Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X 2020 / v.22 / n.4 / 001-008

http://dx.doi.org/10.1590/1806-9061-2020-1329

Original Article

Use of Betaine in Post-Hatch Feed for Broiler Chicks

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■Keywords

Delayed placement, glucose, histomorphometry, poultry.

Submitted: 04/June/2020
Approved: 05/September/2020

ABSTRACT

Post-hatch delayed placement damages the physical and physiological development of broiler chicks. This study was designed to find adequate levels of betaine inclusion in pre-hatching and pre-starter feed, in order to minimize the negative effects of post-hatch delayed placement on broiler chicks. Newly-hatched chicks were allotted in a completely randomized design, with five treatments, five replicates of ten birds each. Five dietetic levels of betaine (control, 0.070, 0.130, 0.200 and 0.260%) were used in the pre-starter ration, offered to the chicks in the transporting box and during the pre-starter phase. Performance, yolk sac retraction, plasma glucose concentration, weight and histomorphometry of the small intestine were evaluated, after 24 hours of feed access and at 7 days of age. A metabolic trial was performed from seven to ten days of age. Betaine supplementation linearly influenced the chick's ileum crypt depth after 24 hours in the transportation box. There was a quadratic effect, with an increase in feed intake up to 0.152% betaine supplementation. There was an improvement in the quality of the jejunum with betaine supplementation above 0.1%. The metabolizable coefficient of the ether extract was improved until reaching 0.163% of betaine supplementation. Betaine supplementation around 0.150% increases feed intake, the use of ether extract and interferes with the intestinal villi of chicks at seven days of age.

INTRODUCTION

The post-hatch period is considered a critical time for broiler chicks. It is a phase of adaptation to the external environment to the egg and specially to feed coming from exogenous sources. What makes this period even more difficult is the fasting that these chicks undergo.

Because of standard incubator procedures, chicks may not have access to feed for approximately 48 hours, from their hatching until their placement, which could mean they do not have access to feed for two or three days (Bhuiyan *et al.*, 2011). Delayed intake of feed can hinder physiological and immunological development, reducing the overall productivity of chick-rearing (Van Den Brand *et al.*, 2010).

Specific formulas were recommended for the post-hatch and pre-starter phases diets of broiler chicks. These formulas include the presence of trophic agents that can attenuate the effects of post-hatch delayed placement, to increase precocity in the physical and physiological development of broiler chicks.

Among these trophic agents is betaine. According to Ahmed *et al.* (2018), betaine is not present in large quantities in poultry feedstuffs such as corn and soybean. Dietary betaine supplementation has many benefits for the productive performance and health status of poultry.

The primary function of betaine is as a donor source of methyl groups, for example, methylation of DNA, RNA and lipid cell membranes to the synthesis of methionine, carnitine and creatine. In broiler diets with methionine deficiency, betaine supplementation improved the metabolizability of ether extract and protein (El-Husseiny *et al.*, 2007).

Also, betaine is an organic osmolyte. Unlike inorganic salts, osmoregulatory substances are soluble and remain in high concentrations inside cells without impairing cell metabolism (Kempf & Bremer, 1998). This mechanism is very important for intestinal cells to maintain the exchange of water and solutes during digestion and absorption of nutrients. There are several molecules considered organic osmolytes, such as proline, taurine, glutamine and glycine, but betaine is considered the most effective (Jahn *et al.,* 2006). Betaine also has the function of preventing dehydration of bacteria, such as enterobacteria and lactobacilli, in hyperosmotic environments (Li *et al.*, 2015). Therefore, betaine supplementation in the broiler diet appears to be an alternative to improve mucosa development in the first post-hatch week, which is also a challenging time for the chick.

The objective of the present study was to ascertain the appropriate levels of betaine inclusion in post-hatch and pre-starter feed, to minimize the negative effects of post-hatch delayed placement and ensuring intestinal integrity and nutritional support for these chicks.

MATERIALS AND METHODS

This research on animals was conducted according to the institutional committee on animal use under protocol nº 008/13. A total of 1,500 Cobb 500 male chicks originated from eggs produced by breeder hens the same age and incubated in the same machine were used.

The treatments consisted of five supplementation levels of Betaine HCl 95% (72% de betaine): Control (no supplementation); 0.070, 0.130, 0.200 and 0.260% inclusion per tonne of pre-starter feed. The chicks were distributed in the transportation boxes in a completely randomized design in five treatments and five replicates of 60 birds each.

The product was added "over the top" replacing starch in a basal pre-starter feed (Table 1) formulated with corn and soybean meal, following the Rostagno *et al.* (2011) nutritional recommendations.

Table 1 – Composition and values calculated (%) for the basal diet.

Ingredients	Pre-starter feed
	1 to 7 days
Corn	51.06
Soybean meal	39.50
Soybean oil	2.35
Calcitic limestone	0.88
Dicalcium phosphate	1.94
L-lysine	0.20
DL-methionine	0.25
Threonine	0.10
Sodium bicarbonate	0.19
Table salt	0.39
Vitamin supplement[1]	0.10
Mineral supplement[2]	0.05
Starch	3.00
Total	100.00
Calculated Composition	
Metabolizable energy (kcal/kg)	2,951.00
Crude protein (%)	22.280
Calcium (%)	0.922
Available phosphorus (%)	0.471
Methionine (%)	0.542
Methionine + cystine (%)	0.850
Lysine (%)	1.312
Threonine (%)	0.852
Sodium (%)	0.223
Chlorine (%)	0.283

1 Vitamin supplement – guaranteed level per kilogram of product: 3.125.000 IU of vitamin A; 550.000 IU of vitamin D3; 3.750 mg of vitamin E; 625 mg of vitamin K3; 250 mg of vitamin B1; 1.125 mg of vitamin B2; 250 mg of vitamin B6; 3.750 mg of vitamin B12; 9.500 mg of niacin; 3.750 mg of calcium pantothenate; 125 mg of folic acid; 350.000 mg of DL-methionine; 150.000 mg of choline chloride 50%; 50 mg of selenium; 2.500 mg of antioxidant; and 1.000 g of vehicle q.s.p.

2 Mineral supplement – guaranteed levels per kilogram of product: manganese, 150.000 mg; zinc, 100.000 mg; iron 100.000 mg; copper, 16.000 mg; and iodine, 1.500 mg.

After hatch, neonates were vaccinated and sexed, following the standard proceedings of the hatchery, and then they were transported to the aviary. All proceedings conducted with the chicks, from hatching until housing, lasted 24 hours.

During this 24-hour period, the chicks received 3 g/bird of pre-starter feed and did not receive water, in order to simulate what happens in the hatchery. After 24 hours of experimental feed intake, ten chicks per replicate (50 chicks per treatment), according to the average plot weight, were transferred to galvanized steel cages equipped with linear feeders and water dispensers. The chicks continued to receive the same experimental feeds in the cages until they reached seven days of age.

Water and feed were offered *ad libitum* over the whole rearing period, with a 24-hour light cycle. The maximum and minimum environmental temperatures

were monitored twice a day (8:00 AM and 5:00 PM) using thermometers within the shed. The mean maximum and minimum temperature during the experiment were 33.47°C and 25.48°C, respectively.

Before the chicks went to the transportation box, 15 chicks were separated as controls and the data on average body weight 44.54g, average yolk sac weight 5.87g, average intestine weight 1.87g and blood glucose 348.2 were collected. After 24 hours in the transportation box, five chicks per treatment were chosen at random. They were evaluated by body weighing, weighing of the yolk sac and intestine, collection of fragments from the duodenum, jejunum and ileum, and measurement of glucose concentration. The rest of the chicks were housed in the batteries according to the treatments. At seven days of age, another five broilers per treatment were separated and body weight and relative weight of yolk sac and total intestine, collected from the duodenum jejunum and ileum fragments for histomorphometric measurements and blood samples for glucose concentration determination were evaluated.

For performance evaluation at seven days of age, weight gain was calculated as the difference between the final and initial weights. Feed intake was calculated as the difference between the feed supplied and the leftovers at the end of each phase. Feed conversion was obtained through the ratio of feed intake/weight gain. Livability was assessed through the formula V (%) = 100 – mortality.

Glucose concentration was assessed through analysis within thirty seconds after drawing blood, using portable glucose metering equipment (Accu-Chek Active Roche®)

Five chicks from each treatment were euthanized to evaluate intestinal development and yolk sac retraction. The yolk sac and intestines (small and large) were then weighed on a scale precision of 0.0001 g.

One chick per replicate was sampled for histomorphometric measurements of the small intestine. Two-centimeter fragments were collected from the duodenum, jejunum and ileum, and were fixed in buffered 10% formaldehyde for 24 hours. These samples were then subjected to standard histological procedures to produce histological slides. The sections were stained with hematoxylin and eosin (Hu *et al.*, 2012; Sousa *et al.*, 2015). The heights of ten villi and the depths of ten crypts were measured in each section.

A metabolic trial was conducted between seven and ten days of age, using the total excreta collection method. Excreta were collected twice a day (8:00 AM and 5:00 PM), stored in plastic bags (which were identified per replicate) and kept in a freezer. At the end of the experimental period, the amount of feed intake and excreta produced were determined. The samples of excreta and experimental feed were used to determine the dry matter, nitrogen and ether extract content, as described by Silva & Queiroz (2002).

The results from bromatological analyses were used to calculate coefficients of metabolizability (MC) for dry matter (MCDM), nitrogen (MCN) and ether extract (MCEE). Nitrogen balance (NB) and ether extract balance (EEB) were also calculated by Sakomura & Rostagno (2007).

All data analysis was evaluated for normality by the Shapiro-Wilk test. Polynomial regression analysis was performed concerning betaine concentrations tested (p<0.05).

RESULTS AND DISCUSSION

When mean live weights of the chicks before and 24 hours after the experimental feed and the amount of feed intake were analyzed (Table 2), it was observed that the birds did not consume a significant amount of feed and therefore experienced mean weight loss of 2.52 g over the 24 hours inside the transportation boxes. The fact that the chicks did not have access to water during this period may contribute to weight loss. The reduction of live weight of the chicks during this phase occurs due to higher use of energy reserves from the yolk sac, digestive and renal excretions, and dehydration (Leu *et al.*, 2002; Carvalho *et al.*, 2013). The treatments in this experiment were not sufficient to reduce these effects (p>0,05).

Table 2 – Mean weight loss and feed intake of pre-starter diet supplemented with betaine of newly hatched chicks, before and after a 24-hour waiting period in transportation boxes.

Product (72% betaine)	Weight before (g)	Weight after 24 hours (g)	Weight loss (g)	Feed intake (g)
Control	45.45	42.90	2.55	0.009
0.070%	45.30	42.90	2.40	0.022
0.130%	45.68	43.70	1.98	0.029
0.200%	45.98	43.85	2.13	0.037
0.260%	45.25	43.06	2.18	0.023
Mean	45.83	43.31	2.52	0.017

The live weight of the chicks, retraction of the yolk sac, small intestine weight and glucose levels (Table 3) were not affected by betaine supplementation, probably due to the low feed intake in the transportation box. Different results were observed by Bhanja *et al.* (2009), who verified more significant reduction of yolk sacs in birds fed immediately after hatch, thus suggesting that feed intake might increase the mechanical activity of the intestine, such that the yolk sac would be absorbed more rapidly.

Fasting chicks go through a critical gluconeogenic metabolism period, with increases in ketosis and dehydration. Quick feed supplying could minimize these effects by altering the glycemic pattern of these birds (Reis, 2018). Gluconeogenesis decreases as the plasma glucose concentrations increase through feed intake. Our results showed that chickens are relatively hyperglycemic compared to mammals. Hyperglycemia is independent of feed intake. In the experiment conducted by Zhao *et al.* (2014), the authors observed blood concentrations higher than 200 mg/dL at hatch (before feed intake).

Table 3 – Evaluation of yolk sac retraction, intestine weight and blood glucose of chicks fed a pre-starter diet supplemented with betaine, after a 24-hour waiting period in transportation boxes.

Product (72% betaine)	Weight (g)	Yolk sac (g)	Intestine (g)	Glucose
Control	43.20	3.97	2.31	296.84
0.070%	43.45	3.22	2.69	288.80
0.130%	43.22	3.11	2.73	339.80
0.200%	44.14	4.01	2.64	328.00
0.260%	42.82	3.33	2.59	312.60
p-value	0.934	0.826	0.775	0.391
[1]CV	5.60	44.37	21.50	15.50

*Polynomial regression ($p<0.05$). [1]Coefficient of variation.

(Table 4) presents the results regarding the development of intestinal villi in the chicks fed pre-starter diet supplemented with betaine. Betaine supplementation influenced linearly (p=0,027) the ileum crypt depth (Y=32.6342 + 0.0401451X, R^2=0.85).

Table 4 – Evaluation of the villi development (villi height, crypt depth and villus/crypt ratio) in the intestine of chicks fed a pre-starter diet supplemented with betaine after a 24-hour waiting period in transportation boxes.

Product (72% betaine)	Duodenum			Jejunum			Ileum		
	Crypts	Villi	V/C	Crypts	Villi	V/C	Crypts	Villi	V/C
Control	35	256	7.19	37	179	4.86	33	164	4.95
0.070%	41	255	6.33	31	163	5.15	35	158	4.57
0.130%	38	222	5.98	34	171	5.17	38	172	4.51
0.200%	33	252	7.66	31	122	3.83	38	145	3.87
0.260%	41	218	5.34	39	189	4.76	45	192	4.28
p-value	0.221	0.911	0.641	0.289	0.271	0.595	0.027*	0.210	0.343
[1]CV	11.98	27.90	30.84	14.89	21.87	23.32	9.84	10.21	13.72

*Polynomial regression ($p<0.05$). [1]Coefficient of variation.

Increasing of crypt depth implies higher rates of cells proliferation and renovation of the intestinal epithelium, thereby increasing the number of villi since these structures originate from the lower part of the crypt (Fernandes *et al.*, 2017). The lumen of the intestine is always hyperosmotic when compared to blood plasma, so osmoregulatory substances are beneficial for intestinal cells (Mongin, 1976). Betaine has the function of controlling osmotic pressure in intestinal cells (Kettunen *et al.*, 2001). This osmoprotective function must have helped the linear increase in the depth of the Ileum crypt with betaine supplementation.

Chick feed intake was altered by betaine supplementation in the pre-starter diet (Table 5). There was a quadratic effect, with an increase in feed intake until a supplementation of 0.152% betaine was reached (Y = 168.242 + 0.12448X - 0.409295x², R^2= 0.62), from that value the feed intake was reduced, but did not interfere in weight at seven days. A similar result was found by Teixeira *et al.* (2006), studying levels of betaine inclusion (0.00; 0.05; 0.10 and 0.15%), who reported that birds that received 0.10% betaine had lower feed intake at seven days of age. However, this reduction in consumption did not affect weight gain. However, betaine supplementation in the feed did not interfere with the chick's feed intake at 7 days (Pereira *et al.*, 2010), but improved the final weight and conversion at 14 days (El-Shinnawy, 2015) and feed conversion at 21 days (Amerah & Ravindran, 2015).

The current experiment evaluated only the pre-starter phase. Therefore, a considerable amount of betaine may be preferentially applied by enterocytes to stimulate intestinal development and function in

Table 5 – Performance in the pre-starter phase (1 to 7 days) among chicks that fed diets supplemented with betaine.

Product (72% betaine)	Initial weight (g)	Final weight (g)	Weight gain (g)	Daily weight gain (g)	Feed intake (g)	Feed conversion (g/g)
Control	43.02	187.07	144.05	20.57	168.14	1.07
0.070%	42.96	191.70	148.74	21.24	176.87	1.09
0.130%	43.60	193.57	149.87	21.24	173.53	1.08
0.200%	43.60	190.66	147.02	21.00	180.23	1.10
0.260%	42.96	187.36	144.40	20.62	171.74	1.09
p-value	0.301	0.091	0.150	0.150	0.040*	0.600
[1]CV	1.54	2.16	2.85	2.85	3.42	3.00

*Polynomial regression ($p<0.05$). [1]Coefficient of variation.

chicks rather than improve growth performance during the starter period. Thus, these effects may affect the overall performance of broilers in the future.

Betaine supplementation did not influence the retraction of the yolk sac, the absolute intestine weight and the plasma glucose concentration at seven days of age (Table 6). During the first-week after hatching, the intestinal weight of these chicks increases dramatically when compared to the total body weight (Uni *et al.*, 2003). Therefore, the effects of substances beneficial to the development of the intestine are more prominent in the first-week of the broiler. Tissues that rely on betaine as an osmolyte include the leukocytes, kidney, liver, brain and intestines (Klasing *et al.*, 2002). In the current study, there was no effect of betaine supplementation on intestinal weight. Regardless of the treatments, the results showed that the intestine increased during the first-week post-hatch, whereas the yolk sac weight decreased rapidly, which is indicative of the maturation of digestive.

Even with stress in the transportation box and reduced feed intake, glucose concentration in chicks at seven days was also not influenced by betaine supplementation. The negative effect of stress on the central nervous system can reduce the metabolic rate and feed intake. This stress reaction process generates higher energy expenditure by the body and osmotic imbalance in the cells. Therefore, betaine supplementation can reestablish the osmotic balance in cells, mitigating the deleterious effects of stress (Klasing *et al.* 2002). Layers created in heat stress showed a higher concentration of glucose when supplemented with 0.10% betaine (220 mg / dL) compared to the control (208 mg / dL) (Attia *et al.* 2016). Contrary to these results, Konca *et al.* (2008), Sayed & Downing (2011) and El-Shinnawy (2015) evaluated betaine supplementation in the diet of broilers and found no difference in serum glucose levels.

Table 6 – Evaluation of yolk sac retraction and intestinal growth among chicks that were given pre-starter feed supplemented with betaine, at the age of seven days.

Product (72% betaine)	Yolk sac (g)	[1]Intestine weight (g)	Glucose (mg/dL)
Control	0.45	15.68	338.25
0.070%	0.35	15.24	308.00
0.130%	0.15	16.49	341.00
0.200%	0.15	16.23	340.40
0.260%	0.56	15.37	348.40
p-value	0.189	0.915	0.188
[2]CV	33.75	13.76	8.01

[1]Weight of the small and large intestines; [2]Coefficient of variation.

Betaine supplementation influenced jejunum villus/crypt ratio (Table 7), presenting quadratic effect ($p=0.049$) with minimum point in 0.087% of supplementation ($Y=5.20261 - 0.0074454X + 0.00425619X^2$, $R^2=0.74$). This fact demonstrated an improvement in the integrity of the jejunum with supplementation above 0.1% betaine.

Table 7 – Evaluation of intestinal villi development in chicks fed a pre-starter diets supplemented with betaine at seven days of age.

Product	Duodenum			Jejunum		
(72% betaine)	Crypts[1]	Villi[1]	V/C[1]	Crypts[1]	Villi[1]	V/C[1]
Control	62	454	7.36	60	314	5.20
0.070%	64	425	6.58	52	261	5.02
0.130%	59	406	6.83	62	284	4.56
0.200%	70	417	6.12	57	333	5.81
0.260%	63	484	7.66	56	333	5.99
p-value	0.628	0.454	0.465	0.393	0.079	0.049*
[2]CV	13.53	12.58	15.64	11.16	10.56	10.21

*Polynomial regression ($p<0.05$). [1]Crypt depth (crypt, µm), villus height (villi, µm), villus/crypt ratio (V/C). [2]Coefficient of variation.

In pigs, betaine decreased the energy expenditure of intestinal cells due to its osmoregulatory effect (Siljander-Rasi *et al.*, 2003). The most significant effects of betaine have been reported when broilers are challenged by pathogens that affect villi, as shown by Klasing *et al.* (2002), who after seven days of inoculating broilers with *Eimeira,* found that those fed 0.10% betaine presented higher villi.

The results from the metabolic trial demonstrated that there were no differences in betaine concentrations, except for the ether extract metabolizability coefficient (Table 8) that was improved until reaching 0.163% supplementation ($Y= 54.3717 + 200.9654X – 671.5212X^2$, $R^2= 0.47$).

Betaine has the function of donating methyl groups mainly for homocysteine that will provide the

Table 8 – Nitrogen balance (NB), ether extract balance (EEB), nitrogen metabolizability coefficient (NMC), ether extract metabolizability coefficient (EEMC) and dry matter metabolizability coefficient (DMMC) of chicks fed a pre-starter diet supplemented with betaine, from seven to ten days of age.

Product (72% betaine)	NB (g)	EEB (g)	NMC (%)	EEMC (%)	DMMC (%)
Control	18,47	54,67	62,42	86,86	73,60
0.070%	17,95	69,52	63,99	90,44	78,09
0.130%	18,20	65,81	63,66	90,47	76,39
0.200%	20,88	67,89	65,14	92,54	76,85
0.260%	17,57	61,63	64,08	91,72	76,13
p-value	0,323	0,033*	0,624	0,089	0,072
[1]CV	10,56	8,01	3,22	2,51	2,16

*Polynomial regression ($p<0.05$).

[1]Coefficient of variation.

formation of methionine, therefore it is involved in protein and energy metabolism (Eklund *et al.*, 2005). In broiler diets with methionine deficiency, betaine supplementation improved the metabolizability of ether extract and protein (El-Husseiny *et al.*, 2007).

Betaine for being osmoregulatory helps to maintain the integrity of the intestinal epithelium, which is important for improving the absorption and digestibility of nutrients. Betaine supplementation has shown an improvement in the use of some nutrients in birds of different ages. Amerah & Ravindran (2015) with supplementation of 0.096% betaine for 21-day-old chickens found better digestibility of starch, ash and ten amino acids; Ratriyanto *et al.* (2014), with 0.14% inclusion of betaine found a higher percentage of dry matter, protein and crude fiber in chickens aged 39 days and Attia *et al.* (2016) challenged layers to heat stress and observed improvement in utilization of protein with betaine supplementation.

In the current study, this osmoprotective effect was not directly evaluated, but there was an improvement in the use of the ether extract. Possibly, chicks that were fed diets containing betaine were favored by the ability of this substance to act as an osmolyte, maintaining the integrity of the intestinal mucosa, improving digestibility and the absorption capacity of nutrients.

CONCLUSIONS

Betaine supplementation around 0.150% increases feed intake, the use of ether extract and interferes with the intestinal villi of chicks at seven days of age.

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