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Single and combined effects of feed supplements on intestinal morphology of broiler chickens

SS Chauhan, RK Sharma, DV Singh, SK Shukla and Jyoti Palod

Abstract
A feeding trial was conducted to evaluate the Influence of organic mineral mixture, probiotics, enzymes, emulsifier and liver stimulants on Haematological Profile of broilers. For this purpose, a total of 396 day old broiler chicks (Cobb) were used and randomly allocated into 11 groups with three replicates of 12 chicks each. The results revealed that at 42ndday,All the supplemented group showed significant (P<0.05) increase in duodenum length than T_0(control) group except T_6 and T_7.All the supplemented group showed significant (P<0.05) increase in jejunum length than T_0(control) group except T_1, T_6, T_7 and T_8.All the supplemented group showed significant (P<0.05) increase inileumlength thanT_0(control) group except T_6 T_7,andT_8.All the supplemented group showed significant (P<0.05) increase in small intestinal length than T_0(control) group except T_6 andT_7. All the supplemented group showed significant (P<0.05) increase in Duodenum villous height than T_0(control) group except T_6 andT_7..All the supplemented group showed significant (P<0.05) decreased in Duodenum crypt depth than T_0(control) group except T_3, T_4, T_5 and T_9.All the supplemented group showed significant (P<0.05) increase in villous height and crypt depth ratio than T_0(control) group.

Keywords: Feed supplements, intestinal morphology, broiler chickens

Introduction
Poultry is one of the fastest growing segments of the agricultural sector in India. India is the third largest egg producer after China and USA and the fourth largest poultry producer after China, Brazil and USA. The annual egg and broiler production of India is 70 billion eggs and 3.8 million tons respectively, with per capita consumption of 68 eggs and 2.5 kg chicken meat against the ICMR recommendations of 180 eggs and 11 kg poultry meat (CARIVISION 2050). Poultry meat has significant role in Indian diet valued at US $ 6.6 billion. Favoured by socio- economic conditions like rising purchasing power and changing food habits of the people this sector is driven by ever increasing domestic demand. Poultry meat is an excellent source of high quality protein, vitamins, and minerals and is not subjected to cultural and religious restrictions. Rising input cost in poultry production has necessitated the need to look for feed supplements which can enhance the nutrient utilization efficiency of feeds thereby improving performance of poultry and resultant increase in profitability. In this context use of organic minerals, probiotics, enzymes, emulsifiers and liver supplements seems promising. Use of organic minerals in poultry diets has been shown to have multiple beneficial effects including higher absorption and increased antibody levels as they may provide alternative pathways for absorption, by decreasing mineral excretion. Similarly, use of probiotics and feed enzymes have been reported to regulate gut integrity, reduce digestive disorders, improve nutrient absorption/feed efficiency, increases production, check the mortality and lowering of feed cost. Poultry produces emulsifiers in the form of bile however, at times it is insufficient in view of added fats and oils. Also, as the digestive tract in young birds is not completely developed, fat absorption from the feed matrix is hampered. Hence, addition of emulsifier into the diet can overcome this problem by reducing the size of the fat globules forming small micelles and increasing the total surface available for enzymatic digestion. The addition of synthetic emulsifier to broiler diets is a recent practice as compared to other dietary supplements. Polyherbal liver stimulants possess hepato – protective, hepatogenic, immunomodulatory and antioxidant properties, which tone up liver resulting in increased utilization of feed and better performance. Keeping the above facts in view, an experiment was conducted to determine the effect of supplementation of organic mineral mixtures, probiotics, enzymes, emulsifier and liver stimulants on intestinal morphology of broilers.

Materials and Methods

The present experiment was carried out to study the effect of feed supplements of organic mineral mixtures, probiotics, enzymes, emulsifier and liver stimulants in broiler chickens. The maximum temperatures may go up to 43 ^{0}C in summers and minimum up to 0 ^{0}C in winters and relative humidity ranges between 45 to 95%.A total of 396 day old commercial broiler chicks (Cobb) were procured for conducting the experiment. All the chicks were individually weighed and randomly allotted to eleven different groups each with three replicates of 12 chicks.A total number of 396 day old commercial broiler chicks (Cobb) were procured for undertaking the experiment. All the chicks' were individually weighed and randomly allotted to eleven different groups each with three replicates of 12 chicks. The groups were designated as T_0; basal diet, T_1; chicks fed basal diet along with organic mineralmixture 1 (Organomin forte) @ 0.5 g per kg feed, T_2; basal dietalong with organic mineral mixture 2 (Vannamin) @ 0.5 g per kgfeed,T_3; basal diet along with probiotics (Microguard) @ 0.1g per kgfeed,T_4; basal diet along with enzymes + probiotics (Brozyme -XPR) @ 0.5 g per kg feed, T_5; basal diet along with emulsifier(Lipigon) @ 0.5 g per kg feed, T_6; basal diet with 3% less energy, T_7;basal diet with 3% less energy along with liver supplement 1(Superlivpremix) @ 0.5 g/kg feed, T_8; basal diet with 3% less energy along with liver supplement 2 (X- liv Pro) @ 0.5 g/kg feed, T_9; basal dietalong with enzymes with probiotics (Brozyme - XPR) and liver supplement 1(Superliv premix) @ 0.5 g/kg feed, and T_{10}; basal dietalong with enzyme with probiotics (Brozyme - XPR), liversupplement 1(Superliv premix) and emulsifier (Lipigon) @ 0.5 g/kgfeed. Average body weight of chicks was similar for all the treatment groups. The broiler chicks were housed in deep litter system. Blood samples were collected from six experimental birds of each group i.e. two broiler chicks from each replicate on 21st and 42nd day of experimental feeding. After evisceration the intestine of the birds were carefully separated and the length of the duodenum (from the ventriculus to the pancreo-biliary duct), jejunum (from the pancreo-biliary duct to Meckel's diverticulum), and ileum (from Meckel's diverticulum to the ileocecal junction) were measured using a measuring tape to study the effects of diet supplementations on the intestinal gross morphology. A sample of two cm from duodenum was collected and preserved in 10 per cent formalin to study the histological changes in the cross section of intestine. Two cross sections (4-5 µm) of 10 per cent formalin preserved and processed segments from each duodenum sample were then prepared using microtome for staining with haematoxylin and eosin using standard paraffin embedding procedures (Uni *et al.*, 1995) [4]. A total of three intact well-oriented villi were selected in two replicates from each jejunum cross section (six measurements for each jejunum sample, with thirty six measurements per treatment). Villus height was measured from the tip of the villus to the bottom of the villus, and crypt depth was measured from the villus bottom to the crypt base. Villus height and crypt depth ratio was also calculated. This was done by instrument Nicon eclipse 80i.

Results and Discussion

Intestinal Morphology

In the present investigation on the effect of supplementation of organic mineral mixtures, probiotics, enzymes, emulsifier and liver stimulants in broilers on intestinal morphological parameters of intestine viz. duodenum length, jejunum length, ileum length, small intestinal length, duodenal villous height, duodenal crypt depth and villous height-crypt depth ratio were studied. The results obtained have been presented inTable1 and Fig1

Intestine length

Themean values of duodenum length as recorded in T_0 to T_{10} groups were30.77 ± 0.16,32.43 ± 0.21,33.40± 0.34,33.25± 0.05, 33.40 ± 0.02, 32.95 ± 0.11, 31.50± 0.23, 31.01 ±0.00, 31.92 ± 0.80, 33.46 ± 0.00 and 33.66 ± 0.09cm respectively. Maximum duodenum length of 33.66 ± 0.09 cm (9.39% more than control) was noticed in the broilers of T_{10} group followed by T_9(33.46 ± 0.00), T_2 (33.40 ± 0.34), T_4 (33.40 ± 0.02), T_5 (32.95 ± 0.11), T_1 (32.43 ± 0.21), T_8 (31.92 ± 0.80), T_6 (31.50 ± 0.23), T_7 (31.01 ± 0.00) and T_0 (30.77 ± 0.16). Duodenum length was significantly increased in the broilers of feed supplemented groups in comparison to control except T_6 and T_7. Minimum duodenum length (30.77 ± 0.16 cm) was noticed in T_0 group. However, duodenum length was non significantly different among T_1, T_3 T_5 and T_8; T_2, T_4, $T_9$and T_{10} groups of broilers.

Table 1: Effect of feed supplements on Intestinal Morphology of broilers

Treatments	Duodenum length (cm)	Jejunum length (cm)	Ileum length (cm)	Small intestinal length (cm)	Duodenum villous height (µm)	Duodenum crypt depth (µm)	Villous height :Crypt depth ratio
T_0	30.77^a± 0.16	55.89^a ± 0.56	55.19^a ± 0.18	141.85^a ± 0.64	1436.84^a ± 0.64	301.65^a± 0.70	4.76^a± 0.00
T_1	32.43^b± 0.21	57.56^a ± 0.46	56.96^b± 0.14	146.95^b ± 0.80	1526.78^b± 0.40	274.83^b ± 0.60	5.55^b ± 0.13
T_2	33.40^c ± 0.34	58.39^b± 0.68	57.49^b± 0.14	149.28^b ± 0.48	1542.74^{bc} ± 0.75	287.49^c ± 0.53	5.36^c ± 0.00
T_3	33.25^b ± 0.05	59.40^b ± 0.65	57.93^b± 0.09	150.58^b ± 0.54	1570.82^c ± 0.61	301.46^a ± 0.82	5.21^d ± 0.07
T_4	33.40^c ± 0.02	59.72^b± 0.47	58.14^b ± 0.02	151.26 ^{c} ± 0.71	1579.47^c ± 0.80	301.61^a ± 0.74	5.23^d ± 0.00
T_5	32.95^b ± 0.11	58.6^b ± 0.63	57.73^b ± 0.00	149.28^b ± 0.50	1558.72^c ± 0.75	297.80^a ± 0.64	5.23^d ± 0.00
T_6	31.50^a±0.23	56.79^a ± 0.65	56.79^a ± 0.65	145.08^a± 0.70	1457.60^a ± 0.39	247.61^d ±0.65	5.88^e ± 0.09
T_7	31.01^a± 0.00	56.57^a ± 0.78	55.75^a ± 0.14	143.33^a± 0.78	1442.77^a ± 0.62	241.56^e ± 0.74	5.97^e ± 0.13
T_8	31.92^b ± 0.80	57.55^a ± 0.66	56.73^a ± 0.00	146.20^b ± 0.76	1488.59^d ± 0.60	257.72^f± 0.71	5.77^f± 0.16
T_9	33.46^c ± 0.00	60.36^c ± 072	58.21^b± 0.00	152.03^c ± 0.63	1607.70^e ± 0.76	301.56^a ± 0.63	5.33^c ± 0.04
T_{10}	33.66^c ± 0.09	60.39^c± 0.62	58.53^b ± 0.16	152.58 ^{c} ±0.74	1613.59^e ± 0.69	237.91^e± 0.57	6.78^g± 0.08

Means bearing different superscripts in a column differ significantly (P<0.05)

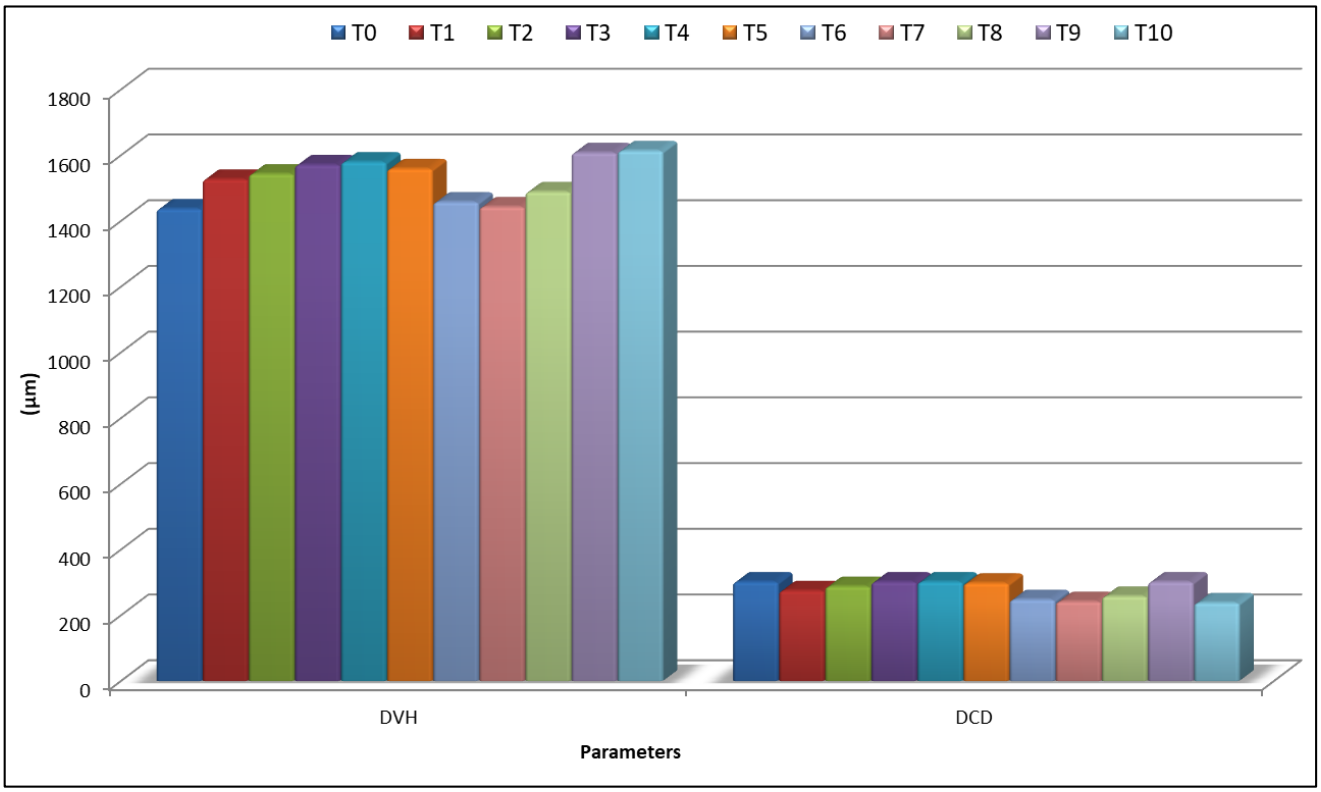

Fig 1: Effect of feed supplements on Intestinal Morphological Values of broilers

Themean values of jejunum length as recorded in T_0 to T_{10} groups were55.89 ± 0.56, 57.56 ± 0.46, 58.39± 0.68,59.40± 0.65, 59.72 ± 0.47, 58.60± 0.63, 56.79 ± 0.65, 56.57 ± 0.78,57.55 ±0.66,60.36 ± 0.72 and60.39±0.62 cm respectively. Maximum jejunum length of 60.39 ± 0.62cm(8.05% more than control) was noticed in the broilers of T_{10} group followed by T_9 (60.36 ± 0.72), T_4 (59.72 ± 0.47), T_3 (59.40 ± 0.65), T_5 (58.60 ± 0.63), T_2 (58.39 ± 0.68), T_1 (57.56 ± 0.46), T_8 (57.55 ± 0.66), T_6 (56.79 ± 0.65), T_7 (56.57 ± 0.78) and T_0 (55.89 ± 0.56). Jejunum length was significantly (P<0.05) increased in broilers of feed supplemented groups in comparison to control except T_1, T_6, T_7 and T_8. There were non significant difference in jejunum length among T_0, T_1, T_6, T_7 and T_8; T_2, T_3, T_4 and T_5; T_9 and T_{10}.

Theme an values of ileum length were recorded in T_0 to T_{10} groups were55.19 ± 0.18, 56.96 ± 0.14, 57.49± 0.14, 57.93 ± 0.09, 58.14 ± 0.02, 57.73 ± 0.00, 56.79 ± 0.65, 55.75 ± 0.14,56.73 ± 0.00,58.21 ± 0.00 and58.53 ± 0.16 cm, respectively. Maximum ileum length of 58.53 ± 0.16 cm (6.05% more than control) was noticed in the broilers of T_{10} group followed by T_9 (58.21 ± 0.00), T_4 (58.14 ± 0.02), T_3 (57.93 ± 0.09), T_5 (57.73 ± 0.00), T_2 (57.49 ± 0.14), T_1(56.96 ± 0.14), T_6 (56.79±0.65), T_8 (56.73±0.00), T_7 (55.75±0.14) andT_0 (55.19 ± 0.18). Ileum length was significantly increased in the broilers of feed supplemented groups in comparison to control except T_6 T_7. And T_8. Minimum ileum length (55.19 ± 0.18 cm) was noticed in T_0 (control) group. However, Ileum lengths were non significantly different among T_1, T_2, T_3, T_4, T_5, T_9 and T_{10}; T_6, T_7 and T_8 groups of broilers.

The mean values of small intestinallengthas recorded in T_0 to T_{10} groups were 141.85 ± 0.64, 146.95 ± 0.80, 149.28 ± 0.48, 150.58± 0.54, 151.26± 0.71, 149.28 ± 0.50, 145.08 ± 0.70, 143.33 ± 0.78,146.20 ± 0.76,152.03 ± 0.63 and 152.58 ± 0.74 cm, respectively. Maximum small intestine length of 152.58 ± 0.74 cm (7.56% more than control) was noticed in the broilers of T_{10} group followed by T_9 (152.03 ± 0.63), T_4 (151.26 ± 0.71), T_3 (150.58 ± 0.54), T_5 (149.28 ± 0.50), T_2 (149.28 ± 0.48), T_1(146.95 ± 0.80), T_8(146.20 ± 0.76), T_6 (145.08 ± 0.70),T_7(143.33 ± 0.78) and T_0(141.85 ± 0.6). Small intestinal length was significantly increased in the broilers of feed supplemented groups in comparison to control except T_6 andT_7. While minimum small intestine length (141.85 ± 0.64) cm was noticed in T_0. However, small intestine lengths were non significantly different among T_1, T_2, T_3, T_5 and T_8; T_0T_6 and T_7; T_4, T_9 and T_{10} groups of broilers.

Duodenum villous height

Mean values of duodenum villous height in T_0 to T_{10} groups were 1436.84 ± 0.64, 1526.78 ± 0.40, 1542.74 ± 0.75, 1570.82 ± 0.61, 1579.47 ± 0.80, 1558.72 ± 0.75, 1457.60 ± 0.39, 1442.77 ± 0.62, 1488.59 ±0.60, 1607.70 ± 0.76 and 1613.59 ± 0.69 µm, respectively. Maximum villous height of 1613.59 ± 0.69 µm (12.30% more than control) was found in T_{10} group broilers followed by T_9 (1607.70 ± 0.76), T_4 (1579.47± 0.80), T_3 (1570.82 ± 0.61), T_5 (1558.72 ± 0.75), T_2 (1542.74 ± 0.75), T_1 (1526.78 ± 0.40), T_8 (1488.59 ±0.60), T_6 (1457.60 ± 0.39), T_7 (1442.77 ± 0.62) and T_0 (1436.84 ± 0.64). Duodenum villous height was significantly increased in the broilers of feed supplemented groups in comparison to control except T_6 and T_7. Minimum duodenum villous height (1436.84 ± 0.64µm) was found in T_0 group broilers. There were non significant differences in the duodenum villous height between T_1 and T_2; T_2, T_3, T_4, and T_5; T_6 and T_7; T_9 and T_{10} groups of broilers.

Duodenum crypt depth

Mean values of duodenum crypt depth in T_0, to T_{10} groups were 301.65 ± 0.70, 274.83 ± 0.60, 287.49 ± 0.53, 301.46 ± 0.82, 301.61 ± 0.74, 297.80 ± 0.64, 247.61 ± 0.65, 241.56 ± 0.74, 257.72 ±0.71, 301.56 ± 0.63and237.91 ± 0.57µm in broilers of T_0 to T_{10} groups respectively. Minimum crypt

depth of 237.91 ± 0.57 µm (21.13% less than control) was noted in broilers of T_{10} group followed by T_7 (241.56 ± 0.74), T_6 (247.61 ± 0.65), T_8 (257.72 ±0.7), T_1 (274.83 ± 0.60), T_2 (287.49 ± 0.53), T_5 (297.80 ± 0.64), T_3 (301.46 ± 0.82), T_9 (301.56 ± 0.63), T_4 (301.61 ± 0.74) and T_0 (301.65±0.70). Duodenum crypt depth was significantly decreased in broilers of feed supplemented groups in comparison to control except T_3, T_4, T_5 and T_9. Maximum crypt depth (301.65 ± 0.70 µm) was recorded in broilers of T_0 group. Duodenum crypt depth of T_3, T_4, T_5 and T_9; T_7 and T_{10} groups were statistically similar.

Villous height: crypt depth ratio

Mean values of villous height and crypt depth ratio in T_0, to T_{10} groups were 4.76 ± 0.00, 5.55 ± 0.13, 5.36 ± 0.00, 5.21 ± 0.07, 5.23 ± 0.00, 5.23 ± 0.00, 5.88 ± 0.09, 5.97 ± 0.13, 5.77 ± 0.16, 5.33 ± 0.04 and 6.78 ± 0.08, respectively. Maximum villous height and crypt depth ratio of 6.78 ± 0.08 (42.43% more than control) was recorded in broilers of T_{10} group followed by T_7 (5.97 ± 0.13), T_6 (5.88 ± 0.09), T_8 (5.77 ± 0.16), T_1 (5.55 ± 0.13), T_2 (5.36 ± 0.00), T_9 (5.33 ± 0.04), T_5 (5.23 ± 0.00), T_4 (5.23 ± 0.00), T_3 (5.21 ± 0.07) and T_0 (4.76 ± 0.00). Villous height: crypt depth ratio was significantly increased in the broilers of feed supplemented groups in comparison to control. Minimum villous height and crypt depth ratio (4.76 ± 0.00) was noted in broilers of T_0 group. There were no significant differences in the villous height and crypt depth ratio among T_3, T_4 and T_5; T_6 and T_7; T_2 and T_9.

Wang *et al.,* (2016) [3] found significant (P<0.05) increase in duodenal, jejunal and illeal length of broilers fed diet supplemented with probiotics. Echeverry *et al.,* (2016) [2] found significantly (P<0.05) lower crypt depth and higher villi height/crypt depth ratio in broilers fed diet supplemented with organic trace minerals. Abdel-Latif *et al.,* (2018) [1] found significant (P<0.05) increase in the villi height and villus height/crypt depth ratio in broilers while crypt depth was significantly (P<0.05) decreased in broilers fed diet supplemented with probiotics.

Conclusion

Thus it may be inferred that supplements had altered the gut morphology by stimulating rapid cell division & elongation and more inter cellular space leading to increased surface area for absorption of nutrients as evidenced by improvements in feed efficiency and growth performance.

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