Effect of in Ovo Injected Methionine on Feather Follicle Formation and its Growth in the Chicken Embryo

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Article info:
Received: 26 Jan. 2015
Accepted: 13 Apr. 2015

Key Words:
Methionine, Hair follicle, Chicken embryo

ABSTRACT

Introduction: Feathers are important body structures that act as surface insulators to reduce maintenance energy requirements and also to protect skin against abrasions and infection. The purpose of this study is to investigate the effects of methionine injection into the yolk sac on development of feather follicle in chicken embryo.

Methods: 50 fertilized eggs (weighting 50±0.4 gr) of Ross×308 broiler chicks were randomly divided into five equal groups of 10 eggs each. On day 4 of incubation, 20, 30, 40 and 50 mg of methionine dissolved in 0.5 ml of phosphate buffered saline (PBS) was injected into the yolk sac of treatment groups 1 to 4, respectively. The control group was only injected with 0.5 ml of PBS. On the day 18 of incubation, the eggs were removed from the incubator and the embryos were killed humanely. Then, the samples were taken from the skin of thoracic region of each embryo for histometrical study.

Results: The results of this study indicated that the density of feather follicles increased significantly ($P<0.05$) in presence of 50 mg methionine. However, other methionine doses caused insignificant ($P>0.05$) increase in the number of feather follicles. A significant increase ($P<0.05$) was also observed in the feather follicle diameter when 40 and 50 mg of methionine were injected.

Conclusion: Administration of 50 mg methionine in 0.5 ml PBS via yolk sac in day 4 of incubation increased both density and diameter of feather follicle in chicken embryo significantly.

1. Introduction

Feathers are the most important body structures that provide heat insulation because they reduce maintenance energy needs and also prevent skin injuries and infection. Ideal feathering is considered important in modern broiler production due to high standards of carcass quality [1]. Poor feathering increases condemnations or downgrading of poultry's carcasses at slaughter process thus reduces benefit. In the broiler chicken, decrease feather growth can provoke feather picking and subsequently cannibalism [2]. Feathering affected by factors such as nutritional deficiencies, pharmaceuticals, toxicity and nutrient concentration [3].

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Essential amino acids, such as valine, arginine, lysine and methionine, have also been reported to affect feather growth and development [4,5]. Methionine is the major amino acid involved in the synthesis of feather keratin [5]. In studies of the feather growth so far reported, most attention has been focused on supplementation of amino acids in post hatch [6,7]. The period time for rearing broiler chicken is very fast, therefore, we need to promote feather follicle in embryonic stage. However the increase of feather follicle in ovo supplementation of amino acid has not been reported, so the aim of this study is to investigate in ovo injection of methionine on development of feather follicle in chicken embryo.

2. Materials and Methods

All experiments were performed in compliance with the Iranian Veterinary Organization ethic rules. According to the law, no specific authorizations needed for work performed in avian embryos before the time of hatching. Our experiments were terminated on developmental day 18, three days before hatching, by chilling the eggs on ice for 20 minutes.

We use Crystalline DL-methionine (99% purity, Evonik Degussa, German). Methionine solution was prepared by dissolving in phosphate buffered saline (PBS) at low heat (30 ˚C) in a water bath [9], then, injected solution was sterile by using a syringe filter (0.20 µm). The solution was protected in 4 ˚C for daily use.

57 Fertile eggs (weighting 50±0.4 gr) of Ross × 308 broiler chicks were obtained from a local commercial hatchery from a maternal flock 41 wk in lay. The eggs were incubated (18-day incubation period) under optimal condition (37.7 ˚C and 60 % relative humidity) in an incubator (Model PLC_ DQSH, V: 4; Belderchin Damavand, Iran). On day 4 of incubation, eggs were candled, and those that were infertile or contained early dead embryos were removed. Then fertile eggs were randomly divided into five equal groups of 10 eggs. The large end of the eggs (injection site) was sterilized prior to incubation with 70% ethanol. At this time, the yolk was identified by candling and was injected with 0/5 mL of in ovo feeding solution [8] using a 24G hypodermic needle (25mm long). Each treatment group received 20, 30, 40 and 50 mg methionine in 0.5 ml PBS respectively, and control group just received 0.5 ml PBS. Preliminary experiments conducted in our laboratory demonstrated that 10 mg methionine in 0.5 ml PBS had similar effect with control group and also doses above 50 mg were lethal for embryo. Needle punctures in the shell were sealed immediately with paraffin wax [8]. After that, eggs were returned to the incubator.

At the end of the experiment, on day 18 of incubation, the eggs were removed from the incubator and the embryos were killed by placing the eggs on the ice and then, eggs were opened at the wider end [10]. The skin samples of approximately 1.5 cm^2 were excised from the right and left of the thoracic region next to the latest rib near the mid line and stored in 10% buffered formalin for further histological processing. Following routine preparation of tissues, serial sections of paraffin embedded tissues of 5 µm thicknesses were cut using a microtome (Slee-Germany) and stained with haematoxylin and eosin and studied under light microscope. The right skin samples were cut in parallel with the skin surface, unlike to the left ones that were cut perpendicular to the surface.

The aim of these slices was to count the number of feather follicles in right skin samples, and to measure the diameter of follicles in left samples. By using a digital lens (Dino-eye, AM-7023, 5Mp, Taiwan), the number of feather follicles was counted from 10 fields of each cross section of the right thoracic skin at a magnification ×100, and an actual field area of 5.25 mm^2 (Figure 1). At a magnification ×400, and an actual field area of 0.325 mm^2, diameter of feather bulbs were measured from 10 fields from longitudinal sections of the left thoracic skin. Diameter of feather follicle was measured from the outermost layer of follicle where the maximum diameter was observed (Figure 2).

Results were analysed by One-way ANOVA using SPSS software (version 16, Chicago, USA). Differences between groups were compared by Tukey test following ANOVA,

Table 1. Feather follicle density in relation to methionine levels.

Group	Number of feather follicles Mean±SE
Control	6.43±1.43[a]
1	6.43±1.33[a]
2	6.86±1.24[a]
3	10.29±1.06[a]
4	15.86±1.14[b]

[a-b] Means within a column with different superscripts are significantly different (P<0.05).

Table 2. Diameter of feather follicles in relation to methionine levels.

Group	Diameter of feather follicle (µm) Mean±SE
Control	186±20.99[a]
1	227.86±6.74[ab]
2	235.87±12.1[ab]
3	280.28±11.76[bc]
4	312.68±0.11[cd]

[a-d] Means within a column with different superscripts are significantly different (P<0.05).

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and a P-value of <0.05 was considered as statistically significant. Results are reported as least squares means with standard errors.

3. Results

Feather follicle density in relation to increasing methionine levels are shown in Table 1. Increasing methionine levels from 20 to 40 mg in 0.5 ml PBS increased density of feather follicles compared with control group, but they were not significant (P>0.05), whereas in group 4 (50 mg Methionine in 0.5 ml PBS) the density of feather follicle increased significantly (P<0.05) compared with other groups.

Diameter changes of feather follicles in relation to different levels of methionine are shown in Table 2. Increasing methionine levels from 20 to 30 mg in 0.5 ml PBS, increased diameter of feather follicles, but were not significant (P>0.05). In group 3 (40 mg Methionine in 0.5

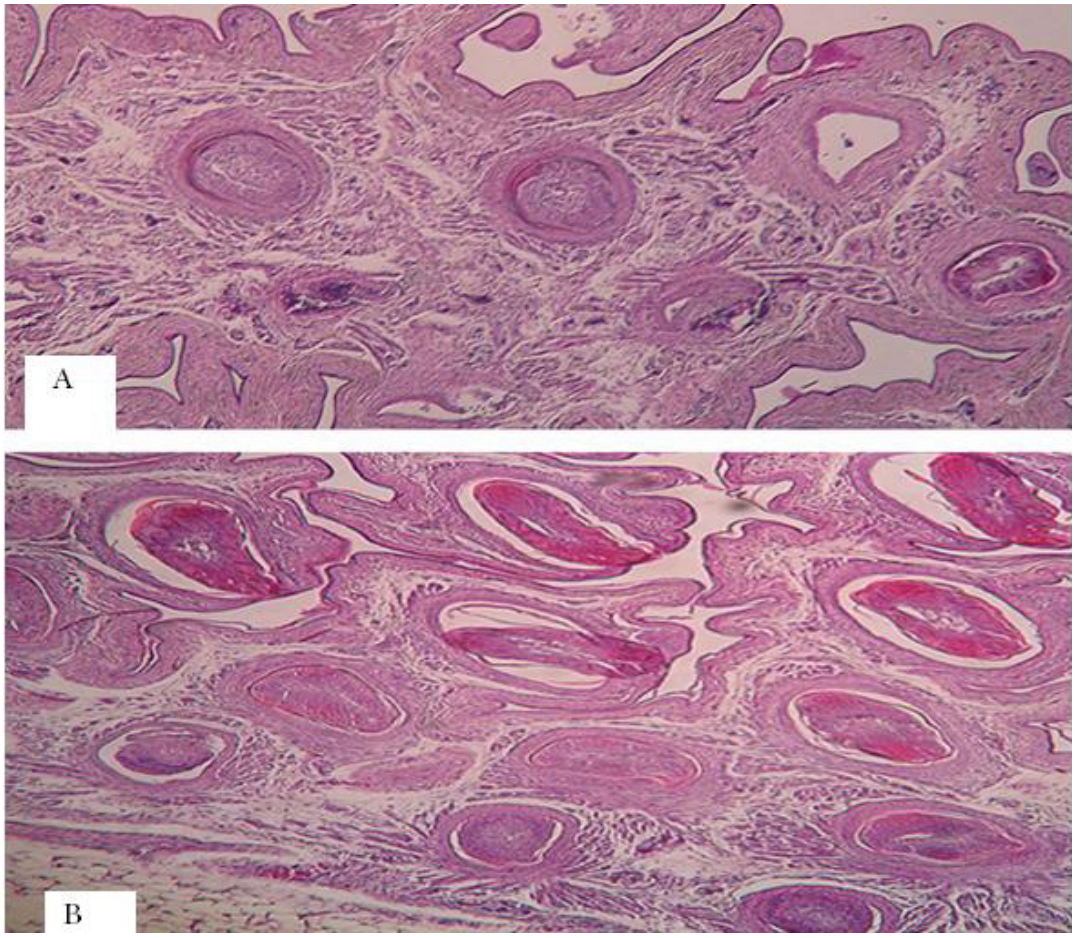

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Figure 1. The cross section of feather follicles. According to this method, the number of feather follicles was counted. A: Control group, B: Treatment group that received 50 mg methionine. × 100 H&E.

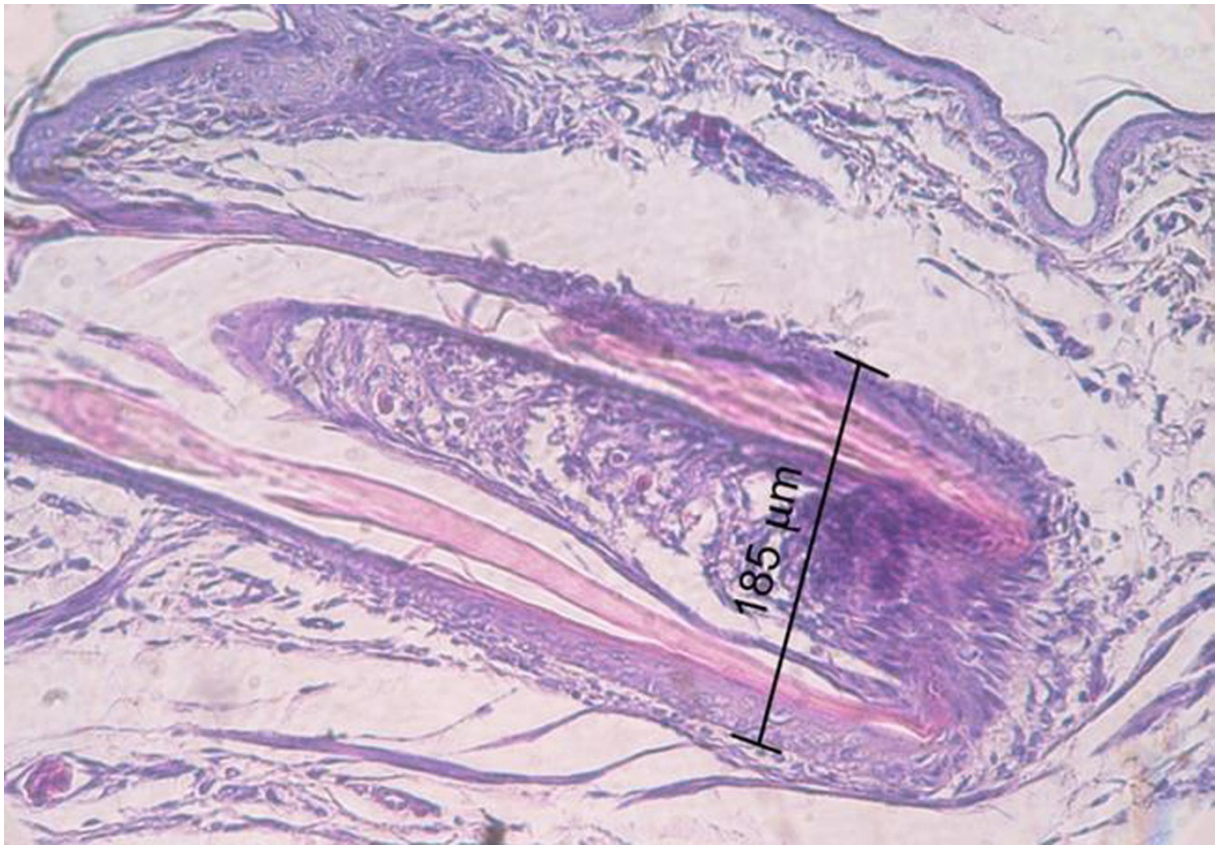

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Figure 2. The longitudinal section of feather follicle. The diameter of feather follicle is indicated by black arrow. × 400 H&E.

ml PBS) diameter of feather follicles increased significantly ($P<0.05$) just compared with control one, whereas that was not significant ($P>0.05$) compared with groups 1 and 2 (20 and 30 mg methionine, respectively). Increasing methionine to 50 mg (group 4), increased the diameter of feather follicles significantly ($P<0.05$) compared with control group and two first treatment groups, whereas it was not significant compared with group 3 (40 mg methionine) ($P>0.05$).

4. Discussion

To the best of our knowledge, this is the first study investigating in ovo administration of methionine on feather follicle development. Our results obviously showed injection of methionine via yolk sac increased feather follicle's density in chicken embryo. Embryonated bird eggs could provide an alternative model, which would fulfill the demand for refinement of animal models. On the other hand, fertilized eggs are readily available from commercial breeders at low cost, are easy to handle, and require little specialized equipment and no specialized facilities or personal. In this study, effect of methionine was investigated in chicken embryos that were alive until day 18 of incubation. Due to lack of researches studying this route of administration, this study cannot be compared with absolutely similar studies, but shared several similarities with the studies conducted to investigate the effect of some nutrients on feather growth in post hatch [1,11,12,13].

Although other researchers have observed an improvement in weight gain with methionine supplementation [5,14]. However, our results were not consistent with those reported by Wylie et al. (2003) that dietary protein in young turkey was not affected by feather follicles density. Imbalances of various amino acids have been reported to result in abnormalities of feather as those observed by deficiencies of specific amino acids [15]. Similarly, supplemental arginine and methionine resulted in increased feather weight, suggesting that both arginine and methionine are required for feather growth. Feather development is also affected by other essential amino acids such as arginine [11], valine [4], leucine [16] and branched amino acids [17]. A marginal deficiency in methionine or cysteine will influence feathering [15,18] because the proportion of the sulfur containing amino acids is high in the integument proteins. Keratin synthesis requires high amounts of cysteine and minimal methionine [19,20].

Thus, increased growth rates would be expected to exert an influence on the absolute methionine requirement. For better feather quality and reduction of feather pecking, DL-methionine can acts efficiently in broiler chicken [17]. Therefore, in the present study we use DL-methionine. Methionine may act as an amino acid in balancing protein or involvement in betaine, choline, vitamin B_{12} and folic acid metabolism [21,22]. Also, methionine is a precursor for cysteine and an important source of dietary sulfur. S-adenosyl methionine is a potent donor of methyl groups which contributes to the synthesis of many important substances including creatine and choline [23]. Ohta et al. (2001) suggested that the best amino acid injection sites in ovo may be the yolk sac and extra-embryonic coleom, hence, in this experiment methionine injected to yolk sac [24].

Feathers are derived from a series of interaction between the mesenchyme and epithelium. Several molecules that mediate inductive signalling during feather tract formation have been identified, including Hedgehog, Notch/Delta, members of the Bone Morphogenetic Protein (BMP) and Fibroblast Growth Factor (FGF) families [25,26]. FGFs can activate feather follicle formation in cultured explants of skin from the chick. Results of the current study suggest that methionine presumably promotes expression of FGFs [25]. Further studies should be conducted to determine that methionine affected which gene or factors exactly.

In conclusion, administration of 50 mg methionine in 0.5 ml PBS via yolk sac in day 4 of incubation, increased density and diameter of feather follicles in chicken embryo. Results of the current study suggest that the lower doses of methionine (under 20 mg in 0.5 ml PBS) did not effect on feather follicle development and also the higher doses (above 50 mg) increased embryonic death. Further studies still need to be undertaken to determine the effects of in ovo injection of different amino acids on feather development in pre hatch chicken.

Acknowledgment

This research was financially supported by a grant (No: 1392/12/1) from the Technology and Research Council of Shahid Bahonar University of Kerman, Iran. The authors would like to thank Mr. Mazhab Jafari for histotechnique lab methods and preparing the histology slides.

References

[1] Lopez-Coello C. Potential causes of broiler feathering problems. Pages 1-46 in feathering manual. St Louis, MO: Novus International; 2003.

[2] Kjaer J, Bessei W. The interrelationships of nutrition and feather pecking in the domestic fowl-A review. European Poultry Science. 2013; 77(1):1-9.

[3] Barbi J, Ribeiro A, Zaviezo D. Factors affecting feather development. Pages 1-41 in feathering manual. St Louis, MO: Novus International; 2003.

[4] Farran M, Thomas O. Valine deficiency. The effect of feeding a valine-deficient diet during the starter period on performance and feather structure of male broiler chicks. Poultry Science. 1992; 71(11):1879-84.

[5] Wheeler K, Latshaw J. Sulfur amino acid requirements and interactions in broilers during two growth periods. Poultry Science. 1981; 60(1):228-36.

[6] Urdaneta-Rincon M, Leeson S. Effect of dietary crude protein and lysine on feather growth in chicks to twenty-one days of age. Poultry Science. 2004; 83(10):1713-7.

[7] Halder G, Roy B. Effect of herbal or synthetic methionine on performance, cost benefit ratio, meat and feather quality of broiler chicken. International Journal of Agricultural Research. 2004; 2(12):987-96.

[8] Ohta Y, Tsushima N, Koide K, Kidd M, Ishibashi T. Effect of amino acid injection in broiler breeder eggs on embryonic growth and hatchability of chicks. Poultry Science. 1999; 78(11):1493-8.

[9] Ohta Y, Kidd M, Ishibashi T. Embryo growth and amino acid concentration profiles of broiler breeder eggs, embryos, and chicks after in ovo administration of amino acids. Poultry Science. 2001; 80(10):1430-6.

[10] Jacobsen ID, Grobe K, Hube B. Embryonated chicken eggs as alternative infection model for pathogenic fungi. Methods in Molecular Biology. 2012; 845:487-96.

[11] Wylie L, Robertson G, Hocking P. Effects of dietary protein concentration and specific amino acids on body weight, body composition and feather growth in young turkeys. British Poultry Science. 2003; 44(1):75-87.

[12] Twining P, Thomas O, Bossard E. The number of feathers on the litter; another criterion for evaluating the adequacy of broiler diets. Poultry Science. 1976; 55(4):1200-7.

[13] Kalinowski A, Moran E, Wyatt C. Methionine and cystine requirements of slow-and fast-feathering male broilers from zero to three weeks of age. Poultry Science. 2003; 82(9):1423-7.

[14] Engler K, Thomas O, Bossard E. The sulfur amino acid requirement of 3-week old broilers. Proceedings of the University of Maryland Nutrition Conference for Feed Manufacturers; 1985 Mar 21-22; College Park, MD, USA; 1985, pp: 5-10.

[15] Deschutter A, Leeson S. Feather growth and development. World's Poultry Science Journal. 1986; 42(3):259-67.

[16] Penz Jr.A, Kratzer F, Rogers Q. Effect of excess leucine on feather structure and feather composition in the chick. Nutrition Reports International. 1984; 29(4):991-5.

[17] Robel E. A feather abnormality in chicks fed diets deficient in certain amino acids. Poultry Science. 1977; 56(6):1968-71.

[18] Urdaneta-Rincon M, Leeson S. Effect of dietary crude protein and lysine on feather growth in chicks to twenty-one days of age. Poultry Science. 2004; 83(10):1713–1717

[19] Nitsan Z, Dvorin A, Nir I. Composition and amino acid content of carcass, skin and feathers of the growing gosling. Poultry Science. 1981; 22(1):79-84.

[20] Hurwitz S, Frisch Y, Bar A, Eisner U, Bengal I, Pines M. The amino acid requirements of growing turkeys: 1. Model construction and parameter estimation. Poultry Science. 1983; 62(11):2208-17.

[21] March B, Biely J. Folic acid supplementation of high protein-high fat diets. Poultry Science. 1956; 35(3):550-1.

[22] Chen F, Noll S, Waibel P, Hawkins D. Effect of folate, vitamin B12 and choline supplementation on turkey breeder performance. Poultry Science. 1993; 72-73.

[23] Bender DA. Amino acid metabolism. 3rd ed. New York: John Wiley & Sons; 2012.

[24] Ohta Y, Kidd M. Optimum site for in ovo amino acid injection in broiler breeder eggs. Poultry Science. 2001; 80(10):1425-9.

[25] Song H, Wang Y, Goetinck PF. Fibroblast growth factor 2 can replace ectodermal signaling for feather development. Proceedings of the National Academy of Sciences. 1996; 93(19):10246-9.

[26] Morgan BA, Orkin RW, Noramly S., Perez A. Stage-specific effects of sonic hedgehog expression in the epidermis. Developmental Biology. 1998; 201(1):1-12.