Journal of Poultry Research 15(1): 1-5, 2018
Print ISSN:1302-3209 - Online ISSN:2147-9003
www.turkishpoultryscience.com
Research Article

Effects of Dietary Butyric Acid Inclusion in Different Source of Grains Based Diets on Growth Performance and Intestinal Histomorphology in Broiler Chickens

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ABSTRACT: This study was conducted to determine the effects of butyric acid addition in corn-based or wheat/barley-based diets on growth performance and intestinal morphology of broilers. Two hundred and forty one-day old male broiler (Ross-308) chicks were assigned to 4 treatments in 2 × 2 factorial arrangement of dietary butyric acid (0 and 0.2%) and grains (corn and wheat/barley) with 4 replicate pens in each treatment, each having 15 birds. Feed and water was provided *ad libitum* throughout the experiment. Results indicated that dietary butyric acid supplementation in wheat/barley based diets had improving effects on body weight, body weight gain and feed intake at day 42 of experiment ($p<0.05$). Moreover, the addition of butyric acid in different source of grain based diets also improved the feed conversion ratio of birds at days 1-21 of the trial ($p<0.001$). Similarly, crypt depth of jejunum was significantly affected dietary butyric acid inclusion in birds at 21 day of age whereas villus height was higher in birds fed wheat/barley based diets ($p<0.05$ and $p<0.01$, respectively). In conclusion, butyric acid supplementation to wheat/ barley based diets seems to be useful practice for broiler chickens.

Keywords: Broiler, Butyric acid, Intestinal histomorphology, Grains, Performance

Received: 29.06.2017 **Accepted:** 06.03.2018

Farklı Tahıl Kaynaklarını İçeren Broyler Rasyonlarına Butirik Asit İlavesinin Büyüme Performansı ve Bağırsak Histomorfolojisi Üzerine Etkileri

ÖZ: Bu çalışma mısır veya buğday/arpa temelli broyler rasyonlarına bütirik asit ilavesinin büyüme performansı ve bağırsak histomorfolojisi üzerine etkilerini araştırmak için yapılmıştır. Toplam 240 adet, bir günlük yaştaki erkek broyler civcivler (Ross-308) 2 farklı butirik asit düzeyi (%0 ve %2) ve 2 farklı tahıl grubu (mısır ve buğday/arpa) olmak üzere 2x2 faktöryel düzende 4 farklı deneme grubuna ayrılmıştır. Deneme grupları her birinde 15 adet civciv bulunanan 4 alt gruptan oluşmuştur. Deneme boyunca yem ve su *ad libitum* olarak sağlanmıştır. Denemenin 42. gününde sonuçlar, buğday/arpa bazlı rasyonlara bütirik asit ilavesinin canlı ağırlık, canlı ağırlık artışı ve yem tüketimini iyileştirdiğini ($p<0.05$) göstermiştir. Ayrıca, farklı tahıl bazlı rasyonlara bütirik asit ilavesi denemenin 1-21 günlük döneminde yemden yararlanma oranını iyileştirmiştir ($p<0.001$). Benzer şekilde, bütirik asit ilavesi 21 günlük yaşta jejunumda kript derinliğini önemli ölçüde etkilerken ($p<0.05$) villus yüksekliği buğday/arpa bazlı rasyonlarla beslenen civcivlerde daha yüksek ($p<0.01$) bulunmuştur. Sonuç olarak, buğday/arpa bazlı broyler rasyonlarına bütikik asit ilavesinin faydalı bir uygulama olduğu görülmüştür.

Anahtar Kelimeler: Broyler, Bütirik asit, Bağırsak histomorfolojisi, Tahıl, Broyler performansı

INTRODUCTION

Short chain fatty acids are used to prevent pathogen contamination in animal feed for years. Today, it is known that these acids have some beneficial effects in the crop and the other parts of gastrointestinal tract. After banning of the antibiotic usage as a growth promoter in poultry feed by the EU in 2006, organic acids and their salts are acknowledged the most prominent potential alternatives (1). Butyrate, short chain fatty acid with four carbon atoms, is derived from the fermentation of non-starch polysaccharides. Many reports showed that butyrate has higher bactericidal activity (2) and also has been considered to play an important role in the development of the intestinal epithelium (3), stimulation of the gut immune system (4), prevention of colon cancer in human (5), modulation of intestinal flora against pathogenic microbes (6,7).

The gastrointestinal tract of newly hatched chicks is immature and sterile, also young chicks have low short chain fatty acid level in their cecum. On the other hand, this level increases with time and reaches optimum levels in around 15 days old (8). Since free form of butyric acid is immediately absorbed from crop, generally protected form of butyric acid, such as mono-, di- and triglycerides, are used in broiler feed (9,10).

In few studies, dose response and/or type of butyric acids have been evaluated in broilers but there are no results about the effect of different type of diets with butyric acid. In this study, it is aimed to evaluate the effect of butyric acid in corn-based and wheat/barley-based diets on broiler performance and intestinal morphology.

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MATERIAL and METHODS

Bird husbandry: Two hundred and forty newly hatched male Ross 308 broiler chicks were obtained from a commercial hatchery (BEYPI Inc., Ankara-Turkey) and transported with in 1 hour to the experimental unit. Chicks were housed in floor pens with fresh wood shavings-based litter at an approximate depth of 8 cm. The study was conducted in a clean environment (properly disinfected experimental facility, clean wood shavings and good management). Each pen (200 x 100 x 100 cm) contained one feeder and two nipple drinkers. Temperature was controlled with electric heater, maintained at 34°C for the first three days and then gradually reduced by 2-3°C per week to final temperature of 22°C. Lighting was provided for 24 h. All experimental conditions and animal care protocols were approved by the Animal Experiments Local Ethics Committee of Ankara University.

Experimental design and diets: The birds were initially weighed and randomly assigned to 4 experimental groups, each with 4 replicate pens of 15 chicks in each pen. The experiment consisted of a 2 × 2 factorial arrangement of dietary butyric acid (0 and 0.2%) and different grain based diets (corn and wheat/barley). The birds were allowed to reach feed and water at the time of arrival to the experimental unit. Monoglycerid form of butyric acid was used in the study. Each diet was formulated to meet or exceed the nutrient requirements of broilers according to NRC (11) recommendations for 1-21 days (22.70% CP and 3030 kcal/kg ME), and 22-42 days (18.50% CP and 3190 kcal/kg ME). Each diet in mash form and water were provided *ad libitum* throughout the experimental period. The ingredient and nutrient composition of the diets are shown in Table 1.

Data collection: Chicks were weighed at the beginning of the experimental period and weekly. At 21 and 42 days of age, mean body weight by pen was evaluated. Feed intake and feed conversion ratios during the same periods were determined. On the day 21, one bird from each pen was slaughtered after killing by cervical dislocation. Tissue samples from jejunum (midway between the point of entry of the bile ducts and Meckel's diverticulum) were immediately removed, washed with physiological saline and fixed in 10% formalin, processed by the routine paraffin sectioning and finally blocked in paraplast and cut into 6 µm thick sections. Mounted sections were stained with Mallory's modified triple staining technique and examined under a light microscope (12). Measurements of villus height were selected from sections in which the villus tops were well oriented and adjacent to crypt mouth. The crypt depth from the crypt mouth to the base was also measured (13). Results were evaluated by using the Image J analysis software (14).

Table 1. The ingredient and nutrient **c**omposition of experimental diets (%).

Ingredients	Starter (1-21 d)				Grower (22-42 d)			
	C	CBA	WB	WB+BA	C	CBA	WB	WB+BA
Corn	50.00	49.80	20.00	19.80	58.60	58.40	2.15	1.95
Wheat	-	-	15.00	15.00	-	-	30.00	30.00
Barley	-	-	15.00	15.00	-	-	30.00	30.00
Soybean meal	33.10	33.10	24.00	24.00	18.00	18.00	11.00	11.00
Full fat soybean	8.00	8.00	16.20	16.25	15.00	15.00	15.00	15.00
Meat-bone meal	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Vegetable oil	3.00	3.00	4.00	4.00	3.00	3.00	6.50	6.50
Dicalcium phosphate	1.10	1.10	1.00	1.00	1.00	1.00	1.00	1.00
Limestone	0.60	0.60	0.50	0.55	0.50	0.50	0.50	0.50
DL-Methionine	0.35	0.35	0.40	0.35	0.20	0.20	0.25	0.25
L-Lysine	0.20	0.20	0.25	0.20	0.20	0.20	0.10	0.10
L-Threonine	0.20	0.20	0.20	0.20	0.05	0.05	0.05	0.05
NaCl	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix[1]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Mineral premix[2]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Butyric acid	-	0.20	-	0.20	-	0.20	-	0.20
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated values								
Dry Mater, %	89.80	89.80	89.70	89.70	89.80	89.80	89.60	89.60
Crude protein, %	22.70	22.70	22.80	22.80	18.50	18.50	18.30	18.30
Ether Extract, %	6.13	6.13	8.42	8.42	7.49	7.49	10.76	10.76
ME, kcal/kg	3027	3027	3038	3038	3190	3190	3180	3180
Calcium, %	1.01	1.01	0.91	0.91	0.92	0.92	0.88	0.88
Phosphorus, %	0.50	0.50	0.50	0.50	0.46	0.46	0.49	0.49
Met+Sist, %	1.06	1.06	1.09	1.09	0.83	0.83	0.80	0.80
Lysine, %	1.42	1.42	1.43	1.43	1.04	1.04	0.99	0.99
Crude fiber	3.9	3.9	4.1	4.3	3.4	3.4	4.2	4.2

C: Corn (Control), CBA: Corn+Butyric acid, WB: Wheat/barley, WB+BA: Wheat/barley+Butyric acid, [1]Content of vitamins provides per kg of diet: vitamin A, 15000 IU; vitamin D3, 5000 IU; vitamin E, 50 mg; vitamin K3, 10 mg; vitamin B1, 4 mg; vitamin B2, 8 mg; vitamin B6, 5mg; vitamin B12, 0.025mg; niacin, 50 mg; pantothenic acid, 20 mg; folic acid, 20 mg; biotin, 0.25 mg; choline,175 mg, [2]Content of minerals provides per kg of diet: manganese, 100 mg; zinc, 150 mg; iron, 100 mg,; cupper, 20 mg; iodine, 1.5 mg; cobalt, 0.5 mg; selenium, 0.2 mg; molybdenum, 1mg; magnesium, 50 mg.

Statistical analysis: Data were analyzed by means of the GLM procedure using Duncan's multiple range test with SAS statistical software. Differences were considered significant at p<0.05 (15). Kolmogorov-Smirnov and Levene tests were used to control parametric test probability of villus length and crypt depth in jejunum. Gabriel test was used for villus length and crypt depth in jejunum as multiple range tests.

RESULTS and DISCUSSION

The performance of the birds is presented in Table 2. During the starter period (0-21), the best weight gain was obtained from the birds receiving butyric acid with either corn-based or wheat/barley-based diets, although there is no statistical difference between the birds given diets with or without butyric acid. However, at the same period, feed intake and feed conversion ratio (feed intake: weight gain) were influenced by dietary treatments and lower feed intake and better feed conversion ratio (p<0.05) was observed in the butyric acid containing diets. Normally, birds consumed significantly less feed when diet contained butyric acid. During the 22-42 days, significantly lower body weight gain and feed intake were obtained from birds fed wheat/barley-based diet without butyric acid compared to the control birds and the birds fed wheat/barley-based diet with butyric acid. When evaluated the overall growing period, dietary treatments generally had no significant (p>0.05) effect on weight gain, feed intake and feed conversion ratio, however body weight gain and feed conversion ratio of birds fed wheat/barley-based diet with butyric acid were numerically improved in compared to birds fed with other diets. The highest weight gain was recorded in the birds given wheat/ barley-based diet with 0.2% butyric acid. But there was no statistical difference among the groups. Similar to these results, Antongiovanni et al. (16) reported that 0.35% butyric acid glycerides addition to the mixed feed resulted in the best weight gain at the end of the first week. But they found that this significant difference was lost in the following periods. In addition, considering the whole rearing period, they found that the best growth performance was achieved with 0.2% butyric acid glycerides in comparison with the control group. In another study, Panda et al. (17) reported that body weight gain and feed conversion ratio was influenced by dietary butyric acid treatments during both the starter and finisher period. Also, they suggested that butyric acid supplementation at 0.2% was not sufficient to maintain the performance. To obtain significant improvement in body weight gain and feed conversion ratio, 0.4% of butyric acid in the diet was adequate.

Table 2. Body weight, body weight gain, feed intake and feed conversion ratio of male broilers fed with barley and wheat based diet and dietary butyric acid inclusion.

Treatments		Body weight (g)				Body weight gain (g)		
Barley&Wheat	Butyric acid	0	7	21	42	0 to 21d	21 to 42d	0 to 42d
0	0	43.23	143.50^{ab}	718.05	2330.50^{a}	674.82	1612.45^{a}	2287.27^{a}
	1	43.24	144.42^{ab}	734.92	2203.75^{b}	691.69	1468.83^{ab}	2160.52^{ab}
1	0	43.13	151.33^{a}	718.09	2145.25^{b}	674.96	1427.16^{b}	2102.12^{b}
	1	43.46	139.17^{b}	751.33	2343.75^{a}	707.86	1592.42^{a}	2300.28^{a}
Pooled SEM		0.32	2.16	12.30	57.06	12.29	48.56	57.04
Barley&Wheat								
0		43.23	143.96	726.48	2267.13	683.25	1540.64	2223.89
1		43.30	145.25	734.71	2244.50	691.41	1509.79	2201.20
Butyric acid								
0		43.18	147.42^{a}	718.07	2237.88	674.89	1519.81	2194.69
1		43.35	141.80^{b}	743.13	2273.75	699.77	1530.63	2230.40
ANOVA		P						
Barley&Wheat		NS	NS	NS	NS	NS	NS	NS
Butyric Acid		NS	*	NS	NS	NS	NS	NS
Barley&Wheat x Butyric acid		NS	**	NS	*	NS	**	*

Treatments		Feed intake (g)			Feed conversion ratio (g:g)[1]		
Barley&Wheat	Butyric acid	0 to 21d	21 to 42d	0 to 42d	0 to 21d	21 to 42d	0 to 42d
0	0	1079.58	2942.50^{a}	4022.08^{a}	1.60	1.83^{b}	1.76
	1	936.16	2892.50^{ab}	3828.66^{b}	1.35	1.98^{a}	1.77
1	0	1051.33	2770.25^{b}	3821.58^{b}	1.56	1.94^{a}	1.82
	1	967.25	2983.75^{a}	3951.00^{a}	1.36	1.88^{ab}	1.71
Pooled SEM		26.08	47.48	59.50	0.04	0.04	0.03
Barley&Wheat							
0		1007.87	2917.50	3925.37	1.48	1.90	1.77
1		1009.29	2877.00	3886.29	1.46	1.91	1.77
Butyric acid							
0		1062.46^{a}	2856.38	3921.83	1.58^{a}	1.88	1.79
1		951.71^{b}	2938.13	3889.83	1.36^{b}	1.93	1.75
ANOVA		P					
Barley&Wheat		NS	NS	NS	NS	NS	NS
Butyric Acid		***	NS	NS	***	NS	NS
Barley&Wheat x Butyric acid		NS	*	*	NS	*	NS

NS=Not significant (p>0.05); *p<0.05; **p<0.01; ***p<0.001, ^{a,b} Means within a treatment and column with different subscripts differ significantly, [1] Feed conversion ratio was calculated by dividing feed intake (g) to BW gain (g) per pen basis.

Table 3. Villus height and crypt depth in jejunum (μm) of male broilers fed with barley and wheat based diet and dietary butyric acid inclusion at 21 day of experiment.

Treatments		Villus height in jejunum, μm	Crypt depth in jejunum, μm
BW	BA		
0	0	778[c]	225
	1	840[bc]	217
1	0	987[a]	248
	1	873[b]	227
Pooled SEM		32.68	6.79
Barley&Wheat			
0		809[b]	221[b]
1		930[a]	238[a]
Butyric acid			
0		883	237[a]
1		856	222[b]
ANOVA		P	
BW		**	*
BA		NS	*
BW x BA		*	NS

BW: Barley&Wheat, BA: Butyric acid, NS=Not significant (p>0.05); *p<0.05; **p<0.01, [a,b] Means within a treatment and column with different subscripts differ significantly.

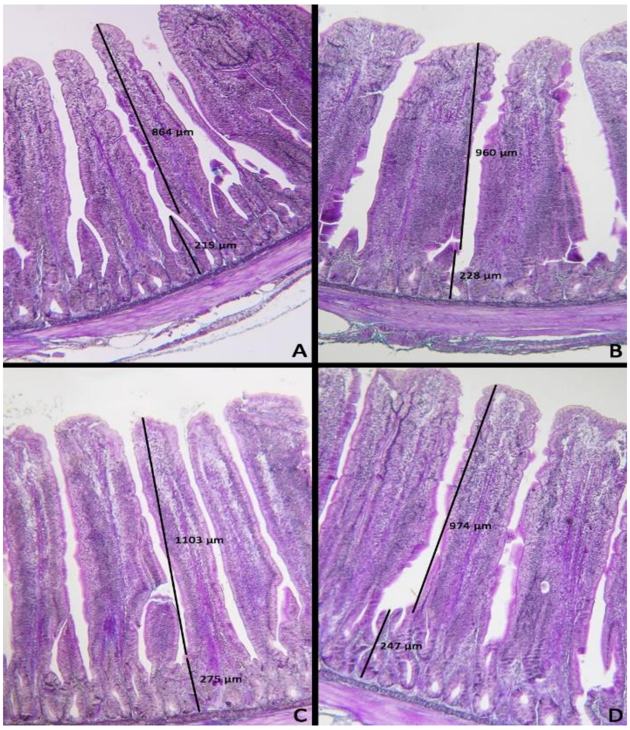

Figure 1. The sections of the jejunum villus height and crypt depth in broilers fed with A) wheat/barley + butyric acid, B) wheat/barley-based C) corn + butyric acid, D) corn-based diets.

Leeson et al. (9) showed that butyric acid addition to the broiler diet generally had no effect on body weight or weight gain in either the starter or grower/finisher periods. Birds consumed less starter feed when diets were supplemented with 0.4% butyrate compared to the control group (p<0.01).

Mansoub et al., (10) evaluated the effects of various levels and forms (powder and oily form) of butyric acid glycerides on performance and serum composition of broiler chickens. They indicated that performance parameters were positively affected by butyric acid treatment, irrespective of the type and level of acid. When considering the results of many studies, generally the effect of butyric acid treatment is seen during the starter period but not following period irrespective of the type and level of butyric acid. From this study, it is understood that there is no difference between diet compositions on arising of the effect of butyric acid on performance.

The results of jejunal morphology in the broiler chicken fed the test diets are shown Table 3. The lowest villus height was observed in jejunum of the birds fed corn-based diet without butyric acid (control group). On the other hand, villus height and crypt depth in jejunum was the highest in the birds consuming wheat/barley-based diet without butyric acid (Figure 1). The main effect on these parameters might have resulted from diet composition regardless of butyric acid supplementation. Furthermore, butyric acid addition to the wheat/barley-based diet reduced villus height and crypt dept compared to wheat/barley-based diet, however, these parameters were better than those of the control group. Recent findings show that insoluble fiber has different roles in gut development as a physical stimulator, improving gut health, enhancing nutrient digestion and modulating the behavior of animals (17-20). In recent study, as low as 15% in the starter period and 30% in the grower period of barley used in the wheat/barley-based diet may have positive effect because of its insoluble fiber content. There are some reports supporting our results. For example, research on rat (21) and pig (22) showed that the mucosal structure and morphology in the small intestine were significantly influenced by feeding and by composition of the diet especially fiber level and type. Furthermore, Yamauchi and Isshiki (23) found that high dietary fiber levels caused broiler chickens to develop higher intestinal villi.

Similarly, Leeson et al. (9) reported that there was no effect of butyric acid on duodenal villus length compared to control diet. On the contrary, Adil et al. (24) found that dietary supplementation of organic acids significantly (p<0.05) increased the villus height in the duodenum and jejunum compared with the control group. The highest duodenal, jejunal, and ileal villus heights were recorded in the birds fed diets supplemented with 3% butyric acid. On the other hand, Panda et al. (17) suggested that the villi length and crypt depth in duodenum increased significantly in all the butyrate treated diets irrespective of the levels (0.2, 0.4, and 0.6%) in the diet. In contrast to these findings, Antongiovanni et al. (16) found that villus length tended to be depressed by butyric acid addition, both in jejunum and in ileum.

CONCLUSIONS

It is concluded that there was no negative effect of butyric acid addition to either corn-based or wheat/barley-based diets on performance parameters during the rearing period of broilers. Also, feeding butyric acid may improve feed conversion ratio during the starter period, since in this period birds obtained higher weight gain with less feed intake with 0.2% butyric acid addition. In addition, diet composition is more effective than butyric acid addition on villus height and crypt depth in jejunum.

ACKNOWLEDGEMENT

We express our thanks to Beypi A.Ş for supplying the chicks used in the experiment. Some parts of these results have been presented in VI. National Animal Nutrition Congress, Samsun, 2011.

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