Acta Scientiarum

http://periodicos.uem.br/ojs/acta
ISSN on-line: 1807-8672
Doi: 10.4025/actascianimsci.v40i1.38423

ANIMAL PRODUCTION

Performance, gut morphometry and enzymatic activity of broilers fed neonatal supplementation in housing

Tatiane Souza dos Santos[1*], Adriano Barbieri[2], Robert Guaracy Aparecido Cardoso Araujo[2], Gustavo do Valle Polycarpo[2], Daniela Felipe Pinheiro[3] and Valquíria Cação Cruz-Polycarpo[2]

*[1]Departamento de Melhoramento e Nutrição Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista, Rua Prof. Doutor Walter Mauricio Correa, s/n, 18618-681, Botucatu, São Paulo, Brasil. [2]Faculdade de Ciências Agrárias e Tecnológicas, Universidade Estadual Paulista, Dracena, São Paulo, Brasil. [3]Departamento de Fisiologia, Instituto de Biociências, Universidade Estadual Paulista, Botucatu, São Paulo, Brasil. *Autor para correspondência. E-mail: tatianesouza.santos@yahoo.com.br*

ABSTRACT. The objective was to evaluate the performance, relative organ weight, morphometry, intestinal length and pancreatic enzyme activity of broilers receiving neonatal supplementation. A total of 900 Cobb 500 broiler chicks was housed in 30 boxes of 2.5 m^2. The treatments were divided in a completely randomized design with six replications, and consisted of five inclusion levels (0.0, 2.5, 5.0, 7.5 and 10 grams per bird). The supplement used was based of amino acids, vitamins and minerals, which was provided on the ration on the first day in the housing. Statistical analysis was performed using SAS (2008) and when significant broken down using a polynomial regression. Due to the inclusion of the neonatal supplement in the period from one to seven and one to 21 days of age, the feed intake presented a linear effect and the weight gain, a quadratic effect. The relative weights of the gizzard and liver showed a quadratic effect, and the inclusions of 4.18 and 3.41 grams/bird provided the lowest weights, respectively. The jejunum villi height decreased with supplementation as well as the activity of pancreatic lipase. It can be concluded that neonatal supplementation benefited the performance of birds up to 21 days of age.

Keywords: post-hatching phase; early nutrition; gastrointestinal tract; pancreatic enzymes.

Desempenho, morfometria intestinal e atividade enzimática de frangos de corte alimentados com suplemento neonatal no alojamento

RESUMO. O objetivo foi avaliar o desempenho, peso relativo de órgãos, morfometria, comprimento intestinal e atividade de enzimas pancreáticas de frangos de corte recebendo suplemento neonatal. Foram utilizados 900 pintos de corte da linhagem Cobb 500, alojados em 30 boxes de 2,5 m^2. Os tratamentos foram divididos em delineamento inteiramente casualizado, com seis repetições, e consistiram em cinco níveis de inclusão (0,0; 2,5; 5,0; 7,5 e 10 gramas por ave). O suplemento utilizado foi à base de aminoácidos, vitaminas e minerais, o qual foi fornecido no primeiro dia de alojamento sobre a ração. A análise estatística foi realizada utilizando o SAS (2008) e quando significativos desdobrados em regressão polinomial. Em função da inclusão do suplemento neonatal no período de um a sete e de um a 21 dias de idade o consumo de ração apresentou efeito linear e o ganho de peso efeito quadrático. Os pesos relativos da moela e do fígado apresentaram efeito quadrático, e as inclusões de 4,18 e 3,41 gramas/ave proporcionaram os menores pesos respectivamente. A altura das vilosidades do jejuno diminuiu com a suplementação assim como a atividade da lipase pancreática. Conclui-se que a suplementação neonatal beneficiou o desempenho das aves até os 21 dias de idade.

Palavras-chave: fase pós eclosão; nutrição precoce; trato gastrintestinal; enzimas pancreáticas.

Introduction

In the last decades, the production of broilers has undergone a great advance, with the objective of obtaining a final product of excellence and better yield of cuts to meet the demand of the consumer. The first week of the bird is representative for its development, and important changes occur in this period, physiological, metabolic and anatomical. Thus, different nutritional and management practices are required for good performance (Panda, Shyam, Rama, & Raju, 2006).

In commercial operations with chicks, the time for vaccination management, sexing and hatchery output can take up to 72 hours without access to water and feed, impairing growth and viability of the flock (Madsen, Su, & Sørensen, 2004). Rapid access to solid food is important for maintaining glucose levels, and prevents glycogen stores from being mobilized to meet physiological needs as they should be used for initial growth, as well as gut development (Geyra, Uni, & Sklan, 2001).

The poultry digestive system is not fully functional soon after hatching, and thus the enzymatic activity that is dependent on substrate will only be initiated with food supply (Vieira & Moran Júnior, 1999). The development of the duodenum is complete from the 7th day after hatching, whereas in the jejunum it reaches development until the 14th day after hatching (Uni, Ganot, & Sklan, 1998). According to Noy and Sklan (2001), birds fed soon after hatching showed up to 200% increase in small intestine weight, while fasting 48 hours provided an increase of only 60%.

The duodenum presents a high cell turnover rate and higher villi height when compared to other segments of the small intestine, and intestinal villi develop from mitotic divisions that occur in large quantities in the initial period of life of the bird, however with passing of age this capacity begins to be reduced and is compensated for by growth in crypt size and width (Geyra et al., 2001).

Neonatal supplementation in the pre-housing period has the purpose of promoting a better homeostasis condition for the chicks, as they can go through a fasting period ranging from 24 to 48 hours until the first access to the food. In this fasting period, the reserves of the yolk sac are utilized, but the longer the access to the first feeding, the performance will be impaired (Uni et al., 1998).

The goal was to evaluate the effect five levels of a neonatal supplement provided on the first day of housing on performance, relative organ weight, morphometry and intestinal length, enzymatic activity of amylase and lipase in broiler chickens.

Material and methods

All procedures were approved by the Ethics Committee on Animal Experimentation of Unesp Dracena, under protocol number 23/2013.

The experiment was conducted in the experimental shed for broilers of the College of Agricultural and Technological Sciences of UNESP – Campus of Dracena. Cobb 500 one-day old male chicks (n = 900) were reared on wood shavings and housed in an experimental shed divided into 30 boxes of 2.5 m^2, with 30 birds/box, at the density of 12 birds m^{-2}. The treatments were divided into a completely randomized design, with six replicates of 180 birds each.

The initial heating was carried out by means of 250-watt infrared lamps, and these were removed in the seventh day of age. The temperature control was performed daily by checking the maximum, minimum and instantaneous temperatures, dry and wet bulb temperatures, black globe temperature and relative humidity.

The treatments consisted of five levels of inclusion of the neonatal supplement (0.0, 2.5, 5.0, 7.5 and 10 grams per bird), which was provided on the first day of housing, on the ration, with supply of water and feed *ad libitum*.

The feeding program was divided into four phases: pre-starter, 1 to 7 days; starter, 8 to 21 days; grower, 22 to 33 days; and finisher, 34 to 42 days. Diets were prepared with corn and soybean meal as recommended by Rostagno et al. (2011), whose levels are listed in Table 1. The chemical composition of the neonatal supplement is presented in Table 2.

Table 1. Composition and calculated values of the experimental diets.

	Diets			
Ingredients (%)	Pre-Starter	Starter	Grower	Finisher
Soybean meal 45%	38.27	34.75	31.54	27.32
Corn 7.88%	55.34	59.50	62.09	66.88
Soybean oil	2.110	2.065	3.061	2.860
Choline Chloride 60%	0.072	0.064	0.058	0.043
Common salt	0.507	0.482	0.457	0.444
Dicalcium phosphate	1.891	1.524	1.334	1.068
Calcitic Limestone	0.924	0.913	0.825	0.771
L-Lysine HCl 99%	0.284	0.215	0.192	0.232
DL-Methionine 99%	0.354	0.283	0.253	0.236
L-Threonine 98,5%	0.104	0.058	0.039	0.047
Mineral Supplement[1]	0.050	0.050	0.050	0.050
Vitamin Supplement[2,3,4]	0.100	0.100	0.100	0.050
	Calculated Composition			
EM (kcal kg^{-1})	2950	3000	3100	3150
Crude protein (%)	22.200	20.800	19.500	18.000
Digestible methionine (dig.) (%)	0.645	0.561	0.517	0.485
Methionine + Cystine dig. (%)	0.944	0.846	0.787	0.737
Lysine dig. (%)	1.310	1.174	1.078	1.010
Threonine dig. (%)	0.852	0.763	0.701	0.656
Tryptophan dig. (%)	0.250	0.231	0.214	0.192
Calcium (%)	0.920	0.819	0.732	0.638
Phosphorus dig. (%)	0.395	0.343	0.313	0.273
Sodium (%)	0.220	0.210	0.200	0.195
Choline (mg kg^{-1})	375	330	300	225

[1]Mineral supplement for broilers (guarantee levels per kg feed)/Multimix®: copper, 18 g; iodine, 2,000 mg; zinc, 120 g; iron, 60 g; manganese, 120 g. [2]Vitamin supplement for broilers at starter phase (guarantee levels per kg feed)/Multimix: vitamin A, 11,000,000 IU; vitamin D3, 2,000,000 IU; vitamin E, 16,000 IU; vitamin K3, 1,500 mg; vitamin B1, 1,200 mg; vitamin B2, 4,500 mg; vitamin B6, 2,000 mg; vitamin B12, 16,000 mcg; folic acid, 400 mg; pantothenic acid, 9,200 mg; biotin, 60 mg; niacin, 35 g; selenium, 250 mg.[3]Vitamin supplement for broilers at growth phase (guarantee levels per kg feed)/Multimix: vitamin A, 9,000,000 IU; vitamin D3, 1,600,000 IU; vitamin E, 14,000 IU; vitamin K3, 1,500 mg; vitamin B1, 1,000 mg; vitamin B2, 4,000 mg; vitamin B6, 1,800 mg; vitamin B12, 12,000 mcg; folic acid, 300 mg; pantothenic acid, 8,280 mg; biotin, 50 mg; niacin, 30 g; selenium, 250 mg.; [4]Vitamin supplement for broilers at finisher phase (guarantee levels per kg feed)/Multimix: vitamin A, 6,000,000IU; vitamin D3, 1,000,000 IU; vitamin E, 10,000 IU; vitamin K3, 1,000 mg; vitamin B1, 600 mg; vitamin B2, 2,000 mg; vitamin B6, 800 mg; vitamin B12, 6,000 mcg; pantothenic acid, 7,360 mg; biotin 30 mg; niacin, 10 g; selenium, 400 mg.

The performance of the birds was evaluated at 7, 21 and 42 days of age, including weight gain (g), feed intake (g), feed conversion (g g^{-1}), viability (%) and production factor.

At 21 days of age, six birds with average weight per treatment were killed by cervical dislocation, after fasting two hours, to determine the relative weight of organs, intestinal morphometry and length. For weighing the organs, the spleen, pancreas, gizzard, proventriculus, liver, small intestine and large intestine were taken and weighed on a semi-analytical scale accurate to 0.001 grams, to determine the relative weight in relation to the post-fast weight. All the weights obtained were used to calculate the relative weight of each organ, using the formula: relative organ weight = (organ weight/post-fast weight) x 100 and expressed as a percentage. Measurements of the length of the small and large intestines were made and expressed in centimeters.

Table 2. Chemical composition of nutrients of the neonatal supplement.

Nutrients	1 kg	10 g
Moisture (g)	256	2.56
Crude protein (g)	210	2.10
Gross fiber (g)	230	2.30
Lysine (g)	11.2	0.112
Methionine (g)	4.2	0.042
Ether extract (g)	17	0.17
Mineral Matter (g)	17	0.17
Calcium (g)	5.4 – 11.4	0.054 – 0.114
Phosphorus (g)	5.4	0.054
Vitamin D3 (UI)	1,570,000	15,700
Vitamin B12 (mg)	11.5	0.115

After weighing the small intestine, three-centimeter segments of the duodenum and jejunum were collected for histological analysis. The segments were washed in saline solution, opened along the mesenteric border, stretched by the tunica serosa, fixed in 10% formaldehyde for 24 hours and later stored in 70% alcohol. Then the material was subjected to dehydration in alcohol series (70-100%). After this procedure, the samples were diaphanized in xylol and included in Paraplast Plus®. Sections were five micrometers thick and were stained with Hematoxylin and Eosin (HE).

Slides were analyzed under an optical microscope coupled to a Leica image analyzer system (Image-Pro Plus version 1.0.0.1). Measurements of height and width of the villi and depth of crypts of both segments were taken, with 15 measurements of each parameter per animal (Pelicano et al., 2003).

The pancreas was weighed, frozen in liquid nitrogen for later analysis of amylase (EC 3.2.1.1) and lipase (EC 3.1.1.3) activity. The activity of pancreatic enzymes was determined after homogenizing the pancreas in 50 mM Tris-HCl buffer, pH 8.0 at 1/20 ratio. Amylase was determined by iodometric method, in which one unit of amylase is the amount of enzyme that will hydrolyze 10 mg starch within 30 minutes. Lipase activity was determined by colorimetric method, in which lipase hydrolyzes the thioester to produce a thioalcohol, which reacts with nitrobenzoic acid releasing a yellow anion. The color intensity is proportional to the concentration of the enzyme. The reading was made in a spectrophotometer at 660 and 420 nm, for amylase and lipase, respectively. Enzymes activity was expressed in international units (I.U.) per milligram of tissue.

The statistical analysis was performed using the statistical analysis system Statistical Analysis System (SAS, 2008). Data were analyzed for normality of residuals and homogeneity of variances and later subjected to analysis of variance (ANOVA) by the MIXED procedure at 5% of significance, and when significant, broken down in polynomial regression analysis. Data that did not meet normal distribution were tested by non-parametric Kruskal-Wallis ANOVA.

Results and discussion

The performance of broilers fed increasing levels of the neonatal supplement is presented below in Table 3.

Feed intake increased linearly in the periods one to seven and one to 21 days of age, when supplying 10 grams of the supplement/bird. In the period from one to 21 days of age, the weight gain showed a quadratic effect, and the inclusion of 2.40 grams supplement/bird provided less weight gain. From these results, it is observed that early feeding is able to improve bird performance, since it provides lower initial weight loss reflecting in greater weight uniformity at 21 days of age (Sklan, 2001). However, this effect was not observed in the period from one to 42 days of age, confirming the authors who also did not find an advantage in the use of this supplementation in this period (Caregui et al., 2005; Pedroso et al., 2005; Peebles et al., 2005).

Batal and Parsons (2002) observed that the weight gain in the one to 21-day-old period of birds that consumed neonatal supplementation within the 24-hour post-hatch period was the same as that of birds that did not consume this supplement, and the worst weight gain was observed for those birds that went through the 48 hour fast. Studies report that immediate access to food causes the yolk sac to be consumed rapidly, as the antiperistaltic movement that moves the yolk from the yolk sac to the duodenum appears to be stimulated by the presence of food in the intestine (Panda & Reddy, 2007).

Table 3. Performance of broilers fed different levels of neonatal supplement at 7, 21 and 42 days of age.

Variables[1]	Neonatal supplement g/bird					Probability				CV[2]	SEM[3]
	0.0	2.5	5.0	7.5	10.0	Linear	Quadratic	Cubic	Quartic		
1-7 days											
AWG	133.4	134.0	138.5	134.2	139.5	0.2004	0.9539	0.5326	0.1965	5.08	1.39
FI	143.6	147.4	151.8	150.7	154.5	0.0017[4]	0.5091	0.5516	0.3868	4.29	1.17
FC	1.078	1.101	1.099	1.133	1.099	0.2279	0.2760	0.4591	0.3001	4.05	0.01
VIAB	99.44	100.00	99.44	98.33	99.44	0.4365	0.8252	0.1263	0.6931	1.62	0.29
1-21 days											
AWG	890.16	889.01	896.19	887.26	900.84	0.0159	0.0461[5]	0.5297	0.4695	2.808	4.99
FI	1229.08	1242.07	1251.13	1228.26	1246.26	0.0282[6]	0.1935	0.2185	0.3665	2.582	6.27
FC	1.386	1.399	1.399	1.394	1.398	0.5998	0.1811	0.4428	0.9838	1.483	0.01
VIAB	98.34	98.89	98.89	96.67	96.11	0.5215	0.7194	0.5367	0.5854	3.008	0.54
1-42 days											
AWG	2545.01	2442.19	2461.45	2555.21	2531.24	0.8714	0.4021	0.1119	0.7127	4.531	20.75
FI	4072.39	4058.59	4109.03	4078.61	4160.44	0.3797	0.5940	0.7606	0.4344	3.040	21.76
FC	1.584	1.622	1.665	1.639	1.634	0.4011	0.1238	0.8435	0.3816	3.342	0.01
VIAB	96.56	96.79	96.40	96.15	95.85	0.3126	0.6398	0.6836	0.7855	1.076	0.18
FEP[3]	370.05	346.98	339.36	357.26	353.63	0.5497	0.1143	0.1542	0.3986	5.054	3.75

[1]AWG, average weight gain (g/bird); FI, feed intake (g/bird); FC, feed conversion (g g^{-1}); VIAB, viability (%). [2]CV, coefficient of variation (%). [3]SEM, standard error of the mean. [4]$Y = 1x + 144.56$; $R^2 = 0.32$; [5]$Y = 0.15x2 - 0.79x + 890.64$; $R^2 = 0.01$. [6]$Y = 0.82x + 1235.3$; $R^2 = 0.01$.

Similar effects were also reported by Henderson, Vicente, Pixley, Hargis, and Tellez (2008), when provided a neonatal supplement immediately after hatching for a period of 24 hours and found a better performance of the birds until the time of slaughter when compared to birds that did not receive any type of feed in this period.

Table 4 lists the values of organ weight and the length of the small and large intestine of birds at 21 days of age. In the analysis of the relative organ weight, only the gizzard and the liver were influenced, presenting a quadratic effect ($p < 0.05$). Inclusions of 4.18 and 3.41 grams supplement/bird provided lower relative weights of the gizzard and liver respectively, according to Table 4.

The gizzard responds rapidly to changes in diet and its size varies according to particle size, which is stimulated by the musculature, and studies show that this organ can increase by up to 100% over its original size depending on diet used (Svihus, 2011). Pelleted diets are rapidly assimilated by the gizzard after consumption, and the highest level of supplementation may have promoted the increase in the relative weight of this organ. The same effect could be observed for the liver, and the group that received 10 grams/bird presented greater relative weight. This is the main metabolic organ of the organism, therefore nutritional factors can influence its relative weight (Marcato et al., 2010) and in this case, the highest level of supplementation led to greater weight of this organ.

The results regarding morphometry of the duodenum and jejunum of broilers at 21 days of age are presented in Table 5. Statistical analysis indicated only effect ($p < 0.05$) for jejunum villi height.

Neonatal supplementation did not affect the intestinal morphometry of the duodenum, although in the jejunum there was a quadratic effect for villus height. These data are in agreement with Gonzales et al. (2003), who observed improvement in the morphometric parameters of the small intestine of birds fed shortly after housing. The higher villus height is related to higher absorption capacity and nutrient digestion, favoring the birds' performance (Viola & Vieira, 2007). These results confirm the finding in this study, where weight gain from one to 21 days of age improved with neonatal supplementation in the same way as jejunal villus height.

According to Sklan (2001), the production of amylase is related to food consumption. Some authors affirm that until the second day of life, the activity of this enzyme is low, since carbohydrate consumption stimulates its secretion (Bigot, Tesseraud, Taouis, & Picard, 2001) and from the moment that the consumption of exogenous food is initiated, the activity tends to increase up to 14 days of age and then stabilizes after 21 days (Lima, Macari, Pizauro Júnior, & Malheiros, 2002).

In the present study, the amylase was evaluated at 21 days of age, when its activity was already stabilized, and the regression equation indicated that 5.72 grams supplement/bird provided a decrease in the enzymatic activity.

The activity of pancreatic enzymes (amylase and lipase) were expressed as U mg^{-1} tissue and are shown in Table 6, evidencing the quartic effect of the neonatal supplement on amylase activity ($p < 0.05$). Lipase activity was also influenced by the neonatal supplement ($p < 0.05$), and the non-parametric Kruskal-Wallis test was used to evaluate the dataStudies indicate that the activity of amylase and lipase oscillates during the life of the bird, and the peak can be reached at eight days of age (Maiorka, Dahlke, & Morgulis, 2006).

Table 4. Relative weight of organs and length of small and large intestine of broilers at 21 days of age, fed with increasing levels of neonatal supplementation in housing.

Variables[1]	Neonatal supplement g/bird					Probability				CV[2]	SEM[3]
	0.0	2.5	5.0	7.5	10.0	Linear	Quadratic	Cubic	Quartic		
Spleen (%)	0.101	0.090	0.083	0.089	0.075	0.1129	0.8211	0.4641	0.6125	28.28	0.01
Gizzard (%)	1.926	1.860	1.829	1.858	2.077	0.212	0.0313[4]	0.5100	0.8629	9.48	0.03
Proventriculus (%)	0.441	0.424	0.439	0.434	0.456	0.3765	0.2724	0.8966	0.3840	7.84	0.01
Liver (%)	2.687	2.667	2.407	2.868	3.057	0.0296	0.0421[5]	0.9381	0.1438	11.50	0.06
Pancreas (%)	0.307	0.344	0.337	0.331	0.319	0.8618	0.1899	0.5294	0.7330	14.33	0.01
SI (%)	6.234	6.263	6.413	6.182	6.495	0.4900	0.8026	0.4940	0.3819	7.41	0.01
LI (%)	0.965	0.931	0.936	0.937	0.887	0.4667	0.8822	0.6454	0.9908	16.04	0.03
SI (cm)	140.500	143.167	144.200	142.167	143.833	0.6950	0.7731	0.7121	0.8380	7.75	1.92
LI (cm)	25.833	24.833	26.667	26.167	25.250	0.9663	0.6432	0.4131	0.4993	11.75	0.53

[1]SI, small intestine; LI, large intestine. [2]CV, coefficient of variation (%). [3]SEM, standard error of the mean. [4]$Y = 0.0072x2 - 0.0602x + 1.9406$; $R^2 = 0.21$; [5]$Y = 0.01x2 - 0.08x + 2.71$; $R^2 = 0.27$.

Table 5. Morphometry of the duodenum and jejunum of broilers at 21 days of age receiving different levels of neonatal supplementation.

Variables[1]	Neonatal supplement g/bird					Probability				CV[2]	SEM[3]
	0.0	2.5	5.0	7.5	10.0	Linear	Quadratic	Cubic	Quartic		
					Duodenum						
VH (µm)	1154.26	1174.70	1312.06	1161.13	1074.59	0.543	0.143	0.853	0.317	18.49	39.51
AW (µm)	84.84	85.14	92.63	72.17	78.42	0.176	0.471	0.302	0.079	17.35	2.75
BW (µm)	108.02	107.30	113.43	102.10	108.95	0.849	0.912	0.522	0.205	12.48	2.38
CD (µm)	147.79	173.70	180.33	202.19	175.56	0.115	0.149	0.572	0.473	22.44	7.43
					Jejunum						
VH (µm)	730.48	595.92	609.52	631.63	655.92	0.340	0.026[4]	0.222	0.669	14.00	17.66
AW (µm)	80.92	78.59	96.39	87.55	77.60	0.923	0.151	0.383	0.264	22.02	3.40
BW (µm)	92.68	99.04	92.75	100.13	97.33	0.655	0.866	0.916	0.418	18.51	3.08
CD (µm)	106.93	105.27	101.14	98.72	116.19	0.638	0.191	0.383	0.834	18.46	3.49

[1]VH, villus height; AW, apex width; BW, base width; CD, crypt depth. [2]CV, coefficient of variation (%). [3]SEM, standard error of the mean. [4]$Y = 3.73x2 - 41.82x + 713.97$; R2=0.20.

Table 6. Activity of pancreatic enzymes of broilers at 21 days of age receiving different levels of neonatal supplementation.

Variables[1]	Neonatal supplement g/bird					Probability				CV[2]	SEM
	0.0	2.5	5.0	7.5	10.0	Linear	Quadratic	Cubic	Quartic		
Amylase	20.74	40.59	25.51	30.09	31.06	0.381	0.191	0.010	0.016[3]	29.81	1.93
Lipase	0.34	0.03	0.01	0.01	0.02	0.0022[4]				29.81	0.02

[1]U mg^{-1} tissue. [2]CV, coefficient of variation (%). [3]$Y = -0.08x4 + 1.83x3 - 12.87x2 + 30x + 20.74$; $R^2=0.40$. [4]Non-parametric *Kruskal-Wallis* test.

The higher activity of pancreatic enzymes is related to the higher relative weight of the pancreas, so the presence of substrate and enzymatic activity are due to maturation of the pancreas (Moraes et al., 2008). The specific activity of lipase presented a distinct behavior when compared to the specific activity of amylase, because through the results obtained here the lipase activity was low. León, Garrido, Castañeda, and Rueda (2014) evaluated the enzymatic activity of pancreatic amylase and lipase from broilers fed post-hatching diet, and found that amylase increased up to 48 hours post-hatching and then decreased, while lipase began to increase after 48 hours post-hatching.

Noy and Sklan (1995) observed that the activity of lipase, trypsin and amylase showed a great increase in the period of four to 21 days of age, but lipase is the enzyme that presents less increase of activity when compared to these other enzymes, which may interfere with bird performance.

Unlike the aforementioned authors who provided supplementation immediately after hatching, in the present study the birds received supplementation in the housing and it may have been one of the reasons for not showing any difference in performance at 42 days of age, since between hatching and arrival at the shed for housing the birds used their yolk sac reserves.

Conclusion

Neonatal supplementation was able to improve poultry performance up to 21 days of age, but did not alter gut morphometry and the pancreatic enzymes activity.

Acknowledgements

To São Paulo Research Foundation (Process: 2013/08637-8) for granting an undergraduate scholarship and to Biogenic for financing this research.

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Received on July 29, 2017.
Accepted on August 31, 2017.