

Effects of Different Levels of Lysine on Small Intestinal Villous Morphology in Starter Diet of Broiler Chickens

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Abstract: The purpose of this study to demonstrate the effects of lysine supplement on small intestinal morphology of broilers chickens. In starter period control group received diet containing 1.2% lysine, Treatment 1[T1] and Treatment 2[T2] groups received diet containing 1.3% and 1.4% lysine, respectively. Intestine villous morphology examination was done by analyzing of villi's height, width, depth of crypts and the type of villous. The finding shown that as diet lysine increased, the villous height had a significant increase compared to the control group. In the starter period, the growth of intestine villous width which depends on lysine density had significant increase ($P<0.05$) compared to control group. As T_2 group lysine increased ($P<0.05$) compared to control Group, Libercuhn crypt depth had a significant increase ($P<0.05$). A lot of intestine villous were in a kind of leaf and tongue and also a little percent were bridge villi, Finger, Ridge and convoluted villi. In duodenum, different levels of lysine on the tongue and leaf villi had not significant effect. In the starter period, lysine increase in T_1 and T_2 lead to significant decrease ($P<0.05$) of leaf villous compared to control but has caused to significant increase ($P<0.05$) of tongue villous in jejunum and ileum.

Key words: Lysine Diet • Intestinal Morphology • Villi • Starter Period

INTRODUCTION

Protein and amino acids are the major nutrients representing a high percentage of total cost of the diets. According to Humphrey *et al.* [1] lysine is of interest for two primary reasons. First, this essential amino acid is often the first or second limiting amino acid in diets based on grain. Secondly, a lysine deficiency creates a nutritional environment where tissues must compete for the limited supply of this essential amino acid. Lysine is generally accepted as the second limiting amino acid following The methionine in corn-soybean meal diets. Additionally, lysine is utilized as the basis for evaluating the content of other essential amino acids for ideal amino acid balance. It has been shown that amino acids are effective on small intestine morphology. The Villous

height depends upon two factors, firstly on cellular multiplication and differentiation from the crypts and secondly on the migration of mature cells along the villous axis, accompanied by the cellular shedding from the apex. The regulation of these factors is widely considered to be way in which the small intestine adapts to nutritional demands [2]. According to Franco *et al.* [3], a trophic effect of amino acids on crypt depth can be considered. Trophic agents stimulate the development of the intestinal mucosa, that is, stimulate mitosis and consequently, promote cell hyperplasia, resulting therefore in higher crypt and villi development. Franco *et al.* [3] indicated in starter period there was no effect of lysine levels on duodenum or jejunum villi height, but an increase in crypt depth in both segments as lysine level increased.

Bartell and Batal [4] reported that the birds fed diets supplemented with glutamine had significantly longer intestinal villi than the intestinal villi of birds fed the control corn- Soybean meal diet. in agreement with Zaefarian *et al.* [5] showed that threonine supplement had significant effect on villous height, epithelial thickness, goblet cell number and crypt depth in duodenum, jejunum and ileum. Soltan [6] showed that the increased glutamine (0.2%) in the diet of chickens, led to an increase height of villous in the duodenum and jejunum and decrease the depth of the crypts. Wang [7] reported that dietary lysine supplementation increased villous height in jejunum and ileum of pigs. This study, considering the lack of information relating to effect of lysine on broiler gut morphology, aiming at evaluated the influence of different levels of dietary lysine supplementation on the small intestinal villous morphology of broiler chickens.

MATERIALS AND METHODS

180 day old male Ross breed broiler chickens purchased from local commercial breeders. After hatch, chicks were provided water and commercial chick starter for ad libitum consumption. Corn-soybean meal basal diets used in present experiment are described in Table 1. Diets were formulated to meet or exceed the nutrient needs of young growing broiler chicks as suggested by NRC (National Research Council) [8] except for lysine. Birds were randomly divided into 3 groups. One group kept as control (C) and given plain ration plus 1.2% lysine and other two were kept as treatment groups T₁ and T₂ which were given 1.3% and 1.4% lysine, respectively. The evaluated intestinal morphology variables were villous sizes included height, width and crypt depth from the duodenum, jejunum and ileum. On day 14, six chicks from

each group were randomly selected and killed by decapitation. Eight centimeter segments were dissected from the midpoint of the duodenum, the midpoint between the bile duct entry and Meckel's diverticulum (jejunum) and the ileum. These segments were flushed with phosphate-buffered saline (PBS, pH 7), Each segment was tied at one end and after injecting fresh Clark fixative tied at other end of the intestine [9]. The other end was also tied and sausage like sample were left in the fixative. After 45 min, samples were cut off and left in ethyl alcohol 50% for longer storage. Each sample was divided into 2 sections along its length (section A and B). Section A was left in Periodic acid Schiff (PAS) reagent for 2-3min, then removed washed and fixed over the paraffin wax by using an ordinary dissecting microscope in four randomly chosen fields, types and number of different villi were recorded for morphological studies. Six villous forms as: tongue like villi, leaf like villi, finger like villi, bridging villi, ridge villi and convoluted like villi [10]. Section B of each sample was left in PAS for staining, washed with cold tap water, muscle layers separated from the mucosa using a cataract knife over a paraffin bed, rows of villi were cut, transferred over the glass slides, few drops of glycerin added and then covered with a cover-slip. The sections were examined with a binocular microscope and measurements were made at a magnification of X50 with an eyepiece graticule. The height of twenty tallest villi was measured from tip to crypt mouth and depths of twenty associated to crypts were measured from crypts mouth to its base. Villous width measurement was performed on the base of these villi. Abbreviated signs are as follows: VH = villous height, VW= villous width and CD = crypt depth. Data were analyzed by SPSS 17 software through one-way variance analysis and Tukey Test.

Table 1: Chemical analysis of broiler starter diet (as-fed basis)

T ₂	T ₁	Control	Metabolizable energy, kcal/kg	
2900	2900	2900		
21	21	21	%	Crude protein
1	1	1	%	Ca
0.5	0.5	0.5	%	P
0.15	0.15	0.15	%	Na
0.93	0.93	0.93	%	Methionine + Cystine
0.59	0.59	0.59	%	DL- Methionie
1.4	1.3	1.2	%	L-Lysine
1.44	1.45	1.46	%	Arginine
0.27	0.27	0.27	%	Tryptophan
0.86	0.87	0.87	%	Threonine

Table 2: Villous parameters measured at different sites along the small intestine (Mean±SE)

		VH (µm)	VW (µm)	CD (µm)	VH/CD	VH/VW
D	C	1072.5±8.6 ^a	460.4±4 ^a	143±0.5 ^a	7.4±0.06 ^a	2.3±0.02 ^a
	T ₁	1163.1±13.6 ^b	472.8±7.6 ^{ac}	147±0.9 ^{ac}	7.9±0.06 ^{bb}	2.4±0.04 ^a
	T ₂	1239.2±8.2 ^c	506.8±7.4 ^{cb}	153.3±1.6 ^{cb}	8 ± 0.06 ^{cb}	2.4±0.04 ^a
J	C	544.01±5.9 ^a	542±4.38 ^a	105.2±0.7 ^a	5.1± 0.04 ^a	1±0.01 ^a
	T ₁	580.9±8.7 ^{bb}	587.3±3.7 ^{bb}	109.05±1 ^b	5.35±0.08 ^a	0.9±0.01 ^a
	T ₂	581.3±7.6 ^{cb}	594.3±4.2 ^b	112.03±0.3	5.1±0.07	0.9±0.01
I	C	434.1±4.62 ^a	547.6±2.1 ^a	101.1±0.5 ^a	4.3± 0.05 ^a	0.7±0.008 ^a
	T ₁	447.5±3.61 ^{ac}	540.0±2.4 ^{ac}	102.3±1.1 ^{ac}	4.37±0.06 ^{ab}	0.8±0.007 ^a
	T ₂	480.1±3.4 ^{cb}	584.1±4.9 ^{cb}	106.1±0.6 ^{cb}	4.5±0.04 ^{cb}	0.8±0.009 ^a

^{abc}Means in a columns without a common superscript are different (P <0.05)

D: duodenum, J: jejunum, I: ileum

Table 3: Villous types at different sites along the small intestine (Mean±SE)

	%	Leaf	Tongue	Finger	Bridge	Ridge	Convuluted
D	C	64.8±2.2 ^a	33.1±1.8 ^a	0.1±0.1 ^a	1.69±0.2 ^a	0.1±0.1 ^a	0.13±0.1 ^a
	T ₁	68.4±2.3 ^a	29.7±2.8 ^a	0.02±0.02 ^a	1.64±0.5 ^a	0.02±0.02 ^a	0.06±0.04 ^a
	T ₂	58.8±4.9 ^a	37.4±5.5 ^a	0.02±0.02 ^a	3.6±0.9 ^a	0.02±0.02 ^a	0.02±0.02 ^a
J	C	66.9±1.5 ^a	28±2.07 ^a	0.05±0.05 ^a	4.3±1.6 ^a	0.4±0.1 ^a	0.2±0.1 ^a
	T ₁	57.5±2.8 ^{bb}	41.3±2.8 ^{bb}	0.02±0.02 ^a	1.07±0.2 ^a	0.02±0.02 ^{ac}	0.02±0.02 ^a
	T ₂	56.5±0.8 ^{cb}	39.6±1.1 ^{cb}	0.02±0.02 ^a	3.6±0.5 ^a	0.02±0.02 ^{cc}	0.05±0.05 ^a
I	C	64.8±2.2 ^a	33.1±1.8 ^a	0.1±0.1 ^a	1.6±0.2 ^a	0.1±0.1 ^a	0.1±0.1 ^a
	T ₁	55.4±2.1 ^{bb}	41.6±2.2 ^{bb}	0.03±0.03 ^a	2.7±0.5 ^{ac}	0.03±0.03 ^a	0.06±0.06 ^a
	T ₂	50.4±2.2 ^{cb}	43.2±2.2 ^{cb}	0.02±0.02 ^a	6.1±0.6 ^{cb}	0.02±0.02 ^a	0.05±0.05 ^a

^{abc}Means in a columns without a common superscript are different (P <0.05)

D: duodenum, J: jejunum, I: ileum

RESULTS

The morphology of the small intestine at day 14 of the experiment is shown in Table 2 and 3. In the duodenum, jejunum and ileum the highest VH, VW and CD were obtained in the T₂ group. The growth of intestine villous width which depends on lysine density had significant increase (P<0.05) compared to control group. As T₂ group lysine increased (P<0.05) compared to control group, Libercuhn crypt depth had a significant increase (P<0.05).

DISCUSSION

Results from the present study contradicted with those obtained by Franco *et al.* [3], who indicated that in starter period there was no effect of lysine levels on duodenum or jejunum villi height.

The higher villi and the deeper crypts in the duodenum, jejunum and the ileum at 14 days of age in birds fed 1.4% lysine could be explained by the negative influence of amino acid unbalance on crypt development.

These results were in agreement with Franco *et al.* [3]. These authors showed that with higher dietary lysine levels increased crypt depth in duodenum and jejunum in starter period. It seems 1.4% lysine was more effective on jejunum than 1.2 and 1.3% lysine. In this study, the birds fed diets supplemented with 1.3 and 1.4% lysine had significantly higher intestinal villi than the intestinal villi of birds the control. If the intestinal villi height can be increased early in the chick's life, then the chick may be able to utilize nutrients more efficiently earlier in life and thus have improved growth performance. Increased villi height has been proposed to increase performance by improving nutrient absorption. The increase in villi height that was observed might indicate that the birds fed diets supplemented with 1.3 and 1.4% lysine might have had greater nutrient absorption and utilization because increases in villi height result in more surface area for nutrient utilization. Bartell and Batal suggest that in fact increased villi height does not necessarily lead to increased nutrient utilization and then increased performance. This may be due an imbalance in amino acids in diet. According to Table 2, increasing level of

dietary lysine, have significant effect on cell renewal in duodenum. The ratio of villous height to crypt depth gives an indication of cell renewal and turnover. The ratio of villous height to villous width is also given in Table 2 the differences between groups were not statistically significant. This study showed that a lot of intestine villous were in a kind of leaf and tongue shape and also a little percentage were bridge villi, Finger, Ridge and Convoluted shape villi. There was an increase in the number of tongue shape villi in T₁ and T₂ and reduction leaf shape villi compared with control group in jejunum and ileum. Diets containing 1.4% lysine, increased bridge shape villous in the ileum.

In this study, the villi height in duodenum was greater than those in the jejunum and ileum and this is consistent with the major role of duodenum in nutrient absorption. This resulted in a change from leaf shaped villi to mainly tongue shaped villi. The origin of tongue and leaf shaped villi is unknown. Possibly they result from villous fusion. In the present study a decrease in the percentage of leaf shaped villi and an increase in tongue shaped villi were observed as a morphological response to the Lysine in the Jejunum and Ileum.

CONCLUSION

The results of this study shown that elevating of dietary lysine level improved the broiler chickens morphology and can affect the Villous and crypt dimensions positively. The results also recommends that the higher levels of lysine may be required for noticeable improvements in intestinal villous characteristics.

REFERENCES

1. Humphrey, B.D., C.B. Stephensen, C.C. Calvert and K.C. Klasing, 2006. Lysine deficiency and feed restriction independently alter cationic amino acid transporter expression in chickens. *science direct, Comparative Biochemistry and Physiology, Part A*, 143: 218-227.
2. Ofusori, D., E. Caxton-Martins, O. Komolafe, K. Oluyemi, O. Adeeyo, S. Ajayi, P. Oluwayinka, E. Adelakun, S. Keji and O. Adesanya, 2008. A comparative study of the ileum in rat (*Rattus norvegicus*), bat (*Eidolon helvum*) and pangolin (*Manis tricuspis*) as investigated using histological method. *International J. Morphol.*, 26: 137-141.
3. Franco, J., A. Murakami, M. Natali, E. Garcia and A. Furlan, 2006. Influence of delayed placement and dietary lysine levels on small intestine morphometrics and performance of broilers. *Rev. Bras. Cienc. Avic.*, pp: 8.
4. Bartell, S.M. and A.B. Batal, 2007. The effect of supplemental glutamine on growth performance, development of the gastrointestinal tract and humoral immune response of broilers. *Poult Sci.*, 86: 1940-7.
5. Zaefarian, F.Z.M. and S.M., 2008. The Threonine Requirements and its Effects on Growth Performance and Gut Morphology of B'roiler Chicken Fed Different Levels of Protein. *International J. Poultry Sci.*, 7: 1207-1215.
6. Soltan, M., 2009. Influence of Dietary Glutamine Supplementation on Growth Performance, Small Intestinal Morphology, Immune Response and Some Blood Parameters of Broiler Chickens. *International J. Poultry Sci.*, 8: 60-68.
7. Wang, W., S. Qiao and D. Li, 2009. Amino acids and gut function. *Amino Acids*, 37: 105-110.
8. National Research Council, 1994. Nutrient requirements of poultry, National Academy Press, Washington, DC.
9. Clarke, R.M., 1977. The effects of age on mucosal morphology and epithelial cell production in rat small intestine. *J. Anat.*, 123: 805-11.
10. Mouwen, J.M., 1971. White scours in piglets. I. Stereomicroscopy of the mucosa of the small intestine. *Vet. Pathol.*, 8: 364-80.