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Use of Prebiotic Supplementation to Diet for Reducing the Negative Effects of Delayed Feed Access on Growth Rate in Broiler Chickens

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Abstract: About 36 h delays in access to feed and water after hatch is a common practice for broiler chicken production in Turkey. In the current study it was aimed to investigate the compensatory affect of dietary prebiotic inclusion on growth rate and gut health in birds delayed to feed and water access after hatch. The study was a factorial arrangement in a complete randomized design and continued for 42 days. One hundred ninety six, one day old male broilers (Ross 308) with an initial weight of 48.39±0.06 g/birds assigned to 4 treatments in a 2 (times of initiation of feeding) x 2 (prebiotic supplemental levels) factorial arrangement with 4 replications, each having 12 birds. Results indicated that delay in feed access had negative effects on body weight, body weight gain and feed intake while dietary prebiotic inclusion improved growth rate and feed conversion ratio in birds. However, almost all other examine parameters, including gut histomorphology and microbiology, showed no significant variances between treatments. As a conclusion, expect feed conversion ratio values, dietary prebiotic inclusion had no beneficial effect on growth rate and gut health in broiler chickens exposed to post hatch holding time.

Key words: Holding time, growth rate, gut health, prebiotic

INTRODUCTION

The first feeding after hatch plays an essential role not only on chick growth but also for gastrointestinal development in the early rearing period of broiler chickens (Nitsan et al., 1991; Nir et al., 1993; Dibner et al., 1998). It is also important to uptake of solid feeds that are necessary for changes in the morphology of the digestive tract and its secretions after hatch. Under practical conditions many birds have access to feed only 36-48 h after hatching (Noy and Uni, 2010). Chicks emerge from hatch at different time intervals which is required for hatchery treatments and transport to the broiler farms (Pinchasov and Noy, 1993). This situation resulted in decreasing of growth rate; immune system weakness, decline of digestive enzyme stimulation and negative effects on organ development (Pinchasov and Noy, 1993; Dibner, 1999; Noy and Sklan, 1999; Bigot et al., 2003; Gonzales et al., 2003). Intestinal growth occurs in delayed fed chicks to a significantly lesser extent then birds given access to feed immediately after hatch (Noy and Sklan, 1999; Bigot et al., 2003). This indicates a preferential intestinal growth immediately after hatch. Insufficient development of intestine results in decreasing of crypt size, the number of crypts/villus, crypt proliferation and villus area (Geyra et al., 2001; Uni et al., 2003). Moreover delaying access to feed and water makes birds more susceptible to pathogens (Dibner et al., 1998), influence to muscle development and retarded marketing weight (Tona et al., 2003).

access. 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). Fluorescent lights provided 24 hours illumination. The temperature was maintained at 34°C for the first three days and then gradually reduced by 2°C/week to final temperature of 22°C. All experimental conditions and animal care protocols were approved by the Adnan Menderes University Animal Care Committee.

MATERIALS AND METHODS

Experimental design and diets: The birds were initially weighed and randomly assigned to 4 experimental groups, with 4 replicates of 12 chicks each. The experiment consisted of a 2x2 arrangement of holding time [with or without 36h post hatch holding time] and prebiotic [with or without prebiotic (Agrimos®) supplementation; 0.1% for starter and grower and 0.05% for finisher diets, respectively]. Agrimos® is a combination of manno-oligosaccharides and β-glucans extracted from the yeast cell walls of Saccharomyces cerevisiae. Some of the birds were allowed to reach feed and water at the time of arrival time to the experimental unit meanwhile others exposure to 36 h holding. They were fed a corn and soybean meal basal diet which nutrient and ingredient composition is shown in Table 1. The experimental diets were provided on a three stage

Table 1: Composition and calculated analysis of experimental diets

		Diets	
	Starter	Grower	Finisher
Ingredients	(0 to 10 d)	(11 to 28 d)	(29 to 35 d)
Corn, ground	53.90	56.90	59.00
Soy bean meal	39.10	36.00	33.50
Vegetable fat	3.00	3.90	4.50
Calcium carbonate	1.20	1.00	1.00
Dicalcium phosphate	1.60	1.30	1.25
Salt	0.35	0.35	0.30
DL-methionine	0.35	0.15	0.10
L-Lysine	0.10	-	-
Vitamin and Mineral premix*	0.30	0.30	0.30
Prebiotic (Agrimos®) **	0.10	0.10	0.05
Calculated analysis			
Metabolizable energy, MJ/kg ***	13.0	13.2	13.4
Crude protein (%)	23.5	22	21
Calcium (%)	0.96	0.80	0.78
Available phosphorus (%)	0.40	0.35	0.32

*Vitamin and mineral premix include per kilogram of diet:

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 Thiamin, 2.7 mg Niacin, 45 mg D-biotin, 0.07 mg Pyridoxine, 5.3 mg Manganese, 90 mg Zinc, 83 mg Iron, 121 ma Copper, 12 mg lodine, 0.5 ma Selenium, 0.3 ma

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

feeding program consisting of starter (23.5% crude protein; 13.0 MJ Metabolizable Energy/kg (ME) for 0-14 d of age), grower (22% crude protein; 13.2 MJ ME/kg diet for 15-28 d of age) and finisher (21% crude protein; 13.4 MJ ME/kg for 29-42 d of age) diets as/Ross 308 recommendations. Metabolizable energy content of the diets was estimated using the equation of Carpenter and Clegg (LEESON and SUMMERS, 2001): ME, kcal/kg = 53+38 [(crude protein, %)+(2.25×ether extract, %)+(1.1×starch, %)+(sugar, %)] (1 kcal = 4.19 kJ).

Data collection: All birds were weighed individually before and after holding time for determined BW loss. Body weight (BW) and accumulative feed intake (FI) was recorded at 14, 28 and 42 days of experiment to calculate BW gain (BWG) and feed conversion ratio (FCR). On the days 8, 12 and 25 of experiment, four randomly selected birds from each treatment were euthanasia after stunned in order to comply with welfare practices. The weight of the gizzard, heart, breast, liver, pancreas, spleen, bursa of Fabricius, intestine were expressed as percentages of the carcass weight.

Intestine samples were collected from four broilers/treatment at 8 and 12 d of age and analyzed for intestinal length, weight, pH and microflora. The intestinal tract was removed aseptically. The GI tract was then divided into sections (i.e., ileum, cecum and colon)

that were ligated with light twine before separating the content from end part of duodenum to initial part of cecum from the small intestine. Intestinal contents from the end part of duodenum to beginning of cecum were collected manually for pH measurement. Intestinal pH was measured after the contents were mixed (as 1/10) and homogenized with deionized water. The content of ileum and jejunum was collected from end part of duodenum to initial part of cecum from the small intestine for microbiological analysis at different days of experiment (8, 12 and 25). For the bacterial enumeration in digesta/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 10fold (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, Enterobactericeae, coliforms, Lactobacillus spp. and Salmonella were enumerated using nutrient agar, violet red bile glucose agar, violet red bile lactose agar, Rogosa agar and Brilliant 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, Germany) in sealed anaerobic jars (Oxoid, Basingstoke, UK). Results were expressed as log10 colony-forming units/g of digesta (Hartemink and Rombouts, 1999).

Whole blood samples containing EDTA were obtained via subcutaneous vein puncture from each chicken into appropriate collection tubes for estimating the heterophil-lymphocyte ratio. Whole blood samples were smeared on to glass slides and stained with May-Grünwald-Giemsa. Briefly, the blood films were thoroughly air-dried in a staining rack and, then, were fixed in%100 methanol for 5 min. After fixation, smears were washed in tap water for 1 minute and were stained with Giemsa (4% diluted deionized water) for 20 minutes. At the end of the staining procedure, smears were washed in slowly running tap water and were dried in upright position at room temperature. Two hundred heterophil and lymphocyte were manually counted on each slide, using a light microscope at x1000 magnification. The heterophil-lymphocyte ratio was determined by dividing the number of heterophil by the number of lymphocyte.

Following the necropsy examinations, tissue samples taken from the jejunum were fixed in 10% neutral buffered formalin, sectioned at 5 μ m and stained with hematoxylin and eosin (H and E) for histomorphological examinations. In addition, in order to calculate goblet cell

^{***}Metabolically energy content of the diets was estimated using the equation of CARPENTER and CLEGG (Leeson and Summers, 2001): ME, kcal/kg = 53+38 [(crude protein, %)+(2.25×ether extract, %)+(1.1×starch, %)+(sugar, %)]

number/villus (at 20X microscope objective) in the jejunum sections, periodic acid-Schiff reaction (PAS) was also used (Culling et al., 1985). For the histopathologic analysis of each parameter (epithelial degeneration and separation in propria mucosa of villus and hyperplasia in crypts), 10 replicate measurements were taken/bird and the average of these values was used for statistical analysis. Villus length and width were measured in at least 10 well-oriented villi at 10X microscope objective. In addition, using 40X magnification, crypt depth of at least 5 well-oriented villi were also measured and recorded.

Statistical analysis: All percentage data from experiment were arcsine transformed before analysis by using the General Linear Models procedure of SAS (SAS, 2003) to determine the effects of delayed access to feed and water. Significant differences among treatment means were separated by Duncan's multiple range tests (Duncan, 1955) with a 5% level of probability.

RESULTS AND DISCUSSION

Birds were in good health and there were no mortality record throughout the entire experimental period. The initial BW of chicks (Table 2) did not differ whereas birds delayed to feed and water access had lower BW (43.82 g) compared with others (62.02 g). Delayed feed access had significant (p<0.001) effect on BW change (28.18% vs., -9.46%) at 36 h post hatch. As it summarized in Table 3, birds exposed to holding time after hatch had significantly lesser BW and BWG results while birds fed with prebiotic supplemented diets showed higher values at day 42 of trial (p<0.05 and p<0.001, respectively). These findings are consistent with some previous studies (Pinchasov and Noy, 1993; Bigot et al., 2003; Saki, 2005), which reported that delayed access to feed after hatch had adverse effects on growth performance of broiler chickens. Beside this, post hatch holding time

Table 2: Body weight (g) and BW change (%) at 0 and 36 hours of male broilers subjected to holding time and dietary prebiotic inclusion

DIO	ners subject	ea to nolaling ti	me and dietary	prebiotic inclusion
Treatments		BV	V (g)	BW change (%)
Holding time	Prebiotic	0 h	36 h	0 to 36 h
0	0	48.45	61.68	27.34
36	0	48.43	43.96	-9.25
0	1	48.33	62.36	29.02
36	1	48.36	43.68	-9.67
Pooled SEM	0.73	0.80	1.28	
Holding time				
0		48.39	62.02°	28.18 ^a
36		48.39	43.82⁵	-9.46⁵
Prebiotic				
0		4844	53.02	9.04
1		48.35	52.82	9.67
ANOVA			p	
Holding time		NS	***	***
Prebiotic		NS	NS	NS
Hold. timexPre	ebiotic	NS	NS	NS

NS: Not significant (p>0.05), *** p<0.001

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Table 3: E	

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Treatments	nts		Body weight (g)		Bod	Body weight gain (g)	(Ē	Fee	Feed consumption (g)	(g)	Fee	Feed efficiency (g:g
Holding time	Prebiotic	14	28	42	0 to 14d	0 to 28d	0 to 42d	0 to 14d	0 to 28d	0 to 42d	0 to 14d	0 to 28d
0	0	479	1526	2474	430⁴	1478	2426	589	2301	4657	1.37	1.56
36	0	438₽	1530	2434	391₺	1482	2386	515	2220	4308	1.32	1.50
0	-	474³	1576	2567	426	1527	2518	573	2301	4466	1.35	1.51
36	-	449₽	1564	2539	401₽	1516	2491	525	2260	4472	1.31	1.49
Pooled SEM	ı	2.4	10.6	16.1	2.4	10.6	16.0	8.4	50.3	118.9	0.01	0.01
Holding time												
		476	1551	2521 ²	428	1503	2472	581	2301	4562	1.36	1.53
98		<u>4</u>	1547	2487⁵	39€₽	1499	2438°	520°	2240	4390⁰	1.31	1.49⁵
Prebiotic												
0		459	1528°	2454₺	410	1480₽	2406⁰	549	2261	4482	1.33	1.53
-		462	1570	2553	413	1522	2504	552	2281	4469	1.34	1.50
ANOVA							d					
Holding time		**	SN	*	**	SN	#	**	SN	*	*	*
Prebiotic		SR	**	* *	SN	**	*	SN	SN	SN	SN	SN
Hold. timexPrebiotic	iotic	**	SN	NS	**	SN	SN	SN	SN	SN	SN	SN

1.32° (1.78° (1.

Table 4: Some intestinal parameters and pH analysis of intestinal contents (ileum) of male broiler chickens subjected to holding time and dietary prebiotic inclusion

					Treat	tments			
		Le	ngth of intestine (cm)	Relative we	ights of intestine (g	/100 g BW)	p	Н
Holding time	Prebiotic	Day 8	Day 12	Day 25	Day 8	Day 12	Day 25	Day 8	Day 12
0	0	104.3	137.7	188.7	12.6	12.4	8.6	6.3	5.9
36	0	100.6	132.6	193.1	15.5	12.6	7.8	6.6	5.8
0	1	108.9	137.1	187.5	13.2	12.9	8.0	6.6	5.2
36	1	103.7	127.8	171.6	14.9	7.9	6.0	5.5	
Pooled SEM	-	3.9	5.9 9.4 0.8 0.4		0.4	0.4	0.2	0.3	
Holding time									
0		106.6	137.4	188.1	12.9⁵	12.6	8.3	6.4	5.5
36		102.1	130.2	182.3	15.2°	12.6	7.8 6.3	6.3	5.6
Prebiotic									
0		102.4	135.2	190.9	14.0	12.5	8.2	6.4	5.8
1		106.3	132.4	179.5	14.1	12.8	7.9	6.3	5.3
ANOVA						- p			
Holding time		NS	NS	NS	*	NS	NS	NS	NS
Prebiotic		NS	NS	NS	NS	NS	NS	NS	NS
Holding timexPre	biotic	NS	NS	NS	NS	NS	NS	NS	NS

NS: Not significant (p>0.05), *p<0.05

Table 5: Microbiological analysis of intestinal contents (ileum) at 8 and 12 d of male broiler chickens subjected to holding time and dietary prebiotic inclusion

						Coliform	bacteria				
Treatments		Total	bacteria	Total anae	robe bacteria	log ₁	₀cfu/g	Enteroba	cteriaceae	Lacto	bacilli
Holding time	Prebiotic	Day 8	Day12	Day 8	Day12	Day 8	Day12	Day 8	Day12	Day 8	Day12
0	0	7.7	6.9	7.8	6.9	4.4	3.7	5.1	3.9	4.9	5.7
36	0	7.8	7.9	7.2	7.0	4.9	4.4	4.6	3.3	4.2	4.8
0	1	7.9	7.3	7.2	7.6	5.3	4.0	5.2	4.1	4.8	5.8
36	1	7.6	7.4	7.9	7.1	5.1	3.5	6.00	3.8	5.3	5.8
Pooled SEM	-	0.4	0.2	0.4	0.4	0.3	0.4	0.4	0.5	0.3	0.5
Holding time											
0		7.7	7.1	7.5	7.3	4.9	3.9	5.1	4.0	4.8	5.7
36		7.7	7.7	7.5	7.1	5.0	3.9	5.3	4.1	4.9	5.3
Prebiotic											
0		7.7	7.4	7.5	7.0	4.6	4.0	4.8	4.1	4.6	5.8
1		7.7	7.3	7.6	7.4	5.2	3.8	5.6	3.9	5.1	5.2
ANOVA						p)				
Holding time		NS	*	NS	NS	NS	NS	NS	NS	NS	NS
Prebiotic		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Holding timex	Prebiotic	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS: Not significant (p>0.05), *(p<0.05)

also had decreasing affect on FI of birds (p<0.001) while prebiotic supplementation had no impact. FCR of the birds delayed to feed access was lower than birds given access to feed and water immediately after hatch (1.31 vs. 1.36 for 14 d and 1.49 vs. 1.53 for 28 d; p<0.01 and p<0.05 for 14 and 28 d). On the other hand, dietary prebiotic inclusion had improved FCR of birds by the end of trial (p<0.05). There was interaction between holding time and prebiotic addition effect on FCR at day 42 (p<0.05). Our findings are in agreement with Gonzales et al. (1999, 2003) who noticed that birds final BW depressed when fasted longer than 24 h posthatch. In contrast, some other studies observed no positive effects of prebiotics on FE in broilers (Waldroup et al., 1993; Williams et al., 2008). There might be a number of causative factors for different results including dose and composition of prebiotics or variances of feedstuffs used in diets.

The lengths of intestine were similar among treatments while weights of intestine were lower in birds delayed to feed access at 8 d of age (p<0.05). On the other hand, prebiotic had no effect on intestine lengths or weights at any examination days of trial (Table 4). The result from present (for d 8) study is relatively similar to previous studies of Bigot et al. (2003) and Moore et al. (2005) who observed that depressive effect of post hatch holding time on intestinal growth was ameliorated after accessing to feed and water. However there are some other contradictional results from different studies (Corless and Sell, 1999; Moore et al., 2005). Moreover, holding time and prebiotic inclusion to diets did not alter pH level of ileocecal digesta of birds at days 8 and 12 of experiment. Rebole et al. (2010) observed that dietary prebiotic supplementation had no significant affect about intestinal pH in broiler chickens. This result may be due to strong buffering capacity of gastrointestinal tract in poultry.

The results of total bacteria, total anaerobe bacteria, Enterobacteriaceae, Coliform and Lactobacilli counts determined in the ileal digesta collected on d 8 and 12 were summarized in Table 5. On d 12, total bacteria counts in ileal content of birds exposed to post hatch holding time were higher (7.65 vs., 7.07 log10cfu/g) than birds accessed to feed and water. Dietary prebiotic inclusion had no effect on microbial environment of ileal content at 8 and 12 d of ages. For our knowledge, there is limited number of study focus on ileal total bacteria count of broiler chickens delayed to feed access. In agreement with report of Alhotan (2011), Lactobacillus and Salmonella, as an index of healthy gut microflora, were not influenced by delay in access to feed and water. On the other hand, there were no significant effects of prebiotic supplementation to diets on microbial populations of broiler chickens in the current study. However, Kim et al. (2011) found that 0.25% fructooligosaccharide (FOS) addition to broiler diets had lowered affect on Escherichia coli populations whereas lactobacilli count in small intestine was increased. Variances between studies could be related to many factors which alter microflora composition of birds (Yegani and Korver, 2008) including age and breed of birds plus composition of diet and prebiotic. Beside this, microbiological analysis of feed samples indicated that prebiotic addition did not influence Total Bacteria, Bacteria, Anaerobe Coliform Bacteria. Enterobacteriaceae, Lactobacilli count (data not shown). In the present experiment there was no Salmonella determined in feed and intestinal content samples. In the present trial, neither delay to feed access nor

prebiotic supplementation changed the histological characteristics of jejunum. However, the interaction of post hatch holding time x prebiotic treatments for villus width and goblet cell counts was significant (p<0.05) on d 25 (Table 6). Even though there were no significant alterations of jejenum histomorphology, delayed feed access had minimal effect on the numerical reduction of villus length, crypt depth and goblet cell count whereas dietary prebiotic addition numerically increased same variables on d 8. This result showed some contradiction with previous studies which determined that delayed feed access caused crypt dept or number (Nov et al., 2001; Uni et al., 2003) decreasing or depression of villus height and length or goblet cell count in broiler chickens (Uni et al., 2002, 2003). Moreover, Sayrafi et al. (2011) noticed that dietary prebiotic supplementation at a level of 0.1% had positive effects on villus height and width of duodenum and ileum. It is not always possible to observe significant correlations between intestinal morphology and growth performance (Vieira et al., 2008). Different results obtained from previous studies might be related to factors including number of sampling or part of intestine sampled for examination. Lack of

prebiotic inclusion	nclusion														
Treatments		Day 8	Day 8					Day 12					Day 25		
HT Prebiotic	otic VL	NΛ	CD	8	သဗ	۸۲	W/	CD	8	၁၁၅	۸۲	ΛV	9	8	ည္ပ
0	718	98	84	3.0	61	533	107	83	2.3	70	878	86	84	3.5	75
36 0	694	101	80	2.5	59	845	8	81	3.0	28	998	148	8	3.0	64
0	99/	100	102	3.0	72	1023	9	9/	3.3	61	1019	121	120	3.5	69
36 1	725	86	06	2.8	62	708	\$	83	3.3	56	865	106	108	3.3	9/
Pooled SEM	53	9	10	4.0	4	28	80	2	9.0	-	82	11	4	0.2	က
노															
0	742	26	93	2.6	99	778	66	79	2.8	99	948	110	101	3.5	72
36	710	100	82	3.0	61	777	5	82	3.1	22	998	127	101	3.1	70
Prebiotic															
0	200	86	82	2.8	09	.689	101	82	5.6	8	872	12	88	9.3 8.3	73
_	745	66	96	2.9	29	[₽] 298	102	80	3.3	29	942	113	114	9.6 6.6	70
ANOVA									d						
노	SN	SN	SN	SN	SN	SN	NS	SN	SN	SN	SN	SN	SN	SN	SS
Pre	SN	SN	တ N	SN	SN	*	SΝ	SΝ	S	SN	SZ SZ	SN	SN	SN	SN
HtxPre.	S N	SN	s N	SN	SN	SN	SN	s N	SN	s N	SN	*	SN	SN N	*
HT: Holding time	NS: I	NS: Not significant at p>0.05, *p<0.05	at p>0.05, *p	≥0.05	VL: Villus length	; length	VW: Vill	vW: Villus width	CD: CJ	CD: Crypt depth	CC: Crypt count	ot count	GCC: Go	GCC: Goblet cell count	±

Table 6: Villus length (µm-10x), villus width (µm-10x), crypt depth (µm-40x), crypt count (40x) and goblet cell count (40x) measured in the jejenum of broiler chickens subjected to holding time

difference for morphology of jejunum among birds held or not held prior to feeding could be caused by similar factors.

Data from relative organ weights and heterophillymphocyte (H:L) ratio showed no significant difference among treatments on 8, 12 and 25 d of ages (data not shown). This result is in contrast with the report of Corless and Sell (1999) who noticed that delay in access to feed and water had adverse effects on relative organ weights. Similarly, Kim et al. (2011) found that 0.05% of dietary mannan-oligosaccharide addition decreased H:L ratio in broiler chickens. On the contrary, Pinchasov and Nov (1993) observed no significant alteration on relative pancreas weight of broiler chickens due to a 24 or 48 h delays in access to feed and water. These contradictions between studies might be related to composition of prebiotic or the examination periods, which is critical for determination of stress effect on accurate time.

Conclusion: As a conclusion, post hatch holding time depressed growth rate and this negative effect cannot be compensatory by dietary prebiotic inclusion in different rearing periods of broiler chickens. For this trial, dietary prebiotic inclusion had improved FCR of birds but other parameters did not show any alteration between treatments. As a result, prebiotic inclusion seems had no beneficial effects on growth rate and gut health in broiler chickens exposed to post hatch holding time. However, further studies required for the confirmation of these results.

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