Effect of exogenous enzymes supplementation on growth performance and histo morphology of duodenum of broilers fed cashew apple waste based diets*

P.Venkatramana[1], S. Senthil Murugan[2] and H.S. Patki[3],
Department of Animal Nutrition,
College of Veterinary and Animal Sciences, Pookode, Wayanad, Kerala, India.

ABSTRACT

The current experiment was conducted to study the histology of duodenum in broilers fed with different levels of inclusion of cashew apple waste (CAW) as well as enzymes. The study was carried out for a period of 42 days. A sum total of two hundred and ten day-old vencobb-400 broiler chicks were randomly divided into seven groups with three replicates of 10 chicks in each group. Group 1 (G1) received control diet prepared as per BIS (2007) recommendations. G2 and G3 birds received diet prepared with 5 and 10 per cent CAW without any enzyme supplementation, respectively. G4 and G5 birds received 5 per cent CAW with supplementation of 500 g/ton and 750 g/ton of enzymes, respectively. G6 and G7 birds received 10 per cent CAW with supplementation of 500 g/ton and 750 g/ton of enzymes, respectively. It was found that, histomorphological features like villus height and thickness of tunica mucosa were found to be higher in group (G4) where 5 per cent CAW with NSP degrading enzymes at 500 g/ton were fed significantly ($p<0.01$) when compared to all other groups. The number of goblet cells was observed to be significantly ($p<0.01$) lesser in the group (G4) when compared to all other groups.

Keywords: Cashew apple waste, duodenum, enzymes, goblet cell, villus

INTRODUCTION

Cashew apple waste (CAW) a by-product of cashew apple processing has been identified as an alternative feed resource for poultry but wasted without commercial exploitation (Murugan *et al.*, 2015). Though, CAW is considered as one of the feed resources and shows promising growth performance in broilers (Bhamare *et al.*, 2016); inclusion levels are limited due to presence of anti nutritional factors. Due consideration were given to identify anti nutritional factors such as level of condensed tannins and non starch polysaccharides present in CAW (Murugan *et al.*, 2015; Bhamare *et al.*, 2016 and Venkatramana *et al*., 2018) and these anti nutritional factors were reported to reduce digestibility and influence the growth performance of poultry (Choct, 2006). There are studies on dietary non starch polysaccharides which increases small intestine fermentation and affects the nutrient digestion and absorption for chickens (Bharathidhasan *et al*., 2010; Nian

Corresponding author email id: *Part of M.V.Sc thesis of the first author submitted to Kerala Veterinary and Animal Sciences University, Pookode, Kerala.
1. MVSc Student 2. Assistant Professor
3. Assistant Professor, Dept. of Anatomy

et al., 2011). However currently exogenous enzymes are used in poultry diets to improve the quality of feed ingredients used. The mechanism by which exogenous enzymes improves the growth performance of broilers could be through direct effect on endogenous enzyme activity (Yuan *et al.*, 2008); partial hydrolysis of the non starch polysaccharides (Zhang *et al*., 2014); enhancing nutrient absorption by increasing the villus height in the small intestine (Panda *et al.,* 2006); modifying mucin biosynthesis and or degradation, which in turn influences gut function resulting in improved nutrient uptake (Smirnov *et al*.,2005). Duodenum is the first segment of the small intestine where the process of digestion and trace amount of absorption occurs. Thus, the changes in the duodenum histomorphology might provide idea about actions of exogenous enzymes in cashew apple waste based diets. Thus this study was undertaken to study the histology of duodenum in broilers fed on dietary supplementation of cashew apple waste and at different inclusion levels of enzymes.

MATERIALS AND METHODS

Birds, diets and experimental design

A total of 210 one-day old commercial broiler chicks (Vencobb-400) were purchased from local hatchery. The chicks were weighed individually, wing banded and randomly distributed to seven groups *viz.,* G1, G2, G3, G4, G5, G6 and G7 with three replicates of ten chicks in each group. The study was conducted in Poultry Farm, Instructional Livestock Farm complex (ILFC), Pookode and facilities available in Department of Animal Nutrition and Department of Veterinary Anatomy and Histology at College of Veterinary and Animal Sciences, Pookode, Wayanad were utilized. The broiler chicks were fed with broiler pre-starter for first seven days then broiler starter feed was fed from 8th day to 20th day and followed by broiler finisher feed. The control diet was corn soya bean based without enzyme. The cocktail exogenous degrading enzymes with composition of amylase (24,00,000 Units/kg), hemicellulase (54,00,000 Units/kg), cellulase (1,20,00,000 Units/kg), beta-glucanase (1,06,000 Units/kg) and protease (24,00,000 Units/kg) were used in this study. All the experimental diets were formulated with CAW to meet nutrient requirements mentioned in BIS (IS: 1374; 2007). The experimental design of this study is presented in table-1. Growth Performance of the experimental birds was measured by recording body weight at weekly interval.

Table1. Experimental design

Group	Inclusion level of CAW (%)	Supplementation level of cocktail enzymes (g/ton)
G1	0	0
G2	5	0
G3	10	0
G4	5	500
G5	5	750
G6	10	500
G7	10	750

Histo-morphological study

At 42nd day of the trial six birds from each group were sacrificed after administrating chloroform anaesthesia and representative pieces from duodenum were collected and fixed using 10 per cent neutral buffered formalin for histo-morphological measurements. The paraffin embedding was done in medium paraffin wax with ceresin and tissue sections were taken at 5 µm thickness by using semi-automatic M-TECH microtome. Haematoxylin and Eosin (H & E) and Per Iodic Acid Schiff (PAS) staining methods were used to study histological and muco-polysaccharides respectively as per Luna (1968). The micrometric measurements were taken using ProgRes® capture 2.8.8.version. JENOPTIKR, Optronics software at 10 X magnification for intestinal villus, thickness of mucosa and 40 X magnification for goblet cells and height of epithelium.

Statistical analysis

The data were analyzed using GLM procedure of statistical Package for social sciences (SPSS) 21st version and comparison of means was done using Ducan's multiple range test and significance was considered at $p < 0.01$.

RESULTS AND DISCUSSION

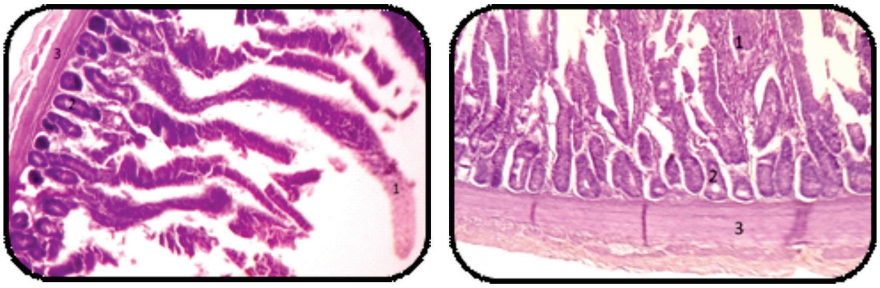
Inclusion of CAW at 5 per cent without NSP degrading enzyme did not show any improved body weight in this study; whereas Bhamare *et al*. (2016) mentioned better body weight compared against 10 and 20 per cent inclusion level in broilers. Significant increase in live body weight (g/bird) of broilers in CAW (5 per cent) with enzyme supplementation (500 g/ton) at 6th week of its age where recorded when compared against 5 and 10 per cent CAW fed without enzyme supplementation. In this study, the enzyme supplemented groups showed improved body weight comparable to control. The mean weekly body weight of the broilers is presented in table-2.

Table 2. Mean weekly body weight of broilers (g/bird) fed CAW with/without enzyme supplementation

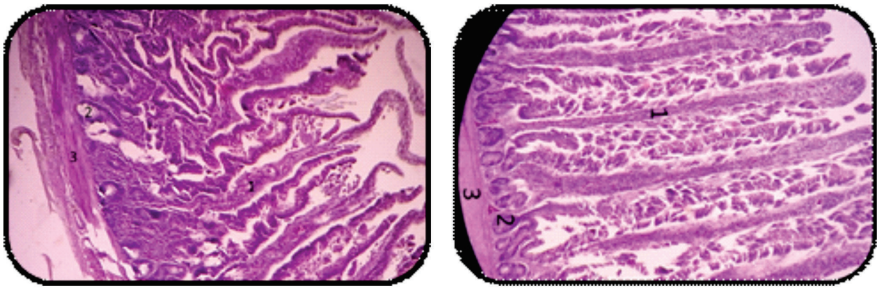
Age (week)	G1	G2	G3	G4	G5	G6	G7	F-value	p-value
Initial B. wt	42.36 ± 0.47	44.72 ± 2.10	44.24 ± 0.68	44.03 ± 0.46	45.48 ± 1.03	43.99 ± 0.64	46.14 ± 0.68	1.42 ns	0.27
I	150.58^{b} ± 0.59	143.39^{c} ± 0.66	161.24^{a} ± 1.71	154.01^{b} ± 0.30	155.34^{b} ± 3.37	165.33^{a} ± 1.63	150.89^{b} ± 2.11	16.52**	<0.001
II	391.82^{a} ± 6.33	319.56^{b} ± 36.44	407.14^{a} ± 15.45	440.00^{a} ± 1.69	417.20^{a} ± 1.11	421.66^{a} ± 6.55	417.31^{a} ± 7.46	6.29**	<0.001
III	742.52^{ab} ± 6.91	671.02^{c} ± 8.28	731.73^{ab} ± 24.58	698.02^{bc} ± 3.67	715.99^{abc} ± 8.97	759.50^{a} ± 27.45	764.40^{a} ± 2.85	5.03**	<0.001
IV	1312.58^{ab} ± 54.78	1268.14^{b} ± 10.78	1322.00^{ab} ± 41.01	1414.37^{a} ± 35.17	1262.52^{b} ± 35.91	1437.75^{a} ± 59.45	1369.88^{ab} ± 26.48	2.84*	<0.05
V	1894.26^{b} ± 4.68	1833.26^{b} ± 10.63	1877.33^{b} ± 38.69	2061.90^{a} ± 4.47	1937.65^{b} ± 27.32	1910.89^{b} ± 47.62	1929.72^{b} ± 56.59	4.54**	<0.001
VI	2290.87^{abc} ± 35.68	2194.99^{bc} ± 56.79	2264.33^{bc} ± 35.57	2395.08^{a} ± 5.99	2365.93^{a} ± 9.61	2268.33^{bc} ± 53.22	2288.89^{abc} ± 20.96	3.40*	<0.02

Mean values with different superscript within a row differ significantly.** Significant at 0.01 level; * significant at 0.05 level; ns-non- significant. G1-without CAW and cocktail enzymes; G2- 5 % CAW without cocktail enzymes; G3- 10% CAW without cocktail enzymes; G4-5 % CAW with cocktail enzymes (500 g/ton); G5-5 % CAW with cocktail enzymes (750 g/ton); G6- 10 % CAW with cocktail enzymes (500 g/ton);G7- 0 % CAW with cocktail enzymes (750 g/ton).

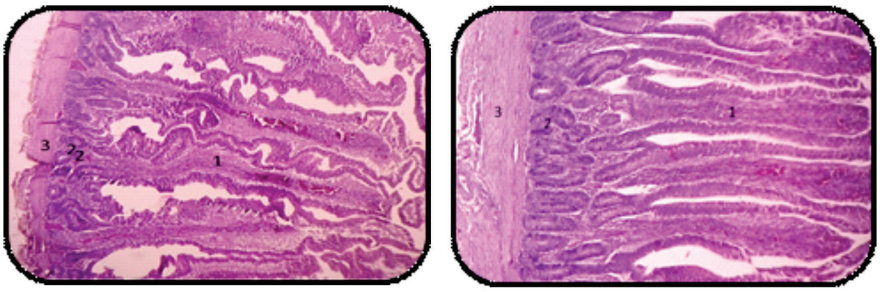
The micrographs depicting histo architecture of villi are as shown in Fig. 1. Per Iodic Acid Schiff stain micrographs of goblets cells are depicted in Fig.2. The micrometric measurements of duodenum length of villus (µm), thickness of tunica mucosa (µm) and goblet cell numbers recorded are presented in table 3.



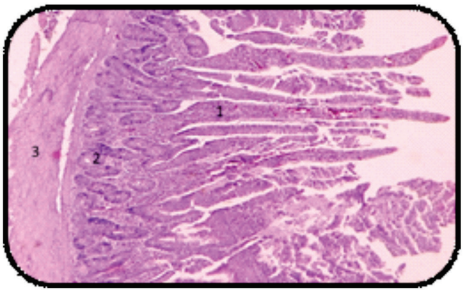
G I G II



G III G IV



G V G VI



G VII

Figure 1: Microphotographs of Duodenum in G I to G VII (Haematoxylin and Eosin method X 100)

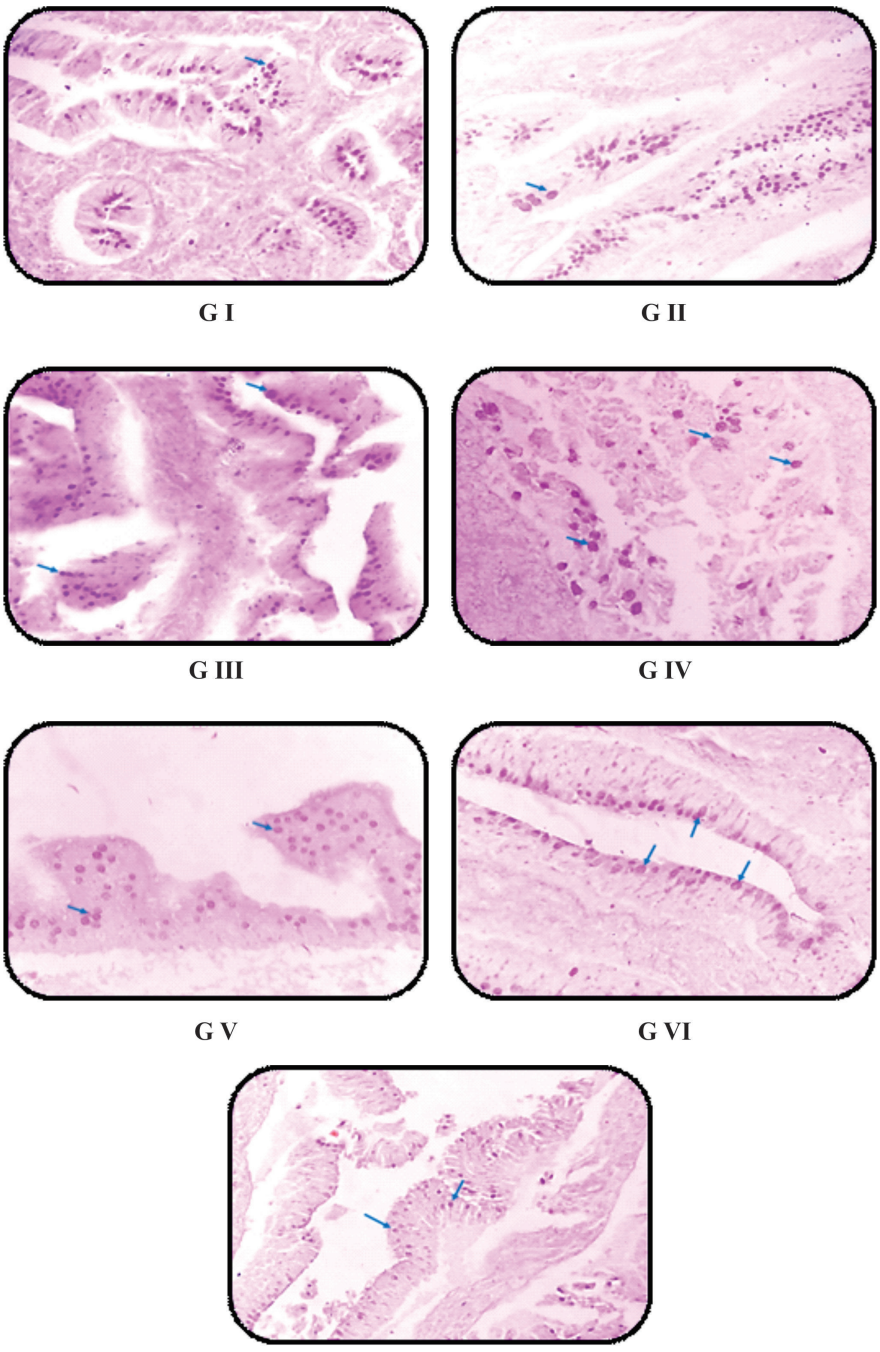


Figure 2: Microphotographs of Duodenum in G I to G VII (Per iodic Acid Schiff method X 400) Arrow indicates goblet cells showing presence of mucopolysaccharides

Table 3.Micrometric parameters of duodenum

Attributes	Group							F-value	p-value
	G1	G2	G3	G4	G5	G6	G7		
Villi height (µm)(10X)	1292.43^{bc} ± 47.55	1389.82^{b} ± 32.45	934.63^{e} ± 24.96	1601.08^{a} ± 71.13	1180.33^{cd} ± 28.79	1262.43^{bcd} ± 34.26	1154.47^{d} ± 50.26	22.22**	<0.001
Thickness of mucosa (µm) (10X)	1522.10^{abc} ± 68.68	1688.08^{ab} ± 18.93	983.60^{d} ± 161.30	1779.43^{a} ± 37.72	1420.6^{bc} ± 31.88	1293.1^{cd} ± 225.84	1413.70^{bc} ± 38.97	5.61**	<0.001
Goblet cell number (40X)	267.00^{a} ± 3.10	203.83^{b} ± 3.82	193.83^{c} ± 3.20	89.17f ± 2.89	107.83^{e} ± 2.41	113.00^{e} ± 2.67	167.67^{d} ± 2.80	448.35**	<0.001
Height of Lining epithelium (µm) (40X)	38.60^{a} ± 0.80	24.83^{d} ± 0.71	31.50^{c} ± 0.67	30.85^{c} ± 0.65	35.46^{b} ± 1.45	33.20^{bc} ± 1.27	35.03^{b} ± 1.43	16.99**	<0.001

Mean values with different superscripts with in a row differ significantly.** Significant at 0.01 level.
G1-without CAW and cocktail enzymes;G2- 5 % CAW without cocktail enzymes;G3- 10% CAW without cocktail enzymes; G4-5 % CAW with cocktail enzymes (500 g/ton);G5-5 % CAW with cocktail enzymes (750 g/ton);G6- 10 % CAW with cocktail enzymes (500 g/ton);G7- 0 % CAW with cocktail enzymes (750 g/ton).

Significant ($p<0.01$) increase in villi length of duodenum in enzyme supplemented group in current study are in concurrence with Balamurugan *et al*.(2011); Mazhari *et al*.(2015) and Thavasiappan *et al*. (2016).The highest duodenal villus length was recorded in group (G4) where 5 per cent CAW with NSP degrading enzymes at 500 g/ton was fed ($p<0.01$). However, there was no linear increase in villi length, but a decrease was recorded in 750 g/ton supplemented group with 5 per cent CAW. Similar, observations were given by Yuan *et al.* (2008) and Luo *et al*. (2009) where higher dose of enzyme might suppress excretion of endogenous enzymes and damage the structure of small intestine.

Significantly ($p<0.01$) minimum number of goblet cells have been recorded in duodenum of birds that received 5 per cent CAW with 500 g/ton of enzyme supplementation. Similarly, Balamurugan *et al*. (2011) recorded reduced goblet cell numbers compared to control in broiler chicks where cellulase, xylanase, pectinase and phytase enzyme were supplemented.

It could be corroborated that, the birds fed with 5 per cent CAW, supplemented with 500 g/ton enzymes showed increase in duodenum villi length which, increased surface area and allows greater absorption of nutrients. Decrease in goblet cells numbers in response to enzyme supplementation evidenced disruption of mucous layers, decrease in mucin secretion, improved digestibility and better body weight.

CONCLUSION

It could be concluded that the cashew apple waste included in broiler diet and enzyme supplementation improves bird's performance which correlated with morphometric measurements of duodenum.

REFERENCES

Balamurugan, R., Chandrasekaran, D. and Kirubakaran, A. 2011. Effects of multi-enzyme supplementation on gut morphology and Histomorphology in broilers. *Indian J. Sci. and Tech.***4**:15-18

Bhamare, K.S., Dildeep, V., Senthil Murugan, S. and Chavan, S.J. 2016. Nutritive evaluation of cashew apple waste in broilers. *Intl. J. Sci. and Nat.***7**: 629-632

Bharathidhasan A., Chandrasekaran D., Natarajan A., Ravi R and Ezhilvalavan S., 2010. Effect of enzyme supplementation to nutrient reduced diet on performance of broilers. *Tamilnadu J. Vet. & Anim. Sci.* **6**:174-178

Choct, M. 2006. Enzymes for the feed industry: past, present and future. *World's Poul. Sci.* 81: 1842-1849

Indian standard for Poultry Feeds, 2007. Bureau of Indian Standards (IS: 1374), 5th revision, New Delhi.

Luna, L.G. 1968. Mannual of Histologic Staining Methods of Armed Forces Institute of Pathology. (3rd Ed.). McGraw Hill Co., New York. **258**

Luo, D., Yang, F., Yang, X., Yao, J., Shi, B. and Zhou, Z. 2009. Effects of xylanase on performance, blood parameters, intestinal morphology, microflora and digestive enzyme activities of broilers fed wheat-based diets. *Asian-Aust J. Anim. Sci*. **22**: 1288-95.

Nian, F., Y. M. Guo, Y. J. Ru, F. D. Li, and A. Péron. 2011. Effect of exogenous xylanase supplementation on the performance, net energy and gut microflora of broiler chickens fed wheat-based diets. *Asian Australas. J. Anim. Sci.* **24**:400-406.

Mazhari, M., Golian, A and Kermanshahi, H. 2015. Effect of corn replacement with graded levels of wheat screening and enzyme supplementation on performance, blood lipids, viscosity and jejunal histomorphology of finisher broilers. *Spanish J. Agric. Res.***13**

Murugan, S.S., Sudhis, K., Rajan, J.K., Varshney, L. and Virendra, K. 2015. Cashew apple *(Anacardiumoccidentale)*: Evaluation of physical and chemical composition. *Ind. J. Nat. Sci.* **5**(29): 4255-4259.

Panda., A.K., Ramarao., S.V., Raju., M.V.L.N. and Sharma., S.R. 2006. Dietary supplementation of probiotic Lactobacillus sporogenes on performance and serum bio-chemicolipid profile of broiler chickens. *Indian Journal of Poultry Science,* **43**: 235-240.

ProgRes® capture 2.8.8.version. JENOPTIKR, Optronics.

Smirnov, A., Tako, E., Ferket, P.R. and Uni, Z. 2006. Mucin gene expression and mucin content in the chicken intestinal goblet cells are affected by in ovo feeding of carbohydrates. *Poult. Sci.* **85**: 669-673.

Snedecor, G.W. and Cochran, W.G. 1989. Statistical Methods, (8th Ed.). Iowa State University, Ames, I.A.

Thavasiappan V., Selvaraj P.., Jayachandran S. Visha P..,.Paramasivan S., Naik. B.R.2016. Influence of enzyme supplementation on intestinal morphology in Broiler Chicken. *J. Agric. Vet. Sci.*9: 25-28

Venkatramana P., Senthil Murugan, S., Shyama, K., Biju Chacko, Patki, H.S. and Sunanda. Qualitative and Quantitative Analysis of Phyto-Chemicals in CashewApple Waste. *Indian. J. Nat. Sci.* **8**: 976-997

Yuan, J., Yao, J., Yang, F., Yang, X., Wan, X., Han, J., Wang, Y., Chen, X., Liu, Y., Zhou, Z. and Zhou, N. 2008. Effects of supplementing different levels of a commercial enzyme complex on performance, nutrient availability, enzyme activity and gut morphology of broilers. *Asian Aust. J. Anim. Sci.* **21**: 692.

Zhang, L., Xu, J., Lei, L., Jiang, Y., Gao, F. and Zhou, G.H. 2014. Effect of xylanase supplementation on growth performance, nutrient digestibility and non-starch polysaccharides degradation in different sections of the gastrointestinal tract of broilers fed wheat-based diets. *Asian Aust. J. Anim. Sci.* **27**: 855-861.