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Effect of Different Dietary Levels of Acid Whey Powder on Growth Performance and Immune Responses of Broiler Chicks

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Abstract: The present study was carried out to evaluate the effect of different levels of acid whey powder (AWP) and low lactose acid whey powder (LLAWP) on growth performance and immunity of broiler chicks. A total of 416 one-day old broiler chicks (Ross 308) were randomly allocated to 1 of 8 isocaloric and isonitrogenous dietary treatments (4 pen replicates; 13 birds per pen) and grown over a 42-d experimental period. The Dietary treatments included control, 1, 2.5, 5, 7.5% AWP and 2.5, 5 and 7.5% LLAWP. Body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were recorded biweekly throughout 42 d. Antibody titers against Newcastle and influenza viruses and sheep red blood cells (SRBC) were determined to evaluate immune responses. In overall growth period, BWG in broilers received 2.5 or 5% LLAWP significantly increased and FCR in those fed 2.5% LLAWP decreased ($p<0.05$). Broilers received 5% LLAWP had the greatest FI in overall growth period ($p<0.05$). Lymphoid organs weight and antibody titers against Newcastle and influenza viruses and SRBC were not affected significantly by dietary treatments ($p>0.05$). It was concluded that, 2.5% dietary LLAWP improved performance and AWP could be used up to 5% in diet without adverse effects on performance and immunity of broiler chicks.

Key words: Broiler, acid whey, lactose, performance, immunity

INTRODUCTION

Whey is a fluid milk product obtained during the manufacture of cheese, casein or similar products by separation from the curd after coagulation of milk and products obtained from milk. In acid whey, Coagulation is obtained principally by acidification and in whey through the action of principally rennet type enzymes. The pH and protein of acid whey is lower than whey (pH: 4 vs 5.1 and protein 7 vs 12%). 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 β-lactoglobulin, α-lactalbumin, immunoglobulins, bovine serum albumin, lactoferrin and lactoperoxidase. Thus, whey can be used as a valuable source of protein for animals (Brunner, 1981; Szczurek *et al*., 2013). Dried whey that is produced from its liquid form can be used in poultry diets (Susmel *et al*., 1995). Supplementing whey to diet, contribute to digestibility and absorption of nutrients in diet. For years, whey powder has been used in nutrition of monogastric animals (Balloun and Khajarern, 1974; Damron *et al*., 1971). Balloun and Khajarern (1974) reported that supplementing whey powder in turkey pouts increased BWG and nitrogen retention. It has been shown that inclusion of 2 to 4 percent whey powder in diet of broilers had beneficial effects on performance (Kermanshahi and Rostami, 2006). Dietary supplementation of whey powder to monogastrics significantly increased protein and fat digestibility, improved feed to gain ratio (Balloun and Khajarern, 1974) and increased the absorption of minerals such as Ca, P, Cu, Fe and Mg (Earl and Salim, 1982).

A major factor potentially affecting the use of whey powder in poultry feeds is the high presence of lactose (60%). In contrast to mammals, birds have no lactase (β-galactosidase) which is required to digest lactose and so they are unable to efficiently digest lactose and about half of ingested lactose passes into the lower intestinal tract (Atkinson *et al*., 1957). Anaerobic micro-biota which are living in the gastrointestinal tract (GIT) of the birds convert lactose to short-chain organic acids, especially lactate and propionate (Rehman *et al*., 2009). Moreover, due to the increasing production of volatile fatty acids (VFAs), pH of the GIT decreases (Ziggers, 2000). Therefore, it seems that acid whey may act as a natural probiotic in birds, improving survival rates and performance of broiler chicks. It also might that both level and lactose content of the whey supplement significantly affect broilers performance.

However, the effect of different dietary levels of low lactose acid whey and acid whey powder in broilers diet is less investigated. Therefore, the objectives of this

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study were to investigate the effects of different dietary levels of low lactose acid whey and acid whey powder on growth performance and immunity in broiler chicks.

MATERIALS AND METHODS

Birds, diets and management: A total of 416 day-old broiler chicks (Ross 308) were weighed individually and assigned to 1 of 8 isonitrogenous and isocaloric dietary treatments (4 pen replicates; 13 birds/cage) in completely randomized design. Dietary treatments included control, 1, 2.5, 5 and 7.5% acid whey powder and 2.5, 5 and 7.5% low lactose acid whey powder. The ingredients and composition of experimental diets are presented in Table 1-3.

The acid whey powder was provided from Isfahan Pegah Milk Company and analyzed for the components (Cp: 8.6, P: 1.52, Ca: 0.43, Na: 1.3, lactose 78% and in low lactose 40%).

Chicks were raised on floor pens for 6 weeks and had free access to feed and water throughout the entire experimental period (0-42 days). The lighting program consisted of a period of 23 h light and 1 h of darkness.

Growth performance: BW of broilers were determined at 1, 14, 28 and 42 days of age. FI and body weight gain were recorded in different periods and FCR was calculated. Mortality was recorded as it occurred. At 42 days of age, two birds per replicate were randomly selected, slaughtered and lymphoid organs such as bursa of Fabrecius and spleen were collected, weighed and calculated as a percentage of live body weight.

Immune system measurements: At 42 days of age, two birds per replicate were randomly selected, slaughtered and lymphoid organs such as bursa of Fabrecius and spleen were collected, weighed and calculated as a percentage of live body weight.

At 10 days of age, Newcastle and influenza antigens were injected to chicks with dual vaccine of Newcastle-influenza. Two chicks per pen were selected randomly for injection with a 1.0 ml of 1% sheep red blood cells (SRBC) suspension on day 25. Five days post immunization, the same wing-banded birds were bled to determine antibody titer against SRBC and also against influenza and Newcastle. Subsequently antibody titer against SRBC was measured by HA method and antibody titer against influenza and Newcastle were separately measured by HI method.

Statistical analysis: All data were subjected to ANOVA using the GLM procedure of SAS software (SAS, 1996) as a completely random design. The treatment means were separated by LSD tests at $p<0.05$ statistical level.

RESULTS AND DISCUSSION

Growth performance: Table 4 shows the growth performance of broiler chickens after. In starter period (1-14 d) BWG depressed significantly in chicks fed control and 1% whey powder compared with those fed whey powder diets ($p<0.05$). In this period FI significantly increased in broilers fed 5 and 7.5% acid whey powder and FCR was increased in control and 1% whey powder ($p<0.05$). FI of broilers in grower and finisher periods were significantly affected by dietary treatments and 5% low lactose had the greatest FI ($p<0.05$). In overall growth performance, BWG significantly increased in groups received 2.5 and 5% and FI increased in broilers given 5% low lactose whey powder ($p<0.05$). FCR significantly improved in broilers fed 2.5% low lactose whey powder ($p<0.05$).

In this experiment improvement in growth performance observed in diet supplemented with low lactose whey powder, especially at level of 2.5%. Lactose is a major component of whey powder and is a disaccharide that naturally occurs in mammalian milk. Lactose or milk sugar can be broken down into its two smaller components of galactose and glucose by lactase enzyme. Lactose content of whey powder is limiting factor for using whey powder in poultry diet because of poultry are lacking enzyme lactase (Harms *et al*., 1977). In the present study, lactose contents were 78 and 40% in whey powder and low lactose whey powder, respectively. Therefore, poultry can not well digest high levels of whey powder in their diets and this may cause diarrhea (Kermanshahi and Rostami, 2006). The improvement in performance of broilers fed low lactose whey powder and low levels of whey powder treatments may also attributed to this effect. Additionally, Waldroup *et al*. (1992) reported that high levels of lactose in broiler diets have been shown to increase the incidence of diarrhea. The improvement in growth performance by whey powder attributed to its balanced amino acid profile, unknown growth factor (UGF) (Al-Ubaidi and Bird, 1964), high quality protein (Szczurek *et al*., 2013; Susmel *et al*., 1995), being rich source of water soluble vitamins (Molder, 1982) and contribution to absorption of minerals like Ca, P, Cu, Fe and Mg (Earl and Salim, 1982).

Our finding regarding the improvement or none adverse effect on growth performance by low levels of whey powder was supported by other researchers (Kermanshahi and Rostami, 2006; Al-Ubaidi and Bird, 1964; Corrier *et al*., 1990; Radfar and Farhoomand, 2008; Majewska *et al*., 2009).

Immune system measurements: The effect of different levels of acid whey powder on lymphoid organs of broiler chicks is presented in Table 5. Dietary treatment had no significant effect on bursa of Fabrecius and spleen weights.

Antibody titer against Newcastle and Influenza viruses and SRBC in broiler chicks was not significantly affected by whey powder (Table 6). We expected that immune

Table 1: Composition of the experimental diets in starter

		Whey powder			
Ingredients (%)	Control	1%	2.5%	5%	7.5%
Whey powder	0	1	2.5	5	7.5
Corn	49.5	48.4	47.5	46	44.4
Soybean meal	38.4	38.4	38.6	38.7	38.8
Soybean oil	2.72	2.86	3.03	3.22	3.45
Wheat bran	5	5	4	3	2
Monocalcium phosphate	1.4	1.35	1.26	1.11	0.95
Calcium carbonate	1.7	1.7	1.74	1.77	1.81
Salt	0.25	0.25	0.25	0.25	0.17
Vitamin premix[1]	0.25	0.25	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.33	0.33	0.33	0.33	0.33
L-Lysine HCl	0.19	0.18	0.17	0.16	0.15
Calculated composition					
Metabolizable energy (Kcal/kg)	2870	2870	2870	2870	2870
Crude protein (%)	21.74	21.74	21.74	21.74	21.74
Methionine+Cysteine (%)					
Lysine (%)	1.36	1.36	1.36	1.36	1.36
Calcium (%)	0.99	0.99	0.99	0.99	0.99
Available phosphorus (%)	0.47	0.47	0.47	0.47	0.47

[a]Vitamin premix provided per kg of diet: vitamin A: 2.7 mg; vitamin D_3: 0.05 mg; vitamin E: 18 mg; vitamin k_3: 2 mg; Thiamine: 1.8 mg; Riboflavin: 6.6 mg; Pantothenic acid: 10 mg; Pyridoxine: 3 mg; Cyanocobalamin: 0.015 mg; Niacin: 30 mg; Biotin: 0.1 mg; Folic acid: 1 mg; Choline chloride: 250 mg and Antioxidant: 100 mg

[b]Mineral premix provided per kg of diet: Fe: 50 mg; Mn: 100 mg; Zn: 100 mg; Cu: 10 mg; I: 1 mg; Se: 0.2 mg

Table 2: Composition of the experimental diets in grower

		Whey powder			
Ingredients (%)	Control	1%	2.5%	5%	7.5%
Whey powder	0	1	2.5	5	7.5
Corn	55.1	54	53.1	51.7	50.2
Soybean meal	35	35	35.2	35.3	35.4
Soybean oil	2.35	2.53	2.69	2.86	3
Wheat bran	4	4	3	2	1
Monocalcium phosphate	1.19	1.13	1.05	0.89	0.74
Calcium carbonate	1.4	1.4	1.42	1.46	1.5
Salt	0.25	0.25	0.25	0.09	0
Vitamin premix[1]	0.25	0.25	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.24	0.24	0.24	0.24	0.24
L-Lysine HCl	0.03	0.03	0.03	0.01	0
Calculated composition					
Metabolizable energy (Kcal/kg)	2930	2930	2930	2930	2930
Crude protein (%)	20.49	20.49	20.49	20.49	20.49
Methionine+Cysteine (%)	0.89	0.89	0.89	0.89	0.89
Lysine (%)	1.15	1.15	1.15	1.15	1.15
Calcium (%)	0.83	0.83	0.83	0.83	0.83
Available phosphorus (%)	0.42	0.42	0.42	0.42	0.42

[a]Vitamin premix provided per kg of diet: vitamin A: 2.7 mg; vitamin D_3: 0.05 mg; vitamin E: 18 mg; vitamin k_3: 2 mg; Thiamine: 1.8 mg; Riboflavin: 6.6 mg; Pantothenic acid: 10 mg; Pyridoxine: 3 mg; Cyanocobalamin: 0.015 mg; Niacin: 30 mg; Biotin: 0.1 mg; Folic acid: 1 mg; Choline chloride: 250 mg and Antioxidant: 100 mg

[b]Mineral premix provided per kg of diet: Fe: 50 mg; Mn: 100 mg; Zn: 100 mg; Cu: 10 mg; I: 1 mg; Se: 0.2 mg

responses of broiler chicks improved by addition of whey powder in diets but maybe because of farm condition, that no disease was observed during experiment, immunity were not affected. However, whey powder might be able to affect immunity of broilers by changing in pH of GIT and microbial population. Lactose has also been shown to have beneficial effects on the GIT microflora. An investigation in day-of-hatch chicks found that dietary lactose decreased *Lactobacillus*, *Clostridium* and *Proteus* species and increased bifidobacteria in the ceca (Morishita *et al*., 1982; Van der Wielen *et al*., 2002). Tellez *et al*. (1993) found that feeding 10% lactose in the diet resulted in a significant increase in VFAs, which resulted in a significant decrease in pH and *Salmonella Enteritidis* invasion of Leghorn organs. Similar results have been reported

Table 3: Composition of the experimental diets in finisher

		Whey powder			
Ingredients (%)	Control	1%	2.5%	5%	7.5%
Whey powder	0	1	2.5	5	7.5
Corn	59.6	58.5	57.6	56.1	54.6
Soybean meal	30.4	30.4	30.6	30.7	30.8
Soybean oil	2.6	2.76	2.93	3.12	3.3
Wheat bran	4	4	3	2	1
Monocalcium phosphate	1.12	1.12	0.97	0.82	0.67
Calcium carbonate	1.34	1.34	1.4	1.42	1.46
Salt	0.25	0.25	0.23	0.06	0
Vitamin premix[1]	0.25	0.25	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.2	0.2	0.2	0.2	0.2
L-Lysine HCl	0	0	0	0	0
Calculated composition					
Metabolizable energy (Kcal/kg)	3000	3000	3000	3000	3000
Crude protein (%)	18.75	18.75	18.75	18.75	18.75
Methionine+Cysteine (%)	0.81	0.81	0.81	0.81	0.81
Lysine (%)	1.02	1.02	1.02	1.02	1.02
Calcium (%)	0.80	0.80	0.80	0.80	0.80
Available phosphorus (%)	0.39	0.39	0.39	0.39	0.39

[a]Vitamin premix provided per kg of diet: vitamin A: 2.7 mg; vitamin D_3: 0.05 mg; vitamin E: 18 mg; vitamin k_3: 2 mg; Thiamine: 1.8 mg; Riboflavin: 6.6 mg; Pantothenic acid: 10 mg; Pyridoxine: 3 mg; Cyanocobalamin: 0.015 mg; Niacin: 30 mg; Biotin: 0.1 mg; Folic acid: 1 mg; Choline chloride: 250 mg and Antioxidant: 100 mg

[b]Mineral premix provided per kg of diet: Fe: 50 mg; Mn: 100 mg; Zn: 100 mg; Cu: 10 mg; I: 1 mg; Se: 0.2 mg

Table 4: Effect of different levels of acid whey powder on growth performance of broiler chicks

		Whey powder				Low lactose whey powder		
Parameter	Control	1%	2.5%	5%	7.5%	2.5%	5%	7.5%
Body weight gain (g/d)								
1-14 d	18.2±0.78^{b}	19.4±0.80^{b}	21.7±0.41^{a}	21.6±0.26^{a}	21.4±0.23^{a}	21.7±0.66^{a}	22.4±0.20^{a}	22.3±0.40^{a}
14-28 d	50.4±1.35^{b}	51.9±3.84^{b}	51.6±2.08^{b}	51.1±2.13^{b}	49.1±0.85^{b}	58.1±2.40^{a}	58.7±1.60^{a}	55.0±0.90^{ab}
28-42 d	88.1±1.61	84.1±2.45	88.8±2.17	87.1±2.90	92.9±0.57	89.4±6.92	87.7±2.99	98.3±12.18
1-42 d	49.2±0.90^{b}	49.9±0.83^{b}	51.7±0.40^{ab}	50.9±0.60^{ab}	49.8±2.24^{b}	53.9±1.82^{a}	53.7±0.65^{a}	51.8±0.32^{ab}
Feed consumption (g/d)								
1-14 d	29.5±0.67^{c}	29.7±0.17^{c}	31.2±0.35^{bc}	32.3±0.29^{ab}	32.4±0.40^{ab}	32.0±0.61^{b}	32.6±0.71^{ab}	34.0±0.21^{a}
14-28 d	83.1±2.85^{c}	90.5±0.31^{ab}	88.9±2.74^{bc}	92.2±2.57^{ab}	91.5±1.00^{ab}	90.1±2.37^{ab}	95.7±1.60^{a}	88.9±0.86^{bc}
28-42 d	174.4±6.57^{ab}	180.7±2.68^{ab}	185.4±2.68^{a}	181.0±1.69^{ab}	166.8±8.99^{b}	174.4±4.10^{ab}	184.1±4.25^{a}	168.7±4.58^{b}
1-42 d	94.7±3.26^{c}	100.3±0.24^{abc}	100.8±1.68^{ab}	100.8±1.21^{ab}	95.9±3.19^{bc}	97.8±2.04^{abc}	103.1±0.89^{a}	96.2±1.58^{bc}
Feed conversion ratio								
1-14 d	1.62±0.05^{a}	1.54±0.06^{ab}	1.43±0.06^{b}	1.49±0.02^{b}	1.52±0.01^{ab}	1.47±0.02^{b}	1.45±0.02^{b}	1.52±0.03^{ab}
14-28 d	1.73±0.04^{abc}	1.69±0.09^{abc}	1.72±0.04^{abc}	1.81±0.08^{ab}	1.87±0.04^{a}	1.56±0.08^{c}	1.63±0.07^{bc}	1.62±0.04^{c}
28-42 d	1.98±0.09^{ab}	2.16±0.09^{a}	2.09±0.06^{ab}	2.09±0.09^{ab}	1.96±0.13^{ab}	1.98±0.16^{ab}	2.11±0.10^{ab}	1.79±0.20^{b}
1-42 d	1.92±0.04^{ab}	1.99±0.03^{a}	1.95±0.03^{a}	1.98±0.03^{a}	1.93±0.07^{ab}	1.82±0.06^{b}	1.91±0.03^{ab}	1.87±0.02^{ab}

[a-b]Means within the same row without common superscripts differ significantly ($p<0.05$)

Table 5: Effect of different levels of acid whey powder on lymphoid organs of broiler chicks

		Whey powder				Low lactose whey powder		
Lymphoid organs (Percentage of LBW)	Control	1%	2.5%	5%	7.5%	2.5%	5%	7.5%
Bursa of fabercius	0.15±0.01	0.12±0.02	0.11±0.01	0.10±0.01	0.13±0.02	0.10±0.01	0.12±0.01	0.09±0.01
Spleen	0.14±0.01	0.11±0.01	0.12±0.01	0.12±0.02	0.11±0.01	0.11±0.01	0.15±0.01	0.11±0.01

[a-b]Means within the same row without common superscripts differ significantly ($p<0.05$)

Table 6: Effect of different levels of acid whey powder on antibody titer against Newcastle and Influenza viruses and sheep red blood cell (SRBC) of broiler chicks at 30 d

		Whey powder				Low lactose whey powder		
Antibody titer (Log_2)	Control	1%	2.5%	5%	7.5%	2.5%	5%	7.5%
Newcastle	5.62±0.32	5.88±0.35	6.13±0.39	5.88±0.25	6.25±0.26	6.38±0.33	5.88±0.35	6.63±0.36
Influenza	6.38±0.32	6.38±0.37	6.75±0.16	6.38±0.32	6.13±0.29	6.25±0.41	6.88±0.13	5.63±0.32
SRBC	7.50±0.33	7.50±0.25	8.25±0.27	7.50±0.19	8.25±0.16	7.88±0.29	7.63±0.18	7.83±0.26

[a-b]Means within the same row without common superscripts differ significantly ($p<0.05$). LBW: Live body weight

when lactose was provided in the drinking water (2.5%) or in the feed (5 or 10%), showing significant increases in bacteriostatic effect of acetic acid and propionic acid due to decrease in cecal pH (Corrier *et al*., 1990).

Conclusion: It could be concluded from this study that lactose content of whey powder affected the growth performance, so that dietary inclusion of low lactose acid whey especially at levels of 2.5% in diet improved growth performance of broiler chicks. Inclusion of acid whey powder up to 5% in diet of broiler chicks had no adverse effect on performance and immunity of broiler chicks.

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