



Research Article

In-ovo Effects of Lysine Amino Acid on Histomorphometry of Thigh Muscles, Cecal Tonsils and pH in Japanese Quail

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Abstract

The current study was carried out to investigate the *in-ovo* effects of lysine on the histology of thigh muscles, their pH and cecal tonsils in the Japanese quail. Total numbers of 240 quail eggs were injected with four different solutions: G-1st was kept as control and injected with only sterile distilled water; G-2nd was injected with 1g/50 ml solution of lysine, G-3rd was injected with 2g/50 ml solution of lysine, G-4th was injected with 3g/50 ml solution of lysine. The eggs were hatched at 17th day after hatching. Three birds slaughter and samples were collected for histological analysis. There observed a significant increase ($p < 0.01$) in the number and diameter of the cecal tonsils and increase ($p < 0.01$) in the diameters of muscle fibers and fascicles of thigh muscles by lysine inoculation at the level of 3g/50 ml compared to the control group, while pH of the thigh muscles was un-affected by any treatment. Hence, it was concluded that *in-ovo* inoculation of lysine at the level of 3g/50 ml sterile distilled water solution has more beneficial effects on the histomorphometry of cecal tonsils; thigh muscles by improving the immunity level, muscles growth and meat quality of birds, respectively.

Keywords: Lysine, Thigh Muscles, Cecal Tonsils, Histo-morphometry, Japanese Quail

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INTRODUCTION

In-ovo feeding is the injection of an experimental material in the chicken egg [15]. It is reported that *in-ovo* inoculation increase the growth performance [5]. Lysine and methionine the amino acids, are reported to enhance the immune system and improve the growth when used in the diet of broilers [3]. Moreover these two amino acids, enhance the absorption of nutrients by increasing the villus height of the ilium [13] increase the weight of hatching chicks [1] and hence weight of the broiler birds [2]. Similarly, it was observed that injection of amino acids into the amniotic membrane increased the growth performance of birds by increasing the villus height of intestinal epithelial [14]. Moreover, the time of *in-ovo* feeding time is the integral instance to obtain the paramount results after hatching [12]. Feeding of embryos via *in-ovo* feeding improved the development of the gut and immune organs in the poultry birds [11]. In the poultry diet, lysine is considered as the second most important amino acid after methionine, hence both are considered as ideal amino acids for protein synthesis in the birds [9]. *In-ovo* injection of amino acids improved the development of muscles fiber and histological characteristics of intestines [4]. Administration of lysine alone and in combination

with arginine and histidine showed the beneficial effects on viability of the embryo, hatchability rate and post-hatch growth performance in domestic pigeons [16,17]. Moreover, lysine is the essential amino acids required for the body metabolism and mainly involved in the protein synthesis [7]. The objectives of the present project were to study the beneficial effects of these amino acids on the development of immune organs to improve the immunity and the development of thigh muscles that could enhance the meat quality and growth performance of the birds.

MATERIAL AND METHODS

A total of 240 freshly laid quail eggs were obtained from the breeder flock. All the eggs were almost of, equal in size and weight and belong to single hatching. The eggs were divided into four groups i.e. Group-1, 2, 3 and 4. Each group has sixty eggs. Group-1st was injected with sterile distilled and was considered as control group. Group-2nd was injected with 1g/50 ml sterile distilled water solution of lysine; group-3rd was injected with 2g/50ml sterile distilled water solution of lysine, group-4th was injected with 3g/50 ml sterile distilled water solution of lysine. Equal quantity of solution (0.2 ml/egg) at the depth of 14mm into the air sac of each egg by the use of 27-



guage needle at 1st day of post-laying. Before the injection, all the eggs were cleaned with pyodine antiseptic and sterile egg driller was used to make hole in the eggs. After the injection, the eggs were sealed air tightly with hot liquid paraffin. The eggs were then left in the incubator at 37°C until hatching.

Rearing of the birds

Before putting the chicks, cleaning and fumigation of the shed was done, before shifting the chicks there. Birds were weight and divided into four groups. All the birds were reared in the standard conditions with feed and water ad libitum. At the 35th day of rearing, birds were slaughter for sample collection of thigh muscles and cecal tonsils. At the time of slaughtering, pH of the fresh meat was noted.

Histomorphometry of thigh muscles and cecal tonsils

The muscle tissues were fixed in 10% formaline solution for 48hours at 4°C under slight agitation and then immediately processed for histo-morphometric analysis. The samples were processed by paraffin sectioning and 4–5µm thick histological sections were stained with Hematoxylin and Eosin (H&E) staining technique as previously described (Ali et al 2014, Hussain et al 2017, Mahmood et al 2017). Pixel Pro software fitted with Bright field microscope (Labomed USA) was used for the morphometry of tissues at 100X and 400X.

pH of muscles

Conventional probe electrode was inserted 1cm deep into the muscles for determining the pH of the fresh meat at the time of slaughtering.

Table 1. Effects of *in-ovo* injection of lysine on histo-morphometry of cecal tonsils

Cecal tonsils	G-1 st (Control)	G-2 nd (1g lysine)	G-3 rd (2g lysine)	G-4 th (3g lysine)	p-value
Number	12.60±0.22 ^c	13.30±0.21 ^c	14.30±0.21 ^b	17.00±0.33 ^a	0.000
Height (mm)	101.30±0.21 ^d	102.70±0.36 ^c	106.30±0.30 ^b	109.80±0.29 ^a	0.000
Width (mm)	61.10±0.43 ^a	61.40±0.33 ^a	61.70±0.36 ^a	64.70±0.30 ^b	0.000
Area (mm ²)	6189.00±38.71 ^c	6305.70±40.14 ^c	6558.80±44.59 ^b	7103.80±32.04 ^a	0.000

Different superscripts in the same row indicate significantly different (p<0.05) means± SEM.

Table 2. Effects of *in-ovo* injection of lysine on histo-morphometry of thigh muscles

Thigh muscles	G-1 st (Control)	G-2 nd (1g lysine)	G-3 rd (2g lysine)	G-4 th (3g lysine)	p-value
Diameter of fascicle (µm)	326.40±0.87 ^d	330.40±1.17 ^c	344.50±0.61 ^b	350.80±0.91 ^a	0.000
Diameter of fiber (µm)	23.40±0.49 ^d	26.60±0.52 ^c	32.90±0.52 ^b	37.60±0.47 ^a	0.000

Different superscripts in the same row indicate significantly different (p<0.05) means± SEM.

Table 3. Effects of *in-ovo* injection of lysine on pH of thigh muscles immediately after slaughtering of the bird

pH of thigh muscles	G-1 st (Control)	G-2 nd (1g lysine)	G-3 rd (2g lysine)	G-4 th (3g lysine)	p-value
pH	6.12±0.005	6.11±0.006	6.12±0.005	6.12±0.006	0.86

Statistical analysis

All data are presented as mean ± SEM (Standard error of mean). SPSS (Version 20) was used for the statistical analysis. One-way analysis of variance (ANOVA) was used to compare the multiple treatments, while group differences were compared by Tukey's test. p<0.05 was considered as statistically significant level.

DISCUSSION

New born chicks have low immunity at the time of hatching, so the high level of immunity during incubation period and just after hatching protects the birds from many pathogens [6]. Stem cells in ducks were activated by *in-ovo* feeding which enhanced the muscle fibers diameter and their cross sectional area, *in-ovo* administration also improved the quality genes of growth

performance [9]. *In-ovo* inoculation enhanced the growth and physiological characteristics in productive birds [10].

CONCLUSION

In-ovo feeding of lysine amino acid improved the immunity and meat quality of birds, which leads to the improvement in the growth performance till the final weight of the birds.

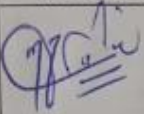
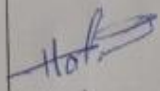
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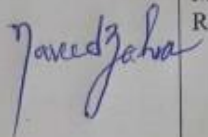
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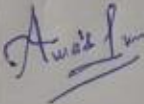


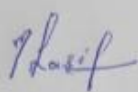
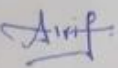
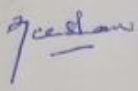
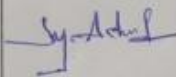
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