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Post Hatch Histo-morphological Studies of Small Intestinal Development in Chicks Fed with Herbal Early Chick Nutritional Supplement

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Abstract: Earlier the food passes through gastrointestinal tract, better is the stimulus for initiating the gut function development. Morphology of the small intestine of Vencobb broiler chick was determined immediately post hatch by comparing between untreated control group fed with standard basal ration 48 h after hatch and the treatment groups (T_2 and T_3) offered Chikimune at 2 different doses of 6 and 8 g/chick/day for early 2 days followed by administration of standard basal ration after 2 days. Pattern of development of the intestinal mucosa, mechanisms underlying the structural changes in small intestine were assessed. The length, weight and diameter of different parts of small intestine developed significantly earlier in treatment groups (II and III) as compared to control (T_1). Crypt depth and villous height increased with age in the duodenum, jejunum and illeum. There were also significant changes in apparent villous surface area in the three regions, while interactions between age and intestinal region were significant in the case of crypt depth and villous height although, the intestinal mucosa of the strain was structurally developed at hatch, there was much change in structure with age, especially over the first 7 day post hatch.

Key words: Post hatch, early chick, intestine, morphology, villous, crypt

INTRODUCTION

Post hatch nutrition in early few days helps in utilization of yolk sac in immunity development and in gut function enhancement. At hatching, the digestive system of chick is anatomically immature and its functional capacity is not fully developed. The gastrointestinal tract undergoes morphological changes (increase in intestinal length, villous height and density) and physiological changes (increased production of pancreatic and digestive enzymes) including increased surface area of digestion and absorption during the post hatch period. During the post hatch period, the small intestine weight increases at a faster rate than the body mass (Katanbaf *et al*., 1988; Sell *et al*., 1991; Sklan, 2001) because of rapid enterocyte proliferation and differentiation (Geyra *et al*., 2001a). Intestinal crypts begin to form at hatch and are clearly defined at several days post-hatch, increasing in both cell numbers and size (Geyra *et al*., 2001a; Uni *et al*., 2000). Previous studies have shown that feeding immediately after hatching accelerates the morphological development of the small intestine (Noy and Sklan, 1998), while delayed access to external feed arrests the development of the small intestine mucosal layer (Geyra *et al*., 2001a; Uni *et al*., 1998; Uni *et al*., 2003). Furthermore, day old chick denied access to first feed for 24-48 h have decreased villi length (Yamauchi *et al*., 1996), decreased crypt size and crypts per villi and decreased enterocytes migration rate (Geyra *et al*., 2001b). In addition, delayed access to feed for 48 h post-hatch resulted in changes in mucin dynamics, which affects the absorptive and protective functions of the small intestine (Uni *et al*., 2003). Early feeding has a great effect in triggering gut development in broiler hatchlings. The previous studies by various scientists showed that the nutrient supply to chick as early as possible can increase the intestinal mechanical activity, faster intestinal development, greater assimilation of feed, development of immunity and thereby overall growth performance. The concept of providing the chicks with additional nutrient source in early stages is termed as early chick nutrition. However, today with our intensive growing system we are losing potential growth if we do not make feed available immediately after hatch. In view of above the present study was undertaken to evaluate the effect of polyherbal formulation Chikimune (supplied by M/S Ayurvet Ltd Baddi (H.P.), India), an early chick nutritional supplement formula, on gut development in broilers.

MATERIALS AND METHODS

The present experiemental study was conducted with an aim to assess the efficacy of early chick nutritional formula 'Chikimune', in accelerating intestinal development and morphogenesis. Chikimune is a composite post hatch balanced nutrient package fortified with carbohydrate, protein, vitamins, fats, trace minerals,

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spirulina and herbs. 150 day-old straight-run "Vencob" broiler chicks were procured from hatchery immediately after hatch and were randomly divided into three groups, control group T_1 and treatment groups; T_2 and T_3 and transferred into 3 different chick boxes. Feeding of Chikimune was initiated immediately after hatch and continued during the transport till brought to the farm and until completion of 48 h of post hatch to the treatment groups T_2 and T_3 in 2 different doses of 6 and 8 g/chick/day for 2 days. After transportation from hatchery, the chicks belonging to three different groups were kept in deep litter system at an organized poultry farm, Shirval, Veterinary College, Maharashtra, India, for the experiemental period of six weeks. Standard Basal ration with similar composition was fed to the chicks in all the 3 groups after 48 h of hatch as per NRC requirements. The chicks were reared in deep litter system under standard managemental practices and environmental conditions for 6 weeks. The biometrical observations of small intestine were carried out on total 54 randomly selected birds, 6 birds/group at the age of 2^{nd}, 4^{th} and 42^{nd} day. Immediately after decapitation, the small intestine of all birds were collected and cleared from fascia and other tissue debris. Digesta were obtained by gentle manipulation from duodenum, jejunum and lower ileum and length, weight and diameter of each segment of the small intestine was measured. The weight of each sample was recorded with the help of electronic weighing balance; length was measured with non stretchable thread and scale while the diameter was measured with electronic Vernier Caliper. Villi height was measured using lamina propria as the base; crypt height was defined as the depth of invagination between adjacent villi. After recording the biometrical observations the tissue samples were fixed in 10% formalin for more than 24 h and the tissue samples (pieces) were treated with routine dehydration and histological staining procedure. A 1-cm segment of the midpoint of the duodenum and the distal end of the lower ileum were removed and fixed in 10% buffered formalin for 72 h. Each segment was then embedded in paraffin and a 2 µm section of each sample was placed on a glass slide and stained with hematoxylin and eosin for examination with a light microscope (Sakamoto *et al*., 2000).

RESULTS AND DISCUSSION

This experiemental study revealed new scientific findings on cryptogenesis, villi development and enterocyte dynamics in the small intestines of the post hatch broiler chick which was accelerated in the present study by Chikimune supplementation in treatment groups T_2 and T_3; however, both the treatment groups do not differ significantly in results.

Length, weight and diameter of the duodenum, Jejunum and Ileum (cm): Development of duodenum, jejunum and ileum was initiated on day 2^{nd} in treatment groups (T_2 and T_3) as compared to control group (T_1) (Table 1). Early maturation of the gut has been shown to be an important factor in raising a healthy chick, as the physiological development of birds is directly related to digestion and nutrient absorption in the small intestine (Aptekmann *et al*., 2001). In this study, significant increases in gut development were observed 4 days post hatch, as previously reported by Uni *et al*., (1999). These dramatic developmental changes occur in the avian gut 2 to 3 day post hatch due to a change in nutrient sources from yolk to an exogenous feed ration rich in carbohydrates (Uni *et al*., 1999). Control group revealed signs of early development on day 4^{th} in different segments of small intestine. The length of the duodenum on day 4^{th} was significantly greater in treated groups T_2 (13.23±00.36 cm) and T_3 (13.47±00.19 cm) while 12.20±00.24 cm in T_1 group. At the end of 6 weeks, a similar trend has been observed; duodenal length was 32.32±01.26 cm in group T_2, 32.90±00.90 cm in T_3 and 27.90±01.53 cm in T_1 group. The results are in concomitance to those of Handerson *et al*. (2008) who reported that supplementation of nutritional supplements immediately after hatching helps in development of digestive system which evokes the nutrient utilization, growth and overall development of chicks. Providing early feed supplements has been shown to improve the digestive system which ultimately reflects with increased body weight (Noy and Sklan, 1999). The weight of duodenum, jejunum and ileum on day 2^{nd}, 4^{th} and 42^{nd} was significantly increased in both the treatments as compared to control group (Table 1), however the two treatments were non-significantly different from each other. The results are in confirmation with those reported by Geyra *et al*. (2001a) that feeding immediately post-hatch results in accelerated intestinal morphogenesis and enterocyte differentiation. According to Pinchasov and Noy (1994); the relative weight of intestine increases post-hatch, reaching a maximum at 3 to 7 days and declines slightly thereafter. Although, chicks intestinal system is anatomically complete in the embryonic stage (Overton and Shoup, 1964; Lim and Low, 1977; Chambers and Grey, 1979), the absorptive surface changes considerably post-hatch and the rate of enterocyte proliferation increases (Cook and Bird, 1973; Moran, 1985); similar findings have been recorded in present investigation. The average diameter of duodenum, jejunum and ileum on day 2^{nd}, 4^{th} and 42^{nd} was significantly higher in Chikimune treated groups (T_2 and T_3) as compared to untreated control group (T_1). The intestinal development in treated groups was at faster rate as compared to untreated group.

Table 1: Length, weight and height of small intestine in group I, II and III at day 2nd, 4th and 42nd of experiemental study

Days of obs.	Duodenal length (cm)			Duodenal weight (gm)			Duodenal diameter (mm)		
	G I	G II	G III	G I	G II	G III	G I	G II	G III
2nd	11.92±00.37a	10.95±00.44a	11.37±0.31a	00.64±0.04a	00.69±00.03b	00.99±0.13b	02.31±0.10a	02.57±0.04b	02.77±0.05b
4th	12.20±00.24a	13.23±00.36b	13.47±00.19b	01.61±0.07a	01.87±0.13b	01.79±0.15b	03.60±0.05a	03.53±0.11a	03.48±0.10a
42nd	27.90±01.53a	32.32±01.26b	32.90±00.90b	10.17±0.33a	12.00±0.52b	11.92±0.27b	09.61±0.78a	09.32±0.57a	09.78±0.70a
	Jejunum length (cm)			Jejunum weight (gm)			Jejunum diameter (mm)		
2nd	28.75±0.52a	31.13±0.30	29.51±0.43	00.76±0.04a	01.07±0.04b	02.28±0.13b	02.15±0.04a	02.03±00.04a	02.54±0.05b
4th	31.57±0.25a	31.83±0.23a	32.25±0.60b	03.13±0.09a	03.52±0.23b	03.37±0.11b	02.89±0.13a	03.75±00.18b	03.53±0.04b
42nd	00.76±0.05a	100.38±6.44a	102.05±2.91b	24.42±01.37a	23.67±01.20a	25.10±00.55b	11.13±0.99a	08.92±00.53b	10.33±0.86a
	Ileum lenght (cm)			Ileum weight (gm)			Ileum diameter (mm)		
2nd	03.72±00.20a	03.71±00.20a	04.40±00.21b	00.16±00.02a	00.20±00.02	00.53±00.02b	01.50±00.05a	01.85±00.05b	02.28±0.02b
4th	04.12±00.09a	04.20±00.11b	04.23±00.16b	00.46±00.01a	00.56±00.03b	00.67±00.04b	02.72±00.03a	03.02±00.14b	02.69±0.09a
42nd	15.80±00.72a	16.28±00.72b	15.28±00.58a	10.50±00.76a	09.83±00.4a	09.63±00.84a	08.20±0.37a	08.84±00.83b	09.28±00.44b

Means bearing different superscripts in a column differ significantly at ($p \leq 0.05$). Obs. = Observation

Table 2: Height of villi, crypt and epithelia of small intestine in group I, II and III at day 2nd, 4th and 42nd of experiemental study

Days of obs.	Duodenal villi height (µm)			Duodenal crypt height (µm)			Duodenal epithelium height (µm)		
	G I	G II	G III	G I	G II	G III	G I	G II	G III
2nd	276.96±10.32a	387.30±11.92b	680.00±17.32b	60.80±04.46a	72.05±06.68b	76.56±09.01b	15.71±1.16a	16.34±1.26b	20.74±0.84b
4th	396.29±16.24a	418.81±20.34b	513.38±07.80b	85.56±04.5a	85.66±04.49b	99.07±04.50b	28.28±1.61a	33.30±1.16a	33.30±1.80b
42nd	1038.02±21.90a	1130.24±26.72b	1549.21±48.24b	65.30±06.45a	99.07±10.27b	150.86±16.88b	33.93±1.38a	42.73±1.59a	45.74±2.06b
	Jejunum villi height (µm)			Jejunum crypt height (µm)			Jejunum epithelium height (µm)		
2nd	132.85±06.45a	202.65±15.60b	234.17±13.81b	45.03±2.85	51.79±4.15b	56.29±4.15b	08.17±0.63	10.05±0.79b	13.20±0.84b
4th	321.98±14.55a	310.73±18.46b	321.99±08.12b	63.05±5.70	49.54±4.50	63.05±7.70b	21.99±1.16	23.25±1.80b	28.90±1.26b
42nd	961.46±14.56a	1096.56±23.57b	1121.66±24.98b	78.82±7.60	81.06±4.43b	108.08±4.93b	23.25±1.16	32.67±1.16b	33.93±1.38b
	Ileum villi height (µm)			Ileum crypt height (µm)			Ileum epithelium height (µm)		
2nd	114.84±05.78a	132.85±06.45b	198.15±11.91b	29.27±2.25a	33.78±3.02b	33.78±3.02b	08.87±0.67a	08.17±1.16a	10.05±0.79a
4th	274.12±08.91a	328.74±22.24b	270.20±07.80a	49.54±4.50a	29.27±2.25b	27.02±0.00b	14.45±1.16a	27.02±1.16b	23.88±1.86b
42nd	400.80±14.24a	438.35±11.91b	775.51±20.64b	54.04±4.93a	87.80±5.78b	67.55±4.93b	19.48±1.16a	26.39±1.38b	22.62±2.18b

Means bearing different superscripts in a column differ significantly at ($p \leq 0.05$). Obs. = Observation

Histological (Micrometrical) observations (Height and weight of villi, crypt depth and epithelium of small intestine (µm)): Six birds per group were taken at the age of 2nd day, 4th day and 42nd for the recording of micrometrical observations. The villi are larger and the degree of organization is significantly higher in treated groups with Chikimune (Fig. 1-6). (Table 2), (Fig. 1 and 3) presents the average height of villi, crypt and epithelium of the duodenum (µm) on day 2nd and 4th in different segments which were significantly increased in Chikimune treatment groups (T_2 and T_3) as compared to untreated control group (T_1). Greatest villous height was recorded in jejunum among treated groups; however, the two treatments did not differ significantly as depicted in histomicrographs (Fig. 2, 3 and 6). Crypt depth was also found to be proportionately increased with the advancement of age. A significant difference among treated and control groups were recorded for crypt and epithelia height at 2nd, 4th and 42nd day. This depicts that providing early nutrition to chick helps in not only villi growth but also enterocyte differentiation (Dibner and Knight, 1999). Villous growth in young chick is stimulated by presence of feed and involves generation of cells in the crypt that attain maturity while ascending the shaft , also the increase in volume of villi with age was greater in jejunum and illeum (Moran, 1985). The constituent herbs *Aegle marmeloes and Vitis vinifera* of polyherbal formulation are scientifically studied for improving gut function and villous development. It has also been reported that supplementation of nutrients during post-hatch period stimulates the increase in size and number of villi during 4 to 10 days (Bayer *et al*., 1975). The results in present study are in concomitance with those reported by (Noy and Sklan, 1995). Birds denied access to first feed for 24-48 h post-hatch have decreased villi length, decreased crypt size, number of crypts per villi and decreased enterocytes migration rate. In addition, delayed access to feed for 48 h post-hatch resulted in changes in mucin dynamics, which affects the absorptive and protective functions of the small intestine (Ferkt, 2009). The constituent herbs present in Chikimune synergistically work with the nutrients to achieve the early development of different parts of small intestine. The major constituent herbs of Chikimune are *Vitis vinifera, Aegle marmelos, Terminalia chebula, Zizyphus mauritiana, Phyllanthus emblica, Terminalia belerica, Spirulina* and many more which are scientifically well proven for their efficacy of improving the digestion and development of digestive system in post hatch chicks (Kadam *et al*., 2009). The overall results of

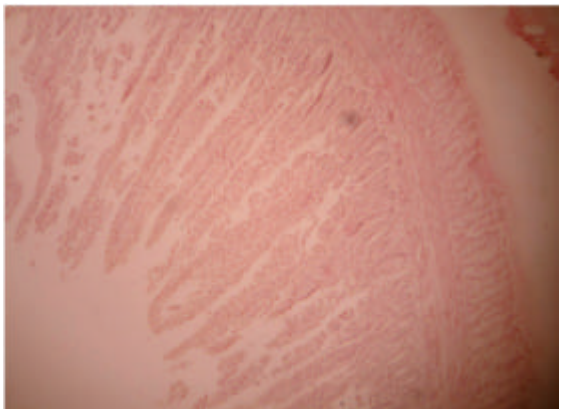

Fig. 1: Photomicrograph of duodenal epithelia, crypt and villi in group I at day 4th

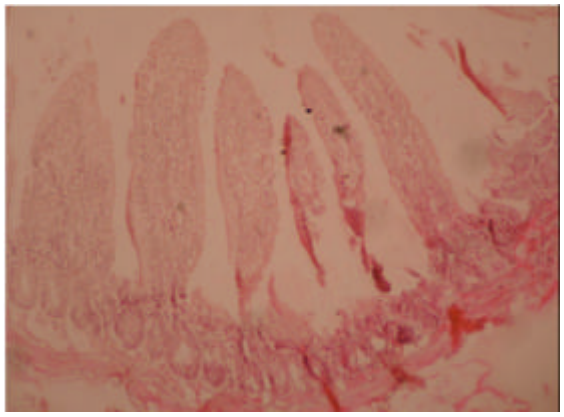

Fig. 2: Photomicrograph of jejunal epithelia, crypt and villi in group I at day 4th

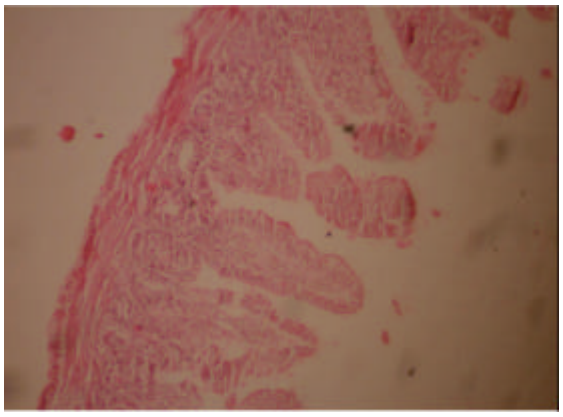

Fig. 3: Photomicrograph of jejunal epithelia, crypt and villi in group II at day 4th

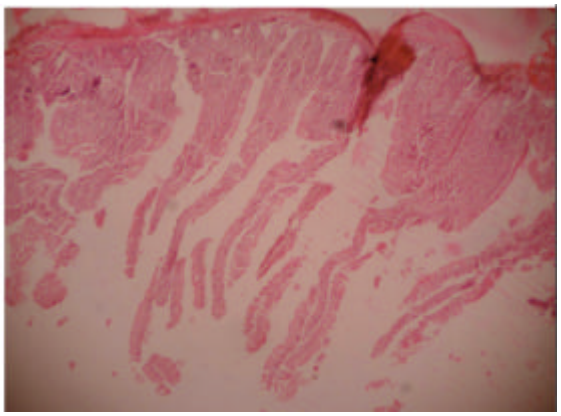

Fig. 4: Photomicrograph of duodenal epithelia, crypt and villi in group I at day 42nd

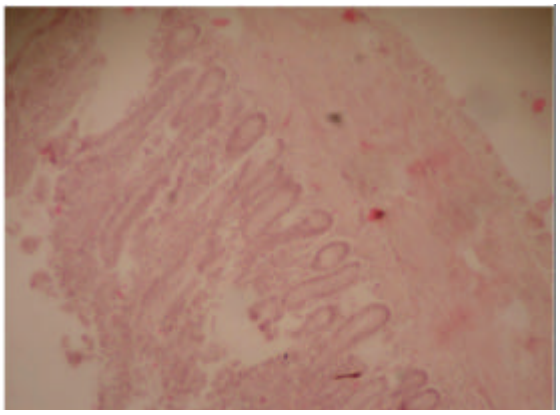

Fig. 5: Photomicrograph of duodenal epithelia, crypt and villi in group II at day 42nd

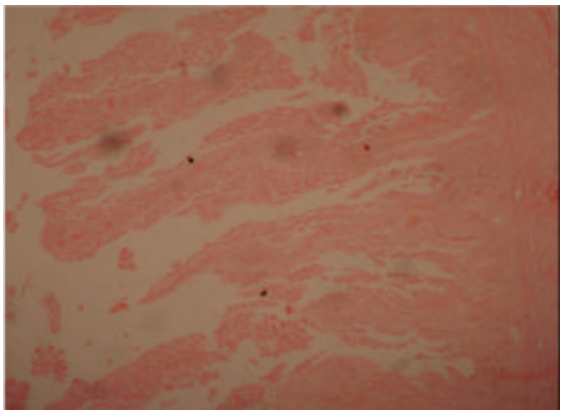

Fig. 6: Photomicrograph of jejunal epithelia, crypt and villi in group I at day 42nd

the study indicated that feeding Chikimune has significant effect upon intestinal morphogenesis, villous growth and development and on overall histomorphology of small intestine of birds (Fig. 1-6).

This may be attributed to the synergistic action of certain herbs responsible for stimulation development of intestinal epithelium, crypts and tissue.

Conclusion: Supplementation of Chikimune containing synergistic herbs alongwith carbohydrate, protein source has prevented the early post-hatch energy deficit in chicks so that yolk can be utilized for initiating intestinal growth and development It can be concluded that a balanced nutrition in chicks during early 24-48 h is important to achieve efficient yolk utilization, better immune response and for overall efficient growth and performance in birds. Supplementation of polyherbal early chick nutritional supplement Chikimune@ 6 g and 8 g/chick to chicks during first 48 h has revealed not only short term but a long term and significant impact on intestinal health and early morphogenesis.

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