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Influence of Dietary Prebiotic Addition on Digestibility and Intestinal Microflora of Young Male Broiler Chickens Exposed to Delayed Feed Access after Hatch

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Abstract: A trial was conducted to investigate the effects of a dietary prebiotic for a period of 14 d on the intestinal microflora, Dry Matter (DM) and Organic Matter (OM) digestibility and growth performance of male broiler chicks Delayed to Feed Access (DFA) after hatch. One hundred forty four 1-d-old broiler chicks (ROSS 308) were randomly distributed into 6 groups with 8 replicate pens having 3 birds in each. A 2 x 3 factorial design was implemented. Six experimental groups were formed by two levels of dietary prebiotic supplementation (Control and Agrimos®, 1 kg/ton) and three periods of DFA (0-, 24- and 48- h). Depending on the time interval between arrival to the experimental site and feeding, holding chicks prior to free access to water and feed had a negative impact ($p<0.001$) on the body weight. At the end of the trial, these differences remained significant for body weight ($p<0.05$) and feed consumption ($p<0.001$) of chicks with DFA. DM digestibility reduced significantly ($p<0.05$) in birds exposed to 24- and 48-hour delay prior to feeding. A significant decrease (6.2 vs. 5.5 log_{10}cfu/g) in *Enterobacteriaceae* ($p<0.01$) and increase (5.5 vs. 5.9 log_{10}cfu/g) in *Lactobacilli* count was noted in prebiotic supplemented groups on d 7. Dietary prebiotic supplementation improved DM ($p<0.05$) and OM ($p<0.05$) digestibility significantly. Relative weight of intestine was reduced ($p<0.05$) in birds fasted for 24- and 48-h after hatch. Overall, dietary prebiotic supplementation helped broiler chicks to develop a healthier intestinal microflora and this may, in turn, inhibit the DFA resulted decrease of dry matter digestibility in early growing period. However, prebiotic inclusion to broiler diets may not be a protective management practice in preventing DFA-related growth depression of broiler chickens.

Key words: Broiler, prebiotic, delayed feed access, intestinal microflora, digestibility

INTRODUCTION

Prebiotics have been shown to alter gastrointestinal microflora, alter the immune system, reduce pathogen invasion including pathogens such as *Salmonella spp.* and *E. coli* (Cummings and Macfarlane, 2002). The major action of prebiotics is to stimulate the growth and/or activate the metabolism of some groups of beneficial bacteria in the intestinal tract. Several studies have shown that addition of prebiotics to the diet of broiler leads to improved performance through improving gut microflora (Xu *et al*., 2003; Spring *et al*., 2000; Pelicano *et al*., 2005).

The composition of the gut microflora plays an important role in digestion, with a beneficial, negative or neutral effect. Modifications to the gastrointestinal microflora which reduce pathogen attachment may have a positive effect on digestibility of nutrients (Hajati and Rezei, 2010).

In practice, hatching and transportation procedures delay the feeding of chicks by 10 to 60 h (Noy and Sklan, 1999). Delayed feeding in the first few days of life span reduces final BW (Noy and Sklan, 1999) and it probably affects immunological capacities (Dibner *et al*., 1998). Indeed, the immune system of the hatchling, particularly the mucosal immune system, requires oral feed intake for its full and rapid development. In addition, the immediate post-hatch period seems to be critical for intestinal development in chicks and fasting often occurs in practice (Uni *et al*., 2003). As brief, this situation resulted with decreasing of growth rate, weaken of immune system, decline of digestive enzyme stimulation and negative effects on organ development (Pinchasov and Noy, 1993; Noy and Sklan, 1999; Dibner, 1999; Gonzales *et al*., 2003; Bigot *et al*., 2003).

Previous reports (Uni *et al*., 2003; Noy and Sklan, 1999; Noy *et al*., 2001; Bigot *et al*., 2003; Gonzales *et al*., 2003) have shown that delayed access to feed and water decreased broiler post hatch performance. However, no studies have been carried out to investigate the response of fasted broiler chickens to inclusion of prebiotic in corn soybean diets. It is thus necessary to understand precisely the consequences of delayed

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feeding and dietary prebiotic supplementation on microbial flora of intestine. Other hypothesis in the present study was to test the influence of dietary prebiotic addition on relative weights of some vital organs and intestine thereby this may help to inhibit detrimental effects of fasting after hatch. In this sense, in addition to the intestinal microflora, the determination of early growth performance and digestibility of experimental feeds (DM and OM digestibilities) were the main objectives of this study.

MATERIALS AND METHODS

Birds, housing, experimental design and diets: One forty four 1 d-old male ROSS 308 broiler chicks were obtained from a commercial hatchery 0 to 4 h posthatch (late hatchers) and transported within 1 hour to experimental unit. Broiler chicks were hold in transportation boxes at a constant temperature (32±1°C) and humidity (65-70%) controlled room to prevent dehydration before feed access. The birds were initially weighed individually so that the pens had similar initial weight and weight distribution and randomly assigned to 6 experimental groups, with 8 replicates of 3 chicks each. Chicks were housed in battery cages with wire mesh bottom. These six treatment groups were formed by supplementation or not of a dietary prebiotic (Control and Agrimos®) and feeding male broiler chicks at the time of arrival to the experimental unit or after a 12-, or 48 h holding time at optimal environmental conditions prior to feeding (2 x 4 factorial arrangement of treatments). Broilers were fed with corn and soybean meal based diet (Table 1) that contained the contents of critical nutrients recommended by ROSS 308 broiler manual up to 14 days. From 1 to 14 d of age, they received a starter diet (23.5% crude protein; 3050 kcal/kg ME, 1 kg/ton prebiotic). Agrimos® is a combination of manno-oligosaccharides and β-glucans extracted from the yeast cell walls of *Saccharomyces cerevisiae*. Each pen (15 x 40 cm) was supplied with metal feeders and nipple drinkers to provide *ad libitum* access to feed and water, whereas lighting was provided on a 24 h light schedule. The experiment was conducted under appropriate animal care regulations.

Sampling and measurements: Broiler chicks were weighed before and after holding period and their BW loss was recorded. Feed Consumption (FC) and pen BWs were recorded on days 0, 7 and 14 and mortality was recorded daily. FC, BW gain and Feed Conversion Ratio (FCR) were adjusted for mortality and calculated for the following growth periods: 0 to 7 d, 7 to 14 d and 0 to 14 d. The probable cause of death or reason for removal was documented.

On d 7, one bird from each pen (8 birds per treatment, 48 birds) was randomly selected and euthanised by cervical dislocation to determine organ weights (heart, gizzard, liver, spleen and intestine) and intestinal microflora. The weights of these parts were expressed as grams of slaughter weight; the entire length of the intestine was measured in cm.

For the intestinal microflora, the carcasses were subsequently opened and the entire gastrointestinal tract was removed aseptically. The gastrointestinal tract was then divided into sections (i.e., ileum, ceca and colon) that were ligated with light twine before separating the content from ileum. For the bacterial enumeration in digesta per bird, appropriately stored samples, frozen at -80°C, were thawed and removed from storage bags. Intestinal contents (ileum) were then aseptically emptied in a new sterile bag and were immediately diluted 10-fold (i.e., 10% wt/vol) with sterile ice-cold anoxic PBS (0.1 M; pH 7.0) and subsequently homogenized for 3 min in a stomacher (Bagmixer 100 Minimix, Interscience, Arpents, France). Each digesta homogenate was serially diluted from 10-1 to 10-7. Dilutions were subsequently plated on duplicate selective agar media for enumeration of target bacterial groups. In particular, total aerobes, total anaerobe bacteria, *Enterobactericeae*, *coliforms*, *Lactobacillus* spp. and *Salmonella* were enumerated using nutrient agar, violet red bile glucose agar, violet red bile lactose agar, Rogosa agar and Brillant green agar according to Hartemink and Rombouts (1999). Plates were then incubated at 37°C for 24 to 72 h aerobically and colonies were counted. Anaerobic incubation was achieved using appropriate catalysts (Anaerocult A, Merck, Darmstadt,

Table 1: Composition and calculated analysis of experimental diets

Ingredients	Starter diet (0 to 14 d)
Corn, ground	53.90
Soy bean meal	39.10
Vegetable fat	3.00
Calcium carbonate	1.20
Dicalcium phosphate	1.60
Salt	0.35
DL-methionine	0.35
L-Lysine	0.10
Vitamin and mineral premix*	0.30
Prebiotic (Agrimos®)**	0.10
Calculated analysis	
Metabolizable energy, kcal/kg	3050.00
Crude protein, %	23.50
Calcium, %	0.96
Available phosphorus, %	0.40

*Vitamin and mineral premix include per kilogram of diet (Kartal Kimya San. ve Tic. AS., Turkey): retinol acetate, 1706 mg, cholecalciferol, 41 mg, DL-α-tocopherol, 27 mg, menadione, 0.99 mg, cobalamin, 0.015 mg, folic acid, 0.8 mg: D-pantothenic acid, 15 mg, riboflavin, 5.4 mg, niacin, 45 mg, thiamin, 2.7 mg, D-biotin, 0.07 mg, pyridoxine, 5.3 mg, manganese, 90 mg, zinc, 83 mg, iron, 121 mg, copper, 12 mg, iodine, 0.5 mg, selenium, 0.3 mg.

**Agrimos® is a combination of manno-oligosaccharides and β-glucans extracted from the yeast cell walls of *Saccharomyces cerevisiae*

Germany) in sealed anaerobic jars (Oxoid, Basingstoke, UK). Results were expressed as log10 colony-forming units per gram of digesta (Hartemink and Rombouts, 1999).

Digestibility trial: At the end of the feeding trial (d 14), 8 birds from each treatments (48 birds totally) were randomly selected and placed into individual battery cages with excreta collection trays. Each cage was equipped 1 feeding and 2 water nipples placed on the front and back sides of the cage respectively. Similar environmental circumstances with feeding trial were provided in digestibility trial. Chicks were fasted for 12 h after 3-d of pre-experimental adaptation period. Experimental diets were offered *ad libitum* for 3 d. Chicks were again fasted for 12 h to unload the digestive tract. Faeces was collected in every 36 h by using aluminum papers placed on metal trays. Remaining feed in the excreta trays was carefully removed and weighed. Feathers were also removed from the excreta. Samples were kept in nylon bags at -20°C before analysis. Faecal samples were weighed and dried at 60°C and then they were grinded with 0.5 mm pore filter to homogenize. Feed and excreta samples were subsequently analyzed for DM, OM and ash using the routine procedures (AOAC, 2003). All analysis were performed in duplicate.

Statistical analyses: Data were analyzed by GLM procedure using Duncan's multiple range test with SAS statistical software (SAS Institute, 2003). Relative organ weight data were subjected to arc sine transformation, which showed a similar statistical trend. Differences were considered significant at $p<0.05$ (Steel *et al*., 1997).

RESULTS

Growth performance: The results for live performance are presented in Table 2 and 3. The present trial indicated that there was a significant ($p<0.001$) effect of DFA on BW prior to feeding. In the present study, depending on the time interval (24- and 48-hour prior to feeding), holding chicks prior to free access to water and feed had adverse impact on day 14 BW and FC whereas FE was not affected presumably due to decreased feed intake (Table 3). This was especially obvious for the treatment group hold for 48-hours prior to feeding. Dietary prebiotic supplementation did not affect live weight and feed consumption in the entire experiment. There were no significant differences between treatments regarding mortality that was generally low and averaged 2.4% for the whole experiment.

Intestinal microbial population: The population of Total Bacteria, Total Anaerobe Bacteria, Coliform Bacteria, *Enterobacteriaceae* and *Lactobacilli* in the digesta content of the ileum is presented in Table 4. Current study revealed that dietary supplementation of prebiotic reduced (6.22 vs. 5.53 log_{10}cfu/g; $p<0.01$) *Enterobacteriaceae* count at 7 d of age. Moreover, *Lactobacilli* count was significantly ($p<0.05$) higher (5.46 vs 5.92 log_{10}cfu/g) in groups fed diet supplemented with prebiotics at d 7 (Table 4). The feed sample analysis

Table 2: Body weight (g) and BW change (%) at 0, 24 and 48 hrs of male broilers subjected to holding time and dietary prebiotic inclusion

Treatments		BW (g)			BW change (%)	
DFA	Prebiotic[1]	0	24	48	0 to 24 hr	0 to 48 hr
0	0	39.88	45.92	51.94	15.19	30.27
24	0	39.96	37.41	46.03	-6.32	16.38
48	0	39.92	38.20	35.67	-4.27	-10.66
0	1	39.71	46.84	53.77	17.97	35.41
24	1	39.88	37.94	47.01	-4.77	17.95
48	1	39.96	38.04	36.12	-4.78	-9.59
Pooled SEM		0.37	0.39	0.70	1.13	1.67
DFA						
0		39.79	46.38[a]	52.85[a]	16.58[a]	32.84[a]
24		39.92	37.67[b]	46.52[b]	-5.54[b]	17.17[b]
48		39.94	38.12[b]	35.89[c]	-4.52[b]	-10.12[b]
Prebiotic						
0		39.92	40.51	44.55	1.54	12.00
1		39.95	40.94	44.63	2.80	14.59
ANOVA		P				
DFA		NS	***	***	***	***
Prebiotic		NS	NS	NS	NS	NS
DFA x Prebiotic		NS	NS	NS	NS	NS

[a,b,c]Means within a treatment and column with different subscripts differ significantly ($p<0.05$). NS: Not significant ($p>0.05$); ***$p<0.001$. [1]Agrimos® (1 kg/ton)

Table 3: Body weight, body weight gain, feed consumption and feed efficiency of male broilers subjected to holding time and dietary prebiotic inclusion

Treatments		Body weight (g)		Body weight gain (g)		Feed consumption (g)		Feed efficiency (g:g)[1]	
DFA	Prebiotic[2]	7	14	0 to 7d	0 to 14d	0 to 7d	0 to 14d	0 to 7d	0 to 14d
0	0	119.50	163.96	79.63	124.08	122.63	281.33	1.60	2.37
24	0	104.75	163.83	63.28	123.43	95.29	243.28	1.51	1.98
48	0	94.42	159.25	54.50	119.33	87.63	241.18	1.63	2.05
0	1	129.09	189.54	89.38	149.83	127.04	298.38	1,45	2.02
24	1	101.50	165.71	61.63	125.83	92.54	253.34	1.54	2.06
48	1	88.46	145.04	48.50	105.08	74.08	214.38	1.55	2.07
Pooled SEM		5.60	9.01	5.55	9.00	6.80	13.00	0.10	0.11
DFA									
0		124.29[a]	176.75[a]	84.50[a]	112.21[b]	124.83[a]	289.85[a]	1.53	2.20
24		103.12[b]	164.57[b]	62.45[b]	124.63[a]	93.91[b]	248.31[b]	1.53	2.02
48		91.44[b]	152.15[b]	51.50[b]	112.21[b]	80.86[b]	227.78[c]	1.59	2.06
Prebiotic									
0		106.22	162.21	65.80	122.28	101.85	255.26	1.58	2.13
1		106.35	166.76	66.50	126.92	97.89	255.36	1.51	2.05
ANOVA		P							
DFA		***	*	***	*	***	***	NS	NS
Prebiotic		NS	NS	NS	NS	NS	NS	NS	NS
DFA x Prebiotic		NS	NS	NS	NS	NS	NS	NS	NS

[a,b,c]Means within a treatment and column with different subscripts differ significantly ($p<0.05$). NS: Not Significant ($p>0.05$); *$p<0.05$; ***$p<0.001$.

[1]Feed efficiency was calculated by dividing feed consumption (g) to BW gain (g) per pen basis.

[2]Agrimos® (1 kg/ton)

Table 4: Microbiological analysis (ileum) and total tract digestibility of male broiler chickens subjected to holding time and dietary prebiotic inclusion at day 7

Treatments		log_{10}cfu/g				Digestibility, %	
DFA	Prebiotic[1]	TAB	Enterobacteriaceae	Coliform	Lactobacilli	Dry matter	Organic matter
0	0	8.30	6.09	6.07	5.48	83.59	75.35
24	0	8.26	6.17	5.82	5.72	80.40	74.97
48	0	8.58	6.40	6.08	5.18	74.94	72.09
0	1	8.05	5.47	5.79	5.74	84.76	79.28
24	1	8.27	5.77	5.79	6.08	81.00	77.04
48	1	8.53	5.37	5.44	5.48	82.85	76.49
Pooled SEM	0.18	0.29	0.33	0.25	1.94	2.08	
DFA							
0		8.18	5.78	5.76	5.61	84.17[a]	77.32
24		8.26	5.97	5.80	5.90	80.70[b]	76.01
48		8.56	5.78	5.75	5.56	78.89[b]	74.29
Prebiotic							
0		8.38	6.22[a]	5.99	5.46[a]	79.64[b]	74.14[b]
1		8.29	5.53[b]	5.67	5.92[b]	82.87[a]	77.60[a]
ANOVA		P					
DFA		NS	NS	NS	NS	*	NS
Prebiotic		NS	**	NS	*	*	*
DFA x Prebiotic		NS	NS	NS	NS	NS	NS

[a,b]Means within a treatment and column with different subscripts differ significantly ($p<0.05$). NS: Not Significant ($p>0.05$), **$p<0.01$.

[1]Agrimos® (1 kg/ton). TAB = Total Aerobe Bacteria

ascertained that dietary prebiotic supplementation did not influence Total Bacteria, Total Anaerobe Bacteria, Coliform Bacteria, *Enterobacteriaceae*, *Lactobacilli* count compared with diet had no prebiotic supplementation (data not shown). During the present investigation also *Salmonella* was absent in feed and intestinal content samples.

Dry matter and organic matter digestibility: As far as DM and OM digestibilities were concerned, it was observed that dry matter digestibility significantly ($p<0.05$) decreased (84.17 vs. 80.70 and 78.89%) depending on the time interval (-24 and 48-h delay) whereas dry matter digestibility increased significantly (79.64 vs. 82.87 %; $p<0.05$) when broiler chickens were supplemented with dietary prebiotic at the end of the first week (Table 4). Similarly OM digestibility was improved significantly ($p<0.05$) in birds fed prebiotic supplemented diets. However, DFA did not affect OM digestibility significantly in this work.

Table 5: Some organ yields of male broiler chickens subjected to holding time and dietary prebiotic inclusion

Treatments		Relative weights of organs (g/100 g BW)			
DFA	Prebiotic[1]	Heart	Gizzard	Liver	Spleen
0	0	0.85	8.25	3.53	0.07
24	0	0.89	8.15	3.96	0.08
48	0	0.82	8.11	4.17	0.08
0	1	0.88	8.28	3.99	0.06
24	1	0.88	8.21	3.90	0.07
48	1	0.93	8.15	4.05	0.08
Pooled SEM		0.06	0.23	0.16	0.01
DFA					
0		0.87	8.27	3.76	0.07
24		0.89	8.18	3.93	0.08
48		0.87	8.13	4.11	0.08
Prebiotic					
0		0.85	8.17	3.89	0.08
1		0.90	8.21	3.98	0.07
ANOVA		P			
DFA		NS	NS	NS	NS
Prebiotic		NS	NS	NS	NS
DFA x Prebiotic		NS	NS	NS	NS

[1]Agrimos® (1 kg/ton). NS = Not significant (p>0.05)

Table 6: Some morphometric intestinal parameters of male broiler chickens subjected to holding time and dietary prebiotic inclusion

		Intestinal part lenght/total intestine length (cm)				Relative weight of intestine	Intestinal ratio (weight/length
DFA	Prebiotic[1]	Duodenum	Jejunum	Ileum	Cecum	(g/100 g BW)	of intestine)
0	0	8.02	38.90	35.70	14.41	15.34	0.16
24	0	6.76	44.73	32.12	13.60	14.68	0.15
48	0	7.46	39.94	34.28	15.01	14.56	0.16
0	1	7.24	39.32	35.75	14.68	15.04	0.18
24	1	8.04	39.78	33.84	15.21	14.46	0.16
48	1	7.43	41.50	33.34	14.33	14.55	0.15
Pooled SEM		0.59	2.80	1.82	0.82	0.29	0.01
DFA							
0		7.63	39.11	35.73	14.54	15.19[a]	0.17
24		7.40	42.25	32.98	14.40	14.57[b]	0.15
48		7.44	40.72	33.81	14.67	14.56[b]	0.15
Prebiotic							
0		7.41	41.19	34.03	14.34	14.85	0.15
1		7.57	40.20	34.31	14.74	14.68	0.16
DFA		NS	NS	NS	NS	*	NS
Prebiotic		NS	NS	NS	NS	NS	NS
DFA x Prebiotic		NS	NS	NS	NS	NS	NS

[a,b]Means within a treatment and column with different subscripts differ significantly (p<0.05). NS: Not Significant (p>0.05), *p<0.05.
[1]Agrimos® (1 kg/ton)

Relative weights of intestine and some organs: Changes in the weight and lenght of the intestine and in relative weight of some organs (heart, gizzard, liver and spleen) are presented in Table 5 and 6. The effects of restriction time and dietary organic acid supplementation on relative weight of organs and lenght of intestine was not significant whereas DFA significantly reduced the relative weight of intestine in the study (p<0.05).

DISCUSSION

Growth performance: The present trial indicated that there was a significant (p<0.001) effect of DFA on BW prior to feeding. Between 0 to 24-h and 0 to 48-h post hatch, chicks with DFA reduced BW by approximately 6 and 10% respectively. Moreover, prebiotic x DFA interaction in BW was not significant for the first 48 hrs (Table 2). In the present study, overall BW (176 vs. 152 g; p<0.05), FC (289.85 vs. 227.78; p<0.001) was significantly lower in birds hold for 48 h prior to feeding than in those access feed immediately (Table 3). Beside this, FE of chicks delayed to feed access was not significant (p<0.05) in 14 d period. Extended posthatch holding (in the hatcher) has been reported to dehydrate chicks, reduce growth performance and depress immune response (Casteel *et al*., 1994). Similarly, Bigot *et al*. (2003) found a significant BW loss (7%) in chicks delayed to feed access for 2 d post-hatching. Moreover,

Saki (2005) reported that BW was decreased by those chicks which were not accessed to feed compared with that groups which was fed by starter diet immediately after hatching. These findings are consistent with the several reports which demonstrate that delay in feed intake after hatch adversely affect posthatch performance of chicks (Bigot *et al*., 2003; Gonzales *et al*., 2003; Pinchasov and Noy, 1993). The present results for initial BW loss following hatch is mainly due to metabolism and possibly some dehydration occurring during holding time in the incubator or postincubator as has been previously reported by Noy *et al*. (2001). In previous report (Noy and Sklan, 1999) and in the current study, BW begins to increase 24 to 48 h after access to feed. This finding confirms that DFA clearly causes a negative energy balance and chicks have invariably loose weight.

In this trial, BW and FC of birds are depressed due to DFA for 14 d period, this negative difference will probably remain till the end of the entire growing period (42-45 d). However, overall performance of chicks till marketing age was not the main subject in the present study. Overall, mortality rates (data not shown) in the present experiment were found similar to some previous studies (Hooshmand, 2006; Kidd *et al*., 2007). Moreover, bird losses due to mortality and culling were at a low level and were not affected by feeding program or feed supplement.

Either no effects or negative effects of dietary prebiotic on chick growth performance were observed in the study. There was no significant prebiotic x DFA interaction for growth performance in the study. Result for overall FI and FE were also not significant and similar with BW and BWG results at the end of trail. Similar to this, Houshmand *et al*. (2012) also noticed no significant effect on growth performance of broiler chickens fed diets supplemented with prebiotic. Similarly, Biggs *et al*. (2007) and Rehman *et al*. (2007, 2008) using inulin at concentrations 4 and 10 g/kg, respectively in their studies and had observed no positive effect of prebiotics on growth performance in broiler chickens. In consistent with our results, some other studies (Waldroup *et al*., 1993; Williams *et al*., 2008) also observed no effects of prebiotics on FE in broiler chickens. However, these results are in disagreement with some previous studies (Li *et al*., 2008; Yang *et al*., 2008). There are a number of possible causes for the differing results among trials subjecting prebiotic supplementation in broiler diets. One of these reasons is the prebiotic dose used in the experimental diets. The results of this study indicate that there was no significant effect on growth performance by suggested doses of prebiotic in diet. Another possible reason is variations in the specific prebiotics used, which may have different impacts on the performance of the birds. Variations in feedstuffs and nutrient levels and age of birds (our study revealed only first 14 d of growing period) may also influence results. Data from this study suggest that the adverse effects of DFA on growth performance cannot be alleviated by dietary prebiotic supplementation in early growing period.

Intestinal microbial population: The population of microbiata in the content of the small intestine is presented in Table 4. Current study results revealed that dietary supplementation of prebiotic inhibited (6.22 vs. 5.53 $\log_{10}$cfu/g; $p<0.01$) *Enterobacteriaceae* count at 7 d of age. Moreover, Lactobacilli in chicks fed dietary prebiotic had higher (5.46 vs. 5.92 $\log_{10}$cfu/g; $p<0.05$) count when compared to chicks fed diets unsupplemented at d 7. Some previous studies are in consistent with the present trial. Kim *et al*. (2011) found that 0.25% Fructo-Oligosaccharide (FOS) addition to broiler diets had lowered *Escherichia coli* count whereas *Lactobacilli* count in small intestine was increased. Differences between studies could be related with many factors which alter microflora composition of birds (Yegani and Korver, 2008) including age and breed of birds plus composition of diet and prebiotic.

Sterile gastro-intestinal tract of chick is altered with the time of hatch and the number and diversity of bacteria increase with age (Verstegen *et al*., 2005; Yegani and Korver, 2008). In the present trial, there were no significant effects of DFA on microbial populations of broiler chickens. For our knowledge, there is a limited number of study focuses on ileal total bacteria count of broiler chickens delayed to feed access. In agreement with report of Alhota (2011), *Lactobacillus* and *Salmonella*, as an index of healthy gut microflora, were not influenced by delay in access to feed and water.

Dry matter and organic matter digestibilities: In this study, the depression in growth performance in treatments delayed to feed access was concomitant with an decreased total tract dry matter digestibility determined in 7-d-old broilers. OM digestibility did not show a significant reduction in birds supplemented with prebiotics compared with unsupplemented groups. On the other hand, dietary prebiotic supplementation improved dry matter digestibility of birds at 7 day of age. Contrary to studies with dietary enzyme supplementation, only a few studies have examined DM and OM digestibility in broilers fed prebiotics. It was shown that, depending on the probiotic inclusion level in the diet, prebiotic intake resulted in an improved ileal DM digestibility in broilers fed corn-soy bean meal based diets at 21 and 42 d of age (Huang *et al*., 2005).

The enhanced total tract digestibility of DM in the broilers fed the prebiotic containing diets might be explained by the following findings. First, prebiotic supplementation reduced the number of pathogenic bacteria (e.g., *Escherichia coli*, *Salmonella typhimurium*) (Choi *et al*., 1994; Wang *et al*., 2003) and increased the beneficial

bacteria (e.g., *Lactobacilli*) numbers (Oli *et al*., 1998) in the intestine. Such changes in the intestinal bacterial population resulted in a decrease in the incidence of diarrhea (Oli *et al*., 1998). Second, dietary prebiotic addition may stimulate the secretion of digestive enzymes from the stomach, pancreas and intestinal mucosa (Hou and Gao, 2001). This effect is expected to reduce local inflammation in the intestinal mucosa, facilitate the breakdown of complex molecules into simpler ones and enhance the integrity of enterocytes, thereby promoting the digestion and absorption of nutrients (Wu, 1998).

Generally, it is very difficult to directly compare different studies using different prebiotics and different administration levels because the efficacy of a prebiotic application will additionally depend on many other factors stated in a recent review (Hajati and Rezei, 2010).

Relative weights of intestine and some organs: The effect of restriction time (24- and 48-h prior to feeding) and dietary organic acid supplementation on lenght of total or parts of intestine was not significant. Moreover, relative weight of organs were not affected by holding time at d 7. However, relative weight of intestine was significantly reduced ($p<0.05$) in birds received feed and water 24- and 48-h after hatch.

As it is known, the first days of life of broiler chickens are a critical stage of development with regard to feeding factors. Several research papers focus on the effect of DFA in broiler production and were motivated by its significant effects on muscle and organ development (Uni *et al*., 2003; Noy and Sklan, 1999). In addition to this, some of the important metabolic pathways prior to hatch are also described in a recent review (De Oliveira *et al*., 2008) which emphasizes the liver, pectoral muscle, hatching muscle and intestine, as most affected by changes toward hatch. In the present study, the relative growth rates differed in the different organs (numerical differences), but relative growth of the intestine depressed more rapidly and dramatically than the other organs. Results for relative weight of intestine were relatively similar with previous studies of Bigot *et al*. (2003) and Moore *et al*. (2005) who pointed out that posthatching starvation impaired intestinal growth, retarded pectoral muscle weight gain and the weight increase occurred only after chicks had access to feed. Neither the relative weight of organs (gizzard, liver, pancreas, spleen, bursa Fabricius) nor the length or weight of intestine evaluated in this study was affected by the addition of prebiotic to broiler diets. Some previous studies subjecting prebiotic supplementation in broiler diets were in agreement with the present trial. Similarly, Piray *et al*. (2007) did not find any significant effect on relative weights of heart, gizzard, liver, pancreas, spleen and bursa of Fabricius by dietary prebiotic supplementation. Moreover, it was also shown that (Pelicia *et al*., 2004) addition of prebiotic and probiotic into the diets of broilers have no effect on the digestive system (liver, proventriculus, gizzard, pancreas, duodenum, jejunum, ileum and cecum). In addition to this, Kalavathy *et al*. (2003) investigated the effects of 12 *Lactobacillus* strains in broiler diets and stated no significant differences in the relative weights of liver, spleen, heart and pancreas among treatments at different ages (21, 28, 35, 42 d).

In conclusion, the prebiotic (Agrimos®) was effective at enhancing DM and OM digestibility when it was included in a corn-SBM-based coccidiostat-free diet at 1 kg/ton. At this inclusion level, the prebiotic also have a significant positive effect on ileal microflora composition. Moreover, our results also revealed that this level of prebiotic supplementation to diets of young chicks may not help in alleviating the inhibitory effects of DFA on growth performance. It is proposed that optimal prebiotic inclusion levels for growth performance in broiler diets should be explicitly examined in context with the feed ingredients and the level of nutrients in diet. Results gathered from this work confirmed that increase in the digestion and absorption of nutrients is a major mechanism responsible for the enhanced DM and OM digestibility of broilers in response to dietary prebiotic (Agrimos®) supplementation. From practical point of view, this study highlights the need for a proper adjustment of feed additive (prebiotic) in the broiler diet to achieve the desired beneficial outcome when birds delayed to feed and water access after hatch.

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