ISSN 1809-4678

# Effect of in ovo feeding of L-glutamine + ISP to chick embryos

*[L-glutamina consorciada com PIS na alimentação in ovo de embriões avícolas]*

**"Artigo Científico/Scientific Article"**

João Paulo Ferreira **Rufino**[1*], Frank George Guimarães **Cruz**[2], Valcely da Rocha **Costa**[1], André Ferreira **Silva**[1], Pedro Alves de **Oliveira Filho**[1], Lucas Duque **Melo**[3], Natalia dos Santos **Bezerra**[3]

[1]Graduate Program in Animal Science, Faculty of Agrarian Sciences, Federal University of Amazonas, Manaus-AM, Brazil.
[2]Department of Animal and Vegetable Production, Faculty of Agrarian Sciences, Federal University of Amazonas, Manaus-AM, Brazil.
[3]Faculty of Agrarian Sciences, Federal University of Amazonas, Manaus-AM, Brazil.
[*]Autor para correspondência/Corresponding author: E-mail: joaopaulorufino@live.com

**Abstract**

In ovo feeding (IOF) is the biotechnology that injects nutrients into the amnion of the avian embryo may cause an increase in hatchability and gastrointestinal development, and affecting the serum metabolism. This hypothesis was evaluated using 5 IOF solutions containing L-glutamine and L-glutamine + ISP (Isolated Soy Protein). 300 fertile eggs Rhode Island Red (breeders with 43-weeks) were distributed in a completely randomized experimental design with the treatments constituted by two controls (intact egg and IOF control with saline solution) and three solutions containing L-glutamine and L-glutamine + ISP, with 60 replicates (eggs) each. Data collected were subjected to the Tukey test at 5% of significance. There was a significantly lower ($p<0.01$) in hatchability from the IOF of L-glutamine + ISP, and a consequent increase in the intermediary embryo mortality. The yolk sac was higher ($p<0.01$) in chicks subjected to solutions containing 0.5% L-glutamine and 0.5% L-glutamine + 1.0% ISP, whereas all in ovo embryos presented great ($p<0.05$) development of heart and pancreas than intact eggs. In ovo fed embryos at 0.5% L-glutamine and 0.5% L-glutamine + 1.0% ISP presented great developed of gastrointestinal tract than intact eggs. In conclusion, the results of this study indicated that 0.5% L-glutamine may be used to IOF in chick embryos without a negative influence on chick weight and gastrointestinal tract development, acting as a metabolism regulator and obtaining better hatchability.

**Keywords**: amino acid; biotechnology; embryo mortality; hatchability; in ovo feeding.

**Resumo**

O objetivo deste estudo foi avaliar soluções contendo L-glutamina e L-glutamina consorciada com Proteína Isolada de Soja (PIS) para fins de alimentação in ovo de embriões avícolas. Foram utilizados 300 ovos férteis da linhagem Rhode Island Red. O delineamento experimental foi o inteiramente casualizado com os tratamentos constituídos de dois controles experimentais (ovo íntegro e inoculação controle) e três soluções contendo L-glutamina e L-glutamina + PIS, com 60 ovos (repetições) cada. Os dados coletados foram submetidos ao teste de Tukey a 5%. Foram observadas diferenças ($p<0,01$) nos resultados da eclodibilidade e mortalidade intermediária, com uma significativa redução de eclodibilidade a partir da utilização da alimentação in ovo de L-glutamina + PIS e, consequentemente, aumento da mortalidade intermediária. O saco vitelínico foi maior ($p<0,01$) após a alimentação in ovo dos embriões com 0,5% de L-glutamina e 0,5% de L-glutamina + 1,0% de PIS, enquanto que todos os embriões que receberam alimentação in ovo demonstraram maior desenvolvimento do coração e pâncreas ($p<0,05$) em comparação aos grupos controle. O desenvolvimento gastrointestinal foi maior ($p<0,05$) após a alimentação in ovo com 0,5% de L-glutamina e 0,5% de L-glutamina + 1,0% de PIS, onde estes embriões apresentaram maior desenvolvimento gastrointestinal em comparação aos grupos controle. Os resultados deste estudo indicam que 0,5% de L-glutamina pode ser utilizada para alimentação in ovo sem influência negativa sobre o peso do pinto e o

Recebido 15 de janeiro de 2018. Aceito 08 de janeiro de 2020.
DOI: https://doi.org/10.26605/medvet-v13n4-3669

desenvolvimento do trato gastrointestinal, atuando como um regulador metabólico e obtendo melhor eclodibilidade.

**Palavras-chave:** aminoácido, biotecnologia, eclodibilidade, mortalidade embrionária.

## Introduction

During the incubation period and in the first hours after hatching, the avian embryos present limited digestive functions, which reduces the nutrient's availability to growth metabolism, restricting its digestive capacity that begins the development when the amniotic fluid is orally consumed at 17 days of incubation (Uni et al., 2005). It is important to mention that even the nutritional egg composition being considered complete, the percentages of amino acids, carbohydrates, vitamins, minerals, and lipids are only sufficient to meet the initial stage of incubation period (1 to 7 days), being low to meet the requirements of intermediary and final stage of incubation period (15 to 21 days), and during the hatching period (Gonzales et al., 2013).

In this sense, the in ovo feeding (IOF) (US Patent 6,592,878) of Uni and Ferket (2003) is a biotechnology that aim to help the incubation process, being an alternative method for poultry industry to improve their results, especially because a simple increase in hatchability may represent a great economic impact (Ipek et al., 2004). The IOF involves the administration of exogenous nutrients into the amnion region of embryos of chickens and turkeys at 17 and 23 days of incubation, respectively (Foye et al., 2006). These substances may act as nutritional supplements, increasing the activity of digestive enzymes and the size of villi, and improving the development of the gastrointestinal tract during the embryo development (Geyra et al., 2001).

The use of substances to IOF such as amino acids and extracted/isolated proteins may be beneficial to the embryo due to its function to growth metabolism. Physiologically, these substances are not considered the main energy source to muscle contraction, but act as an important energy source for the skeletal muscle during metabolic stress (Nelson and Cox, 2014; Damasceno et al., 2017), providing nutritional support to the embryo and assisting in the final stage of embryo development.

The present study examined the hypothesis that IOF of L-glutamine and L-glutamine + ISP may positively affect the hatchability and provide a better development of the gastrointestinal tract of chick embryos.

## Material and Methods

This study was conducted in the Poultry Technology Laboratory, Poultry Sector of Faculty of Agrarian Sciences, Federal University of Amazonas, Manaus, Amazonas, Brazil. 300 fertile eggs of Rhode Island Red strain (breeders with 42-weeks) were distributed in a completely randomized design, where the treatments (Table 1) were constituted by intact egg, IOF control (saline solution), and three solutions containing L-glutamine and L-glutamine + ISP with 60 replicates (eggs) each.

**Table 1.** Experimental solutions with L-glutamine and L-glutamine + ISP.

| Treatments | Solutions |
|---|---|
| Intact egg | - |
| IOF Control | 0.5% of NaCl |
| Solution 1 | 0.5% of NaCl + 0.5% of L-glutamine |
| Solution 2 | 0.5% of NaCl + 0.5% of L-glutamine + 1.0% of ISP |
| Solution 3 | 0.5% of NaCl + 0.5 % of L-glutamine + 2.0% of ISP |

The eggs were identified, 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 16 days of incubation, the fertile eggs were sanitized and drilled in the air chamber. The solutions (0.5 mL) were injected into the amniotic fluid using needle syringes (7 x 2.5 mm). The hole in the eggshell was closed using melted paraffin. 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).

Immediately post-hatch, the chicks were weighed before sacrifice for sample collection, and hatch weight was recorded. Was evaluated the hatchability (percentage of birth chicks per fertile eggs injected), intermediary mortality (percentage

of dead embryos among 16 and 18 days of incubation), late mortality (percentage of dead embryos among 19 and 21 days of incubation without pecked the eggshell), pipped eggs (percentage of dead embryos among 19 and 21 days of incubation that pecked the eggshell), and proportion of chick weight per its respective egg weight.

From hatchability results, five able 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 + oesophagus, duodenal loop, jejunum + ileum, cecum, and colon + rectum).

A completely randomized block design was applied in this study. Data collected were subjected to one-way ANONA in Statistical Analysis System (SAS Inc., Cary, NC), and subsequently Tukey test. Significant differences were found when the possibility value (p-value) was less than 0.05.

## Results

Differences ($p<0.01$) were observed in hatchability and intermediary mortality, where hatchability was lower in eggs injected with L-glutamine + ISP (1 and 2 %). There was a significant increase in embryo mortality, especially in the intermediate mortality (embryos that died hours after IOF procedures) (Table 2). From these results, it was possible to determine the point of embryo physiological limit in a graphic representation using the curves of hatchability and intermediary embryo mortality (Figure 1).

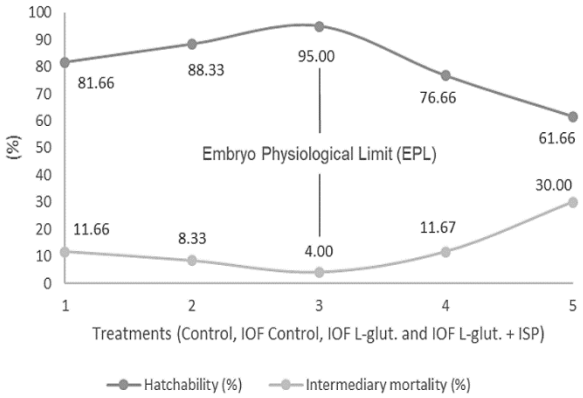


**Figure 1**. The behavior of IOF using L-glutamine and L-glutamine + ISP on hatchability and intermediary mortality. All data represent the mean value per treatment. The most distance of the curves determined the graphic point of embryo physiological limit.

However, embryos subjected to solutions of IOF Control and with 0.5% of L-glutamine presented higher hatchability than the control group (intact egg), with lower ($p<0.05$) late mortality and better ($p<0.01$) results in chick weight and chick-egg correlation.

**Table 2**. The effects of IOF of L-glutamine and L-glutamine + ISP on hatchability and embryo mortality.

| Treatments | Hatchability (%) | Intermediary mortality (%) | Late mortality (%) | Pipped eggs (%) | Chick weight (g) | Chick-Egg Correlation |
|---|---|---|---|---|---|---|
| Control | 81.66$^{b}$ | 11.66$^{b}$ | 3.35$^{b}$ | 3.33 | 33.13$^{b}$ | 0.61$^{b}$ |
| IOF Control | 88.33$^{ab}$ | 8.33$^{ab}$ | 1.66$^{ab}$ | 1.68 | 34.37$^{ab}$ | 0.64$^{ab}$ |
| Solution 1 | 95.00$^{a}$ | 4.00$^{a}$ | 0.00$^{a}$ | 1.00 | 35.47$^{a}$ | 0.65$^{a}$ |
| Solution 2 | 76.66$^{b}$ | 11.67$^{b}$ | 6.67$^{c}$ | 5.00 | 32.63$^{bc}$ | 0.60$^{bc}$ |
| Solution 3 | 61.66$^{c}$ | 30.00$^{c}$ | 6.68$^{c}$ | 1.66 | 31.49$^{c}$ | 0.58$^{c}$ |
| p-value | 0.01 | 0.01 | 0.04 | 0.57 | 0.01 | 0.01 |
| Effect | * | * | ** | ns | * | * |
| CV (%) | 3.70 | 12.35 | 14.75 | 18.29 | 3.87 | 3.84 |

CV - Coefficient of variation. p-value - Coefficient of probability. * Means followed by lowercase letters in column differ in 1% by Tukey test (P<0.01); ** Means followed by lowercase letters in column differ in 5% by Tukey test (P<0.05); ns – non-significant.

The yolk sac was higher ($p<0.01$) in chicks subjected to solutions containing 0.5% L-glutamine and 0.5% L-glutamine + 1.0% ISP, whereas all in ovo embryos presented great ($p<0.05$) development of heart and pancreas than intact eggs (Table 3).

The development of gastrointestinal tract was higher ($p<0.05$) in embryos subjected to IOF (control, L-glutamine or L-glutamine + ISP), where in ovo fed embryos at 0.5% L-glutamine and 0.5% L-glutamine + 1.0% ISP presented great development in oropharynx + oesophagus ($p<0.05$), duodenal loop ($p<0.01$) and colon + rectum ($p<0.05$) than chicks from intact eggs (Table 4).

The other regions of the gastrointestinal tract were not affected by (p>0.05) by IOF. However, was observed an increase in several organs (Table 3) and regions (Table 4) of the gastrointestinal tract of embryos subjected to IOF.

**Table 3**. The effects of IOF of L-glutamine and L-glutamine + ISP on gastrointestinal development (organs).

| Treatments | Yolk sac (g) | Heart (g) | Liver (g) | Pancreas (g) | Pro-ventricle (g) | Gizzard (g) |
|---|---|---|---|---|---|---|
| Control | 4.45$^{b}$ | 0.28$^{b}$ | 0.89 | 0.02$^{b}$ | 0.37 | 2.36 |
| IOF Control | 4.78$^{b}$ | 0.29$^{ab}$ | 0.91 | 0.03$^{ab}$ | 0.38 | 1.93 |
| Solution 1 | 6.03$^{a}$ | 0.30$^{a}$ | 0.90 | 0.04$^{a}$ | 0.32 | 2.24 |
| Solution 2 | 5.05$^{ab}$ | 0.29$^{ab}$ | 1.02 | 0.03$^{ab}$ | 0.33 | 1.89 |
| Solution 3 | 3.14$^{c}$ | 0.26$^{c}$ | 0.90 | 0.02$^{b}$ | 0.32 | 2.02 |
| p-value | 0.04 | 0.03 | 0.78 | 0.02 | 0.39 | 0.10 |
| Effect | * | ** | ns | ** | ns | ns |
| CV (%) | 19.05 | 13.97 | 18.11 | 14.76 | 18.12 | 14.65 |

CV - Coefficient of variation. p-value - Coefficient of probability. * Means followed by lowercase letters in column differ in 1% by Tukey test (P<0.01); ** Means followed by lowercase letters in column differ in 5% by Tukey test (P<0.05); ns – non-significant.

**Table 4**. The effects of IOF of L-glutamine and L-glutamine + ISP on gastrointestinal development (regions).

| Treatments | Gastrointestinal tract (cm) | Oropharynx + oesophagus (cm) | Duodenal loop (cm) | Jejunum + ileum (cm) | Cecum (cm) | Colon + rectum (cm) |
|---|---|---|---|---|---|---|
| Control | 43.20$^{bc}$ | 6.80$^{b}$ | 6.30$^{b}$ | 26.60 | 7.40 | 4.00$^{a}$ |
| IOF Control | 47.60$^{ab}$ | 7.20$^{ab}$ | 6.70$^{ab}$ | 32.30 | 7.70 | 3.60$^{ab}$ |
| Solution 1 | 47.70$^{a}$ | 7.40$^{a}$ | 7.50$^{a}$ | 28.00 | 7.80 | 2.90$^{b}$ |
| Solution 2 | 43.60$^{b}$ | 6.60$^{b}$ | 6.10$^{bc}$ | 26.50 | 7.10 | 2.70$^{b}$ |
| Solution 3 | 42.80$^{c}$ | 6.10$^{bc}$ | 5.90$^{c}$ | 24.60 | 7.20 | 2.64$^{b}$ |
| p-value | 0.05 | 0.05 | 0.01 | 0.09 | 0.24 | 0.05 |
| Effect | ** | ** | * | ns | ns | ** |
| CV (%) | 8.54 | 9.62 | 15.00 | 15.57 | 10.32 | 11.72 |

CV - Coefficient of variation. p-value - Coefficient of probability. * Means followed by lowercase letters in column differ in 1% by Tukey test (P<0.01); ** Means followed by lowercase letters in column differ in 5% by Tukey test (P<0.05); ns – non-significant.

## Discussion

Our results pointed that the use of IOF increased the hatchability (IOF Control and at 0.5% L-glutamine) and the development of the gastrointestinal tract, attributing these results to the increase in available nutrients to the chicks during the embryo development. Based on positive preliminary studies using amino acids and proteins (Pedroso et al., 2006; Damasceno et al., 2017), the first objective of IOF is to increase the concentration of available nutrients and improve the embryo development of chicks (Foye et al., 2007). However, Damasceno et al. (2017) reported that high levels of substances using to IOF may cause a gradual reduction in hatchability, and a subsequent increase in embryo mortality, especially the intermediary embryo mortality (shortly after inoculation).

The IOF may solve growth restrictions imposed by limited intestinal function in the neonate avian, increasing the nutrients that stimulate the consumption of enteric modulators (compounds that stimulate the development and metabolism of the digestive system cells) in the amnionic fluid, and enhancing the capacity of gut to digest and absorb dietary nutrients in the final stage of embryo development (Uni and Ferket, 2003; Tako et al., 2004; Foye et al., 2007). According to Ohta and Kidd (2001) and Jochemsen and Jeurissen (2002), several factors may cause high embryo mortality after IOF, like age, weight and time of egg storage, egg place to IOF, age of breeder among others. And depending on the profile of nutrients supplied to the embryo, there will be different embryos responses reflected in this post-IOF development.

Several studies about IOF using CHO presented a better development and functional maturity of gut, where the IOF work as a tool to overcome growth problems imposed by limited digestive capacity in hatchlings, enhancing intestinal function and maturation prior to hatching

(Tako et al., 2004; Foye et al., 2006; Leitão et al., 2010; Leitão et al., 2014). On the other hand, the use of amino acids to IOF may present a positive relationship with the protein synthesis, available amino acid concentration (Jepson et al., 1988, Welborne, 1995; Maiorka et al., 2000; Silva et al., 2007), and an affinity with the growth hormone (Ray et al., 2003) that starting it synthesis during the embryo development (Harvey et al., 2001).

Our data imply that the use of 0.5% of L-glutamine to IOF may act as an enteric modulator that enhances intestinal absorption due to the significant development of organs and regions of the gastrointestinal tract. Some studies have demonstrated that the intestinal amino acids activity (Karasov et al., 1987; Torras-Llort et al., 1998) and glucose transporters (Diamond and Karasov, 1987; Karasov et al., 1987; Solberg and Diamond, 1987; Buddington and Diamond, 1989; Ferraris and Diamond, 1989; Ferraris et al., 1992) are regulated by the presence of increasing concentrations of their specific precursors or substrate(s).

Another important question related to IOF is the embryo physiological limit, which corresponds to the meeting of graphic curves of hatchability and intermediary embryo mortality. This point represents the limit of the embryo organism in accepting the exogenous nutrient, substance or solution, mainly due to the clash among their osmolarities. However, this meeting does not need to be an intersection between the curves. The simple behavior (in the opposite way) of the curves can determine a maximum limit of the relationship between hatchability and intermediary embryo mortality, according to was observed in this study.

Uni and Ferket (2003) also affirmed that solutions that present high concentrations of exogenous nutrients may affect the egg osmotic balance, and consequently, the embryo development, suggesting that the lower hatchability and high intermediary mortality of embryos subjected to high levels of amino acid or protein substances, as was verified in the results obtained using ISP, could be caused by osmotic balance changes. It was observed that the use of ISP was unfavorable to the metabolism of the embryos. The results pointed that the organism of this embryos has reached its physiological limit, not supporting this concentration of exogenous nutrients, causing high embryo mortality.

## Conclusion

In conclusion, the results of this study indicated that 0.5% L-glutamine may be used to IOF in chick embryos without negative influence on chick weight and gastrointestinal tract development, acting as metabolism regulator and obtaining better hatchability.

## Conflict of Interest

The authors declare no conflict of interest.

## Ethics Committee

Animal care procedures followed guidelines established by the Federal University of Amazonas Ethical Committee for Animal Research (016/2016).

## References

Buddington, R.K.; Diamond, J.M. Ontogenetic development of intestinal nutrient transporters. **Annual Review of Physiology**, 51: 601–619, 1989.

Damasceno, J.L.; Cruz, F.G.G.; Melo, R.D.; Feijo, J.C.; Rufino, J.P.F.; Valentim, F.M.; Oliveira, J.P.C. Inoculação de proteína isolada de soja em ovos embrionados oriundos de matrizes semipesadas com diferentes idades. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, 69(5): 1259-1266, 2017.

Diamond, J.M.; Karasov, W.H. Adaptive regulation of intestinal nutrient transporters. **Proceedings of the National Academy of Sciences USA**, 84: 2242–2245, 1987.

Ferraris, R.P.; Diamond, J.M. Specific regulation of intestinal nutrient transporters by their dietary substrates. **Annual Review of Physiology**, 51: 125–141, 1989.

Ferraris, R.P.; Villenas, S.A.; Hirayama, B.A.; Diamond, J.M. Effect of diet on glucose transporter site density along the intestinal crypt-villus axis. **American Journal of Physiology**, 262: 1060-1068, 1992.

Foye, O.T.; Uni, Z.; Ferket, P.R. Effect of In Ovo Feeding Egg White Protein, β-Hydroxy-β-Methylbutyrate, and Carbohydrates on Glycogen Status and Neonatal Growth of Turkeys. **Poultry Science**, 85(7): 1185-1192, 2006.

Foye, O.T.; Uni, Z.; Ferket, P.R. 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. **Poultry Science**, 86(12): 2343–2349, 2007.

Geyra, A.; Uni, Z.; Sklan, D. Enterocyte dynamics and mucosal development in the posthatch chick. **Poultry Science**, 80(6): 776-782, 2001.

Gonzales, E.; Cruz, C.P.; Leandro, N.S.M.; Strighini, J.H.; Brito, A.B. In Ovo Supplementation of 25(OH)$D_3$ to Broiler Embryos. **Brazilian Journal of Poultry Science**, 15(3): 169-286, 2013.

Harvey, S.; Johnson, C.D.M.; Sanders, E.J. Growth hormone in neural tissue of the chick embryo. **Journal of Endocrinology**, 169(3): 487-496, 2001.

Ipek, A.; Sahan, U.; Yilmaz, B. The effects of in ovo ascorbic acid and glucose injection in broiler breeder eggs on hatchability and chick weight. **Archives Geflugelk**, 68: 132-135, 2004.

Jepson, M.M.; Bates, P.C.; Broadbent, P.; Pell, J.M.; Millward, D.J. Relationship between glutamine concentration and protein synthesis in rat skeletal muscle. **American Journal of Physiology**, 255(2): 166-172, 1988.

Jochemsen, P.; Jeurissen, S.H.M. The location and uptake of in ovo injected soluble and particulate substances in the chicken. **Poultry Science**, 81: 1811-1817, 2002.

Karasov, W.H.; Solberg, D.H.; Diamond, J.M. Dependence of intestinal amino acid uptake on dietary protein or amino acid levels. **American Journal of Physiology**, 5: 252: 614-625, 1987.

Leitão, R.A.; Leandro, N.S.M.; Stringhini, J.H.; Café, M.B.; Andrade, M.A. Inoculação de maltose, sacarose ou glicose em ovos embrionados de baixo peso. **Acta Scientiarum. Animal Sciences**, 32(1): 93-100, 2010.

Leitão, R.A.; Leandro, N.S.M.; Stringhini, J.H.; Café, M.B.; Matos, M.S.; Andrade, M.A. Inoculação de maltose e/ou sacarose em ovos leves embrionados. **Ciência Animal Brasileira**, 15(1): 55-63, 2014.

Maiorka, A.; Silva, A.V.F.; Santin, E.; Borges, S.A.; Boleli, I.C.; Macari, M. Influência da suplementação de glutamina sobre o desempenho e o desenvolvimento de vilos e criptas do intestino delgado de frangos. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, 52(5): 487-490, 2000.

Nelson, D.L.; Cox, M.M. **Princípios de Bioquímica de Lehninger**. 6ª Ed. Porto Alegre: Artmed, 2014. 1328p.

Ohta, Y.; Kidd, M.T. Optimum site for in ovo amino acids injection in broiler breeders' eggs. **Poultry Science**, 80: 1425-1429, 2001.

Pedroso, A.A.; Chaves, L.S.; Lopes, K.L.A.M.; Leandro, N.S.M.; Café, M.B.; Stringhini, J.H. Inoculação de nutrientes em ovos de matrizes pesadas. **Brazilian Journal of Poultry Science**, 35(5): 2018-2026, 2006.

Ray, E.C.; Avissar, N.E.; Vukcevic, D.; Toia, L.; Ryan, C.K.; Berlanga-Acosta, J.; Sax, H.C. Growth hormone and epidermal growth factor together enhance amino acid transport systems B and A is Remnant small intestine after massive enterectomy. **Journal of Surgical Research**, 115(1): 164-170, 2003.

Silva, A.V.F.; Borges, S.A.; Maiorka, A.; Giviziez, P.E.N.; Rocha, C.; Macari, M. Ornitine Decarboxylase expression in the small intestine of broilers submitted to feed restriction and glutamine supplementation. **Brazilian Journal of Poultry Science**, 9(2): 111-116, 2007.

Solberg, D.H.; Diamond, J.M. Comparison of different dietary sugars as inducers of intestinal sugar transporters. **American Journal of Physiology**, 252(4): 574-584, 1987.

Tako, E.; Ferket, P.R.; Uni, Z. Effects of in ovo feeding of carbohydrates and $\beta$-hydroxy-$\beta$-methylbutyrate on the development of chicken intestine. **Poultry Science**, 83(12): 2023-2028, 2004.

Torras-Llort, M.; Soriano-Garcia, J.F.; Ferrer, R.; Moreto, M. Effect of a lysine-enriched diet on L-lysine transport by the brush-border membrane of the chicken jejunum. **American Journal of Physiology**, 274(1): 69-75, 1998.

Uni, Z.; Ferket, P. **Enhancement of development of oviparous species by in ovo feeding**. US Regular Patent US 6,592,878 B2. North Carolina State Univ., Raleigh and Yissum Res. Dev. Co., Hebrew Univ. Jerusalem, Israel, 2003.

Uni, Z.; Ferket, P.R.; Tako, E.; Kedar, O. In ovo feeding improves energy status of late-term chicken embryos. **Poultry Science**, 84(5): 764-770, 2005.

Welborne, T.C. Increased plasma bicarbonate and growth hormone after an oral glutamine load. **American Journal of Clinical Nutrition**, 61(5): 1058-1061, 1995.