	210.36 ^{ab}	9.71
±MSE ³	35.60	3.731	0.173	9.382	0.358

¹DW, dried whey; CW, carcass weight; FPW, fat pad weight; LBW, live body weight; GITW, gastrointestinal tract weight.

² Means in each column with different superscripts are significantly different (P<0.05).

³ Mean standard error.

Table 5: Effect of dietary supplementation of dried whey on cecal microbial population of broiler chickens at 7, 14 and 35 days of age

DW (%) ¹	7 days		14 days		35 days	
	Total counts	Coli forms	Total counts	Coli forms	Total count	Coli forms
	Log ₁₀ CFU ² per gram cecal contents					
0	7.19 ^{bc}	6.35 ^b	5.92 ^b	4.19 ^c	5.86	4.82
2	7.31 ^b	6.25 ^b	6.18 ^b	5.21 ^b	5.52	5.00
4	8.16 ^a	7.31 ^a	7.32 ^a	5.47 ^{ab}	5.51	5.29
8	7.91 ^a	6.62 ^b	7.04 ^a	6.06 ^a	5.36	5.50
±MSE ³	0.138	0.138	0.274	0.240	0.188	0.243

¹DW, dried whey. ²CFU, Colony forming unit. ³Means in each column with different superscripts are significantly different (P<0.05).

³Mean standard error.

was also similar in all 3 periods. The highest BWG was observed in treatments with 2 and 4 percent DW. This clearly shows beneficial effect of DW on BWG. These results are in agreement with those of Al-Ubaidi and Bird (1964) in chickens and Potter and Shelton (1976) in turkeys. Researchers attributed the UGF of DW to its balanced amino acids (Al-Ubaidi and Bird, 1964), high protein efficiency ratio (Susmel *et al.*, 1995), rich source of water soluble vitamins (Modler, 1982). Poultry are lacking lactase enzyme (Harms *et al.*, 1977) and DW in this experiment had 67 percent lactose. Therefore high levels of DW to poultry diets can not digested well and may cause some osmotic diarrhea that we saw in the chickens fed 8 percent DW. The lower weight at 49 days

of age may also attribute to this effect. The overall improvement of F/G in this study was supported by others (Barnett and Bird, 1956). There are some reports showing whey powder up to 4 percent increases fat and protein digestibility (Balloun and Khajareem, 1974; Susmel *et al.*, 1995) and increases absorption of some minerals like calcium and phosphorous (Al-Ubaidi and Bird, 1964). These factors can improve the efficiency of feed consumed by birds. Partial improvement might be related to the beneficial effect of lactose on gut microorganisms like lactobacillus and bifidobacteria in chickens (Al-Ubaidi and Bird, 1964; Barnett and Bird, 1956; Corrier *et al.*, 1990a, b; Waldroup *et al.*, 1992). The presence of DW in the diet increased the total count

of microorganisms in the ceca of chickens in early ages and increased the ceca weight and volume and also the foamy form of ceca are all the evidences clearly indicating lactose in DW is fermented by lactobacillus and/or coliforms (Tellez *et al.*, 1993). Heavier gastrointestinal tract of the chickens fed higher levels of DW is also related to the heavier ceca. In this study, by increasing the level of DW, the ceca of chickens were bigger and foamier. This result was also confirmed by others (Corrier *et al.*, 1991). Since microflora in the ceca of younger chickens (0-21) are not completed yet, therefore the chickens fed more DW in comparison with control group, feed to gain ratio (F/G) should be higher and feed is less digested. Lactobacillus and coliforms can use lactose as a source of fermentable carbohydrate and produce lactic acid and volatile fatty acids like acetic, propionic and butyric acid (Corrier *et al.*, 1990b; Hume *et al.*, 1992). Increasing iodized VFAs in more acidic conditions of ceca have bacteriostatic and bactericidal effect on pathogenic bacteria like salmonella (Chambers *et al.*, 1997; Hinton *et al.*, 1990). Higher susceptibility of the chickens to salmonella colonization is mostly related to the lower concentration of VFAs and higher pH of GIT in early ages (Nurmi and Rantala, 1973). Therefore, use of DW at early ages decreases pH of GIT and ceca and inhibits salmonella colonization (Lloyd *et al.*, 1977; Soerjadi *et al.*, 1982). In this experiment, the number of coliforms that are mostly fecal *E. coli*, increased with increasing DW in the diet at week one and two. Cecal microflora dramatically changes and becomes complete by age (Corrier *et al.*, 1991; Mead and Adams, 1975). That is why at 35 days of age, total counts and the number of coliforms among treatments were not significant. The lack of salmonella in cecal contents may attribute to the lack of salmonella in the feed and possibly changes in cecal microflora indicates an environment more hostile to salmonella establishment from 0-21 days of age. Under the conditions of this study, use of dried whey at the level of 2-4 percent had beneficial effects on bird performance at rearing period and cecal microflora at starter period.

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