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Effect of Prebiotic Fermacto® on Gut Development and Performance of Broiler Chickens Fed Diet Low in Digestible Amino Acids

[1]V. Khaksar, [1]A. Golian, [1]H. Kermanshahi, [2]A.R. Movasseghiand and [2]A. Jamshidi
[1]Department of Animal Science, Excellence Center of Animal Research,
Ferdowsi University of Mashhad, Mashhad, P. O. Box: 91775-1163, Iran
[2]Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

Abstract: A complete randomized design experiment with 9 dietary treatments was conducted to evaluate the effect of prebiotic Fermacto® and digestible amino acid levels on performance, gut development and transit time of feed of Ross male broiler chicks. Each diet was fed to five pens of 12 chicks each, during starter (0-21 day), grower (22-41 day) and finisher (42-47 day) periods. Three diets were provided to meet 100 (control), 95 and 90% of Recommended Digestible Amino Acids (RDAA) of starter, grower and finisher periods. The starter and grower diets were further subdivided into 3 parts and supplemented with 0.0, 0.0; 2.0, 2.0 and 2.0, 1.0 g kg^{-1} Fermacto®, respectively. Body Weight Gain (BWG) and feed intake (FI) was recorded and Feed Conversion Ratio (FCR) was calculated during 1-21, 22-41, 42-47 day. The carcass yield and cuts of broiler chicks were measured at the end of the experiment. Chick weight was recorded individually to determine the uniformity at day 7, 21 and 47. Chromic oxide at the rate of 0.3% was added to each diet to determine the Gastrointestinal Transit Time (GTT) at day 16 and 32. Tissues from the midpoint of ileum and jejunum was excised to assay the lactobacillus bacteria counts in ileum and villi height, surface area and lamina propria thickness in jejunum of chicks at 21 day of age. Fermacto® supplementation of starter and grower diets improved BWG, FCR, breast yield and gut development regardless of DAA levels in diet. Addition of more than 1.0 g kg^{-1} Fermacto® in the grower diet did not further improve the BWG or FCR. Flock uniformity was increased ($p<0.05$) by the inclusion of Fermacto® in diet as compared to non-Fermacto® treated at day 21 when fed 100 or 90% RDAA diets. Fermacto® prolonged the GTT of feed and increased ($p<0.05$) the ileal lactobacillus counts and improved the jejunal histological traits. Performance and breast yield of broiler chickens fed 95% RDAA diet was similar to those fed control diet, whereas birds fed diet contained 90% RDAA were significantly smaller and less efficient as compared to control birds. Formulating diet based on 95% RDAA did not have a negative effect on performance, but may decrease environmental pollution and cost of broiler chicken production. A 10% decrease in RDAA increased AF, lowered GTT, ileal lactobacillus counts and weakened jejunum morphometry.

Key words: Prebiotic Fermacto®, gut development, feed transit time, digestible amino acids, broiler performance

INTRODUCTION

The currently used feed additives in broiler diets aim to enhance nutrient utilization by means of diverse mechanisms. It is suggested that enhance effect of nutrient uptake of feed additives might be detected, when a marginal digestible amino acid deficient diet is fed to broiler chickens. In the modern intensive poultry production, newly hatched chicks have little chance to contact with hen, thereby normal microflora is slowly colonized in the intestine. Due to possible hazards and risks of antibiotics in poultry production, the importance of using prebiotics as an alternative has increased more than ever (Fuller, 1989). Fermacto® is a microbial feed supplement derived from Aspergillus Mycelium (AM) has been used as an alternative tool for helping newly-hatched chicks. Fermacto is shown to affect the host animal through stimulation of growth and/or improvement in intestinal microbial balance (Mamiek, 1993; Tangendjaja, 1993). Kim *et al*. (2003) reported that feeding Fermacto supplemented diet to broiler chickens significantly enhanced feed intake and body weight gain. Supplementation of 2 g kg^{-1} AM to normal and insufficient protein diets for both young and old laying hens resulted in a longer transit time of feed (212 vs. 196±4.6 min) in gastrointestinal tract (Grimes *et al*., 1997).

Corresponding Author: A. Golian, Department of Animal Science, Excellence Center of Animal Research, Ferdowsi University of Mashhad, Mashhad, P. O. Box: 91775-1163, Iran

Fermacto has shown to enhance gut development and nutrient digestibility through an increase in gut beneficial microflora, short chain fatty acids and doudenal and jejunal villi height (Gomez-Alacon *et al*., 1990; Beharka and Nagaraja, 1998; Hirayama *et al*., 2000). Beneficial microflora promotes gut health by influencing enterocyte turnover, competing with pathogenic bacteria for nutrients and binding sites and producing bacteriostatic compounds that limit the growth of pathogenic bacteria (Farthing, 2004).

In the past, poultry producers and nutritionists have formulated diets based on total amino acids (NRC, 1994); however, the DAA might be lower or higher than of required levels (Creswell and Swick, 2001). The digestible amino acid levels in a diet may more closely present the portion of available protein for bird maintenance, growth and development. Previous studies on cottonseed meal (Fernandez *et al*., 1995) and several byproduct ingredients (Rostagno *et al*., 1995) have shown that diet formulation based on digestible amino acids is superior to those based on total AA, in particular when using ingredients with lower digestible AA than those in corn and soybean meal. In chicks, the Gastrointestinal Tract (GIT) develops rapidly during the first few days post hatch due to a transition in nutrient sources when the GIT switches from utilizing the lipid-rich yolk in the yolk sac to an exogenous feed ration that is rich in carbohydrates (Uni *et al*., 1999). Feeding diets with more available nutrients such as DAA reduce the passage rate of feed through the GIT and provides long-term increase in feed efficiency in birds.

The present study was conducted to evaluate the effect of dietary Fermacto® supplementation on performance, transit time and gut development of broiler chicks fed diets with various digestible amino acid levels.

MATERIALS AND METHODS

Five hundred forty day old male Ross broiler chicks were divided into 45 groups of 12 birds each. Every 5 groups were randomly assigned to one of the nine dietary treatments. Three diets were first provided to meet 100 (control), 95 and 90% of Recommended Digestible Amino Acids (RDAA) of starter, grower and finisher periods (Creswell and Swick, 2001). The starter and grower of each indicated diet was subdivided into 3 parts and were supplemented with 0.0, 0.0; 2.0, 2.0 and 2.0, 1.0 g kg^{-1} Fermacto®, respectively. The composition and nutrient values of experimental diets are shown in Table 1. The chicks were raised on floor pens (1×1 m) covered with wood shaving. Birds were provided with 23: 1 h light: Dark cycle and had free access to feed and water throughout the 47 day experiment.

The Body Weight Gain (BWG) and Feed Intake (FI) of each group of birds were determined 4 h after feed removal and Feed Conversion Ratio (FCR) was calculated during 1-21, 22-41 and 42-47 day of age. Daily mortalities

Table 1: Composition and digestible amino acid (Dig. amino acid) levels of starter, grower and finisher diets

	Recommended digestible amino acids (%)								
	Starter			Grower			Finisher		
Ingredients (%)	100%	95%	90%	100%	95%	90%	100%	95%	90%
Corn	45.70	48.35	50.00	55.00	58.41	59.50	59.64	62.63	64.33
Soybean meal	32.00	28.00	27.40	30.95	29.00	28.00	28.00	25.50	24.50
Wheat	2.15	2.15	2.15	-	-	-	-	--	
Wheat bran	2.57	3.28	3.28	-	-	-	-	-	-
Sunflower meal	6.00	6.00	6.00	4.50	4.50	4.50	2.50	2.50	2.50
Corn gluten meal	5.00	..6.00	5.00	2.50	1.20	1.20	1.80	1.60	1.00
Sunflower oil	2.80	2.40	2.40	3.37	3.20	3.06	4.40	4.04	3.95
Limestone	1.95	1.95	1.95	1.84	1.79	1.84	1.80	1.89	1.86
DiCa- phosphate	0.95	1.00	1.00	1.00	1.02	1.05	1.00	1.00	1.00
Sodium chloride	0.23	0.23	0.23	0.29	0.32	0.32	0.31	0.33	0.33
Vit. and min[1]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Hcl-lysine	0.09	0.12	0.08	-	-	-	-	-	-
DL-methionine	0.06	0.02	0.01	0.05	0.06	0.03	0.05	0.04	0.03
Calculated nutrients									
ME (Kcal kg^{-1})	3000	3000	3000	3100	3100	3100	3200	3200	3200
CP %	21.43	20.71	20.02	19.26	18.07	17.74	17.39	16.53	15.94
Ca %	0.95	0.95	0.95	0.91	0.91	0.91	0.89	0.89	0.89
Av. P. %	0.42	0.42	0.42	0.40	0.40	0.40	0.38	0.38	
0.38Dig.lys.%	1.07	1.02	0.96	0.93	0.88	0.86	0.84	0.79	0.76
Dig. Met. %	0.44	0.40	0.37	0.38	0.37	0.33	0.35	0.32	0.30
Dig.Met+Cys.%	0.78	0.73	0.69	0.69	0.66	0.62	0.63	0.60	0.57
Dig. Thr. %	0.69	0.66	0.64	0.62	0.58	0.56	0.56	0.53	0.50

[1]Supplied per kilogram of diet: vitamin A, 10000 IU; vitamin D_3, 9790 IU; vitamin E, 121 IU; vitamin K_2, 2 mg; vitamin B_{12}, 0.02 mg; thiamine, 4 mg; riboflavin, 0.0044 mg; niacin, 22 mg; pyridoxine, 4 mg; biotin, 0.03 mg; folic acid, 1 mg; Ca-panthotenate, 40 mg; choline chloride, 840 mg; etoxycoin, 0.125 mg; Zn-sulfate, 60 mg; Mn-sulfate, 100 mg; Cu-sulfate, 100 mg; Se, 0.2 mg; I, 1 mg; Fe, 50 mg

were recorded and used to correct performance criteria. One chick from each group of birds close to the average pen weight after 12 h fasting was selected, weighed and slaughtered to determine the carcass, breast, thigh, drum sticks, empty gut, gizzard, ceca and abdominal fat weight at the end of experiment. Birds were weighed individually within each pen and the percent of birds within 10% of the mean was used as flock uniformity at 7, 21 and 47 day of age. Gastrointestinal Transit Time (GTT) of feed was measured by covering the pens with clean paper 4 h after feed withdrawal and recoding the time between offering the chromic oxide (0.3%) diet and appearance of average three spotted green excreta in each pen at 16 and 32 day. One bird close to the average pen weight was randomly, euthanized and approximately 10 cm length of the jejunum and ileum were removed, placed in 10% buffered formalin and stored in fridge for later gut criteria determination. For lactobacillus population count in the ileum, one gram of ileal content homogenized in 9 mL sterile water and the colony-forming units per gram was determined by plating serial dilutions of the homogenates on lactobacillus deMan, Rogosa, Sharpe (MRS) agar plates and counting colonies, differentially according to colony morphology, after 48 h of incubation at 37°C under anaerobic conditions (Guban *et al*., 2006). Jejunum samples were fixed in 10% buffered solution for 72 h and about 0.5 cm^2 was immediately excised and washed by physiological saline. The samples were treated in tissue processor apparatus, blocked in paraffin, 5-6 μm tissue sections stained with hematoxylin and eosin for histological examination of samples using the light microscope (Sakamoto *et al*., 2000). The villi height, villi surface area and lamina propria thickness of jejunum were determined. The villi height was measured from the top of the villi to the top of the lamina propria. The villi surface area was calculated the following formula: $(2\pi)(VW/2)(VL)$, where VW = villi width and VL = villi length (Sakamoto *et al*., 2000). The lamina propria thickness was measured from the base of the villi to the top of the muscularis mocosa. The project was reviewed and approved by the Animal care Committee of the Ferdowsi University of Mashhad.

The data were subjected to ANOVA as a completely randomized design using the GLM procedure of SAS Institute (1999). The least significant difference test (Steel and Torrie, 1980) was applied to compare the treatment means when the treatment effect was significant at p<0.05.

RESULTS AND DISCUSSION

The inclusion of Fermacto® in starter and grower diets improved (p<0.05) the final BWG and FCR of broiler chicks as compared to those without Fermacto® supplementation when fed 100 or 90% of RDAA diets (Table 2). Similar improvement in the final BWG and FCR

Table 2: Body weight gain, feed conversion ratio, carcass yield and cuts and flock uniformity of broiler chicks fed Fermacto® supplemented diet with 100, 95 and 90% recommended digestible amino acids (RDAA)

	Dietary treatments									
	100% RDAA			95% RDAA			90% RDAA			
Periods (days)	Fermact®o (g kg^{-1})			Fermacto® (g kg^{-1})			Fermacto® (g kg^{-1})			
Starter	0.0	2.0	2.0	0.0	2.0	2.0	0.0	2.0	2.0	
Grower	0.0	2.0	1.0	0.0	2.0	1.0	0.0	2.0	1.0	
Finisher	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	MSE
Body weight gain (g/b/period)										
Starter	470^{b}	510^{a}	505^{a}	451^{bc}	485^{ab}	487^{ab}	428^{c}	468^{b}	468^{b}	20.1
Grower	1060^{b}	1202^{a}	1190^{a}	1010^{bc}	1125^{ab}	1119^{ab}	900^{c}	1o50^{b}	1050^{b}	40.2
Finisher	535^{a}	561^{a}	556^{a}	486^{b}	500^{b}	492^{b}	466^{b}	482^{b}	470^{b}	34.6
Total	2065^{b}	2273^{a}	2251^{a}	1947^{bc}	2110^{ab}	2098^{ab}	1792^{c}	2000^{b}	1980^{b}	74.6
Feed conversion ratio (g feed/ g gain)										
Starter	1.75^{b}	1.65^{c}	1.66^{c}	1.80^{ab}	1.70^{bc}	1.70^{bc}	1.86^{a}	1.82^{ab}	1.82^{ab}	0.054
Grower	2.20^{b}	2.00^{c}	2.02^{c}	2.27^{ab}	2.09^{bc}	2.10^{bc}	2.38^{a}	2.28^{ab}	2.28^{ab}	0.096
Finisher	2.24^{b}	2.19^{b}	2.20^{b}	2.44^{a}	2.41^{a}	2.42^{a}	2.42^{a}	2.43^{a}	2.43^{a}	0.040
Total	2.14^{b}	1.91^{c}	1.92^{c}	2.22^{ab}	2.09^{bc}	2.11b^{c}	2.30^{a}	2.20^{ab}	2.21^{ab}	0.043
Carcass cuts										
LBW[1] (g)	2259^{b}	2443^{a}	2400^{a}	2095^{bc}	2348^{ab}	2300^{ab}	2000c	2210^{b}	2210^{b}	122.9
Carcass[2] (%)	72.3	73.6	73.5	72.0	7.4	73.6	72.2	72.5	72.3	0.16
Breast[3] (%)	33.5^{b}	35.7^{a}	35.7^{a}	32.9^{bc}	34.2^{ab}	34.0^{ab}	31.0c	33.5^{b}	33.4^{b}	0.15
Legs[4] (%)	28.3	28.4	28.4	27.5	28.5	28.5	27.2	27.2	27.0	0.08
AF[5] (g)	41.0^{c}	41.4^{c}	41.2^{c}	53.5^{ab}	49.3^{b}	50.2^{b}	58.4^{a}	59.5^{a}	58.6^{a}	4.69
Flock uniformity (% birds within 10% of the mean weight)										
Day1	59.8	62.6	59.6	58.9	59.6	58.8	59.6	60.0	59.0	7.58
Day 21	63.0^{b}	74.1^{a}	74.9^{a}	57.0c	69.2^{ab}	68.2^{ab}	59.2^{c}	61.0^{b}	61.0^{b}	6.43
Day 47	61.2	62.2	62.0	59.8	60.8	61.2	59.2	59.8	60.8	5.46

$^{a-c}$ means within each row with different superscript are significantly different (p<0.05), [1]Live body weight, [2] Carcass weight excluding skin, head, feet and edible organs, given as % of live body weight, [3] Breast skinless with bone, (% of carcass weight), [4] Legs, (% of carcass weight), [5] Abdominal fat was shown when grower diet supplemented with 1.0 or 2.0 g kg^{-1} Fermacto. It may be justified that birds fed grower diet with 1.0 or 2.0 g kg^{-1} Fermacto had similar intestine microflora during growing and finishing periods. Similar results have obtained other researchers (Meinz, 1993; Tangendjaja, 1993). When they found that Fermacto supplementation of broiler diet resulted in a higher weight gain than those fed diet without Fermacto supplementation. Rodriguez *et al.* (2005) designed an experiment with three treatments as High Protein (HP, more than 19%), Low protein (LP, less than 19%) and LP + Fermacto. The BWG was significantly different among 3 experimental groups ($p<0.05$) with HP as the heaviest, LP as the lightest and LP + Fermacto intermediate. Harms and Miles (1988) reported the effect of adding 0.48 g kg^{-1} Fermacto to diets of laying hens with various levels of supplemental methionine. They reported a significant improvement in feed consumption and feed efficiency when basal diet was supplemented with Fermacto or methionine. In the present study the positive effect of Fermacto inclusion on BWG was similar in all birds regardless of dietary DAA levels. The Carcass yield was not influenced by Fermacto inclusion or level of digestible amino acids in diet. However, breast yield was significantly increased with Fermacto supplementation when birds fed diet contained 100, 95 or 90% of RDAA (Table 2). The higher breast yield of chickens fed diet with Fermacto supplementation might be associated with the partial replacement of intestinal microflora by lactobacillus and more efficient uptake of essential amino acids. Although, Maiorka *et al.* (2001) evaluated probiotics in broiler diets and reported no effects on carcass, leg and breast yields.

The BWG and FCR of broiler chicks were negatively influenced as the level of dietary DAA decreased. A 10% decrease in dietary digestible amino acid requirements significantly suppressed ($p<0.05$) BWG and increased ($p<0.05$) the FCR of broiler chicks throughout the study. The differences between the recommended and low protein diets were expected because lower protein and/or DAA (in particular lysine, methionine and threonine) contents affect weight gain and performance (Auckland and Fulton, 1972; NRC, 1994). Abdominal fat was unaffected by dietary Fermacto supplementation. A decrease in RDAA in diet significantly ($p<0.05$) increased abdominal fat of birds over those fed 100% RDAA diet. The results for AF observed in our study are in accordance with those reported by Ghaffari *et al.* (2007). Diet formulation based on RDAA may help to more accurately supply the amino acids at the cellular level and an increase in protein accretion instead of fat deposition. Rostagno *et al.* (1995) observed that breast meat yield, clearly responded to difference in digestible amino acid supply and carcasses from birds on diet low in DAA (LD) had 29.0% breast portion including skin and bone and was significantly less than 30.1% of those fed higher level of DAA (HD) diet Carcass fatness, as measured by the abdominal fat pad, was slightly higher in LD than HD fed birds and remained unchanged by added amino acids.

The flock uniformity of birds fed 100% RDAA diet improved as compared to those fed diets deficient in DAA when measured at 21 day, but was similar at 47 day of age (Table 2). Lordelo *et al.* (2004) conducted an experiment to evaluate the effect of Cottonseed Meal (CSM) on uniformity in broiler breeder pullets. They reported that despite of low CP and digestible lysine contents, given the lower nutrient density of CSM; it is possible to formulate diets based on this ingredient so that the severity of feed restriction can be reduced and improved body weight uniformity attained. Peak *et al.* (2000) investigated the effect of 2 planes of nutrition (Standard = NRC-recommended level; or low = 66.1-69.5% as much protein while maintaining the same amino acid balance at similar metabolizable energy levels) on performance and uniformity of 4 strains of broiler chicks. They observed low plane of nutrition decreased flock uniformity and uniformity of carcass cut yields, with the effect greater on males than on females. The flock uniformity at day 21 was improved ($p<0.05$) by the inclusion of Fermacto regardless of dietary DAA levels (Table 2). The flock uniformity was about 60% at 47 day of age and was not influenced by supplemental Fermacto or dietary DAA levels. A common consensus among processors is that uniformity among birds is necessary to control the size and quality of parts; although, it is difficult to achieve high uniformity (60-63% >). The significant increase in empty gut weight and numerical increase in ceca weights may be due to enhanced functions of this organ with higher subsequent nutrient uptake (Table 3). Djouvinov *et al.* (2005) reported that added prepared probiotic tended to influence the microbial populations in the cecal digesta. In our experiment, the higher ceca weight was observed in birds fed diet with 100% RDAA and Fermacto supplementation as compared to those fed diet with 95 or 90% of RDAA with/without Fermacto content. Similar effect of Fermacto on ceca weight was reported by Djouvinov *et al.* (2005). The gizzard weight did not change by Fermacto supplementation and/or digestible amino acids levels in diet indicating that Aspergillus mycelium may not affect the function of this part of gut. It is reported that the addition of probiotics to diet of broiler chicks had no effect on percentage of liver and pancreas, although higher gizzard percentage was seen (Loddi *et al.*, 2000).

Table 3: Whole and segments of gastrointestinal weight, ileum Lactobacillus counts, jejunum histological traits and gastrointestinal transit time of broiler chicks fed Fermacto® supplemented diet with 100, 95 and 90% recommended digestible amino acids (RDAA)

	Dietary treatments									
	100% RDAA			95% RDAA			90% RDAA			
Periods (days)	Fermact®o (g kg^{-1})			Fermacto® (g kg^{-1})			Fermacto® (g kg^{-1})			
Starter	0.0	2.0	2.0	0.0	2.0	2.0	0.0	2.0	2.0	
Grower	0.0	2.0	1.0	0.0	2.0	1.0	0.0	2.0	1.0	
Finisher	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	MSE
Whole and segments of gastrointestinal tract weight (g)										
Whole tract	180^{b}	197^{a}	197^{a}	150^{c}	178^{b}	175^{b}	140^{c}	170^{b}	169^{b}	9.42
Gizzard	34.6	35.3	35.3	34.9	35.4	35.5	34.4	34.8	34.5	2.11
Ceca	8.0^{ab}	9.4^{a}	9.4^{a}	6.7^{b}	7.2^{b}	6.7^{b}	6.5^{b}	7.0^{b}	6.6	0.59
Ileum lactobacillus count (Log CFU g^{-1})										
Lactobacillus	7.40^{b}	8.78^{a}	8.74^{a}	5.98^{c}	7.42^{b}	7.40^{b}	6.00^{c}	7.39^{b}	7.41^{b}	0.22
Jejunum (μm)										
Villi hight	25.0^{b}	26.0^{a}	25.7^{a}	21.5^{c}	25.1^{b}	24.6^{b}	18.7^{a}	21.7^{c}	21.8^{c}	0.75
LPT[1]	5.1^{b}	6.6^{a}	6.4^{a}	4.0^{c}	5.1^{b}	5.2^{b}	3.0^{d}	4.0^{c}	4.0^{c}	0.17
Villi surface area	268^{b}	303^{a}	294^{a}	230^{c}	270^{b}	260^{b}	200^{d}	233^{c}	235^{c}	10.3
Gastrointestinal transit time of feed (min)										
Day 16	155^{b}	181^{a}	179^{a}	145^{bc}	168^{ab}	165^{ab}	112^{d}	130^{c}	131^{c}	0.52
Day 32	193^{b}	220^{a}	196^{b}	175^{bc}	215^{a}	193^{b}	128^{d}	162^{c}	133^{d}	0.57

[1]Lamina properia thickness, $^{a-d}$ Means within each row with different superscript are significantly different ($p<0.05$)

Adding Fermacto to diet improved($p<0.05$) gut performance which is probably related to the effect of this prebiotic on physiology and morphology of gut tissues. It is reported that lactobacillus has increased villi hight and crypt depth (Dunham *et al*., 1993). The improved morphometry (villi height and wall thickness) and increased beneficial microflora specifically lactobacillus in jejunum and ileum by supplemental Fermacto at the rate of 2 g kg^{-1} in the starter and 1 or 2 g kg^{-1} in the grower diet as compared to non Fermacto-fed birds may be the cause of longer ($p<0.05$) transit time of feed in gut at day 16 and 32 of age (Table 3). Generally, the GTT of feed increased as birds aged and this could be the reason that increasing the inclusion rate of Fermacto over 1 g kg^{-1} in the grower diet may not influence transit time of feed. Angel *et al*. (2005) reported that the Inclusion of direct fed microbial in diet increased nutrient retention and allows for the feeding of low nutrient diets without negative impact on performance. Feeding Fermacto for 21 day as expected increased ($p<0.05$) ileum lactobacillus bacteria counts, villi height, villi surface area and lamina propria thickness in jejunum of broiler chicks (Table 3). Fermacto supplementation increases population of beneficial bacteria by providing nutrients and mycelial fiber for the proliferation of intestinal bacteria. Similar effect of this prebiotic on ileal lactobacillus population and histological examinations in duodenum and ileum was found in broilers (Han *et al*., 1999; Kim *et al*., 2003). In contrast, Djouvinov *et al*. (2005) reported no significant differences in ileum lactobacillus counts and jejunum villi height and thickness of control birds and those fed prebiotic (Lactina) supplemented diet for 42 days. Most likely, the inconsistent results in using prebiotics or probiotics are due to differences in species and strains of microbes, in combination with inclusion rate and way of application.

The lower activity of cecum was also observed through a decrease in ceca weight ($p<0.05$) of birds fed 5 or 10% deficient DAA diets as compared to those fed control diet. Our results showed that transit time of feed was reduced ($p<0.05$) when birds fed diet low in RDAA (90%) as compared to control birds (Table 3). A significant difference in ileal lactobacillus population and jejonal villi height, villi surface area and lomina propria thickness were noticed between birds fed recommended and low DAA diets (Table 3). This elucidated that the necessity of meeting bioavailable amino acids to produce a proper microflora in small intestine. The gastrointestinal tract is a highly metabolically active organ that has considerable nutrient requirements (Yen *et al*., 1989). This organ can be negatively influenced by nutrients, additives, or pathogens (Ivatury *et al*., 1996). Early maturation of the gut has been shown to be an important factor in raising a healthy chick, as the physiological development of birds is directly related to digestion and nutrient absorption in the small intestine (Aptekmann *et al*., 2001).

The villi play a crucial role in the digestion and absorption processes of the small intestine and its surface area is the first to make contact with nutrients in the lumen (Gartner and Hiatt, 2001). The lamina propria contains connective tissue in the mucosa that supports the delicate enterocyte of the villi and is an essential component of the immune system, as a thin or scattered lamina propria is more readily infiltrated by pathogens.

CONCLUSION

Supplementation of starter and grower diets with Fermacto improved BWG, FCR, breast yield and gut development regardless of dietary DAA levels. Addition of more than 1.0 g kg^{-1} Fermacto in the grower diet did not further enhance BWG or FCR. The flock uniformity was positively influenced by inclusion of Fermacto as compared to non-Fermacto treated birds fed 100 or 90% of DAA requirements at 21 day. Fermacto prolonged transit time of feed, increased the ileal lactobacillus counts and improved jejunal histological traits. Performance and breast yield of broiler chickens fed 95% of DAA requirements was similar to those fed control diet, whereas birds fed diet contained 90% of DAA requirements were significantly smaller and less efficient as compared to control birds. Formulating diets based on 95% DAA requirements did not have any negative effect on performance, but may decrease environmental pollution and cost of broiler chicken production. A 10% decrease in DAA requirements increased AF, reduced transit time of feed and ileal lactobacillus counts and weakened jejunum morphometry.

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