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The effect of in ovo albumin feeding to Rhode Island Red poultry embryos on chick weight, hatchability, and embryo mortality immediately post-hatch

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Abstract: This study aimed to evaluate the effects of *in ovo feeding* using albumin on hatchability, hatching weight, embryo mortality, as well as on organ development of Rhode Island Red chicks. A total of 245 fertile eggs with viable embryos were randomly distributed in seven treatments with 35 replicates (eggs) each. The treatments were untreated eggs and eggs treated with a buffer solution (0.50% saline) or solutions containing increased levels of albumin (0.5, 1.0, 1.5, 2.0, and 2.5%). On 17 days of incubation, eggs were injected with solutions containing albumin dissolved in 200 µL of 0.5% sterile saline. After the solutions were injected, the pinholes in the eggs were sealed with molten paraffin and moved to a hatcher machine. Evaluation of the chicks' weight, hatchability, and embryo mortality was made immediately post-hatch. Five viable chicks of each treatment were randomly selected and slaughtered by cervical dislocation to evaluate the heart, gastrointestinal organs and regional development. Data collected were subjected to polynomial regression (P≤0.05) after a significant ANOVA result. *In ovo* feeding using albumin directly affected (P≤0.05) the hatching characteristics of injected eggs. When eggs were injected with increased levels of albumin, there was a linear decrease (P≤0.05) in hatchability and an increase in embryo mortality. The increased supplementation of albumin resulted in a linear growth (P≤0.05) of gastrointestinal tract areas responsible for digestion (oropharynx plus esophagus, duodenal loop and cecum), and a linear decrease of heart, liver, pro-ventricle, gizzard and gastrointestinal tract areas responsible to absorption (jejunum plus ileum and colon plus rectum).
Keywords: amino acid, biotechnology, embryo, hatchability, *in ovo*.

1. Introduction

During the incubation period and in the first hours after hatching, birds have limited digestive functions. This can reduce nutrient availability and restricts the digestive capacity when the amniotic fluid is orally consumed at 17 days of incubation (Uni *et al*., 2005). Unlike mammals, the growth and development of avian embryos and hatchlings during incubation have been dependent on the defined nutrient deposits in the fertile egg (Uni *et al*., 2012).

Although the egg is considered nutritionally complete, modern poultry strains demonstrated that the percentages of amino acids, carbohydrates, vitamins, minerals, and lipids are sufficient only for the initial stage (1 to 8 days) of embryo development, being below to ideal requirements for an intermediary (9 to 17 days) and final (18 to 21 days) stages and during the hatching (Gonzales *et al*., 2013). Front this, *in ovo* feeding may serve as an effective tool to improve embryo development in oviparous species to meet this nutrient requirement (Zhang *et al*., 2017).

The *in ovo* feeding in the pre-hatching phase (US Patent (6,592,878) of Uni and Ferket, 2003) is a technology in the poultry industry that involves the administration of exogenous nutrients into the amnion of the developing embryo of chickens and turkeys at about 17 and 23 days of incubation, respectively (Foye *et al*., 2006). And the *in ovo* feeding using amino acids already proved viable (Ohta *et al*., 1999), and could enhance the chick weight at hatch, energy status, intestinal function and growth performance (Al-Murrani, 1982; Ohta *et al*., 2001; Yang *et al*., 2019).

Albumin is the most abundant circulating protein found in the blood plasma. It represents half of the total protein content of plasma, being synthesized by liver hepatocytes and rapidly excreted into the bloodstream. Very little albumin is stored in the liver, and most of it rapidly excretes into the bloodstream. Physiologically, serum albumin functions as a significant modulator of plasma pressure and a transporter of endogenous and exogenous ligands (Moman *et al*., 2020).

The albumin molecule has several characteristics that make it a unique protein, presenting a singular importance to any metabolic functions, such as the synthesis and degradation of biomolecules (Throop *et al*., 2004). Thus, this study aimed to evaluate the effects of albumin *in ovo* injection on hatchability, hatching weight, embryo mortality, as well as on organ development of Rhode Island Red chicks.

2. Materials e Methods

All experimental procedures were performed in the Poultry Sector of the Federal University of Amazonas, Manaus, Amazonas, Brazil. The experimental protocols applied in this study were in accordance with the Brazilian guidelines for animal welfare and approved by the Animal Care and Use Committee of the Federal University of Amazonas (protocol number 013/2019).

Standard-size fertile eggs (45.89±3.91 g, n = 300) were obtained from Rhode Island Red breeders (with 65 weeks of age) housed in an experimental aviary located in the Research Poultry Farm itself. Breeders were fed the same diet and water *ad libitum*, and the diet was calculated according to Rostagno *et al*. (2017). All eggs were collected at one time, weighed at room temperature, and distributed in trays into a hatcher machine (PETERSIME 168, Coopermaq Incubation Equipment Co. Ltd., Urussanga, Brazil) within 24 hours of lay under optimal incubation conditions (37.6 ºC and 66% RH). Before incubation, the eggs were left at room temperature for approximately 2 hours, where the temperature (preheated) was controlled to be close to what would be used in the hatcher machine. The hatcher machine was previously disinfected using ultraviolet light and the eggs in it were turned every hour. Distilled water was added to a tank in the incubator twice, daily, by hand. The trays were positioned to avoid incubational environment positional effects.

On 17 days of embryonic development, eggs were candled with a lamp, and those unfertilized or containing dead embryos were removed from the incubator. The remaining eggs with viable embryos (n = 245) were marked with a soft-lead pencil. These

Treatments	Solutions	Osmolarity (mOsm/L)[2]
Control	Intact egg	-
Saline	0.5% NaCl	170.94
Solution 1	0.5% NaCl + 0.5 % Alb	185.43
Solution 2	0.5% NaCl + 1.0 % Alb	199.92
Solution 3	0.5% NaCl + 1.5 % Alb	214.41
Solution 4	0.5% NaCl + 2.0 % Alb	228.91
Solution 5	0.5% NaCl + 2.5 % Alb	243.40

Table 1 – Experimental treatments containing controls groups and solutions with different levels of albumin (Alb) to in *ovo* injection. The injected experimental solutions were calculated in 100 mL of solution base. Determined by the calculation of the molecular weight and the concentration of the substances in each solution.

fertile eggs were distributed in a completely randomized experimental design, where the experimental groups, as shown in Table 1, were composed of untreated control (control), a sterile buffer solution (0.50% saline), and five solutions containing increased levels of albumin (0.5, 1.0, 1.5, 2.0, and 2.5%), with 35 eggs (replicates).

Albumin (purity>99.5%) in the free base was obtained from a commercial company (MF-MuscleFull Suplementos Ltda, Marília, Brazil). The feeding solution was prepared according to the method reported by Bhanja *et al.* (2004). The concentration of the amino acid that was injected into the egg was reported by Murphy (1994) (Table 1). The albumin was dissolved in 200 µl of 0.50% sterile saline before injection. The solutions were adjusted to pH 7.0 adding NaOH and nullified for the microbial count through a sterile filter. Before *in ovo* feeding procedure, the solutions were warmed up to 29 ºC in a water bath.

Fertile eggs were sanitized and drilled in the air chamber region (avoiding drilling the inner membrane of the eggshell). The solutions (manufactured with sterilized materials) were injected (0.5 ml) into the amniotic fluid (methodology previously tested) using needle syringes (7 x 2.5 mm), following the method described by Uni and Ferket (2003). All these procedures were performed in a closed room with a controlled environment replicating the conditions observed in the hatcher machine. All treatments were injected at the same time, presenting an average difference of 14 minutes between the first and the last egg injected per treatment.

After injection, the pinholes in the eggs were closed using melted paraffin and the eggs were transferred to the hatcher machine (PETERSIME 168, Coopemaq Incubation Equipment Co. Ltd., Urussanga, Brazil) under optimal conditions (36.6 ºC and 76% RH) until complete the 21 days of incubation (504±2 h). Immediately post-hatch, the chicks were weighed prior to sacrifice for sample collection, and hatch weight (HW) was recorded. The percentage of hatchability was evaluated by birth chicks per fertile eggs injected, percentage of intermediary mortality (dead embryos between 16 and 18 days of incubation), percentage of late mortality (dead embryos between 19 and 21 days of incubation without pecked the eggshell), percentage of pipped eggs (dead embryos between 19 and 21 days of incubation that pecked the eggshell), and proportion of chick weight per its respective egg weight according to Damasceno *et al.* (2017).

For the hatchability results, five viable chicks of each treatment were randomly selected, slaughtered by cervical dislocation and the yolk sac, heart, liver, pro-ventricle, and gizzard were dissected, drained out of blood, and weighed. The same procedure was used to evaluate the length of the gastrointestinal tract and its regions (oropharynx plus esophagus, duodenal loop, jejunum plus ileum, cecum, and colon plus rectum) according to the method described by Damasceno *et al.* (2017).

Data collected were subjected to a one-way analysis of variance (SAS Inc., Cary, NC). Polynomial regression was utilized for injection effect determination. The regression procedure of curve estimation was applied in the determination of the regression model. A linear or quadratic model was decided, derived from the scatter diagram plot. Hatchability was determined as the proportion of hatched egg numbers to total fertile egg numbers (Bhanja *et al.*, 2004). Significant differences were found when the possibility value (p-value) was less than 0.05.

3. Results and Discussion

The use of albumin *in ovo* significantly affected ($p<0.05$) hatchability ($Y = -1.1244x^2 + 4.5963x + 12.05$; $R^2 = 0.84$) and intermediary mortality ($Y = -0.4433x^2 + 1.85252x + 45.7257$; $R^2 = 0.81$), where embryos subjected to albumin presented worst results. The buffer saline solution presented better ($P\leq0.05$) hatchability and lower intermediary embryo mortality. There was also

Treatment	Hatchability (%)	Intermediary mortality (%)	Late mortality (%)	Pipped eggs (%)
Control	65.69	17.16	8.66	8.49
Saline	71.40	17.16	8.66	2.78
0.5% Alb.	70.90	17.16	9.16	2.78
1.0% Alb.	57.21	25.81	11.43	5.55
1.5% Alb.	41.47	37.09	15.72	5.72
2.0% Alb.	19.95	45.75	25.81	8.49
2.5% Alb.	28.61	28.59	31.37	11.43
P-value	0.01	0.01	0.01	0.03
Effect	Q	Q	PL	PL
CV (%)	4.13	8.70	8.07	6.41

Table 2 – Effects of in ovo feeding of albumin at 17 days of embryonic development on hatchability and embryo mortality of Rhode Island Red chicks. All data represent the mean of 35 replicates per treatment. Control is the non-injected treatment; buffer saline is 0.50% sterile saline; Alb. = albumin concentration. All in ovo feeding test albumin was dissolved in buffer saline. Q = Quadratic. PL = Positive Linear. CV = Coefficient of variation.

a positive linear effect (P≤0.05) on late mortality (Y = 3.8925x + 0.26; R^2 = 0.83) and pipped eggs (Y = 0.8279x + 3.1514; R^2 = 0.81) of embryos subjected to albumin (Table 2).

These results corroborated those observed by Pedroso *et al.* (2006), who reported greater embryo mortality when a glucose solution was injected into the amniotic fluid of 16-d-old embryos. According to those authors, nutrients injected *in ovo* that present high free energy may cause high osmolarity (above 200-300 mOsm/L) in the inoculated solution. Thus, these solutions directly affect embryonic development, suggesting that the lower hatchability and high intermediary mortality of the high levels of amino acid-injected eggs verified in our results could be caused by osmotic balance disequilibrium (Uni *et al.*, 2003).

On the other hand, Pedroso *et al.* (2006) and Santos *et al.* (2010) did not report the effect of *in ovo* feeding of glutamine in the amniotic fluid of embryos on hatchability. However, Salmanzadeh *et al.* (2016) showed that the hatchability was significantly reduced when injecting glutamine on day 7 of incubation. Studies reported that *in ovo* feeding at the final stage of incubation, how used in our method, may provide better effects than at the initial stage, including increased hatchability (Uni *et al.*, 2005). The significant difference in hatchability among the *in ovo* injected groups observed in this study and other studies (Al-Murrani, 1982; Ohta *et al.*, 1999; Salmanzadeh *et al.*, 2016; El-Kholy *et al.*, 2019; Kop-Bozbay and Ocak, 2019), indicate that the type of nutrient, its metabolic function, and how it affects the osmolarity of the inject solution are the main causes for changes on hatchability and intermediary embryo mortality. In addition, another factor that may help explain the relatively low hatchability results observed in this study is the age of the breeders. Once breeders at 65 weeks of age are close to the end of their productive cycle, there is a natural drop in the percentage of viable embryos produced and, consequently, the number of chicks that hatch (Machado *et al.*, 2020).

The use *in ovo* injection of albumin did not significantly affect (P>0.05) the chick weight and the chick-egg correlation

Treatment[2]	Hatching weight (g)	Proportion of chick weight per its respective egg weight
Control	38.97	0.77
Saline	39.16	0.78
0.5% Alb.	37.70	0.75
1.0% Alb.	36.63	0.77
1.5% Alb.	36.72	0.76
2.0% Alb.	36.72	0.72
2.5% Alb.	38.41	0.75
p-value	0.54	0.89
Effect[3]	Ns	Ns
CV (%)[4]	7.09	9.26

Table 3 – Effects of in ovo feeding of albumin at 17 days of embryonic development on hatching weight of 1-day old Rhode Island Red chicks. All data represent the mean of 35 replicates per treatment. Control is the non-injected treatment; buffer saline is 0.50% sterile saline; Alb. = albumin concentration. All in ovo feeding test albumin was dissolved in buffer saline. ns – non significant. CV = Coefficient of variation.

between different albumin levels and the control treatments (Table 3). Based on preliminary studies using amino acids and proteins (Pedroso *et al.*, 2006; Damasceno *et al.*, 2017), the first objective of *in ovo* feeding is to increase the concentration of available nutrients and improve the embryo development of chicks aiming to increase the body weight of chicks (Foye *et al.*, 2007). However, Damasceno *et al.* (2017) reported that the use of amino acids *in ovo* feeding in some cases did not cause a significant effect at short-term on the body weight of chicks.

The process of growth and development in chickens, especially in broilers, is a very intensive period accompanied by great metabolic changes. Thus, there is an increase of body mass and accumulation of enormous amounts of muscle in a very short time (Stojević *et al.*, 2000). In chickens, the influence of age on the concentrations of pre-albumin during growth is not described. But it

seems that, similarly to mammals, its serum concentrations are associated with the recent nutritional status and reflect the balance between protein synthesis and degradation (Tóthová *et al.*, 2017; Tóthová and Nagy, 2018).

Considering these factors, more or fewer nutrients can be allocated to the egg, giving the developing embryo a more or less

Treatment	Yolk sac (g)	Heart (g)	Liver (g)	Pro-ventricle (g)	Gizzard (g)
Control	6.01	1.06	1.65	1.18	2.38
Saline	5.14	1.03	1.53	1.05	2.62
0.5% Alb.	7.85	1.13	1.48	1.13	2.49
1.0% Alb.	5.92	1.13	1.42	1.11	2.38
1.5% Alb.	6.01	1.11	1.42	1.08	2.32
2.0% Alb.	6.56	1.11	1.39	1.08	2.28
2.5% Alb.	7.37	1.09	1.38	1.03	2.08
p-value	0.12	0.05	0.03	0.04	0.05
Effect	ns	Q	NL	NL	NL
CV (%)	8.19	4.89	8.95	8.90	8.33

Table 4 - Effects of in ovo feeding of albumin at 17 days of embryonic development on organ weigh of Rhode Island Red chicks. All data represent the mean of 35 replicates per treatment. Control is the non-injected treatment; buffer saline is 0.50% sterile saline; Alb. = albumin concentration. All in ovo feeding test albumin was dissolved in buffer saline. Q = Quadratic. NL = Negative Linear. ns = non significant. CV = Coefficient of variation.

favorable 'nutritional' environment to start post-hatch development (Willems *et al.*, 2014). In this sense, egg weight and the amount of albumin present are influenced by many of factors that may have an impact on the success and survival of the hatchling (Christians, 2002; Willems *et al.*, 2014). However, the results of this study demonstrated that the use of albumin *in ovo* feeding did not influence the chick's weight, indicating that its use did not affect the nutritional content available to the embryos.

In the results of the heart and main organs of the gastrointestinal tract, there was no effect ($P>0.05$) of albumin use *in ovo* feeding on the yolk sac. However, the levels of 0.5 and 1.0% caused a larger ($P\leq0.05$) development in the heart ($y = -0.0058x^2 + 0.01049x + 1.1414$ $R^2 = 0.86$). In addition, there was a negative linear effect ($P\leq0.05$) on the liver ($y = -0.0411x + 1.6314$ $R^2 = 0.85$), pro-ventricle ($y = -0.0157x + 1.1571$ $R^2 = 0.84$) and gizzard ($y = -0.0625x + 2.6143$ $R^2 = 0.83$) (Table 4).

These results indicated that high levels of albumin injected *in ovo* tend to cause a significant reduction in the development of the main organs of the gastrointestinal tract. A comprehensive understanding of embryo growth and development trajectory might shed light on the possible albumin contribution (Zhang *et al.*, 2017). In the present study, we observed that changes in digestive organs due to albumin injection are very pronounced. After the injection of the nutrient solutions into the amniotic fluid, the embryo orally consumes the amniotic contents before to piping which leads to exposure of supplemented nutrients to the digestive tract. This may contribute to the negative effects on the development of the digestive tract before, at the moment, and after the chicks pippen the eggshell (Chen *et al.*, 2017; Xu *et al.*, 2019).

The development of digestive organs is critical during the post-hatch period when birds switch to enteral nutrition as they play a vital role in digestion and absorption of nutrients (Uni *et al.*, 1999). Furthermore, the profile of the exogenous nutrients supplied to the embryo provides different answers to embryonic development. Normally, the *in ovo* feeding using energy sources (carbohydrates and some amino acids) may accelerate the gut development and its functional maturity, acting as a tool to overcome the growth constraints imposed by limited digestive capacity in modern embryos, enhancing its intestinal function and maturation before hatching. However, in some situations of disbalance in osmolarity of the *in ovo* feeding solution, may occur a negative effect on gastrointestinal organs development (Tako *et al.*, 2004; Foye *et al.*, 2006; Leitão *et al.*, 2010; Leitão *et al.*, 2014).

However, when analyzing the results of gastrointestinal areas, we observed a significant effect ($P\leq0.05$) on all areas of the gastrointestinal, where there was a positive linear effect of albumin use *in ovo* feeding on oropharynx + esophagus ($y = 0.2139x + 15.286$; $R^2 = 0.80$), duodenal loop ($y = 0.5293x + 14.814$; $R^2 = 0.87$) and cecum ($y = 0.9546x + 14.767$; $R^2 = 0.94$). However, this albumin uses also caused a negative linear effect on jejunum plus ileum ($y = -1.4418x + 47.003$; $R^2 = 0.95$) and colon plus rectum ($y = -0.2561x + 8.13$; $R^2 = 0.85$) (Table 5).

It was observed that the high concentrations of albumin injected *in ovo* used a positive effect on the main areas of the gastrointestinal tract responsible for digestion. However, a negative effect was observed on areas responsible for nutrient absorption. Rufino *et al.* (2019) reported that depending on the profile of the exogenous nutrients supplied to the embryo, there will be different embryo responses reflected in this post-*in ovo* feeding development. It is important to mention that the use of amino acids *in ovo* feeding affected the structural development of the gastrointestinal tract, mainly in the intestinal area. Hence, the *in ovo*-fed avian neonate may have a greater capacity to digest and absorb nutrients from an exogenous diet relative to the conventional hatchling (Tako *et al.*, 2004).

On the other hand, numerous studies have demonstrated that the intestinal amino acid (Karasov *et al.*, 1987; Torras-Llort *et al.*, 1998) and glucose transporters (Diamond and Karasov, 1987; Karasov *et al*., 1987; Solberg and Diamond, 1987; Ferraris and Diamond, 1989; Ferraris *et al*., 1992) are upregulated in the presence of increasing concentrations of their specific dietary substrate(s). The increase of these glucose transporters, once there is osmotic equilibrium between the inoculated solution and the organism of the embryo, can provide an increase in glucose concentration in the bloodstream (Lu *et al*., 2005; Uni *et al*., 2005), as it was verified in our results for inoculation of high levels of albumin. This same relation applies to other transporters and nutrients that act directly on energy metabolism (Uni *et al*., 2005).

4. Conclusion

In conclusion, between the albumin levels tested, the injection *in ovo* of 0.5% albumin provided better results. In general, *in ovo* albumin injection affected hatching characteristics, where increasing levels caused a linear decrease in hatchability and a proportional increase in embryo mortality. The increased supplementation *in ovo* of albumin caused a linear increase in gastrointestinal tract areas responsible for digestion (oropharynx plus esophagus, duodenal loop and cecum), and a linear decrease in heart, liver, pro-ventricle, gizzard and gastrointestinal tract areas responsible to absorption (jejunum plus ileum and colon plus rectum).

5. References

Al-Murrani WK. Effect of injecting amino acids into the egg on embryonic and subsequent growth in the domestic fowl. Br. Poult. Sci., 23;171-174, 1982.

Bhanja SK, Mandal AB, Johri TS. Standardization of injection site, needle site, needle length, embryonic age and concentration of amino acids for in ovo injection in broiler breeder eggs. Indian J. Poult. Sci., 39;105-111, 2004.

Chen W, Wang R, Xiong XL, Wan HF, Xu J, Peng J. Influence of in ovo injection of disaccharides, glutamine and β-hydroxy-β-methylbutyrate on the development of small intestine in duck embryos and neonates. Br. Poult. Sci., 51; 592-601, 2010.

Christians JK. Avian egg size: variation within species and inflexibility within individuals. Biol. Rev., 77;1-26, 2002.

Damasceno JL, Cruz FGG, Melo RD, Feijó JC, Rufino JPF, Valentim FM, Oliveira JPC. Inoculação de proteína isolada de soja em ovos embrionados oriundos de matrizes semipesadas com diferentes idades. Arq. Bras. Med. Vet. Zootec., 69;1259-1266, 2017.

Diamond JM, Karasov WH. Adaptive regulation of intestinal nutrient transporters. Proc. Natl. Acad. Sci. USA, 84;2242-2245, 1987.

El-Kholy MS, Ibrahim ZAEG, El-Mekkawy MM, Alagawany M. Influence of in ovo administration of some water-soluble vitamins on hatchability traits, growth, carcass traits and blood chemistry of Japanese quails. Ann. Anim. Sci., 19;97-111, 2019.

Ferraris RP, Diamond JM. Specific regulation of intestinal nutrient transporters by their dietary substrates. Annu. Rev. Physiol., 51;125-141, 1989.

Ferraris RP, Villenas SA, Hirayama BA, Diamond J. Effect of diet on glucose transporter site density along the intestinal crypt-villus axis. Am. J. Physiol., 262;1060-1068, 1992.

Treatment	Total length (cm)	Oropharynx + oesophagus (%)	Duodenal loop (%)	Jejunum + ileum (%)	Cecum (%)	Colon + rectum (%)
Control	46.50	15.12	16.04	44.56	16.00	8.28
Saline	51.00	15.89	16.15	44.24	16.63	7.09
0.5% Alb.	50.00	16.17	16.23	43.54	16.91	7.15
1.0% Alb.	48.00	16.19	16.28	41.94	18.52	7.07
1.5% Alb.	44.40	16.50	16.40	39.68	20.38	7.04
2.0% Alb.	44.40	16.56	17.19	38.42	20.46	7.37
2.5% Alb.	43.90	16.56	20.23	36.27	21.20	5.74
p-value	0.05	0.05	0.04	0.04	0.05	0.03
Effect	Q	PL	PL	NL	PL	NL
CV (%)	9.79	6.32	7.27	5.02	9.29	6.66

Table 5 - Effects of in ovo feeding of albumin at 17 days of embryonic development on gastrointestinal tract length of Rhode Island Red chicks. All data represent the mean of 35 replicates per treatment. Control is the non-injected treatment; buffer saline is 0.50% sterile saline; Alb. = albumin concentration. All in ovo feeding test albumin was dissolved in buffer saline. Q = Quadratic. PL = Positive Linear. NL = Negative Linear. ns = non significant. CV = Coefficient of variation.

Foye OT, Uni Z, Ferket PR. Effect of in ovo feeding egg white protein, β-hydroxy-β-methylbutyrate, and carbohydrates on glycogen status and neonatal growth of turkeys. Poult. Sci., 85.1185-1192, 2006.

Foye OT, Uni Z, Ferket PR. The effects of in ovo feeding arginine, β-Hydroxy-β-Methyl-Butyrate, and protein on jejunal digestive and absorptive activity in embryonic and neonatal turkey poults. Poult. Sci., 86(12); 2343-2349, 2007.

Gonzales E, Cruz CP, Leandro NSM, Stringhini JH, Brito AB. In ovo supplementation of 25(OH)D3 to broiler embryos. Braz. J. Poult. Sci., 15;169-286, 2013.

Karasov WH, Solberg DH, Diamond JM. Dependence of intestinal amino acid uptake on dietary protein or amino acid levels. Am. J. Physiol., 252(5);614-625, 1987.

Kop-Bozbay C, Ocak N. In ovo injection of branched-chain amino acids: Embryonic development, hatchability and hatching quality of turkey poults. J. Anim. Physiol. Anim. Nutr., 103;1135-1142, 2019.

Leitão RA, Leandro NSM, Stringhini JH, Café MB, Andrade MA. Inoculação de maltose, sacarose ou glicose em ovos embrionados de baixo peso. Acta Sci. Anim. Sci., v. 32, p. 93-100, 2010.

Leitão RA, Leandro NSM, Stringhini JH, Café MB, Matos MS, Andrade MA. Inoculação de maltose e/ou sacarose em ovos leves embrionados. Ciênc. Anim. Bras., v. 15, p. 55-63, 2014.

Lu JW, Mcmurtry JP, Coon CN. Developmental changes of plasma insulin, glucagon, insulin-like growth factors, thyroid hormones, and glucose concentrations in chick embryos and hatched chicks. Poult. Sci., 86;673-683, 2007.

Machado JP, Mesquita MA, Café MB, Assis SD, Veríssimo S, Santos RR, Leandro NSM, Araújo ICS. Effects of breeder age on embryonic development, hatching results, chick quality, and growing performance of the slow-growing genotype. Poult. Sci., 99(12);6697-6704, 2020.

Murphy ME. Amino acid compositions of avian eggs and tissues: Nutritional implications. J. Avian Biol., 25;27-38, 1994.

Ohta Y, Tsushima N, Koide K, Kidd MT, Ishibashi T. Effects of amino acid injection in broiler breeder eggs on embryonic growth and hatchability of chicks. Poult. Sci., 78;1493-1498, 1999.

Ohta Y, Kidd MT, Ishibashi T. Embryos growth and amino acid concentration profiles of broiler breeder eggs, embryos and chicks after in ovo administration of amino acids. Poult. Sci., 80;1430-1436, 2001.

Pedroso AA, Chaves LS, Café MB, Leandro NSM, Stringhini JH, Menten JFM. Glutamine as broilers embryos nutrient. Braz. J. Poult. Sci., v. 8, p. 43-49, 2006.

Rostagno HS, Albino LFT, Hannas MI, Donzele JL, Sakomura NK, Costa FGP, Saraiva A, Teixeira ML, Rodrigues PB, Oliveira RF, Barreto SLT, Brito CO. Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. Viçosa: Universidade Federal de Viçosa, 2017. 488p.

Rufino JPF, Cruz FGG, Costa VR, Silva AF, Melo LD, Bezerra NS. Effect of In Ovo Feeding of L-Glutamine to Chick Embryos. Braz. J. Poult. Sci., 21(4); eRBCA-2018-0852, 2019.

Salmanzadeh M, Ebrahimnezhad Y, Shahryar HA, Ghaleh-Kandi JG. The effects of in ovo feeding of glutamine in broiler breeder eggs on hatchability, development of the gastrointestinal tract, growth performance and carcass characteristics of broiler chickens. Arch. Anim. Breed.; 59, 235-242, 2016.

Santos TT, Corzo A, Kidd MT, McDaniel CD, Torres Filho RA, Araújo LF. Influence of in ovo inoculation with various nutrients and egg size on broiler performance. J. Appl. Poult. Res., 19;1-12, 2010.

Solberg DH, Diamond JM. Comparison of different dietary sugars as inducers of intestinal sugar transporters. Am. J. Physiol., 252(4);574-584, 1987.

Stojević Z, Milinković-Tur S, Ćurčija K. Changes in thyroid hormones concentrations in chicken blood plasma during fattening. Vet. Arh., 70(1);31-37, 2000.

Tako E, Ferket PR, Uni Z. Effects of in ovo feeding of carbohydrates and β-hydroxy-β-methylbutyrate on the development of chicken intestine. Poult. Sci., 83;2023-2028, 2004.

Thoop JL, Kerl ME, Cohn LA. Albumin in Health and Disease:Protein Metabolism and Function. Compend. Contin. Educ. Vet., 26;932-939, 2004.

Torras-Llort M, Soriano-Garcia JF, Ferrer R, Moretó M. Effect of a lysine-enriched diet on L-lysine transport by the brush-border membrane of the chicken jejunum. Am. J. Physiol., 274(1);69-75, 1998.

Tóthová CS, Major P, Molnár L, Nagy O. Protein electrophoresis in avian medicine. In: Mitchell GH. (ed). Gel Electrophoresis: Types, Applications and Research. New York: Nova Science Publishers, Inc., p. 157-187, 2017.

Tóthová CS, Nagy O. Transthyretin in the evaluation of health and disease in human and veterinary medicine. In: Gaze DC. (ed). Pathophysiology Altered Physiological States. London, United Kingdom: In Tech Open, p. 51-67, 2018.

Uni Z, Yael N, Sklan D. Post hatch development of small intestinal function in the poult. Poult. Sci., 78; 215-222, 1999.

Uni Z, Ferket P. Enhancement of development of oviparous species by in ovo feeding. North Carolina State Univ., Raleigh and Yissum Res. Dev. Co., Hebrew Univ. Jerusalem, Israel. US Pat. No. 6,592,878 B2, 2003.

Uni Z, Ferket PR, Tako E, Kedar O. In ovo feeding improves energy status of late-term chicken embryos. Poult. Sci., 84;764-770, 2005.

Uni Z, Yadgary L, Yair R. Nutritional limitations during poultry embryonic development. J. Appl. Poult. Res., 21;175-184, 2012.

Willems E, Decuypere E, Buyse J, Everaert N. Importance of albumen during embryonic development in avian species, with emphasis on domestic chicken. World's Poult. Sci. J., 70(3);503-518, 2014.

Xu QQ, Zhang XY, Zou XT, Dong XY. Effects of in ovo injection of L-histidine on hatch performance and post-hatch development in domestic pigeons (Columba livia). Poult. Sci., 0;1-10, 2019.

Yang T, Zhao M, Li J, Zhang L, Jiang Y, Zhou G, Gao F. In ovo feeding of creatine pyruvate alters energy metabolism in muscle of embryos and post-hatch broilers. Asian-Australas. J. Anim. Sci., 32;834-841, 2019.

Zhang XY, Wan XP, Miao LP, Zou XT, Dong XY. Effects of in ovo feeding of l-arginine on hatchability, hatching time, early post hatch development, and carcass traits in domestic pigeons (Columba livia). J. Anim. Sci., 95;4462-4471, 2017.