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Effect of neonatal hydrating supplementation on the absorptive surface area of the jejunum in the early stage of Cobb500™

Efeito da suplementação hidratante neonatal sobre a área de superfície absortiva do jejuno na fase inicial de frangos de corte Cobb500™

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ABSTRACT: The effect of neonatal supplementation on the absorptive surface area of the jejunum up to 14 days of age was investigated in 320 male Cobb500™ chicks. After vaccination, the neonatal hydrating product was added to the transport box. Four fasted groups (A - D) contained chicks housed at 8, 24, 48, and 72 hours post-hatching and four treated groups (E - H) also contained chicks housed at 8, 24, 48, and 72 hours post-hatching. Samples from four animals per group were collected at 8 h, 24 h, 48 h, and 72 h post-hatching as well as on the 7th and 14th days of production. The following measurements were taken at 10 points along the jejunum of each animal: villus height, mean width, distance between villi, and crypt diameter, followed by the calculation of the absorptive area of the jejunum. Statistical analyses were performed using ANOVA and Tukey's post-hoc test at a 5% significance level ($p<0.05$). Group E had the largest mean absorptive area throughout the experiment: 853.26 μ^2 and 1,098 μ^2 at 24 h and 72 h, respectively. This corresponds to approximately 50% more than that of the control group in the same post-hatching period. The approach described in this study offers a novel option for neonatal poultry nutrition, promoting animal welfare and the commercial viability of newly hatched chicks.

Keywords: post-hatch nutrition; absorption; poultry.

RESUMO: O efeito da suplementação neonatal sobre a área de superfície absortiva do jejuno até os 14 dias de idade foi investigado em 320 pintos machos da linhagem Cobb500™. Após a vacinação, o produto hidratante neonatal foi adicionado à caixa de transporte. Quatro grupos em jejum (A – D) continham pintos alojados às 8, 24, 48 e 72 horas pós-eclosão e quatro grupos tratados (E – H) também continham pintos alojados às 8, 24, 48 e 72 horas pós-eclosão. Amostras de quatro animais por grupo foram coletadas às 8 h, 24 h, 48 h e 72 h pós-eclosão, bem como no 7º e 14º dias de produção. As seguintes mensurações foram realizadas em 10 pontos ao longo do jejuno de cada animal: altura das vilosidades, largura média, distância entre vilosidades e diâmetro das criptas, seguidas do cálculo da área absortiva do jejuno. As análises estatísticas foram realizadas por ANOVA e teste post-hoc de Tukey, com nível de significância de 5% ($p<0,05$). O grupo E apresentou a maior média de área absortiva ao longo do experimento: 853,26 μ^2 e 1.098 μ^2 às 24 h e 72 h, respectivamente. Isso corresponde a aproximadamente 50% a mais que o grupo controle no mesmo período pós-eclosão. A abordagem descrita neste estudo oferece uma nova opção para a nutrição neonatal de aves, promovendo o bem-estar animal e a viabilidade comercial de pintos recém-eclodidos pelos autores.

Palavras-chave: nutrição pós-eclosão; absorção; avicultura.

INTRODUCTION

The feeding of chicks prior to lodging stimulates digestive enzymes and the greater development of intestinal villi (Geyra; Uni; Sklan, 2001; Obun; Osaguona, 2013; Oliveira *et al*., 2015). This phase is characterized by the transition from the lipid-rich yolk sac as the source of nutrients to exogenous feeding rich in carbohydrates and proteins (Sklan, 2001). The digestive and absorptive capacities and protection of the intestinal mucosa of poultry are related to the density, size, degree of renewal and preservation of the villi, height of the mucosal epithelium, density of goblet cells, as well as the production and secretion of mucins by these cells (Macari; Maiorka, 2000).

According to Scottá *et al.* (2014), the use of pre-initial diets is based on the recognition of the importance of nutrition to the growth of modern breeds in the first days following hatching. Morphological and morphometric aspects are of the utmost importance to the development of poultry farming (Barros, 2017). These aspects are indispensable to the understanding of the functioning of the digestive process, especially in broiler chickens, offering information on the extent to which different ingredients in the diet exert an influence on the functions of this system and the health status of the birds (Wang *et al*., 2014).

Therefore, the aim of the present study was to investigate the effects of a neonatal diet on the absorptive surface area of the jejunum in broiler chickens and the use of such as diet on the intestinal integrity and quality of neonatal chicks.

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MATERIAL AND METHODS

This experiment received approval from the Animal Use Ethics Committee of Universidade Federal Rural de Pernambuco (UFRPE) (license number: 74/2018) and was developed in two steps: 1- post-hatch period conducted in a commercial incubator; 2- initial rearing phase on a private property (Brejo Santo ranch) in the municipality of Garanhuns, state of Pernambuco, Brazil. The post-hatch experiment was conducted with 320 male chicks from Cobb500™ matrices 45 weeks of age. Immediately after vaccination, the neonatal hydrating supplement was added to the transport box based on the groups established and delineated in Table 1. Groups A - D were considered control groups, while groups E - H were treatment groups.

The birds were housed in eight experimental boxes measuring 1.5 m2, with 40 chicks per box (density: 26 chicks/m^2). The chicks were placed on a new bedding (first lot) and submitted to 24 hours of light per day until the seventh day of age and 23 hours of light per day from the eighth to the 14th days of age, with free access to water and feed. Two types of commercial feed were offered: bran based and isocaloric (Tables 2 and 3). The nutritional levels of the feeds were in accordance with those normally used in the market and established by Rostagno, Albino, and Donzele (2011) for the different phases of development [pre-initial phase (one to seven days) and initial phase (eight to 14 days)].

On the day of hatching (8 h, 24 h, 48 h and 72 h post-hatching) as well as on the 7^{th} and 14^{th} days of production, four chicks per group were weighed and sacrificed by cervical dislocation, following the method cited by Pelicano *et al.* (2003).

Histometric analyses were performed in each of the experimental periods. Fragments of the jejunum measuring approximately 2 cm in length were collected and immediately fixed in 10% formol in phosphate buffer 0.1 M, pH 7.4, for 24 hours. After this period, the fragments were cleaved and fixed for another 24 hours. The fragments were then placed in 70% alcohol and histological processing was performed following the routine protocol of the Histology Laboratory of the Department of Animal Morphology and Physiology of the UFRPE. Slices were cut on a Minot rotating microtome (model RM2125RT, Leica®) to a thickness of five micrometers (µm), placed on glass slides, and kept at 37°C in a laboratory oven for 24 hours for drying and adherence, followed by staining with hematoxylin-eosin (H.E.) for the subsequent histometric analyses.

Histological analyses were performed with the aid of a trinocular microscope with an HD camera (model ICC50, Leica®). Images were captured and digitized using the Leica LAS EZ software program, followed by analysis using the Image J® program. Ten villus heights and widths, 10 distances between villi (measured at the base), and 10 crypt diameters were measured in each chick, following the method described by Alvarenga *et al*. (2004). The intestinal absorptive surface area was calculated using the equation described by Kisielinski, Willis, and Prescher (2002):

$$M = \frac{\text{villus width} \times \text{villus height} + (\text{villus width}/2 + \text{crypt width}/2)^2 \times (\text{villus width}/2)}{(\text{villus width}/2 + \text{crypt width}/2)2}$$

The experimental design was entirely randomized and divided into two treatments (experimental and control), with lodging 8 h, 24 h, 48 h, and 72 h after hatching and assessments performed on the 7^{th} and 14^{th} days of production. The data were submitted to analysis of variance (ANOVA) and Tukey's post hoc test with the aid of the Assistat 7.7 Beta program. The significance level was set at 5% ($p < 0.05$).

Table 1 – Experimental groups of chicks submitted to fasting and neonatal hydrating supplementation for 8, 24, 48 and 72 hours after hatching.

	Post-hatching period	Without feed (fasting)	With neonatal hydrating supplementation
Treatment	8 h	A	E
	24 h	B	F
	48 h	C	G
	72 h	D	H

Table 2 – Composition of pre-initial commercial feed (one to seven days of life).

Ingredients	Levels per kg of product (%)
Moisture (max.)	12.50
Crude protein (min.)	23.00
Ether extract (min.)	4.00
Fibrous matter (max.)	6.00
Mineral matter (max.)	9.00
Calcium (max.)	1.30
Phosphorus (min.)	0.65

Ground whole corn, corn gluten bran 60, soy bran, meat and bone flour, degummed soybean oil, calcium (min.) 7500 mg, calcium (max.) 14 g, phosphorus (min.) 5500 mg, lysine (min.) 12.5g, methionine (min.) 5500 mg, sodium (min.) 2500 mg, iron (min.) 40 mg, copper (min.) 90 mg, manganese (min.) 80 mg, zinc (min.) 90 mg, iodine (min.) 1.7 mg, selenium (min.) 0.35 mg, vitamin A (min.) 9500 UI, vitamin D3 (min.) 3400 UI, vitamin E (min.) 22 UI, vitamin K3 (min.) 1.5 mg, vitamin B1 (min.) 2 mg, vitamin B2 (min.) 7.2 mg, vitamin B6 (min.) 3 mg, vitamin B12 (min.) 18 mg, niacin (min.) 48 mg, calcium pantothenate (min.) 12 mg, folic acid (min.) 1.2 mg, biotin (min.) 0.08 mg, choline chloride (min.) 1550 mg, diclazuril (min.) 1mg, zinc bacitracin (min.) 55 mg.

Table 3 – Composition of initial commercial feed (eight to 14 days of life).

Ingredients	Levels per kg of product
Macro	
Cornmeal	607.1600
Soybean flour	147.000
Extruded soy	137.000
Meat flour	51.000
Gross limestone	3.000
Micro	
Salt	1.9600
Sodium bicarbonate	2.1900
Dl- methionine	3.8900
L- lysine	3.9200
L- threonine	1.5000
Initial ProAct	0.2000
Hiphos	0.0500
Histarch	0.1300
Biobond	2.0000
Pre-Initial FC SM3Kg	3.0000

RESULTS AND DISCUSSION

Table 4 displays the mean area of intestinal absorption of the portion of the jejunum at the time of lodging and on the different collection days. No statistically significant differences among the groups were found in the variables of interest for lodging eight hours after hatching. The chicks in Group A were statistically similar to those in Group E, which received the neonatal hydrating supplement.

As the transit of feed in the gastrointestinal tract of birds can be influenced by structure and composition, the passage of liquid foods is expected to be faster than that of solid foods, fiber is expected to move slower than solid foods, and pelletized feed is expected to move faster than bran-based feed (Furlan; Macari; Luquetti, 2004). Thus, eight hours is considered insufficient to assess the influence of the hydrating supplement on the absorptive area of the jejunum.

Differences were found in the variables of interest when lodging was performed 24 hours after hatching. Group E had a larger mean absorptive area compared to the other groups with or without neonatal supplementation. The absorptive area in Group E was 50% larger compared to that in Group F (Table 4). However, a significant effect on the variables of interest was found in Group F compared to Group B ($p < 0.05$) and was similar to that found in Group A, demonstrating the importance of post-hatch nutrition and a shorter period prior to lodging. These findings are in agreement with data described by Uni, Ganot, and Sklan (1998), who emphasized the supply of nutrients in the first hours of the lives of chicks to facilitate the functional capacity of the intestinal mucosa (optimization of the enzymatic contribution) and the development of organs associated with the assimilation of nutrients.

More significant results in terms of the mean absorptive area were found when lodging was performed 48 hours after hatching in Group E compared to the other groups (with and without supplementation), as seen in Table 4. Compared to Group A, no significant difference was found in Group F, which received the hydrating supplement, leading to greater prolongation of the area of absorption to the point of being similar. During the first week of life, the small intestine of birds increases its absorptive area by approximately four times relative to body weight. Early administration of the hydrating supplement stimulates

Table 4 – Mean intestinal absorptive area (μm^2) of portion of jejunum in chicks that received neonatal hydrating supplementation and control groups at periods of 8 h, 24 h, 48 h, 72 h, 7 days and 14 days after hatching.

Treatment	Group	8h	24h	48h	72h	7th d	14th d
Control groups	A	246.1 a	349.1 b	375.9 b	554.0 b	775.0 bc	1.176 a
	B		200.3 c	255.97 b	318.9 c	762.4 bc	759.8 c
	C			281.6 b	318.9 c	692.8 c	725.3 c
Neonatal hydrating supplementation	D				214.6 d	811.6 bc	776.5 bc
	E	391.2 a	853.26 a	926.9 a	1.098 a	1.415 a	1.198 a
	F		352.2 b	298.8 b	995.4 a	607.9 c	976.5 b
	G			348.3 b	365.7 c	960.2 b	778.2 bc
	H				246.1 cd	865.8 bc	744.7 bc

*Different letters in same column denote significant differences between groups (Tukey's test at 5% significance level).

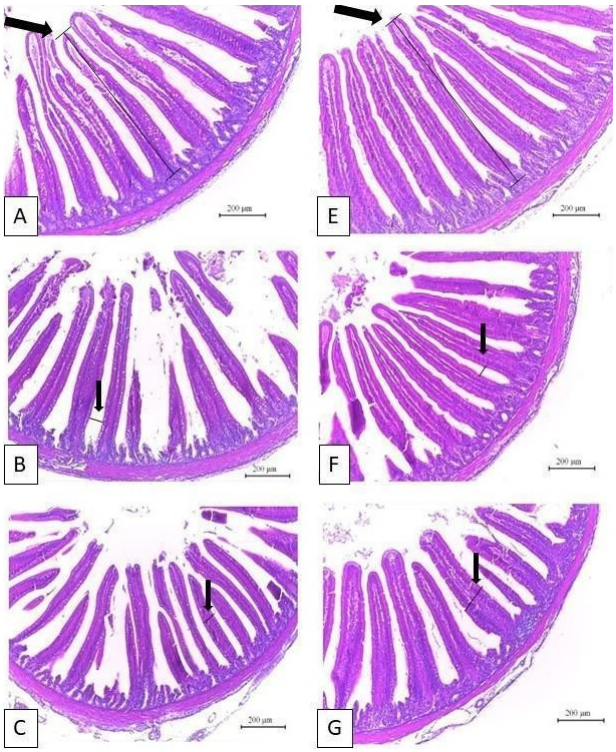
and facilitates this process, promoting better intestinal development (Amoroso *et al*., 2015). Improved intestinal morphometry can lead to enhanced performance under challenging field conditions and optimize feed conversion, thereby increasing production efficiency and profitability for poultry farmers (Fathanah *et al*., 2024).

Compensatory gain was found in Group B compared to Group F. Greater growth in the height of the villi is believed to have occurred due to physiological need, which contributed to the similarity between the two groups. According to Pelicano *et al.* (2003), the absorptive capacity of the intestinal segment in the presence of nutrients is directly proportional to the number and size of the villi and the surface area available for absorption.

The same occurred between Groups C and G. Fernandes *et al*. (2017) draws attention to the fact that the manipulation of the post-hatch diet can lead to improvements in energy efficiency in the intestine, especially in situations of enteric challenges. This is due to the fact that fasting can lead to a reduction in the proliferation and the migration of enterocytes during the development of the crypts and villi, especially in the duodenum and jejunum (Geyra; Uni; Sklan, 2001), can contribute to a substantial increase in mucus and the cellular extrusion of the absorptive epithelium of the small intestine (Maiorka; Luquetti; Almeida, 2003), stimulate the formation of clusters of microvilli on the apical surface of the jejunum in chicks, and reduce the absorption of nutrients (Uni; Ganot; Sklan, 1998).

The influence of neonatal supplementation on morphological aspects of the surface of the jejunum can be seen in the photomicrographs of the jejunum in fasted and non-fasted chicks in the experimental period (Figure 1).

Figure 1 – Photomicrographs of intestinal villi of jejunum in broiler chickens 48 h after hatching. HE, obj. 10x (A-E, B-F and C-G): Note height of villi in Groups A-E. Note distance between villi in Groups B-F. Note mean width (m) of villi in Groups C-G.



Source: Own authorship.

At 72 hours after hatching, Group E had the largest mean absorptive area among all groups. Moreover, the chicks in Group F had a significantly larger mean absorptive area compared to chicks housed at 24 h, 48 h, and 72 h with or without neonatal hydrating supplementation. Group G had a larger mean absorptive area compared to Group D. These findings agree with data described in the study by Barros (2017), in which larger morphometrics of the villi and a larger absorptive area were found in chicks that received a post-hatch diet.

At seven and 14 days of life, the adaptive response of the intestine and digestion was influenced by the fasting time to which the chicks were submitted (Table 4). Chicks fed at an earlier time had a larger mean absorptive area and possibly better use of nutrients from the feed. These results were found in the analysis of the difference in Groups A and E compared to the other groups throughout this period. Similar results are reported by Fernandes *et al*. (2017), who studied post-hatch supplementation with glycine and taurine irrespective of the oil source and found changes in the morphology of the intestinal mucosa, resulting in a greater length of the villi and larger villus/crypt ratio.

CONCLUSIONS

The inclusion of the neonatal hydrating supplement favored the development of the absorptive area of the jejunum in broiler chickens, enhancing nutrient absorption in the early stages of growth. This method constitutes a promising strategy for neonatal bird nutrition, contributing to improved intestinal health and overall performance. By promoting better initial development, this supplementation may reduce early mortality rates and enhance feed efficiency, leading to stronger and more uniform flocks. In a commercial setting, these benefits could translate into optimized production outcomes and improved animal welfare. Therefore, the adoption of neonatal hydrating supplement strategies could be a valuable tool for enhancing the sustainability and profitability of broiler production.

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