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Efeito da L-lisina na alimentação *in ovo* de embriões avícolas

Effect of in ovo feeding of L-lysine to chick embryos

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RESUMO

O presente estudo teve como objetivo avaliar os efeitos da alimentação in ovo utilizando L-lisina sobre a eclodibilidade, relação pinto/ovo, desenvolvimento do trato gastrointestinal (do nascimento aos sete dias pós-eclosão) e desempenho dos pintos de um a sete dias. Foram utilizados 350 ovos férteis da linhagem Rhode Island Red (matrizes com 47 semanas). O delineamento experimental foi o completamente casualizado com os tratamentos constituídos por dois controles e cinco níveis crescentes de L-lisina com 50 repetições (ovos) cada. Os dados coletados foram submetidos a regressão polinomial a 5%. Houve efeito significativo ($p<0,05$) sobre a eclodibilidade, mortalidade embrionária intermediária, mortalidade intermediária tardia e ovos bicados, com uma gradual queda na eclodibilidade a partir do aumento dos níveis de L-lisina utilizados. Foram observadas diferenças significativas ($p<0,05$) sobre o peso do coração e do pâncreas de pintos com um dia; e no consumo de ração, percentual de ganho de peso e comprimento do ceco dos pintos aos sete dias. Os resultados deste estudo indicam que a alimentação in ovo utilizando L-lisina afeta diretamente a eclodibilidade dos ovos inoculados. Usando 0,5% e 1,0% de L-lisina, houve um efeito positivo no peso do pinto, do coração, do pâncreas e sobre o desempenho dos pintinhos de um a 7 dias, sem afetar negativamente a relação pintinho/ovo e o trato gastrointestinal.

Palavras-chave: alimentação in ovo, aminoácido, desempenho, mortalidade embrionária, trato gastrointestinal.

ABSTRACT

The present study aimed to evaluated the effects of IOF (in ovo feeding) of L-lysine on hatchability, chick/egg relation, development of the gastrointestinal tract (at birth and at seven days post-hatch) and performance at seven days of chicks. 350 fertile eggs Rhode Island Red (breeders with 47-weeks) were used. The experimental design was completely randomized with the treatments constituted by two controls and five solutions containing L-lysine levels with 50 replicates (eggs) each. Data collected were subjected to polynomial regression at 5% of significance. Differences ($p<0.05$) were observed in hatchability, intermediate mortality, late mortality and pipped eggs, with a gradual lower of hatchability from the IOF of 1.0% L-lysine. Differences ($p<0.05$) were observed in heart and pancreas weight in chicks post-hatch; and feed intake, weight gain percentage and cecum length in chicks with seven days. The results of this study indicated that IOF using L-lysine directly affect the hatching characteristics of injected eggs. Using 0.5% and 1.0% of L-lysine to IOF there was a positive effect on chick weight, heart, pancreas, and performance from one to 7 days post-hatch, without negative affect chick/egg relation, and gastrointestinal tract development.

Keywords: amino acid, embryo mortality, gastrointestinal tract, in ovo feeding, performance.

INTRODUCTION

During the incubation period and in the first hours after hatching, the embryos of poultry modern strains presents limited digestive functions, which reduces the nutrients availability to growth metabolism, and restricts the digestive capacity, that begins the development when the amniotic fluid is orally consumed at 17 d of incubation (UNI *et al*., 2005).

In ovo feeding (IOF) in the pre-hatching stage is an interesting technology in poultry science, being performed from the drilling of eggshell and injection of nutrients in the amniotic fluid or in allantoic cavity by graduated syringes, aiming to improve the development of gastrointestinal tract, digestive enzymes, intestinal villi (GEYRA *et al*., 2001), body organs, and performance (LEITÃO *et al*., 2005).

According to Kidd (2004), the researches using IOF needs be developed to evaluate its impact on chick growth and increase of economic viability for poultry industry. Currently, the in ovo technology is veery used in incubation centrals of several countries such as United States, Japan, France, Netherlands among others, being majority used to in ovo vaccination of chicks and turkeys embryos (JOCHEMSEN & JEURISSEN, 2002).

The researchers also affirm that, depending of some factors as embryo development stage and solution composition, is possible determined the ideal volume to be applied in different egg places, concentrations, osmolarities, and others parameters. But, the lack of information about the IOF effects of several substances on chicks embryo physiology prevents the full development of this technology in industrial level (GEYRA *et al*., 2001; JOCHEMSEN & JEURISSEN, 2002; LEITÃO *et al*., 2014).

Considering the above, the present study aimed to evaluated the effects of IOF using L-lysine on hatchability, chick/egg relation, development of the gastrointestinal tract (at birth and at seven days post-hatch), and performance from one to seven days of chicks.

MATERIAL AND METHODS

This study was conducted at the Laboratory of Poultry Technology, Poultry Sector, Faculty of Agrarian Sciences, Federal University of Amazonas, Manaus, Amazonas State, Brazil. The experimental protocols applied in this study were complied with the Brazilian guidelines for animal welfare and approved by the Animal Care and Use Committee of College of Agrarian Science of Federal University of Amazonas (protocol n. 010/2015).

350 fertile eggs Rhode Island Red (breeders with 47-weeks) were used. A completely randomized design was applied, where the treatments were constituted by two controls and five solutions containing L-

lysine levels with 50 replicates (eggs) each (Table 1).

Table 1. Experimental solutions with L-lysine.

Treatments	Solutions	Osmolarity (mOsm/L)
Control	0.0% NaCl + 0.0 % L- lysine	-
IOF Control	0.5% NaCl + 0.0 % L- lysine	170.94
Solution 1	0.5% NaCl + 0.5 % L- lysine	239.34
Solution 2	0.5% NaCl + 1.0 % L- lysine	307.75
Solution 3	0.5% NaCl + 1.5 % L- lysine	376.16
Solution 4	0.5% NaCl + 2.0 % L- lysine	444.57
Solution 5	0.5% NaCl + 2.5 % L-lysine	512.98

The eggs were identified, disinfected, weighed, and distributed in an incubator machine PETERSIME 168 with 37.6 ºC of temperature, 66% of relative humidity, and turn of eggs at one-hour intervals. At 17 days of incubation, the fertile eggs were sanitized and drilled in the air chamber region (avoiding to driller the inner membrane of the eggshell). The solutions (0.5 mL) were injected into the amniotic fluid using needle syringes (7 x 2.5 mm). The hole in the egg shell was closed using melted paraffin and the eggs transferred to hatching machine PETERSIME 168 with 36.6 ºC of temperature, 76% of relative humidity at 21 days of incubation (504±2 hours), without turning.

After birth, were evaluated the percentage of hatchability (birth chicks/fertile eggs), percentage of intermediary mortality (dead embryos among 16 and 18 days of incubation), percentage of late mortality (dead embryos among 19 and 21 days of incubation without pecked the eggshell), and percentage of pipped eggs (dead embryos among 19 and 21 days of incubation that pecked the eggshell). From hatchability results, five able hatch chicks of each treatment were euthanized by cervical dislocation to evaluate the gastrointestinal tract development (yolk sac (g), heart (g), liver (g), pancreas (g), pro-ventricle (g), gizzard (g), digestive system length (cm), oropharynx + oesophagus (cm), duodenal loop (cm), jejunum + ileum (cm), cecum (cm) and colon + rectum (cm)).

12 chicks of each treatment were selected to performance analysis of progeny in pre-initial stage (from one to seven days). The chicks were housed in cages three compartments (four chicks per compartment), and weekly weighted. The variables evaluated were feed intake (g/bird), weight gain (g/bird), and weight gain percentage (%). After seven days, five able chicks of each treatment were randomly selected and slaughtered to evaluate gastrointestinal tract development using the same variables previous evaluated, except yolk sac.

Statistical analysis was performed using the software Statistical Analysis System (2008), and estimates of treatments were subjected to polynomial regression at 5% of significance.

RESULTS AND DISCUSSION

Differences ($p<0.05$) were observed in hatchability ($y = -13,143x + 107,43$ $R^2 = 0,82$) and intermediary mortality ($y = -13.286x + 15.714$, $R^2 = 0.84$) results, with a gradual lower of hatchability from the increase of levels of L-lysine, and consequently, increase in the embryo mortality, mainly in intermediary stage (few hours after IOF procedures) (Table 2). In this study, it was possible to observe the embryo physiological limit point from IOF of L-lysine (Figure 1) between 0.5 and 1.0% levels.

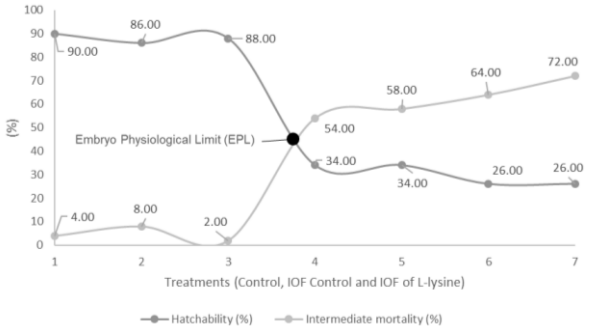

Figure 1. The behaviour of IOF of L-lysine on hatchability and intermediary mortality. All data represent the mean value per treatment. The meeting of curves between 0.5 and 1.0% in ovo feeding of L-lysine determines the embryo physiological limit point.

These results corroborate with Leitão *et al*. (2014), that observed an increase in embryo mortality from IOF using increase levels of glucose into the amniotic fluid at 16 days of incubation. According to Ohta and Kidd (2001), and Jochemsen and Jeurissen (2002), some factors may cause a high embryo mortality in IOF eggs, stand out the age of the breeder, weight and storage time of the eggs; the injection place; the concentration of the substance in the solution, among others. Leitão *et al*. (2014) also affirm that osmolarity and own biochemical composition of the substances used to IOF may be factors that contributed to the embryo mortality, mainly in intermediary stage.

No differences ($p>0.05$) were observed in egg weight, egg weight loss, chick weight and in chick/egg correlation (Table 3). Uni *et al*. (2003) and Leitão *et al*. (2014) also did not observe effect on chick/egg correlation after using carbohydrates to IOF. But, IOF of L-lysine, as other biomolecules, may provide little increases in chicks weight, mainly because the lysine is the principal essential amino acid for the formation of muscle tissue, and have direct relation with the growth metabolism of birds.

IOF of amino acids, same obtaining similar results as IOF of carbohydrates, have potential to promote better results in muscular development due its action on metabolic routes of muscle tissues (GEYRA *et al*., 2001; FOYE *et al*., 2006; CAMPOS *et al*., 2010). In this sense, the heart weight ($y = 0.045x^2 - 0.0455x + 0.4086$, $R^2 = 0.75$) and pancreas weight ($Y= - 0.0043x^2 + 0.035x + 0.0114$, $R^2 = 0.77$) were higher ($p<0.05$) after IOF (control and 1.0% L-lysine) (Table 4).

According Tako *et al*. (2004) and Foye *et al*. (2006), the supplementation of substances in ovo, besides providing nutrients to complete the hatching, also may provide an

Table 2. Effect of in ovo feeding using L-lysine on hatchability and embryo mortality.

Treatments	Variables			
	Hatchability (%)	Intermediate mortality (%)	Late mortality (%)	Pipped eggs (%)
Control	90.00	4.00	6.00	0.00
IOF Control	88.00	8.00	2.00	4.00
0.5 NaCl + 0.5 Lysine	86.00	2.00	8.00	2.00
0.5 NaCl + 1.0 Lysine	34.00	54.00	12.00	0.00
0.5 NaCl + 1.5 Lysine	34.00	58.00	8.00	0.00
0.5 NaCl + 2.0 Lysine	26.00	64.00	2.00	0.00
0.5 NaCl + 2.5 Lysine	26.00	72.00	6.00	4.00
p-value	0.01	0.01	0.06	0.06
Effect	NL	PL	ns	ns
CV (%)	11.36	15.90	8.32	24.50

CV – Coefficient of variation. P-value – Coefficient of probability. Q – Quadratic ($p<0.05$). NL – Negative Linear ($p<0.05$). PL – Positive Linear ($p<0.05$). ns – non significant ($p>0.05$).

Table 3. Effect of in ovo feeding using L-lysine on egg-chick relation.

Treatments	Variables			
	Egg weight (g)	Egg weight loss (%)	Chick weight (g)	Egg-chick relation
Control	49.12	12.57	32.11	0.65
IOF Control	49.54	12.32	33.35	0.67
0.5 NaCl + 0.5 Lysine	49.78	13,03	33.07	0.66
0.5 NaCl + 1.0 Lysine	50.12	11.96	32.70	0.65
0.5 NaCl + 1.5 Lysine	49.22	12.71	31.88	0.64
0.5 NaCl + 2.0 Lysine	49.10	12.04	33.46	0.68
0.5 NaCl + 2.5 Lysine	50.14	12.85	33.60	0.66
p-value	0.47	0.90	0.70	0.80
Effect	ns	ns	ns	ns
CV (%)	2.08	20.07	5.77	6.06

CV – Coefficient of variation. p-value – Coefficient of probability. ns – non significant.

Table 4. Effect of in ovo feeding using L-lysine on gastrointestinal development (organs).

Treatments	Variables					
	Yolk sac (g)	Heart (g)	Liver (g)	Pancreas (g)	Pro-ventricle (g)	Gizzard (g)
Control	2.57	0.32	1.09	0.03	0.33	2.26
IOF Control	3.44	0.36	1.07	0.04	0.35	2.10
0.5 NaCl + 0.5 Lysine	3.03	0.39	1.09	0.02	0.31	1.94
0.5 NaCl + 1.0 Lysine	2.83	0.42	1.04	0.07	0.37	2.24
0.5 NaCl + 1.5 Lysine	2.63	0.34	1.25	0.09	0.33	2.12
0.5 NaCl + 2.0 Lysine	2.97	0.30	1.10	0.03	0.29	1.71
0.5 NaCl + 2.5 Lysine	2.75	0.29	0.98	0.02	0.36	1.92
p-value	0.91	0.01	0.78	0.02	0.52	0.09
Effect	ns	Q	ns	Q	ns	ns
CV (%)	3.96	17.77	20.43	6.05	18.59	13.13

CV – Coefficient of variation. p-value – Coefficient of probability. Q – Quadratic ($p<0.05$). ns – non significant ($p>0.05$).

increase both development of vital organs of the gastrointestinal tract and enzymes production, and in other organs that also have market potential. The supplementation in ovo also can stimulate the early development of the small intestinal mucosa structure.

According Tarachai and Yamauchi (2000), the intestinal mucosa not only responds to physical stimuli, but primarily to the chemical characteristics of nutrients and, physiologically, may present development according is stimulated. However, in this study, there was not a significant effect of IOF of lysine on development of gastrointestinal tract regions (Table 5).

Differences ($p<0.05$) were observed in feed intake ($Y = 0.326x^2 - 0.756x + 12.011$, $R^2 = 0.71$) and weight gain percentage ($Y= 1.1846x^2 - 0.7354x + 11.067$, $R^2 = 0.79$), where better feed intake (11.57 g/bird/day) and weight gain percentage (10.95%) were obtained from IOF (control and 1.0% L-lysine) (Table 6). Leitão et al. (2005) did not observed effect in weight gain between 0 and 10 days in IOF chicks supplemented with glucose at 16 days of incubation. The IOF of some substances, mainly carbohydrates, protein and mixed of these, has demonstrated improved in chicks weight at birth and in the post-hatch development. Researchers attributed this result to increase in gastrointestinal tract development and in enzyme expression at birth promoted by the IOF of exogenous nutrients, causing an effective post-hatch development in a short time (TAKO *et al*., 2004; UNI & FERKET, 2004; UNI *et al*., 2005; FOYE *et al*., 2006).

Differences ($p<0.05$) were observed in cecum ($Y = 0.1952x^2 - 1.039x + 10.927$, $R^2 = 0.78$), with IOF chicks at 7 days presented better cecum development (Table 7). This great development of cecum may be associated to microbiota development from the gradual stimuli provided by exogenous diet (UNI *et al*., 2005).

Tako *et al*. (2004) affirm that the supplementation in ovo improve the post-hatch development of chicks, mainly in the intestinal region after 48 hours at seven days post-hatch. Previous studies reported that the IOF has its most significant results on development of the gastrointestinal tract. This supplementation may increase the digestion capacity and intestinal absorption from chemical stimuli, causing and additional development of muscle tissues and, consequently, the body conformation (FOYE *et al*., 2007; LEITÃO *et al*., 2008).

CONCLUSIONS

The results of this study indicated that IOF using L-lysine directly affect the hatching characteristics of injected eggs. Using 0.5% and 1.0% of L-lysine to IOF there was a positive effect on chick weight, heart, pancreas, and performance from one to 7 days post-hatch, without negative affect chick/egg relation, and gastrointestinal tract development.

Table 5. Effect of in ovo feeding using L-lysine on gastrointestinal development (regions).

Treatments	Variables				
	Oropharynges + Oesophagus (cm)	Duodenal Handle (cm)	Jejunum + Ileum (cm)	Cecum (cm)	Colon + Rectum (cm)
Control	7.82	5.87	26.00	6.30	5.32
IOF Control	7.85	4.90	26.62	6.32	5.60
0.5 NaCl + 0.5 Lysine	7.12	6.12	22.87	7.75	7.12
0.5 NaCl + 1.0 Lysine	7.87	6.87	24.87	7.12	8.12
0.5 NaCl + 1.5 Lysine	8.00	6.25	26.00	6.85	6.12
0.5 NaCl + 2.0 Lysine	6.50	5.12	21.75	6.50	7.50
0.5 NaCl + 2.5 Lysine	6.70	4.77	25.45	6.37	6.85
p-value	0.25	0.13	0.52	0.27	0.13
Effect	ns	ns	ns	ns	ns
CV (%)	14.24	20.21	15.54	13.65	22.52

CV – Coefficient of variation. p-value – Coefficient of probability. ns – non significant.

Table 6. Effect of in ovo feeding using L-lysine on performance of chicks from one to seven days.

Treatments	Variables		
	Feed Intake (g/bird/day)	Weight gain (g/bird)	Weight gain percentage (%)
Control	9.50	5.26	10.70
IOF Control	11.68	4.46	9.23
0.5 NaCl + 0.5 Lysine	10.31	4.73	9.73
0.5 NaCl + 1.0 Lysine	9.92	5.09	10.26
0.5 NaCl + 1.5 Lysine	9.81	4.30	9.32
0.5 NaCl + 2.0 Lysine	7.43	4.46	9.13
0.5 NaCl + 2.5 Lysine	7.33	5.20	10.36
p-value	0.01	0.65	0.01
Effect	Q	ns	Q
CV (%)	10.16	13.97	11.26

CV – Coefficient of variation. p-value – Coefficient of probability. Q – Quadratic ($p<0.05$). ns – non significant ($p>0.05$).

Table 7. Effect of in ovo feeding using L-lysine on gastrointestinal tract of chicks at seven days (organs).

Treatments	Variables				
	Heart (g)	Liver (g)	Pancreas (g)	Pro-ventricle (g)	Gizzard (g)
Control	0.65	2.97	0.34	0.67	4.43
IOF Control	0.63	3.17	0.30	0.71	4.83
0.5 NaCl + 0.5 Lysine	0.60	3.42	0.30	0.83	3.72
0.5 NaCl + 1.0 Lysine	0.61	3.64	0.34	0.77	4.89
0.5 NaCl + 1.5 Lysine	0.62	3.11	0.25	0.71	4.32
0.5 NaCl + 2.0 Lysine	0.58	3.73	0.40	0.71	5.39
0.5 NaCl + 2.5 Lysine	0.64	3.59	0.37	0.67	5.03
p-value	0.90	0.45	0.28	0.77	0.36
Effect	ns	ns	ns	ns	ns
CV (%)	17.54	17.51	25.27	22.48	21.90

CV – Coefficient of variation. p-value – Coefficient of probability. ns – non significant ($p>0.05$).

Table 8. Effect of in ovo feeding using L-lysine on gastrointestinal tract of chicks at seven days (regions).

Treatments	Variables				
	Oropharynges + Oesophagus (cm)	Duodenal Handle (cm)	Jejunum + Ileum (cm)	Cecum (cm)	Colon + Rectum (cm)
Control	10.87	11.62	55.12	10.87	6.37
IOF Control	10.75	11.62	51.50	8.25	8.87
0.5 NaCl + 0.5 Lysine	10.62	11.75	57.25	10.37	8.75
0.5 NaCl + 1.0 Lysine	10.50	9.50	61.25	8.87	8.25
0.5 NaCl + 1.5 Lysine	9.87	11.62	55.50	10.87	6.75
0.5 NaCl + 2.0 Lysine	8.75	11.12	59.00	13.25	7.25
0.5 NaCl + 2.5 Lysine	10.00	12.25	57.75	12.25	6.00
p-value	0.14	0.35	0.31	0.01	0.06
Effect	ns	ns	ns	Q	ns
CV (%)	10.82	14.35	9.80	14.88	20.02

CV – Coefficient of variation. p-value – Coefficient of probability. Q – Quadratic ($p<0.05$). ns – non significant ($p>0.05$).

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