EFFECT OF *IN EGG* PREBIOTIC AND BUTYRIC ACID ON PERFORMANCE, DIET NUTRIENTS DIGESTIBILITY AND BIOMETRY OF THE GASTOINTESTINAL TRACT OF CHICKS UNDER FASTING

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ABSTRACT -

Two experiments were conducted to evaluate the performance, digestibility of diet nutrients and biometry of the gastrointestinal tract of chicks from eggs inoculated with prebiotic or butyric acid, submitted to water and food fasting. The fertile eggs were inoculated with a solution containing prebiotic (experiment I) or water (placebo group), in the allantoic sac on the 16^{th} day of the incubation. In experiment II, the fertile eggs were inoculated with water (placebo group) or organic acid (sodium butyrate). After hatching, 200 chicks were selected and classified by weight and distributed according to the treatments in battery cages. A complete randomized block design in a 2x2 factorial arrangement (inoculants x fasting time) was used, totaling four treatments and 10 replicates. The fasting times evaluated

were eight (control group) and 36 hours after hatching. The inoculation of butyric acid did not affect the performance of chicks nor nutrients digestibility, but gastrointestinal biometry was increased (P<0.05). The prebiotc reduced the weight gain (P<0.05) and did not affect the nutrients digestibility or the gastrointestinal biometry. The inoculation in the eggs did not harm the initial weight of the chicks as well as there was no interaction between the inoculant and the fasting time. The supplementation with prebiotic or butyric acid neither improved the performance of chicks until ten days of age nor the nutrients digestibility of the diet, irrespective of the chicks being under fasting or not; however, the butyric acid enhanced the gastrointestinal tract development.

KEYWORDS: Broilers, inoculated eggs, mannan, nutrients digestibility, organic acid.

EFEITO DO PREBIÓTICO E DO ÁCIDO BUTÍRICO *IN OVO* SOBRE O DESEMPENHO, A DIGESTIBILDADE DOS NUTRIENTES DA RAÇÃO E A BIOMETRIA DO TRATO GASTRINTESTINAL DE PINTOS SUBMETIDOS AO JEJUM

RESUMO

Desenvolveram-se dois experimentos para avaliar o desempenho, a digestibilidade de nutrientes da ração e a biometria de órgãos do trato gastrintestinal de pintos,

oriundos de ovos inoculados com prebiótico ou ácido orgânico, submetidos a jejum hídrico e alimentar. Os ovos férteis foram inoculados com uma solução contendo prebiótico (experimento I) ou água (grupo placebo), no saco allantoide aos dezesseis dias de incubação. No experimento II, os ovos embrionados foram inoculados com água (placebo) ou ácido orgânico (butirato de sódio). Depois da eclosão, selecionaram-se duzentos pintos pelo peso, sendo distribuídos de acordo com os tratamentos em baterias. O delineamento foi em blocos casualizados, em esquema fatorial 2x2 (inóculos x períodos de jejum), totalizando quatro tratamentos e dez repetições. Os períodos de jejum estudados foram de oito (grupocontrole) e 36 horas após a eclosão. O prebiótico inoculado reduziu o ganho de peso (P<0,05) e não afetou a digestibilidade e a biometria dos órgãos. A inoculação in ovo não prejudicou o peso inicial dos pintos e não houve interação entre o inóculo e o período de jejum. A inoculação com ácido orgânico não afetou o desempenho das aves e a digestibilidade dos nutrientes, mas aumentou a biometria intestinal (P<0,05). A suplementação de prebiótico ou butirato de sódio não melhorou o desempenho dos pintos até os dez dias de idade, tampouco a digestibilidade dos nutrientes da ração, independentemente se submetidos ou não ao jejum inicial. Porém, o butirato de sódio favoreceu o desenvolvimento intestinal.

PALAVRAS-CHAVES: Ácido orgânico, digestibilidade de nutrientes, ovos, pinto de corte, manano.

INTRODUCTION

Post-hatching fasting negatively affects the performance of broiler chicks until the final phase of breeding (NIR & LEVANON, 1993; PINCHASOV & NOY, 1993). Similarly, fasting affects the development of gastrointestinal tract (GIT) of birds. GONZALES et al. (2008) observed that birds fed soon after hatching showed greater intestine and pancreas development, and concluded that the period of food restriction during post-hatching affects the GIT maturity. GONZALES et al. (1999, 2008) reported that chicks submitted to 36 hours of fasting after hatching had difficulties in absorbing nutrients from the yolk sac and showed lower weights of secreting organs (liver, pancreas and intestine), reflecting losses in performance at 42 days of age. Almeida et al. (2006) also found poorer performance of birds at 42 days of age, which fasted for 48 hours. UNI et al. (1998) and MAIORKA et al. (2000) observed that food restriction after hatching impaired intestinal development of broiler chicks.

Chicks are usually submitted to water and food restriction between hatching and housing, a period that can range from 24 to 48 hours, due to the time for preparation and rest of the chicks in the hatchery along with transportation to the farm. As a result of such fast, the chicks may suffer dehydration and weight loss of up to 10% (BAIÃO & CANÇADO, 1998). However, GIT maturity and the early establishment of the intestinal microbiota can be stimulated by exogenous supplementation of nutrients or additives (GUILLOT, 2000; LEANDRO et al., 2010), improving the performance of broiler chicks. Among the products that can be considered as trophic factors to induce GIT maturity are probiotics and prebiotics (MAIORKA, 2001) and organic acidifiers (DIBNER & BUTTIN, 2002; RICKE, 2003).

Prebiotics are defined as food ingredients that are not hydrolyzable by the endogenous enzymes, which benefit the animals by selectively stimulating the growth and / or activity of a limited number of bacteria in the intestine, improving animal's health and performance. Oligo and polysaccharide carbohydrates, certain peptides and proteins and certain lipids and fibers are considered as prebiotics (GIBSON & ROBERFROID, 1995). Nevertheless, oligosaccharides, especially fructoligosaccharides (FOS), glucoligosaccharides (GOS) and mannan-oligosaccharides (MOS), are the most studied substances used as additives in animal feed.

MOS is not digestible by birds, but it is specific food for useful bacteria (ROBERFROID, 1998). Besides acting as a substrate for useful bacteria, stimulating its growth and / or activating the metabolism, MOS acts as a blocker of adhesion sites of certain pathogenic bacteria, immobilizing and reducing the ability of these agents to remain in the GIT (COLLINS & GIBSON, 1999).

Studies on performance of broilers supplemented with prebiotics present conflicting results. Some researches show performance improvements with the use of the product (TOLEDO et al., 2003; PELICANO et al., 2004; ALBINO et al., 2006; SILVA et al., 2009). One study demonstrated the product positive effect on the intestinal development of chickens reared in high temperature environments (SILVA et al., 2010). On the other hand, another study indicates that there was no significant effect on weight gain and feed conversion (PEACE et al., 2010).

The organic acid is another product studied with the aim of promoting the balance of the intestinal tract. It is a substance that facilitates the GIT colonization by useful bacteria promoting a faster maturation of the small intestine mucosa in chicks (JANSSENS & NOLLET, 2002). The inhibition of enterobacteria proliferation in the digestive tract caused by the use of organic acid in the diet is due to pH reduction in the upper part of the small intestine, increasing nutrient availability and enhancing the diet's nutritional gains (FREZZA, 2008; FARIA et al., 2009). The organic acids used in poultry breeding are formic, acetic, propionic, butyric, lactic, citric and fumaric presenting, respectively, the following pKa: 3.75; 4.76; 4.87; 4.81; 3.86; 3.09/4.75/5.41; 3.03/4.54.

Garcia et al. (2000) found no effect of organic acids on weight gain and feed conversion of broilers until slaughtering (42 days old). However, studies in which birds were challenged showed positive results with the use of organic acids, such as reduction of colony-forming units of *Campylobacter* (CHAVEERACH et al., 2002), total coliforms and *Escherichia coli* in the duodenum, jejunum and ileum (IZAT et al., 1990), besides contributing to the development of aerobic bacteria in the water (CHAVEERACH et al., 2004).

According to PEDROSO et al. (2005), gastrointestinal tract colonization by the natural microflora in birds begins even before the hatch. Thus, inoculation of prebiotic and organic acid via egg can promote the colonization of useful bacteria, anticipating the GIT maturity in birds.

The aim of this study was to evaluate the effect of prebiotic or organic acid inoculated in embryonated eggs as well as the effect of the period of fasting after hatching on the development of TGI and the performance of broiler chicks up to ten days of age.

MATERIAL AND METHODS

Two experiments were carried out, the

inoculation of prebiotic and of butyric acid in embryonated eggs, studied in experiments I and II, respectively. In each experiment, 300 eggs of 46week-old Cobb-500 breeders were used. The eggs came from the commercial hatchery of the region. The eggs were transferred to the Veterinary School of UFG on the 15th day of incubation. Then they were distributed into four hatcheries, each one with a 120-eggs capacity, to complete the incubation period.

All the hatcheries were placed in the same room. The eggs were weighed, uniformed and distributed into the hatcheries, which were adjusted and monitored daily to keep temperature and relative unidity at 37.5° C and 55%, respectively, until the end of incubation.

On the 16th day of incubation, (experiment I) eggs were inoculated with 0.30 mL of destilled water (placebo) or with the same volume of solution with 40 mg of commercial prebiotic MOS^{®1}. This product was constituted of lactose (15%) and mannanoligosaccharides (85%). Inoculation was done in allantoid cavity, by means of a sterile 1.0 mL syringe and a 13 mm needle for each egg, according to methodology described by LEITÃO et al. (2008).

After hatch and selection of broiler chicks according to body weight, 200 non-sexed chicks coming from both inoculations (water x prebiotic) were placed in batery cages with linear feeders and drinkers and metallic trays. Half of the chicks, inoculated with water or prebiotic, underwent hydric and feed fasting of eight or 36 hours. The combination between the type of inoculum (placebo or MOS) and the time of fasting after hatch (eight or 36 hours) was studied, totaling four treatments. The treatment with eight-hour fasting was considered the control group, because this is the least a commercial hatchery needs to deliver 1-day old chicks.

The same procedure but the inoculum was used in experiment II. The inoculation of 0.30 ml of destilled water or the same volume of a solution with 8.0 mg of sodium butyrate at 98% \pm 2% (Adimix TM Butyrate-C, Invenutri-AD) was performed. For housing, 240 Cobb 500 non-sexed 1-day-old chicks were used. The chicks came from the 300 eggs inoculated with water or butyric acid, according to

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¹ Mannan-ooligosaccharide, BIOCAMP

the treatmetns. Half of the chicks inoculated with water or butyric acid (Adimix TM Butyrate-C, Invenutri-AD) underwent hydric and feed fasting of eight and 36 hours. Thus, the combination between kind of inoculum (placebo or butyric acid) and fasting periods (eight or 36 hours) were studied, similarly to experiment I.

The ration used during the experimental period was constituted of vegetable ingredients (corn soybean based diet), without anticoccidian agents and antibiotics as growth promoters. Ration was mashed and formulated to present 21% of crude protein and 3,000 kcal ME/kg of ration. Ration intake, weight gain, feed conversion (corrected to mortality) and mortality were considered to assess the performance.

Digestibility essay was caried out by the excreta total collection method, in chicks from six to ten days of age. Ration intake was controled and two total excreta collections were performed daily (in the morning and afternoon). The excreta was keept in plastic bags and conserved in freezer for posterior analysis.

Bromatological analysis of dry matter, crude protein, ether extract and nitrogen were carried out according to methodology by SILVA & QUEIROZ (2002). Digestibility coefficients of dry matter (DCDM), crude protein (DMCP), ether extract (DCEE) and nitrogen balance were calculated.

The biometric analysis of the proventriculus, gizzard, liver and intestine were carried out with one to ten-day-old chicks. Ten chicks per treatment were slaughtered by cervical dislocation and individually weighed to collect the organs.

The mortality percentage data were transformed into arcsine, and statistical analysis for all variables were performed by the GLM procedure of SAS (© (2001).

RESULTS AND DISCUSSION

There was no interaction between the factors prebiotic inoculation and fasting period after hatching (Table Thus, the prebiotic 1). supplementation on embryo did not improve the performance of chicks fasted for 36 hours compared to embryos from eggs inoculated with no prebiotic (placebo group). Weight gain and feed intake were lower (p < 0.05) for chicks that received in egg prebiotic and for those undergoing 36-hour period of post-hatching fasting.

Treatments	Initial weight (g)	Weight gain (g)	Feed intake (g)	Feed conversion	(Transformed) Mortality
Inoculum					
Placebo	49	213a	260a	1.217	0.258
Prebiotic	48	196b	240b	1.219	0.255
Fasting					
Control (8h)	48	211a	259a	1.223	0.258
36 hours	48	199b	241b	1.212	0.255
CV(%)	2.5	4.18	8.71	7.63	36.29

TABLE 1. Performance of broiler chicks during the pre-starter phase from eggs inoculated with placebo or prebiotic on the 16th day of incubation and submitted to eight or 36 hours of fasting after hatching

Means followed by different letters in same column differ by F test (0.05).

* Transformed mortality = arcsine ((% Mort. / 100) +0.05) $^{0.5}$ ns = not significant (p> 0.05).

The lowest values for weight gain, observed in chicks from eggs inoculated with prebiotics, disagree with the findings obtained by IJI & TIVEY

(1998), who found that chickens supplemented with prebiotics showed higher feed intake and weight gain in relation to control group (no additives). In the same way, TOLEDO et al. (2003) and ALBINO et al. (2006), who found positive results for performance in broilers supplemented with prebiotics; however, all these authors studied the use of probiotic supplementation in the diet.

The good hygienic conditions of the experiment provided a low sanitary challenge of the facilities. According to the literature, additives such as probiotics are most effective under greater challenge conditions. SPRING et al. (2000) explain that MOS works by blocking the binding sites of pathogenic bacteria in the intestinal mucosa, thus reducing the injuries to the GI tract, which reduces the mucosal cell turnover, providing better utilization of the dietary ingredients and better performance. These authors, by testing MOS as growth promoter, found that the prebiotic promoted the development of the intestinal flora, improving the performance of birds. SANTIN et al. (2003) observed that birds challenged and fed with prebiotics showed greater weight gain and better feed conversion than the birds in the control group.

For the period of fasting, it was observed that chicks submitted to 36 hours of food and water restriction after hatching showed poorer weight gain (Table 1) compared with control (eight hours of fasting). Corroborating this study, PINCHASOV & Noy (1993) and BAIÃO & CANÇADO (1998) reported that chicks submitted to a period of 24 and 48 hours of fasting after hatching may suffer dehydration and weight loss of up to 10%. Similarly, NIR & Levanon (1993) observed a retarded growth of chicks, caused by fasting for 24 and 48 hours, equivalent to one or two days of weight gain, respectively, and GONZALES et al. (2008) observed negative effects on body weight of broilers when the chicks underwent 24 hours of fasting after birth.

The results regarding digestibility of dry matter, crude protein, ether extract and nitrogen balance are presented in Table 2. Statistical analysis showed that the results of digestibility coefficients were similar between control and the group supplemented with prebiotics. However, the prebiotic impaired nitrogen balance (p < 0.05).

Treatment	Digestibility coefficient				
	Dry matter (%)	Crude protein (%)	Ether extract (%)	Nitrogen balance (g)	
Inoculum					
Placebo	74.00	68.60	95.85	1.72a	
Prebiotic	73.79	68.26	95.60	1.32b	
Fasting period					
Control (8h)	74.15	68.74	95.90	1.66	
36 hours	73.64	68.13	95.60	1.37	
CV(%)	1.82	2.60	1.68	27.50	

TABLE 2. Digestibility coefficient of nutrients and nitrogen balance in the diet of chicks from eggs inoculated with placebo or prebiotic at 36 hours of fasting after hatching

Means followed by different letters in same column differ by F test (0.05).

ns = not significant (p > 0.05).

Results obtained by LODDI et al. (2000) also showed no difference in digestibility of CP and DM of the ration between chicks receiving prebiotics in the diet or not. However, FERES et al. (2002), verifying the effects of mannan-oligosaccharides, concluded that the additive improved the digestibility

coefficients of dry matter and digestible protein, besides improving the apparent metabolizable energy and apparent metabolizable energy corrected for nitrogen balance, by the ileal collection method, when it was compared with diet control.

The results of GIT biometry showed no

differences (p> 0.05) among treatments on the first (data not shown) or the tenth day of life (Table 3). The prebiotic did not influence the development of the GIT of chicks during the late embryonic stage, as

in pre-initial phase of growth. Fasting for 36 hours after the outbreak had a negative influence only on the absolute weight of the gizzard (p <0.05).

TABLE 3. Absolute and relative weight of the components of the GIT of chicks, from eggs inoculated with placebo or prebiotic and submitted to 36 hours of fasting after hatching, at ten days of age

		Fasting period						
Variable	Inoculum	Contro	Control (8h)		36 h		Mean	
		(g)	$\%^{1}$	(g)	% ¹	(g)	% ²	
	Placebo	2.2	0.8	2.0	0.8	2.1	0.8	
Proventriculus, g	Prebiotic	2.2	0.9	2.0	0.8	2.1	0.8	
	Mean	2.2	0.8	2.0	0.8			
	Placebo	13.0a	5.0	11.6b	4.6	12.3	4.8	
Gizzard, g	Prebiotic	12.1a	4.8	10.9b	4.5	11.5	4.6	
	Mean	12.6	4.9	11.3	4.6			
	Placebo	9.6	3.6	8.8	3.5	9.2	3.6	
Liver, g	Prebiotic	9.5	3.7	9.1	3.7	9.3	3.7	
	Mean	9.5	3.7	9.0	3.6			
	Placebo	16.2	6.2	16.4	6.6	16.3	6.4	
Intestine weight, g	Prebiotic	16.4	6.4	16.2	6.7	16.3	6.5	
	Mean	16.3	6.3	16.3	6.6			
	Placebo	94.2		92.0		93.1		
Intestine length/cm	Prebiotic	91.4		90.4		90.9		
	Mean	92.8		91.2				
	Placebo	0.17		0.17		0.17		
g/cm of intestine	Prebiotic	0.17		0.17		0.17		
	Mean	0.17		0.17				

¹ In relation to live weight of the bird;² transformed into arcsine before statistical analysis.

Means followed by letters a and b in same column differ by F test (0.05).

These data indicate that the losses observed for weight gain in fasted chicks did not result in developmental disorders of the GI tract of chicks, which were not affected by the fasting period. Thus, the lower weight gain observed in chicks submitted to 36 hours of fasting must be related to low feed intake by chicks. Contrary data were observed by GONZALES et al. (2008), who reported that broiler chicks which underwent 36 hours of fasting showed lower weight for the secreting organs (liver, pancreas and intestine). Maiorka et al. (2000, 2001) observed that the length and weight of the intestine of chicks were influenced by the 24-hour period of food restriction after hatching. The results of the pre-starter phase performance of broiler chicks from the embryonated eggs inoculated with placebo or sodium butyrate (experiment II) are shown in Table 4. It can be observed that there was no interaction between inoculant treatments and fasting for all variables. In egg supplementation of butyrate acid did not influence performance, which is not in agreement with data from RUNHO et al. (1997), GARCIA et al. (2000) and SILVA et al. (2009), who showed that organic acid administered in the diet improved feed conversion of broilers in the initial phase. However, VALE et al. (2004) found deterioration in weight gain at the early stage of the development of broilers

	Variable					
Treatments	Initial weight (g)	Weight gain (g)	Feed intake (g)	Feed conversion (kg/kg)	(Transformed) Mortality	
Inoculum						
Placebo	49.72	190	243	1.280	0.251	
Organic acid	49.14	187	236	1.270	0.238	
Fasting period						
Control (8h)	49.41	199 a	241	1.220 b	0.238	
36 horrs	49.46	179 b	238	1.340 a	0.238	
CV(%)	2.2	4.22	8.61	7.63	36.00	

TABLE 4. Performance of chicks in the pre-initial phase, hatched from eggs inoculated with butyric acid or placebo and submitted to 8 or 36 hours of fasting after hatching

Means followed by different letters in same column differ by F test (0.05).

* M=Transformed mortality:arcsine ((% Mort. / 100) +0.05)^{0.5}.ns = not significant (p>0.05).

Just as in experiment I, the environmental conditions possibly allowed low sanitary challenge for the chicks reared in cages with low density of birds. Organic acids are substances with the potential to change the pH of the birds GIT, favoring the natural microflora, and when birds are submitted to conditions of challenge, the provision of organic acids improves their performance (DIBNER & BUTTIN, 2002; RICKE, 2003).

conversion (p <0.05). Nevertheless, the nitrogen balance (Table 5) was positively influenced (p <0.05) by fasting after hatching, which may be related to a compensatory gain in the utilization of dietary nitrogen, not accompanied by an increase in endogenous nitrogen excretion, although this result was not observed in Experiment I. Corroborating these results, ROSTAGNO et al. (2004) explained that the digestion of certain molecules is enhanced when they are in lower concentration in the diet.

Fasting impaired weight gain and feed

TABLE 5. Digestibility coefficient and nitrogen balance of the diet of chicks from eggs inoculated with butyric acid or placebo and submitted to eight or 36 hours of fasting after hatching

Traatmont	Digestibility coefficient (%)				
Treatment -	Dry matter (%)	Crude protein (%)	Ether extract (%)	Nitrogen balance (g)	
		Inoculum			
Placebo	77.13	66.94	95.80	25.11	
Organic acid	74.22 63.23 94.81 2		24.30		
		Fasting period			
Control	73.32	62.48	94.87	22.67b	
36 hours	78.03	67.70	95.74	26.75a	
CV(%)	10.06 19.64 1.94 24.15				

Means followed by different letters in same column differ by F test (p<0.05). ns = not significant (p>0.05).

The fact that the organic acid is a product that acts as an antibacterial agent (JANSSENS & NOLLET, 2002) can explain the data of lesser weight to the GIT organs combined with the greater length of intestine, found in this experiment for chicks from eggs inoculated with butyric acid (Table 6). These results suggest that the intestinal mucosa was thinner due to a TGI protection conferred by the acidifying. According to FURLAN et al. (2001), the lowest ratio between weight and length of the

intestine indicates a lesser thickness of the intestinal mucosa, which is probably due to enhanced protection to minor injuries to which the chicks were submitted, otherwise, the mucosa would become thicker, resulting in higher weight and increased

demand for nutrients. However, data on nutrient digestibility and feed conversion did not differ between chicks that received butyric acid and chicks from control group.

TABLE 6. Absolute and relative weight of the components of the GIT and intestine length of chicks, at ten days of age, originated from eggs inoculated with placebo or organic acid and submitted to eight or 36 hours of fasting.

37 11	Y 1	Fasting peroid					
Variables	Inoculum	Control		36 h		Mean	
		(g)	$\%^1$	(g)	$\%^{1}$	(g)	% ²
Proventriculum	Placebo	2.3 A	1.0 A	2.0	0.9	2.2	1.0
	Butyric acid	2.0 B	0.8 B	2.1	0.9	2.0	0.9
	Mean	2.2	0.9	2.0	0.9		
Gizzard, g	Placebo	12.2	5.6	11.0	5.0	11.6	5.3a
	Butyric acid	10.8	4.7	10.4	4.7	10.6	4.7 b
	Mean	11.5	5.1	10.7	4.8		
Liver, g	Placebo	7.9	3.6	8.4	3.8	8.2	3.7
	Butyric acid	7.8	3.4	8.3	3.7	8.1	3.5
	Mean	7.9	3.5	8.4	3.8		
Intestine, g	Placebo	17.5	7.9	17.1	7.8	17.3	7.9 a
	Butyric acid	17.2	7.5	16.6	7.4	16.9	7.5 b
	Mean	17.4	7.7	16.8	7.6		
Intestine, cm	Placebo	87.7	-	79.9	-	83.8 a	-
	Butyric acid	88.1	-	80.2	-	84.2 b	-
	Mean	87.9	-	80.0	-		-

¹ In relation to the BIRD live weight.

² transformed into arcsine before stastistical analysis.

A, B, in the column, shows the effect of inoculation within fasting, by F test (P<0.05).

a,b, in the column, shows the effect of inoculation regardless of fasting, by F test (P<0.05).

The performance results with in egg application of prebiotics or organic acid in chicks submitted to fasting did not differ from results of chicks supplemented with no additives that have suffered similar fasting. Thus, inoculation of such additives before the hatching did not accelerate the process of establishing stable and beneficial bacterial population in the GIT of birds. Besides, because of a lack of experimental challenge, it could not be concluded that these additives excluded or limited the colonization of pathogens.

CONCLUSIONS

The supplementation of prebiotics (MOS®) or sodium butyrate (Adimix TM Butyrate-C, Invenutri-AD[®]) in broiler chicken embryos did not improve the chicks' performance or the digestibility of the nutrients in the ration during the pre-starter phase. These additives inoculated in embryonated eggs did not accelerate the maturity of GIT of chicks submited or not to fasting for 36 hours after hatching.

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