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# Effects of Dietary Inclusion of Enzymes and Probiotic on Organ Weights and Intestinal Morphology of Broiler Chickens

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**ABSTRACT**

The present experiment was conducted to examine the effect of Enzymes (Enzymex) and Probiotic (Yeamark) on organ weights and intestinal histomorphology parameters in Ven Cobb$^{400}$ broilers. Three hundred and sixty chicks were divided into eight groups viz. control ($T_1$) in which no supplement was added to the feed, while in treatments $T_2$, $T_3$ and $T_4$ cocktail of enzymes was provided as 0.25, 0.50 and 0.75 g per kg of feed, respectively, in treatment $T_5$ probiotic was added as 0.25 g per kg and in treatment $T_6$, $T_7$ and $T_8$ cocktail of enzymes as in $T_2$, $T_3$ and $T_4$ with probiotic as 0.25 g per kg in the basal diet from 1$^{st}$ to 6$^{th}$ weeks of age respectively. The liver and gizzard weights were greater ($P < 0.05$) for probiotic compared with enzyme supplemented birds. Furthermore, dietary treatments influenced the morphological measurements of small intestine. The addition of enzyme, probiotic and their combinations increased ($P < 0.05$) the villus height to crypt depth ratio and villus height in duodenum. The increase in the villus height to crypt depth ratio was associated with improvement of growth performance for both probiotic and enzymes and their combination. This indicates that the probiotic and enzymes and their combinations can be used as a growth promoter in broiler diets and can improve the gut health. These products show promising effects as alternatives for antibiotics as pressure to eliminate growth-promotant antibiotic use increases.

**Keywords:** Broiler, enzymes, probiotic, organ weights

Poultry production has garnered significant landmark in animal production. From a backyard venture five decades ago (Singh *et al.,* 2014), the Indian Poultry industry has evolved as the most vibrant fast growing and dynamic sub-sector of agriculture with 7.3% growth in poultry population, has witnessed one of the fastest annual growth of about 6% in egg and 10% in meat production over the last decade amongst all animal based sectors (CARI VISION 2050). The industry has not only grown in size but also in productivity. With rapidly changing lifestyles, affluent culture, and a conscious need for general wellness, Indian consumers are now opting for a more protein-rich diet (CARI VISION 2050). The changing trends are definitely a boon for the poultry sector in India. Feed additives or growth promoters have been used to improve growth rate, feed efficiency, and product quality and to reduce the production cost in poultry Craig *et al.* (2008). Various antibiotics, anthelmintics, anti-coccidials and hepato-protectants are used for increasing production. They not only increase the cost of production but have adverse effects on long term usage. Due to prohibition of most of the antimicrobial feed additives in animal feed and their residual effects in animals, enzymes and probiotic are becoming more popular Chuka (2014). The effects of enzymes and a probiotic on the intestinal morphology in association to their organs weights of broilers are still unclear. Therefore, the present investigation was undertaken to study organ weights and intestinal morphological values.

## MATERIALS AND METHODS

### Experimental birds and dietary treatments

The present study was undertaken at the Instructional

Poultry Farm (IPF), of Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, U.S. Nagar from September to December 2012. The place is located between 28° 53' 23" to 30° 27' 50" N and 77° 34' 27" to 81° 02' 22" E at 243.84 m MSL (mean sea level) in the Tarai region of Uttarakhand State (India). The climate is humid subtropical. Winters are very severe and summers are hot and humid. Temperatures may rise to a maximum of 43°C in the summer and fall to a minimum of 2°C in the winter. Relative humidity ranges between 15 to 95% (Singh *et al.,* 2015). The study was conducted on 360 day-old straight run Ven Cobb$^{400}$ broiler chicks for a period of 6 weeks under standard management conditions. Feed and water were provided *ad libitum*. The first treatment was considered as control $T_0$ in which no supplement was added to the feed, while in treatments $T_2$, $T_3$ and $T_4$ cocktail of enzymes was provided as 0.25, 0.50 and 0.75 g per kg of feed, respectively, in treatment $T_5$ probiotic was added as 0.25 g per kg and in treatment $T_6$, $T_7$ and $T_8$ cocktail of enzymes as in $T_2$, $T_3$ and $T_4$ with probiotic as 0.25 g per kg in the basal diet at the end of feeding trial on 42$^{nd}$ day, two birds from each replicate were randomly selected and there organ weights were taken.

### Intestinal morphology

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, villus height was measured from the tip of the villus to the bottom of the villus, crypt depth was measured from the villus bottom to the crypt base and villus height and crypt depth ratio was also calculated to study the effect of enzymes and probiotic supplementation on the intestinal gross morphology.

### Statistical analysis

The data were analysed statistically by using SPSS 19 and significant mean differences between the treatments were determined at $P<0.05$ using Duncan's Multiple Range Test as modified by Kramer (1957).

## RESULTS AND DISCUSSION

### Organ weights

The effect of enzymes and probiotic supplementation on organ weights of the broilers has been shown in Table 1. Liver weight was significantly increased in the broilers of enzymes and probiotic supplemented i.e. groups $T_3$, $T_4$, $T_6$, $T_7$ and $T_8$ in comparison to other groups. The effect of higher inclusion levels of enzymes and probiotic and their combination on liver weight increase was more pronounced as compared to lower level. Maximum (2.72 ± 0.01 per cent) liver weight was noticed in the birds of $T_8$ group which was statistically similar to the liver weight of $T_7$ group while minimum liver weight (2.53 ± 0.01 per cent) was noticed in control group broilers which was statistically similar to the liver weight of $T_2$ and $T_5$ groups. However, the probiotic product displayed a greater growth-promoting effect than the enzymes.

Significantly higher and maximum (2.21 ± 0.01 per cent) gizzard weight was found in $T_8$ group broilers while minimum gizzard weight (2.02 ± 0.01 per cent) was found in control group ($T_1$) broilers. There were no significant differences in the gizzard weight among $T_1$, $T_2$ and $T_5$, $T_3$, $T_4$ and $T_6$ and between $T_7$ and $T_8$ groups of broilers. The effect of higher inclusion levels of enzymes and probiotic and their combination on gizzard weight increase was more pronounced as compared to lower level.

Results of present investigation revealed that weights of heart, pancreas and spleen were statistically not affected by enzymes and probiotic supplementation in broilers.

Narasimha (2013) observed that weights of visceral organs were not affected by supplementation of enzymes and probiotics of broilers. Zakaria *et al*. (2010) also found no significant effects on the heart weight in birds fed diet supplemented with enzyme.

Abudabos (2010) did not report any significant effect on the weights of the liver and gizzard in broilers supplemented with enzymes. Shareef and Al-Dabbagh (2009) reported that there was no significant difference in liver, gizzard, heart, spleen and pancreas weights in all the *Saccharomyces cerevisiae* (probiotic) supplemented groups of broilers.

### Intestinal morphological values

The effect of enzymes and probiotic supplementation on intestinal morphology of the broilers has been shown in Table 2.

**Table 1:** Organ weights (% of live weight) of broilers (Mean±SE) in different treatment at 42nd days

| Treatments | Liver | Gizzard | Heart | Pancreas | Spleen |
|---|---|---|---|---|---|
| $T_1$ | $2.53^{c} \pm 0.01$ | $2.02^{c} \pm 0.01$ | $0.57 \pm 0.00$ | $0.26 \pm 0.00$ | $0.22 \pm 0.01$ |
| $T_2$ | $2.55^{c} \pm 0.01$ | $2.05^{c} \pm 0.01$ | $0.56 \pm 0.01$ | $0.26 \pm 0.00$ | $0.21 \pm 0.01$ |
| $T_3$ | $2.62^{b} \pm 0.01$ | $2.10^{b} \pm 0.01$ | $0.55 \pm 0.01$ | $0.26 \pm 0.00$ | $0.21 \pm 0.01$ |
| $T_4$ | $2.62^{b} \pm 0.01$ | $2.10^{b} \pm 0.02$ | $0.55 \pm 0.02$ | $0.26 \pm 0.01$ | $0.22 \pm 0.01$ |
| $T_5$ | $2.54^{c} \pm 0.01$ | $2.04^{c} \pm 0.02$ | $0.56 \pm 0.01$ | $0.26 \pm 0.01$ | $0.22 \pm 0.01$ |
| $T_6$ | $2.63^{b} \pm 0.01$ | $2.12^{b} \pm 0.00$ | $0.55 \pm 0.01$ | $0.25 \pm 0.00$ | $0.21 \pm 0.01$ |
| $T_7$ | $2.71^{a} \pm 0.02$ | $2.20^{a} \pm 0.00$ | $0.54 \pm 0.01$ | $0.25 \pm 0.01$ | $0.21 \pm 0.00$ |
| $T_8$ | $2.72^{a} \pm 0.01$ | $2.21^{a} \pm 0.01$ | $0.55 \pm 0.01$ | $0.25 \pm 0.00$ | $0.21 \pm 0.01$ |

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

**Table 2:** Intestinal morphological values of broilers (Mean±SE) in different treatment group at 21st and 42nd days

| Treatments | Intestinal morphological values | | | | | | |
|---|---|---|---|---|---|---|---|
| | cm | | | | µm | | — |
| | Duodenum length | Jejunum length | Ileum length | Small intestinal length | Duodenum villous height (DVH) | Duodenum crypt depth (DCD) | DVH/DCD |
| $T_1$ | $32.00 \pm 0.77$ | $82.70 \pm 0.79$ | $84.10 \pm 0.78$ | $198.80 \pm 0.95$ | $1370.4^{f} \pm 2.85$ | $171.4^{a} \pm 1.39$ | $8.00^{e} \pm 0.05$ |
| $T_2$ | $31.70 \pm 0.33$ | $82.50 \pm 0.37$ | $83.80 \pm 0.51$ | $198.00 \pm 0.43$ | $1394.8^{e} \pm 1.68$ | $165.9^{a} \pm 1.46$ | $8.41d^{e} \pm 0.06$ |
| $T_3$ | $31.80 \pm 0.37$ | $82.00 \pm 1.02$ | $83.90 \pm 0.66$ | $197.70 \pm 1.41$ | $1511.2^{c} \pm 6.17$ | $156.4^{b} \pm 3.25$ | $9.68^{c} \pm 0.16$ |
| $T_4$ | $31.50 \pm 0.42$ | $82.10 \pm 0.60$ | $83.50 \pm 1.18$ | $197.10 \pm 1.30$ | $1493.4^{d} \pm 2.23$ | $167.8^{a} \pm 2.30$ | $8.91^{d} \pm 0.11$ |
| $T_5$ | $31.90 \pm 0.72$ | $82.30 \pm 0.96$ | $83.60 \pm 0.32$ | $197.80 \pm 0.90$ | $1376.3^{f} \pm 3.14$ | $170.1^{a} \pm 1.46$ | $8.09^{e} \pm 0.05$ |
| $T_6$ | $31.30 \pm 0.44$ | $81.80 \pm 0.81$ | $83.10 \pm 0.82$ | $196.20 \pm 1.40$ | $1573.2^{b} \pm 2.70$ | $153.8^{b} \pm 3.29$ | $10.25^{b} \pm 0.20$ |
| $T_7$ | $31.10 \pm 0.33$ | $81.60 \pm 0.68$ | $82.90 \pm 0.96$ | $195.60 \pm 1.47$ | $1631.7^{a} \pm 4.31$ | $143.3^{c} \pm 4.31$ | $11.43^{a} \pm 0.31$ |
| $T_8$ | $30.90 \pm 0.33$ | $81.50 \pm 0.57$ | $82.70 \pm 0.59$ | $195.10 \pm 1.45$ | $1640.6^{a} \pm 6.29$ | $138.8^{c} \pm 3.80$ | $11.86^{a} \pm 0.29$ |

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

### Intestine length

The results of the present investigation indicated that the intestine length was not affected due to enzymes and probiotic supplementation. The above results regarding the intestine length revealed a numerical variation between the groups supplemented with enzymes and probiotic. Therefore, it may be concluded that the enzymes and probiotic supplementation did not affect the intestine length of broilers.

Momtazan *et al.* (2011) noted that inclusion of Anvenzyme complex and probiotics (*L. acidophilus*, *L. casei*, *Bifidobacterium bifidum*, *Enterococcus faecium*) mixture of broilers reduced the relative weight of duodenum and length of the jejunum.

### Duodenal villous height

Broilers of enzymes and probiotic supplemented groups $T_2$, $T_3$, $T_4$, $T_6$, $T_7$ and $T_8$ noted significant higher duodenal villous height. There were no significant differences in the villous height between $T_1$, $T_5$, and $T_6$, $T_7$ groups of broilers.

Study done by Gao *et al.* (2008) revealed that supplementation of broilers with *Saccharomyces cerevisiae* increased the height of duodenal villi significantly. Luo *et al.* (2009) found that supplementation of xylanase improved the villus height in the duodenum. Chuka (2014) found that increased villous height in probiotic supplemented groups may be due to the mannan oligosaccharide content of yeast**,** a naturally derived extract from the cell wall of *Saccharomyces cerevisiae* having a trophic effect on

intestinal wall thereby increasing the villi height in broiler chicken.

### Duodenal crypt depth

Supplementation of enzymes and probiotic in broilers showed a significant ($P<0.05$) impact on duodenal crypt depth. Maximum (171.4 ± 1.39 µm) crypt depth was recorded in broilers of $T_1$ group and minimum (138.8 ± 3.80 µm) crypt depth was noted in broilers of $T_8$ group. Duodenal crypt depth of $T_1$, $T_2$, $T_4$ and $T_5$, $T_3$ and $T_6$ groups as well as $T_7$ and $T_8$ groups were statistically similar.

The findings of the present experiment were supported by Awad *et al.* (2009) who found that supplementation of probiotics decrease the crypt depth in the small intestine of broilers.

### Villous height-crypt depth ratio

Maximum (11.86 ± 0.29) villous height and crypt depth ratio was recorded in broilers of $T_8$ group while minimum (8.00 ± 0.05) villous height and crypt depth ratio was noted in broilers of $T_1$ group. There were no significant differences in the villous height-crypt depth ratio among $T_1$, $T_2$ and $T_5$ groups as well as $T_7$ and $T_8$ groups of broilers.

Awad *et al*. (2009) showed that supplementation of *Lactobacillus species* increased the villus height: crypt depth ratio both duodenum and ileum. Similarly, Luo *et al.* (2009) found that supplementation of xylanase improved the villus height: crypt depth ratio in the small intestine of broilers. The increase in the villus height and villus height: crypt depth ratio was associated with improvement in feed efficiency and growth performance by probiotic and it can be used as a growth promoter in broiler diets and can improve the gut health.

## SUMMARY

The liver and gizzard weights were greater ($P < 0.05$) for probiotic compared with enzyme supplemented birds. Furthermore, dietary treatments influenced the morphological measurements of small intestine. The addition of enzyme, probiotic and their combinations increased ($P < 0.05$) the villus height to crypt depth ratio and villus height in duodenum. The increase in the villus height to crypt depth ratio was associated with improvement of growth performance for both probiotic and enzymes and their combination. This indicates that the probiotic and enzymes and their combinations can be used as a growth promoter in broiler diets and can improve the gut health. These products show promising effects as alternatives for antibiotics as pressure to eliminate growth-promotant antibiotic use increases.

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