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Effect of Supplementation of Various Sources of Methionine on Nutrient Digestibility and Intestinal Morphometry in Broiler Chicken

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A B S T R A C T

An experiment was conducted to study the effect of various sources of Methionine on Nutrient Digestibility and Intestinal Morphometry in broiler chicken. In a CRD model, 375 broiler chicks (Vencobb) were randomly divided into five groups (T_1, T_2, T_3, T_4 and T_5), each containing 3 replicates with 25 birds in each replicate. The T_1 group served as control group, T_2 group was supplemented with synthetic Methionine (Nutrient requirements of ICAR 2013), T_3 and T_4 groups were supplemented with Methionine producing microbes (MPM) and T_5 group was supplemented with combination of T_2 and T_3, respectively for a period of 42 days. The results of the experiment revealed that CP digestibility (%) was found to be higher (P<0.01) in synthetic Methionine (T_2) treated group. Whereas, digestibility (%) of DM, EE and CF was found to be non-significant among all the groups. Similarly villi height, villi width, crypt depth of duodenum, jejunum and ileum were found to be higher (P<0.01) in synthetic Methionine (T_2) treated group. Whereas, villi/crypt depth ratio of duodenum, jejunum and ileum was more (P<0.01) in Control group.

Introduction

The Poultry Industry has emerged as the fastest growing segment of the livestock sector globally due to a number of favorable reasons. Among all essential amino acids Lysine and Methionine are considered as critical amino acids (FAO, 2010).

Methionine acts as a lipotropic agent through its role as an amino acid in balancing protein and as methyl donor and is involved in the metabolism of Choline, Betaine, Folic acid and Vitamin B_{12} (Young *et al.,* 1955; March and Biely, 1956). Methionine supplementation in broiler diets leads to change in small intestinal morphology via two mechanisms: (i) Methionine directly stimulates cell proliferation and/or cell number as amino acid precursor of protein synthesis, (ii) high derivatives of Methionine such as Taurine or Glutathione which is an antioxidant, protect villous from damage caused by oxidative stress in the small intestines (Roig- Pérez *et al.,* 2005; Shoveller *et al.*, 2005). Synthetic Methionine appears to be absorbed faster by the intestinal epithelium than dietary protein-bound amino acids (Batterham and Murison, 1981; Cowey and Walton, 1988; Tantikittii and March, 1995; Schumacher *et al.,* 1997; Zarate and Lovell, 1999).

The most common source of Methionine in poultry diets is DL-Met produced by synthetic chemistry from acrolein, methyl mercaptan and hydrogen cyanide. Common forms of synthetic Methionine are crystalline form (DL-Methionine with 99% bioavailability), and liquid form- (Methionine hydroxyl analogue is 88% bioavailable). The synthetic Methionine can be metabolized into highly toxic compounds such as methyl thiopropionate, thereby adversely altering the performance of poultry birds (Baker, 1991). Similarly, Methionine producing microbes have been isolated from soil and from various sources and screened for the amount of Methionine produced from the microorganisms (Thomas, 2014).

Keeping in view, the present investigation was carried out to study the effect of Methionine producing microbes (*Bacillus subtilis, Corynebacterium glutamicum, Lactobacillus plantarum, Leuconostoc sp., Saccharomyces sake*) live microbial cultures with a TVC of 6000 Million CFU/g. and synthetic Methionine in broiler diets. Methionine producing microbes (MPM) is an commercial by product supplied by M/s DVS BIOLIFE Pvt Ltd.

Materials and Methods

Experimental location

The present experiment was carried out at Livestock Farm Complex, College of veterinary science Tirupati, Sri Venkateswara Veterinary University, Andhra Pradesh.

Experimental design

The present study was carried out with three hundred and seventy five, day old broiler chicks obtained from a local hatchery. These chicks were randomly allotted to five experimental groups with each group having three replicates and with twenty five birds per replicate in a Completely Randomized Design. The T_1 group served as control group, T_2 group was supplemented with synthetic Methionine (Nutrient requirements of ICAR, 2013), T_3 and T_4 group were supplemented with MPM and the T_5 group was supplemented with combination of T_2 and T_3 (half the dose of T_2 and T_3) respectively for a period of 42 days was presented in the Table 1.

Experimental diets

The broiler diets were formulated in three phases i.e., pre-starter (0-14 days), starter (15-28 days) and finisher (29-42 days). Basal diet was prepared as per the Nutrient requirements of Poultry ICAR (2013). Representative samples of experimental diets were analyzed for proximate composition as per AOAC (2005).

Health management

The chicks were vaccinated with HVT vaccine, F_1 vaccine, IBD vaccine and Lasota vaccine on the 1^{st}, 6^{th}, 14^{th} and 23^{rd} days respectively.

Nutrient digestibility study

Digestibility trials were conducted during the starter and finisher phases of the biological trial. Two birds from each replicate, thus a total of six birds per treatment were kept separately in six metabolic cages. Birds in the cages were fed with the respective experimental diets consecutively for 3 days and the total feed offered was weighed and recorded for each cage. Similarly feces voided and feed left over in each cage was carefully collected, weighed and recorded. The representative samples of experimental diets offered and fecal samples from each cage were collected separately and analyzed for Dry matter (DM), Crude protein (CP), Ether extract (EE) and Crude fiber (CF) as per AOAC, (2005).

Intestinal morphometry study

The duodenum, jejunum and Ileum segments of the small intestine were identified and separated by dissection at the end of experimental period (42 days of age). Each sample was externally and internally washed with 0.9% NaCl to remove the intestinal contents and individually transferred to jars containing 10% buffered formalin for fixation. After 12-24 h fixation period, samples were embedded in paraffin, sectioned to a 2-5 μm thickness, mounted on glass slides, and stained with hematoxylin - eosin (Prophet *et al.*, 1992).

Villi height and crypt depth were then measured. Villus height was defined as the length between the villus basal lamina (which coincides with the upper crypt end) and the villus apex. Crypts were measured between the base and the crypt: villus transition zone (Pelicano *et al.*, 2007). Measurements were carried out using a trinocular stereoscopic microscope (Quimis™) under 10 × magnifications.

Images were captured by a camera coupled to the microscope and connected to an image analyzer (Leica Software™), and measured using the Paint Brush™ software. Between five and 20 villi and crypts were scored for each bird, and means calculated there from were used in the statistical analysis (Ribeiro *et al.*, 2007).

Analysis of data

The data obtained was subjected to one- way ANOVA. Differences between means were tested at the 1% probability level using Duncan's LSD test. All the statistical analysis were done using SPSS programmer version 16 (SPSS, Richmond, VA, USA) as described by DYtham (2011).

Results and Discussion

The results of the current study revealed that, CP digestibility (%) was found to be higher ($P<0.01$) in synthetic Methionine treated group. Whereas, digestibility (%) of DM, EE and CF was found to be non-significant among all the groups (Table 2 and 3). Higher digestibility (%) of CP in synthetic Methionine treated group might be due to faster absorption by the intestinal epithelium than dietary protein-bound amino acids (Batterham and Murison, 1981; Cowey and Walton, 1988; Tantikittii and March, 1995; Schumacher *et al.,* 1997; Zarate and Lovell, 1999) These results were in congruence with the findings of (Halder and Roy, 2007) who reported superior performances of both protein and energy utilization ability in both synthetic and herbal Methionine supplemented group than control group (Fig. 1).

The villi height, villi width, crypt depth of duodenum, jejunum and ileum was significantly ($P<0.01$) higher in birds fed with synthetic Methionine group (Table 4). Whereas villi/crypt depth ratio of duodenum, jejunum and ileum was more ($P<0.01$) in Control group. Increased villi height, villi width, crypt depth in synthetic Methionine supplemented diet in broiler diets might be attributed to the change of small intestinal morphology via two mechanisms:

Table.1 Inclusion levels of synthetic Methionine, MPM and combination (gram/ton of feed) at various phases of growth in broiler chicken

Phases	T1	T2	T3	T4	T5 (T2+T3)
Pre-starter (0-14 days)	-	2000	500	1000	1000+250
Starter (15-28 days)	-	1700	500	1000	850+250
Finisher (29-42 days)	-	1300	500	1000	650+250

Table.2 The Mean ± SE and analysis of variance of digestibility (%) of nutrients in broilers supplemented with various sources of Methionine in diet during Starter phase

Nutrient	T_1	T_2	T_3	T_4	T_5
Dry matter (DM)[NS]	64.10 ± 0.46	64.78 ± 0.29	64.65 ± 0.23	64.40 ± 0.25	64.08 ± 0.93
Crude protein (CP)[**]	66.00 ± 0.43[b]	70.55 ± 0.41[a]	66.68 ± 0.40[b]	66.79 ± 0.46[b]	69.51 ± 0.54[ab]
Ether extract (EE)[NS]	77.28 ± 0.57	77.98 ± 0.55	77.37 ± 0.27	77.73 ± 0.40	77.08 ± 0.26
Crude Fiber (CF)[NS]	29.59 ± 0.32	29.57 ± 0.32	29.62 ± 0.22	29.35 ± 0.18	29.05 ± 0.64

[abc] Values in a row bearing different superscripts differ significantly ** ($P<0.01$)
NS- Non-significant

Table.3 The Mean ± SE and analysis of variance of digestibility (%) of nutrients in broilers supplemented with various sources of Methionine in diet during Finisher phase

Nutrient	T_1	T_2	T_3	T_4	T_5
Dry matter (DM)[NS]	68.75 ± 0.46	68.65 ± 0.53	68.72 ± 0.24	68.89 ± 0.91	68.65 ± 0.84
Crude protein (CP)[**]	65.24 ± 0.05[b]	70.04 ± 0.32[a]	65.43 ± 0.15[b]	65.97 ± 0.23[b]	68.06 ± 0.72[ab]
Ether extract (EE)[NS]	76.16 ± 0.31	76.37 ± 0.21	76.11 ± 0.26	76.22 ± 0.44	76.53 ± 0.61
Crude Fiber (CF)[NS]	29.44 ± 0.28	29.49 ± 0.25	29.52 ± 0.31	29.48 ± 0.11	29.21 ± 0.68

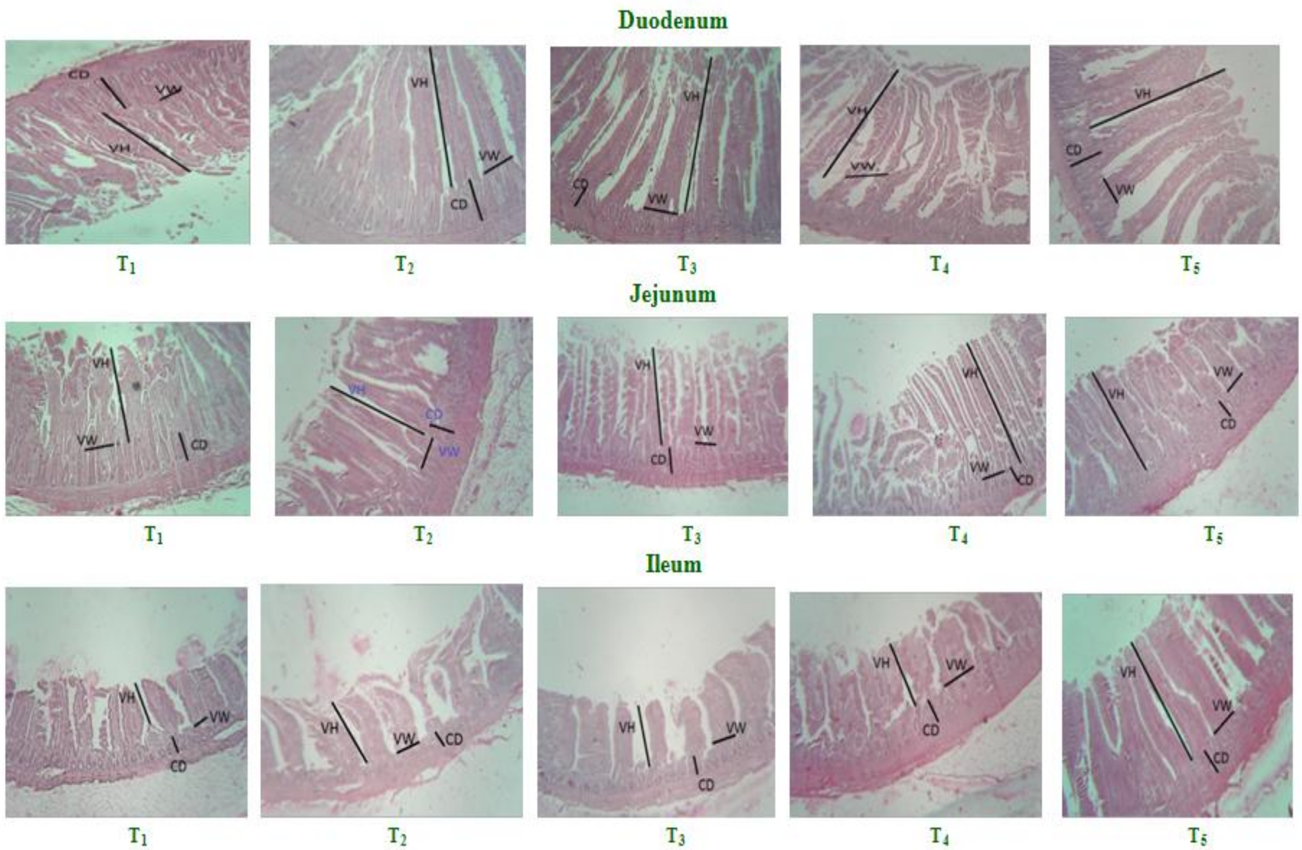
[abc] Values in a row bearing different superscripts differ significantly ** ($P<0.01$)
NS- Non-significant

Table.4 The Mean ± SE and analysis of variance on intestinal morphometry in broilers supplemented with various sources of Methionine in diet at the end of experimental period (42 days of age)

		Duodenum			
	T_1	**T_2**	**T_3**	**T_4**	**T_5**
Villi height (μm)**	2352.68 ±1.63^{d}	2796.30 ±1.84^{a}	2426.71 ±1.59^{c}	2410.33 ±2.56^{c}	2588.42 ±1.62^{b}
Villli width (μm)**	256.76 ± 0.97^{c}	335.48 ± 2.08^{a}	288.64 ± 2.37^{bc}	272.58 ± 193^{bc}	305.12 ± 2.42^{ab}
Crypt depth (μm)**	267.94 ± 1.37^{c}	388.50 ± 2.14^{a}	296.47 ± 1.68^{c}	281.19 ± 0.90^{c}	340.24 ± 1.55^{b}
Villi / crypt depth ratio	8.80	7.20	8.19	8.57	7.61
		Jejunum			
Villi height (μm)**	1710.62 ±2.47^{e}	1864.20 ±1.34^{a}	1775.38 ±0.96^{c}	1752.91 ±1.61^{d}	1812.45 ±1.07^{b}
Villli width (μm)**	231.13 ±1.82^{e}	301.14 ±2.63^{a}	269.45 ±1.78^{c}	251.28 ±2.07^{d}	283.57 ±1.59^{b}
Crypt depth (μm)**	209.73 ±1.96^{e}	272.46 ±2.94^{a}	238.34 ±1.92^{c}	224.93 ±1.67^{d}	251.68 ±2.34^{b}
Villi /crypt depth ratio	8.18	6.85	7.45	7.82	7.21
		Ileum			
Villi height (μm)**	1114.46 ± 2.46^{e}	1218.90 ± 1.62^{a}	1153.92 ± 2.37^{c}	1141.38 ± 2.56^{d}	1181.33 ± 0.93^{b}
Villli width (μm)**	153.76 ± 2.95^{e}	229.44 ± 1.81^{a}	183.49 ± 1.64^{c}	171.94 ± 1.94^{d}	210.58 ± 1.52^{b}
Crypt depth (μm)**	144.11 ± 1.66^{e}	215.39 ± 1.33^{a}	175.44 ± 2.67^{c}	161.33 ± 1.83^{d}	203.52 ± 1.64^{b}
Villi /crypt depth ratio	7.73	5.66	6.58	7.08	5.81

^{abcde} Values in a row bearing different superscripts differ significantly ** (P<0.01)

Fig.1 Effect of supplementation of various sources of methionine on intestinal morphometry in broiler chicken at the end of experimental period (42 days of age)



VH- villi height, VW- villi width, CD- crypt depth

(i) Methionine directly stimulates cell proliferation and/or cell number as amino acid precursor of protein synthesis, (ii) high derivatives of Methionine such as Taurine or Glutathione which is an antioxidant, protect villous from damage caused by oxidative stress in the small intestines (Roig- Pérez *et al.,* 2005; Shoveller *et al.,* 2005). The results were in agreement with Adeniji *et al.,* (2014) who reported that, supplementation of Methionine Hydoxy Analogue with Formic acid significantly ($P<0.05$) reduced gut wall thickness and increased villus height, villus width and crypt depth.

Based on the present results it can be concluded that dietary supplementation of synthetic Methionine had better significant impact on Crude protein digestibility (%) and Intestinal Morphometry compared to MPM treated groups and control group.

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