Biological Forum – An International Journal ***(Special Issue 2015)*** **7**(2): 23-26(2015)

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Effect of in ovo injection of Methionine on the histomorphometry of Jejunum of chicken embryo

Hamideh Mohammadrezaei*, Mohammadnaser Nazem** and Maryam Mohammadrezaei***
**DVM, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.*
***Assistant Professor in Department of Basic Science,*
Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran
****Ph.D. student, Department of Chemistry, Islamic Azad University, Yazd, Iran*

(Corresponding author: Maryam Mohammadrezaei)
(Received 15May, 2015, Accepted 07 July, 2015)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: In this study, the effect of injected methionine in the yolk sac, on the histomorphometry of chicken embryonic Jejunum, is investigated. Poultry industry has gotten advantage from various dimensions such as nutrition, genetics and management in order to reach the maximum rate of growth and efficiency of meat production. Today, in poultry, in order to enhance hatchability, improved growth performance, immune response, birth weight and organogenesis, different materials are injected into embrocated eggs. Fifty eggs of broiler chicken (ROSS 308) with equal average weight were randomly divided into five groups and entered the incubation machine at the same time. After carrying out a series of Steps, 1cm of Jejunum was sampled in order to have a histomorphometric study of the length and width of villi and depth of crypt. The results expressed that in ovo injection of this essential amino acid can make some changes in height and width of villi and accordingly absorption.

Key words: Methionine, Jejunum, Chicken embryo, villi, small intestine

INTRODUCTION

Because the intestine is a major supporting organ, the sooner it canachieve its maximum functional capacity, the more quickly the capability and efficiency of using nutrients and resistance to metabolic and infectious diseases will increase in birds (Uni *et al*., 2004).

In recent decades, in order to enhance the speed and performance of broiler growth, substantial progress has occurred.

Today, the 21-day incubation period and the first week after hatching, include about 50 percent of the total life cycle of broiler, while 20 years ago this period was about 25 to 30 percent (Hulet, 2007).

One of these methods is adding up growth stimulating antibiotics in the diet which are consumed to increase intestinal development, chicken growth and uniformity of flockweight.

However, in recent years due to antibiotic resistance as well as increased demand for antibiotic free products, the requirement for researching alternatives affecting non-antibiotic growth, has increased.

Methionine is one of non-antibiotic alternatives in the diet, which leads to an increase in growth. (Engster *et al*., 2002 and Huyghebaert *et al*., 2010).

But the effectiveness procedure of this amino acid on the intestinal villi, especially in poultry is not defined.

Since the intestine is the first absorptive area of nutrients and therefore will be sensitive to diet, changes and on the other hand, no studies has been conducted in this field in chicken embryo, the recent study was conducted.

Proteins and amino acids are essential components of growth and survival in embryonic period (Mcdonald *et al*. 2002).

It has been shown that amino acids are effective on the small intestine morphology (Vaezi *et al*., 2011).Amino acids are units of proteins formation which are divided to essential and non-essential groups.

Methionine is one of the most essential amino acids that contains sulfur.(Kalinowski *et al*., 2003 and Nazem *et al*.,)

In this study, the effect of injected methionine in the yolk sac, on the histomorphometry of Jejunum of chicken embryo is investigated.

MATERIALS AND METHODS

Fifty fertile eggs of broiler chicken (ROSS 308) with equal average weight were randomly divided into five groups and entered the incubation machine at the same time .At 4 d of incubation and after confirming the fertilization of eggs by candling them, the wide end of the eggs were disinfected with 70% Ethyl Alcohol and then a hole was created in every egg shell using a special needle.

Each group was then injected with suitable IO feed treatment solution, in accordance with standard procedures, with a 21-ga needle inserted into the yolk sac, which was identified by candling (Ohta 2001). 0.5 ml of sterile PBS was injected into the eggs of control group and the other 4 treatment groups received 0.5 ml of PBS solution containing the following: Treatment 1) 20 mg of methionine, Treatment 2) 30 mg of methionine, Treatment 3) 40 mg of methionine, Treatment 4) 50mg of methionine. After the eggs were injected, the injection holes were sealed withmolten paraffin, and eggs were placed in hatching trays. This is based on studies of other researchers (Al-Murrani, 1982; Dooley *et al*., 2011; Tako *et al*., 2004 and Ohta, 2001).

At 18 d of incubation, eggs were voided from embryos and then 1cm long segment of Jejunum, about 1cm before Meckel's diverticulum was sampled in order to have a histomorphometric study of the length and width of villi and depth of crypt (Fasina and Olowo, 2013; Yaghobfar, 2006; Uni *et al*., 1999; Shira *et al*., 2005 and Tako *et al*., 2004).

Samples were left in 10% buffered neutral formaldehyde solution and after 24 hours formaldehyde solution was replaced with new 10% formalin and samples were left in formalin for 10 days.

Then, after the process of fixation, Segments were taken from the jejunum and according to the method of Uni *et al*.,(1998) and Uni *et al*., (2001), Villus height (from the tip of the villi to the villus crypt junction) and villus width (at half height), were evaluated but the crypt depth could not be evaluated according to the embryonic nature of samples.

All data were compared with Control group with statistical software SPSS (16 Chicago. U.S.A) and ONE WAY ANOVA statistical method.

RESULTS AND DISCUSSION

According to the results, the average villus height (from the tip of villi to the villus crypt junction according to Uni *et al*., (1998) and (Uni *et al*., (1999)), of the jejunal samples of control, treatment 1 (receiving 20 mg methionine), treatment 2 (receiving 30 mg methionine), treatment 3 (receiving 40 mg methionine) and treatment 4 (receiving 50 mg methionine), were 214.48±2.42, 209.06±3.53, 223.04±3.52, 282.71±13.5 and 190.66±4.36 µm respectively. Accordingly, the most increase in villus height of jejunum was observed in treatment 3, which states the optimal dose for increased villus height.

As shown in Table 1, changes in height between groups, was only significant in treatment 3 compared to control and other groups (Table1). According to the results, the average maximum villus width of jejunum samples of control, treatment1, treatment 2, treatment3 and treatment 4 were respectively 51.06±3.87, 60.58±1.91, 69.64±2.01, 104.8±7.47 and 68.42±6.85 µm. Accordingly, the most width have been observed in treatment 3 which was significant compared to control and other groups (P<0.05) (Table 2).

Table 1: Villous height of Jejunum in different groups (micrometer).

(Mean±SE)	Groups
214.48±2.42[a]	Control
209.06±3.53[a]	Treatment1
223.04±3.52[a]	Treatment2
282.71±13.5[b]	Treatment3
190.66±4.36[a]	Treatment4

[abc] Means with different letters are significantly different (P<0.05)

Table 2: Villous width of Jejunum in different groups (micrometer).

(Mean±SE)	Groups
51.06±3.87[a]	Control
60.58±1.91[a]	Treatment1
69.64±2.01[a]	Treatment2
104.8±7.47[b]	Treatment3
68.42±6.85[a]	Treatment4

[abc] Means with different letters are significantly different (P<0.05)

DISCUSSION

In this study, IO injection of Methionine, enhanced jejunal development. Chick growth and development are dependent upon nutrient digestion and absorption, which is a direct result of functional and morphological development of the small intestine (Baranylova and Holman, 1976).

(In chickens, the first days after hatch are a critical period for development because a major change occurs in the source of nutrients as the yolk is replaced by an exogenous diet (Noy and Sklan, 1998). Intestinal tissue development can significantly improve chicken performance and also reduce bird diseases. In this case transmission of infectious diseases from birds to humans will decrease (Baranylova and Holman, 1976). Intestine, as the main interface between an organism and it's nutrition environment, plays a vital role in the after birth development of a new born animal (Noy and Sklan, 1995).

The growth of the chickens, is dependent on the digestion and absorption of nutrients, which is a direct result of the morphological and functional development of the small intestine(Baranylova and Holman, 1976).

Today, in poultry, in order to enhance hatchability, improved growth performance, immune response, birth weight and organogenesis, various nutrients are injected into the embryonated eggs (Dooley *et al*., 2011; Kadam *et al*., 2008; Kornasio *et al*. 2011and Zhai *et al*., 2011).

Many previous studies have been conducted about the effects of nutrients like dietary methionine and their effects on birds.In some of these studies, the effects of nutrition on digestive system have been demonstrated more precisely.

Measuring villus height, villus width, crypt depth and calculating villus length/crypt depth ratio are essential methods to study intestinal morphology and its functional capacity (fasina *et al*., 2010; Potten and Loeffler, 1990).

It has been proposed that a deeper crypt is indicative of a faster tissue turnover and perhaps, a higher demand for new tissue (Yanson *et al*., 1987). Furthermore, it has been certified that a high intestinal villus is associated with a well-differentiated intestinal mucosa with high digestive and absorptive capabilities (Jeurissen *et al*., 2002).

Schald *et al*., (1968) have studied and reported the absorption of methionine in the human small intestine.

Cook (1972) and Cummins (1952), have evaluated intestinal absorption of glucose and methionine and also glucose concentration in blood and lumen of small intestine.

Yang *et al*., (2013) have evaluated effects of different diets on growth performance,physiological parameters of digestive tract and apparent digestibility in geese.

Fasinaand Olowo (2013), have studied effects of a yeast (Maxigen) on intestinal villi morphology and growth performance of broiler chickens.

Foye *et al*., (2007), have studied Effects of Arginine, beta hydroxysteroid and betamethylbutyrate and protein absorption in the jejunum and its effect on activity in infants and fetuses in turkey poults.

Tako *et al*., (2004), have evaluated the effects of IOF of Carbohydrates and β-Hydroxy-β-Methylbutyrate,on the development of chicken intestine.

Bhattacharyya *et al*., (2007), have studied the effect of in ovo injection of glucose on growth, immunocompetence and development of digestive organs in turkey poults.

Morphology and development of small intestine is also studied in white Roman gosling.

In our study, the best morphological changes which actually isincreased villus height and width and villus area as a result, was seen in the amount of 40 mg methionine. But 50 mg methionine had no more effective result in spite of being more in terms of amount.

The reason for this situation may be related to toxic nature of high levels of methionine.

Previous studies have also shown that high levels of methionine, have negative effects on development of musculoskeletal system of chicken embryo (Asadollahzadeh, 2015)

It seems injecting essential amino acids into the yolk sac of chicken embryos, can have an increase in intestinal villus length and width and thus an increase in absorption and efficiency.

Few studies have been carried out to determine the time and region of injecting amino acids into embryonated eggs.

In these studies it has been declared that injections into the yolk sac of an embryo not only increases size of the embryo but also increases hatchability (Ohta, 2001).

Considering the height and width of jejunal villus, it seems that the usage of methionine in the yolk sac at 4 d of incubation, has caused an increase in the overall volume of jejunum.

Since enhancement in villus dimension leads to increased absorptive area, it seems that the optimal amount of methionine for injecting into the yolk sac to reach optimal morphology changes, is about 40 mg at d 4 of incubation.

ACKNOWLEDGEMENT

The authorsare thankful to department of Basic Science, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, for providing necessary laboratory facilities.

REFERENCES

Al-Murrani, W.K. (1982). Effect of injecting amino acids into the egg on embryonic and subsequent growth in the domestic fowl. *Journal of Br. Poult. Science.* **23**: 171-174.

Asadollahzadeh, M. (2015). A thesis of faculty of veterinary medicine, Shahid Bahonar University of Kerman, Iran. Effect of Methionine on histomprphometry and histopatholog of Tibia bone of chicken embryo. Number: 459.

Baranylova, E. & Holman, J. (1976). Morphological changes in the intestinal wall in fed and fasted chickens in the first week after hatching. *Acta. Vet. (Brno).* **45**: 151-158.

Bhattacharyya, A., Majumdar, S., Bhanja, S. K., Mandal, A. B., Dash, B. B. & Agarwal, S. K. (2007). Effect of in ovo injection of glucose on growth, immunocompetence and development of digestive organs in turkey poults. 16th European symposium on poultry nutrition.

Cook, G. C. (1972). Intestinal absorption rate of L-methionine in man and the effect of glucose in the perfusing fluid. *Journal of Physiol.* **221**: 707-714.

Cummins, Alvin J. (1952). Absorption of glucose and methionine from the human intestine; The influence of the glucose concentration in the blood and in the intestinal lumen. 928-937.

Dooley, M., Peebles, E.D., Zhai, W., Mejia, L., Zumwalt, C.D. & Corzo, A. (2011). Effects of L-carnitine via in ovo injection with or without L-carnitine feed supplementation on broiler hatchability and post-hatch performance. *Journal of Appl Poultry Res.* **20**: 491-497.

Engster, H.M., Marvil, D. & Stewart-Brown, B. (2002). The effect of withdrawing growth promoting antibiotics from broiler chickens: A long-term commercial industry study. *Journal of Appl. Poult. Res*. **11**: 431-143.

Fasina, Y.O. &Olowo, Y.L. (2013). Effect of commercial yeast-based product (Maxigen) on intestinal villi morphology and growth performance of broiler chickens. *International Journal of Poultry Science* **12**(1): 09-14.

Fasina, Y.O., Hoerr, J., McKee, S.R. & Conner, D.E. (2010). Influence of Salmonella entericaserovar Typhimurium Infection on Intestinal Goblet Cells and Villous Morphology in Broiler Chicks. *Avian Dis*. **54**: 841-847.

Foye, O. T., Ferket, P. R. & Uni, Z. (2007). 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. Journal of Poultry Science. 86:2343-2349.

Hulet, R. M. (2007). Managing incubation: where are we and why? *Journal of Poult. Sci.* **86**: 1017-1019.

Huyghebaert, G., Ducatelle, R. & Van Immerseel, F. (2010). An update on alternatives to antimicrobial growth promoters for broilers. *Vet. Journal.* 182-188.

Jeurissen, S.H.M., Lewis, F., Van der Klis, J.D., Mroz, Z., Rebel, J.M.J. & Huurne, A.A.H.M. (2002). Parameters and techniques to determine intestinal health of poultry as constituted by immunity, integrity and functionality. *Curr. Issues Intest. Microbiol.* **3**: 1- 14.

Kadam, M.M., Bhanja, S.K., Manda, A.B., Thakur, R., Vasan, P., Bhattacharyya, A. & Tyagi, J.S. (2008). Effect of in ovo threonine supplementation on early growth, immunological responses and digestive enzyme activities in broiler chickens. *Journal of Br. Poult. Science*. **49**: 736-741.

Kalinowski, A., Moran, E.T., Wyatt. Jr. and C. (2003). Methionine and Cysteine requirements of slow- and fast-feathering male broilers from zero to three weeks of age. *Journal of Poultry Science.* **82**: 1423-1427.

Kornasio, R., Halevy, O., Kedar, O., & Uni, Z. Effect of in ovo feeding and its interaction with timing of first feed on glycogen reserves, muscle growth, and body weight. (2011). *Poultry Science.* **90**: 1467-1477.

McDonald, P., Edwards, R. A., Greenhalgh, J. F. D. and Morgan, C. A. (2002). Animal Nutrition. 6th edition.

Nazem, M.N., Rezaian, M., Adib Moradi, M., Asadi Fuzi M., Kyaei, S.M.M and Razavi Ebrahim, P. (2013). The effect of oral administration of coated methionine on the female goats and suckling kids hair follicles. J.Vet . Res. 68(1): 61-68.

Noy, Y., Sklan, D. (1995). Digestion and absorption in the young chicks. *Poult Sci.* **74**: 366-373.

Noy, Y., Sklan, D. (1998). Yolk utilisation in the newly hatched poult. *Br. Poult Sci.* **39**: 446-451.

Ohta, Y. & Kidd, M. T. (2001). Optimum Site for In Ovo Amino Acid Injection in Broiler Breeder Eggs. *Poultry Science*. **80**: 1425-1429.

Potten, C.S. & Loeffler, M. (1990). Stem cells: Attributes, cycles, spirals, pitfalls and uncertainties. *Lessons from the crypt. Development.* **110**: 1001-1020.

Schald, P. Hiarold, Pierce Charles E., Ruer Alan, & Clifton James A. (1968). Absorption of L-Methionine from the Human Small Intestine. *The Journal of Clinical Investigation*, Vol. **47**: 417-425.

Shira, E.B., Sklan, D. & Friedman, A. (2005). Impaired immune responses in broiler hatchling hindgut following delayed access to feed. *Veterinary immunology and immunopathology*, **105**: 33-45.

Tako, E., Ferket, P. R. & Uni, Z. (2004). Effects of In Ovo Feeding of Carbohydrates and β-Hydroxy-β-Methylbutyrate on the Development of Chicken Intestine. *Poul Sci.* **83**: 2023-2028.

Uni, Z. &Ferket, P.R. (2004). Methods for early nutrition and their potential. World's Poult. *Science journal.* **60**: 101-111.

Uni, Z., Gal-Garber, O., Geyra, A., Sklan, D. & Yahav, S. (2001). Changes in growth and shihfunction of chick small intestine epithelium due to early thermal conditioning. *Journal of Poult. Science*. **80**: 438-445.

Uni, Z., Ganot, S. &Sklan, D. (1998). Post hatch development of mucosal function in the broiler small intestine. *Poult. Sci.* **77**: 75-82.

Uni, Z., Noy, Y., and Sklan, D. (1999). Post hatch development of small intestinal function in the poult. Poult. Sci. **78**: 215-222.

Vaezi, Gh., Teshfam, M., Bahadoran, Sh., Farazyan H. and Hosseini, S. (2011). Effects of different levels of lysine on small intestinal villous morphology in starter diet of broiler chickens. *Journal of Global Veterinaria* **7**(6): 523-526.

Yaghobfar, A., Rezaian, M., Ashrafi-Helan, J., Barin, J. & Fazaeli, H. (2006). The effect of hull less barley dietary on the activity of gut micro flora and morphology of small intestine of layer hens. *Pakistan Journal of Biological Sciences*. **9**(4): 659-666.

Yang, H. M., Zhou, X. L., Wang, Z. Y., Zou, J. M. & Cao, Y. J. (2013). Effects of different diets on growth performance, physiological parameters of digestive tract and apparent digestibility in geese. *African Journal of Biotechnology*. ***12***(11): 1288-1296.

Yanson, C.V. Summers, B.A. &Schat, K.A. (1987). Pathogenesis of notavirus infection in various age groups of chickens and turkeys: Pathology. *Am. Journal of Vet. Res.* **6**: 927-938.

Zhai, W., Rowe, D. E. & Peebles, E. D. (2011). Effects of commercial in ovo injection of carbohydrates on broiler embryogenesis. *Journal of Poultry Science.* **90**:1295-1301.