Effects of Exogenous Multi-enzyme Feed Additive (Kemzyme) on the Activities of Certain Digestive Enzymes and Intestinal Morphology in Growing Rabbits

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Abstract

The study aimed to investigate the effects of exogenous digestive enzymes supplement on the activity of endogenous digestive enzymes and the histomorphology of the intestinal mucosa in growing rabbits. Two groups of growing rabbits (n=10 / each) were used, control and supplemented groups. Animals of supplemented group were given 1 % Kemzyme mixed in the ration for 8 successive weeks. Results revealed (1) significant increase in the final body weight and blood levels of glucose, total lipids and total protein, (2) significant increase in the activities of amylase, lipase and protease of serum, pancreatic tissues and intestinal contents, and (3) significant improvement in the villus length and crypt depth of the intestinal mucosa. Conclusively, it was obvious that the higher growth rate in kemzyme-supplemented rabbits was associated with improved digestive enzymatic activities and intestinal morphology. Also, it seemed that the exogenous enzymes did not get affected by the endogenous proteolytic activity of the gastro intestinal tract.

Keywords: Multi-enzyme feed additive, Digestive enzyme, Intestinal histomorphology, Rabbit

1. Introduction

Rabbit is single stomach herbivore. Its digestive system is suitable for high cellulose diet. Many experiments have indicated that high cellulose diet is favorable for depressing death rate by the mechanism, appendix vigor, which stimulates ileum. Considering the health of intestinal tract, crude protein concentration in diet for the growing rabbits should be lower than that for fattening rabbits (16 and 16.5 respectively), *Xiangmei* (*2008).* Simple

biological characteristics, short breeding cycle, high prolificacy and better feed conversion efficiency logically place ranks rabbit just below poultry (*Hasanat et al., 2006*). The poor and green grass *Ad-libitum* unbalanced quality of forage based diets, usually provided to rabbits in developing countries was described by *(Cheeke et al., 1985)*. Despite these obvious advantages, improved feed formulation and strategies for enhancing the production and reproduction potentials of rabbit especially in tropical and subtropical regions of the world have not been fully exploited.

The benefits of adding enzymes to diets of non ruminant animals particularly poultry, has become more common in recent years *(Campell and Bedford, 1992)*. Current developments in this area include digestibility of starch and non-starch polysaccharides in cereals. The exogenous enzyme supplementations are well documented *(Bedford and Classen 1992)*. These enzymes can partially hydrolyze non soluble protein (NSP), reduce the viscosity of gut contents, and result in improvements in nutrient absorption. Several studies have also demonstrated that the intestinal morphology was affected beneficially in birds fed barley-based diets *(Brenes et al., 1993*) or decrease the small intestinal fermentation attributed to high NSP diets *(Choct et al., 1996)*.

Several studies have been attempted for incorporating exogenous enzymes into rabbit diets to improve nutrients availability, however in most trials, rabbits appeared lessble responsive and variable effects were observed on their performances *(Remóis et al., 1996; Fernandez et al., 1996; Pinheiro and Almeida, 2000; Falcão-e-Cunha et al., 2004; Garcia et al., 2005; Falcao-e-Cunha et al., 2007)*. The decrease in mortality *(García et al., 2005)* found with proteases and proteases + xylanases (probably reducing protein flow to the caecum) was the most prominent finding. Some positive results were also obtained by other researchers.

Eiben et al., (2004), testing cellulase, got improvements in FCR and mortality of rabbits weaned at 23 days of age, whereas ADG was unaffected. It is interesting to note that in some trials enzymes improved fibre digestibility. *Fernandez et al., (1996) and Bolis et al., (1996).* The latter authors got significant improvements when cellulase and enzyme pool (xylanase, b-glucanase, b-gluccosidase, pentosanase, myloglucosidase, acid and neutral protease) was added on NDF (+5%) and ADF (+13%) digestibilities, yet at the same time getting reductions of digestible and metabolizable energies, and nitrogen balance, in comparison with the control diets.

However, relatively few studies have been conducted on intestinal morphology and endogenous digestive enzyme activities in rabbits fed on multi-enzyme feed additives during growing period. Thus, the objective of this investigation was to examine the effects of exogenous enzyme preparation on the activities of endogenous digestive enzymes, either in serum, tissues or intestinal contents and its effects on the histomorphology of GIT in growing rabbits.

2. Materials and Methods

2.1 Animals and Location

The study was conducted in the experimental rabbitry of Physiology Department, Faculty of Veterinary Medicine, Cairo University, Egypt from March to June, 2011 in accordance with the Chinese guidelines for animal welfare and approved by the animal welfare committee of Animal Science College, Zhejiang University. Twenty male rabbits of the New Zealand white breed, (8 weeks old) and approximately (1000 g body weight) were equally and randomly divided into two groups (n =10 rabbits per group). The rabbits were housed individually in commercial cages (55×60×34cm), equipped with automatic drinkers and j-feeders. Daily lighting regime was 10-12 hour photoperiod /day through both natural and fluorescent lighting. A commercial pelleted diet of 16.7% crude protein, 13.7% crude fiber and 2590 kcal of digestible energy per kg (Atmida Feed Company, Egypt) was offered *ad libitum*. The diet was subjected to chemical analysis according to AOAC (1999). Rabbits of group (1) were kept untreated and served as a control. Group (2), kemzyme group, was supplemented with (0.1%) *"Kemzyme",* a multi-enzyme blend of *Kemin Agrifoods Europe,* containing 3000 µ/g beta-glucanase, 5000 µ/g cellulase, 450 µ/g alfa-amylase and 450 µ/g protease and lipase. Dose of supplemented additive was given mixed with the basal ration in the form of pellets. The experiment lasted for 8 weeks during which fresh, clean water was available at all times. Body weight was determined at the beginning and at the end of the experimental period.

2.2 Samples Collection and Analysis

At the end of 8 weeks of the experimental period blood samples were collected from 10 rabbits by slaughtering. Serum was obtained by centrifugation of samples at 860×g for 20 min for determination of serum amylase, lipase, protease, glucose, total protein and total lipids.

The rabbits were immediately eviscerated for collection of pancreas, tissue segments of GIT, including duodenum, jejunum and ilium. Samples of intestinal digesta from different segments of GIT were also collected. The pancreas and GIT segments sampling procedure was conducted according to the method described by *Uni et al. (1999)*. The pancreatic and tissue samples were homogenized in ice-cold 0.2 *M*Tris -HCl buffer, pH 8.0, containing 0.05

*M*NaCl in the ratio 1:4 (wt. /vol.). The homogenate was centrifuged at 3,000x *g* for 15 min at 4°C, and the supernatant was stored frozen (–70°C) for enzymes assay.

Homogeneous intestinal digesta samples were collected by massaging the tract from the distal end of the duodenum to the ileo-cecal junction using the method of *Jin et al. (2000)*. Immediately, samples were diluted 10-fold, based on the sample weight, with ice -cold PBS (pH 7.0), homogenized for 1 min, and sonicated for 1 min with 3 cycles at 30-s intervals. The samples were then centrifuged at 18,000x *g* for 20 min at 4°C. The supernatants were divided into small portions and stored at –70°C for enzymes assay.

Amylase activity in sera, tissues and digesta was determined using the method of *Somogyi (1960)*. Lipase activity was assayed using the method described by *Tietz and Fiereck (1966).* Protease activity was analyzed using the method of *Lynn and Clevette-Radford (1984).* Blood glucose level was determined according to *Tietz and Fiereck (1966)*, total lipids according to *Allain (1974)* and total protein according to *Doumas et al., (1981)*.

2.3 Histomorphometry

An intestinal segment of 5 cm was taken from every different segment of the intestine; duodenum and jejunum samples were taken 10 cm and 70 cm from the pyloric junction, an ileum segment was sampled 20 cm from the proximal end of the ileal- cecal junction, flushed with physiological saline and fixed in10% formalin. Three cross sections for each sample were prepared after staining with hematoxylin and eosin using standard paraffin embedding procedures. Villus height was measured by averaging the height of 10 intact villi,from the tip of the villus to the villus crypt junction; crypt depth was defined as the depth of the invagination between adjacent villi by averaging 30 measurements. Morphological indices were measured using image processing and analysis system (Version 1, Leica Imaging System Ltd, Cambridge,UK).

2.4 Statistical Analysis

Statistical analysis was performed using the GLM procedure of SAS software (*SAS Institute, 1988*). A significance level of $P< 0.05$ was used.

3. Results

Table (1) shows results of the average body weight and some blood biochemical parameters. Values indicate significant increase in average body weight of rabbits supplemented with kemzyme as compared to controls. Moreover, significant elevations in blood glucose levels, total lipids and total protein were also observed in rabbits supplemented with kemzyme in comparison to rabbits in control group.

Table (2) indicates activities of certain digestive enzymes in serum, pancreatic tissue and intestinal contents. Data identify shows a significant increase in the activities of serum amylase, lipase and protease in rabbits supplemented with kemzyme for 8 weeks as compared to controls. Meanwhile, activities of amylase, lipase, and protease in pancreatic tissue homogenates were observed to be significantly higher as compared to control rabbits. In addition, the activities of amylase, lipase and protease in intestinal content of kemzyme-supplemented rabbits were recorded to be significantly higher as compared to control animals.

Results tabulated in table (3) and figures (1, 2, 3 and 4) indicate villus length (µm) and crypt depth (µm) of small intestinal wall in rabbits supplemented with kemzyme for 8 successive weeks. There was a significant increase in crypt depth measurement in kemzyme supplemented rabbits as compared with control animals.

At the same time the villus length of the kemzyme groups indicated a mathematical increase without statistical significance with high figures of standard error (high individual variation).

4. Discussion

The rabbit's gastrointestinal physiology is a complex system that centers around separation of digestible and indigestible components of the diet. The benefits of exogenous enzyme supplementation are well documented *(Bedford and Classen, 1992).* These enzymes can partially hydrolyze the non starch polysaccharides (NSP), reduce the viscosity of gut contents, and result in improvements in nutrient absorption. Several studies have been attempted for incorporating exogenous enzymes into rabbit diets to improve nutrients availability (Falcao-e-Cunha *et al.,* 2007). Some of them could not detect any significant effect of enzymes on rabbit performances (*Falcão-e-Cunha et al*, 2004), Others, showed that dietary addition of proteases reduced the ileal flow and mortality in fattening period of rabbits(*Garcíaet al.,* 2004, 2005, and 2006) of rabbits besides improving feed conversion ratio *(Eiben et al., 2004)*. In some trials when cellulase and enzyme pool (xylanase, b-glucanase, b-gluccosidase, pentosanase, myloglucosidase, acid and neutral protease) were added, the authors got significant improvements in NDF and ADF digestibilities. In the present study, a significant increase in the average body weight, blood glucose levels, total lipids and total protein were observed in growing rabbits supplemented with kemzyme for 8 weeks. These finding corroborated earlier observations of *Saleh et al., (2006)* who found that kemzyme supplementation to the ration of growing turkey toms resulted in a significant increased level of blood

glucose, total proteins and lipids. They attributed such finding to the improved digestibility and absorbability of different dietary elements, including carbohydrates, fat and proteins that was induced by the addition of exogenous enzymes. Such enrichment of bird's metabolic pools actively affects the blood levels of basic nutrients *(Mathlouthi et al., 2003)*. Moreover, *Abdl-Rahman et al., (2010)* suggests that, multi-enzyme feed additives for rabbits could lead to favorable modifications in GIT environment presumably, acidification of cecal contents and stabilization of ammonia nitrogen concentrations. These alterations should be considered as an advantage, as they improve the impact of the supplemented enzymes on cecal fermentation pattern and rabbit metabolism that might lead to the higher body weight obtained in rabbits supplemented with kemzyme.

According to the obtained data in the present study, the activities of amylase, lipase and protease were significantly increased in serum, pancreatic homogenate and intestinal contents of rabbits supplemented with kemzyme for 8 weeks. Until recently, it was assumed that the proteolytic activity in the GIT ecosystem would rapidly inactivate unprotected enzyme feed additives *(Chesson, 1994; Kung, 1996).* This was conceded in the results of *Kopecny´ et al., (1987),* who reported rapid inactivation of a cellulase preparation by *Trichodermareesei* by proteases from ruminal bacteria. More recently, different feed enzyme additives were reported to be more stable in the rumen than were previously thought possible, and this stability has been reported to depend on origin and type of activity *(Hristov et al., 1998 a, b).* The results obtained by *Morgavi et al., (2001)* suggest that exogenous enzymes can survive in the intestine and exert their action on available substrates. The increased enzymatic activities in the intestinal content might be contributed to the sum of exogenous and endogenous enzymes action. Another possible factor responsible for such an increased enzymatic activity could be the 'favorable change in pH of GI tract' mediated by exogenous enzymes *(Abdl-Rahman et al., 2010)*.

Moreover, the increased availability of nutrients (substrates) inside the GIT, most probably, the cause of increased activity and secretion of such digestive enzymes from the glandular mother cells (exocrine pancreatic cells) as a positive feedback response which is mainly neural factors instead of gut hormones *(Murai et al., 2000)*. Another explanation is based on the conservation model of digestive enzymes which was proposed by *Rotheman et al., (2002)*, and has been proved by *Onderci et al., (2006)* who pointed out that a large portion of the digestive enzymes secreted by the pancreas are absorbed into blood and recycled in an enteropancreatic circulation.

The morphological changes recorded in the intestinal mucosa (increased villus length and crypt depth) might be complementary changes to meet the increased rates of digestion and absorption mediated through the coupled activities of exogenous and endogenous digestive enzymes or due to elimination of toxic molecules and degradation of large-size diet protein.

Many studies have suggested that the morphological changes observed in the villi area so due to transient hypersensitivity to antigenic components of the diet *(Lalles et al., 1993; Hong et al., 2004).* Antigenic materials in diet proteins are associated with villus atrophy, increased crypt cell mitosis, and crypt hyperplasia, and there by cause a malabsorption syndrome *(Kenworthy and Allen, 1966; Miller et al., 1984a, b).*Similarly, the improvement of intestinal morphology may be associated with the degradation of antigenic materials after enzymatic fermentation. It was reported that increased enzymatic fermentation could degrade large-size protein to small-size peptides *(Kiers et al., 2003; Hong et al., 2004).* The improvement of digestive enzyme activity and intestinal mucosa morphology in rabbits supplemented by kemzyme may be partially responsible for the higher growth rate obtained in the present study.

5. Conclusion

In conclusion, supplementing growing rabbits with exogenous digestive enzymes (Kemzyme) was associated with improved digestive enzyme activities and intestinal mucosa morphology coupled with higher growth rate. It also seemed that the exogenous enzymes did not adversely affected with the endogenous proteolytic activity and might be mediate their action either locally or through activating the rate of synthesis and secretion of endogenous digestive enzymes.

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Table 1. Body weight, blood glucose total lipids and total protein levels of rabbits supplemented with Kemzyme at the end of experimental period (8 weeks)

Groups / Parameters	Control	Kemzyme
Body weight (g)	2200±42.12	2450±45.30 *
Glucose (mg/dl)	88.20±8.02	122.10±9.22*
T. lipids (g/dl)	220.10±18.19	295.20±22.20*
T. protein (g/dl)	4.02±0.66	5.93±1.02*

Values are means ± SE, (n : 10 rabbits / group).
Values having the mark (*) are significantly different from the corresponding control values at (P < 0.05).

Table 2. Activities of digestive enzymes in rabbits supplemented with Kemzyme at the end of experimental period (8 weeks)

Groups / Samples	Control			Kemzyme		
	Amylase	Lipase	Protease	Amylase	Lipase	Protease
Serum (U/L)	117.0±8.3	172.0±18.3	72.0±6.2	211.0±17.2*	221.0±13.0*	99.0±5.0*
Pancreas (U/g)	295.0±19.4	90.0±11.3	90.0±12.4	333.0±20.1*	148.0±15.8*	118.4±14.3**
Intestinal content (U/g)	105.3±12.4	68.3±5.3	62.6±6.5	185.0±13.0*	98.2±7.40*	102.0±4.0*

Values are means ± SE (n : 10 rabbits / group).

Values having the mark (*) are significantly different from the corresponding control values at (P < 0.05).

Table 3. Villus length (µm) and crypt depth (µm) of small intestinal segments in rabbits supplemented with Kemzyme for 8 weeks

Groups / Segment	Control		Kemzyme	
	Villus length	Crypt depth	Villus length	Crypt depth
Duodenum	84.00 ± 5.77	21.66 ± 2.28	104.00 ± 16.65	44.25 ± 3.08*
Jejunum	135.00 ± 18.16	44.25 ± 7.56	138.75 ± 18.30	72.00 ± 10.23*
Ileum	166.87 ± 18.88	39.68 ± 0.01	236.19 ± 14.41	57.91 ± 4.42*

Values are means ± SE, (n : 10 rabbits / group).

Values having the mark (*) are significantly different from the corresponding control values at (P<0.05).

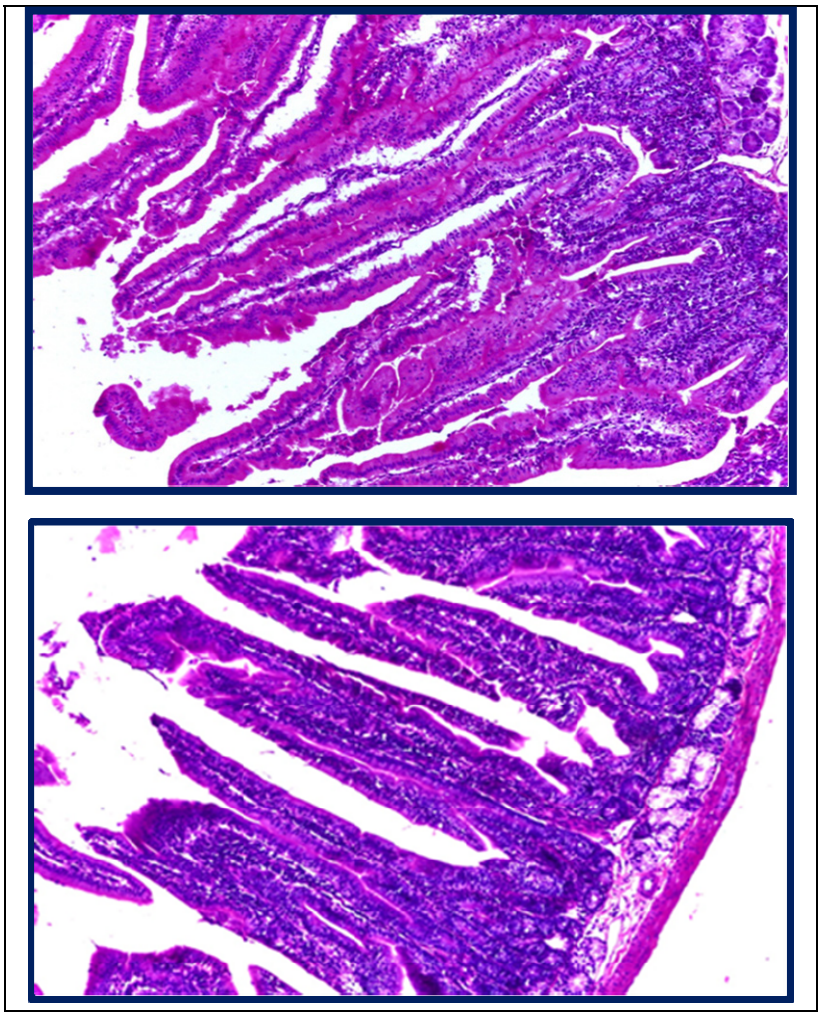

Figure 1. Villus of duodenum wall (Upper) control and (Lower) kemzyme supplemented rabbits (x 40)

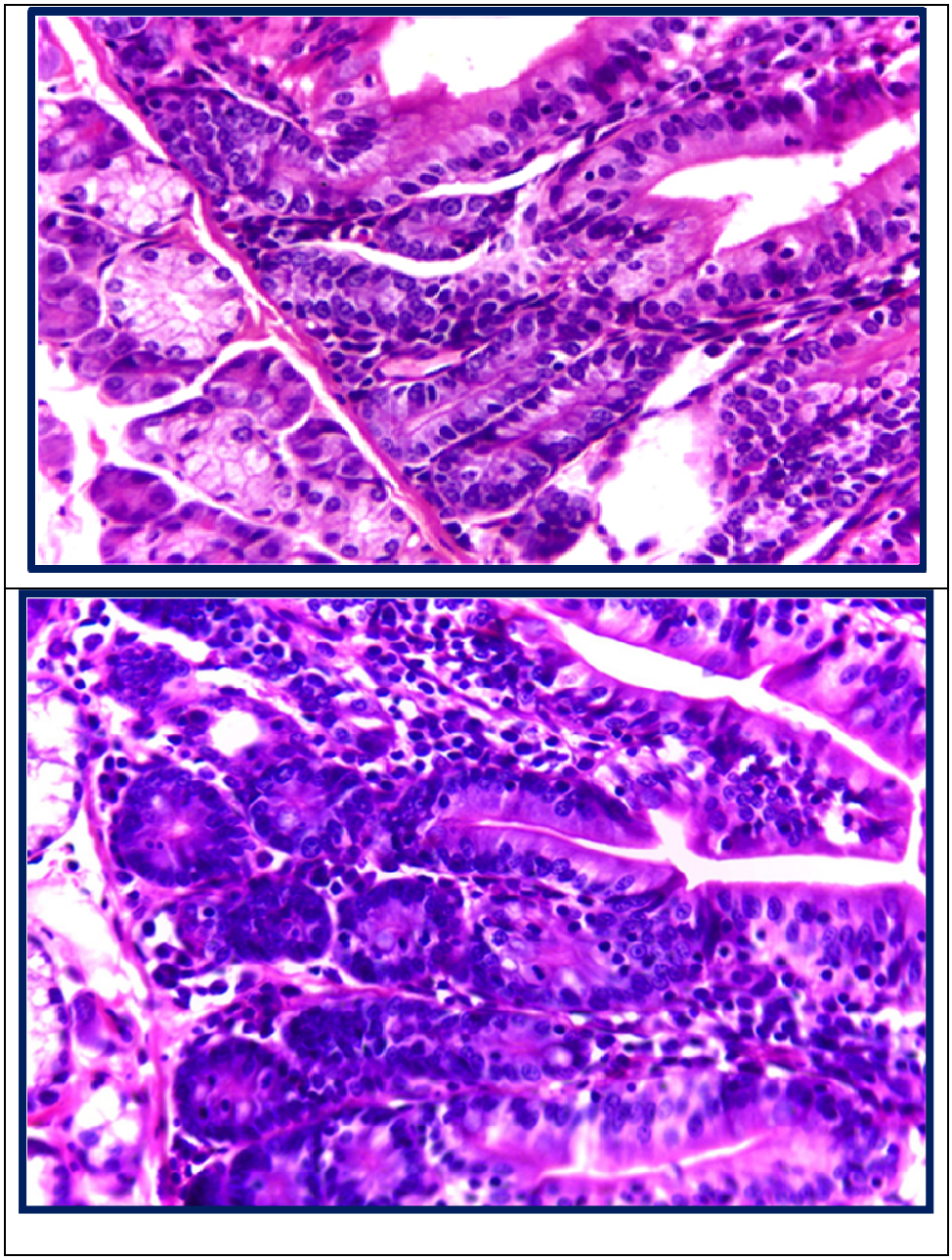

Figure 2. Crypt depth of duodenum wall (Upper) control and (Lower) kemzyme supplemented rabbits (x 80)

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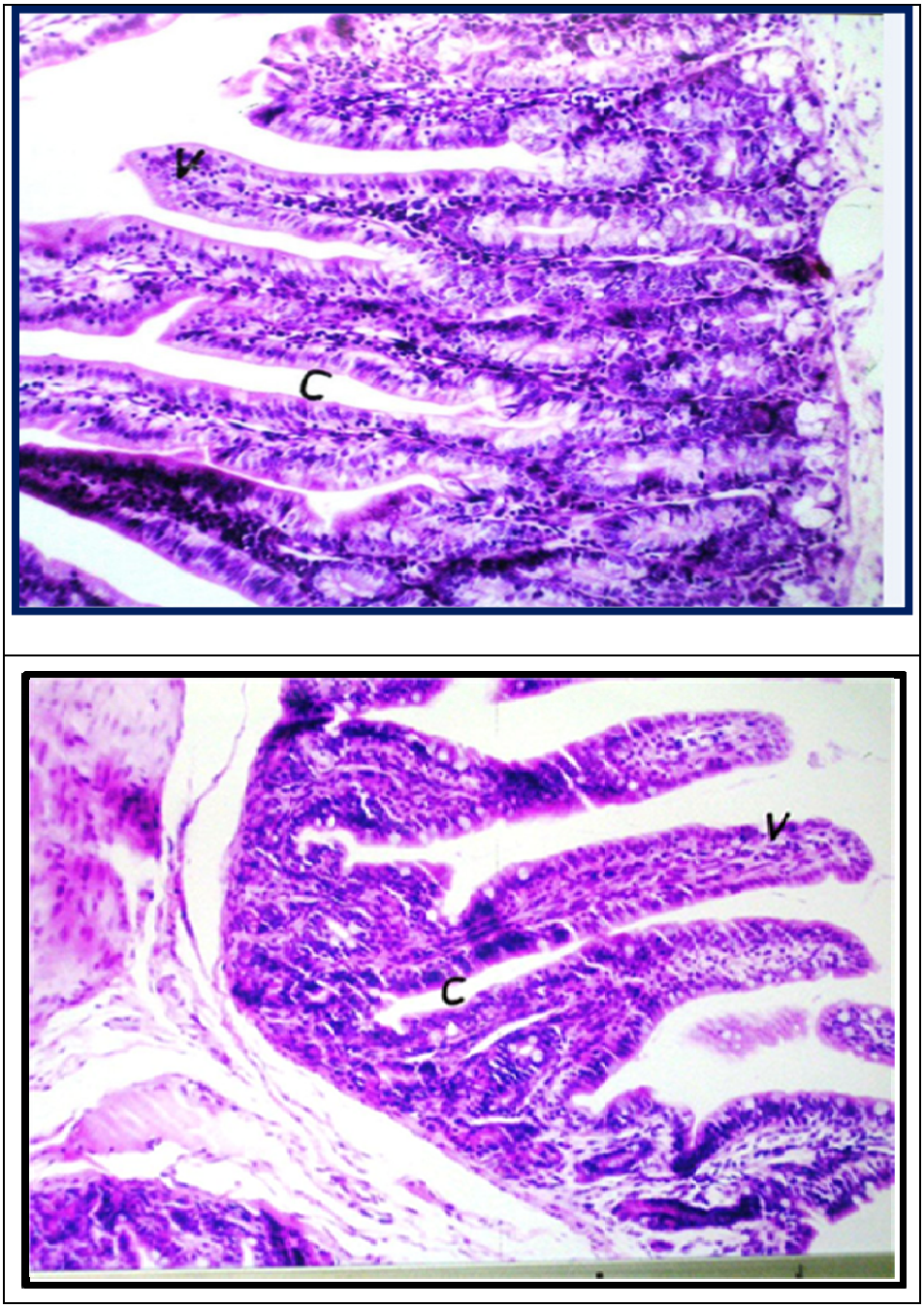

Figure 3. Villus and crypt depth of jejunum wall (Upper) control and (Lower) kemzyme supplemented rabbits (x60)

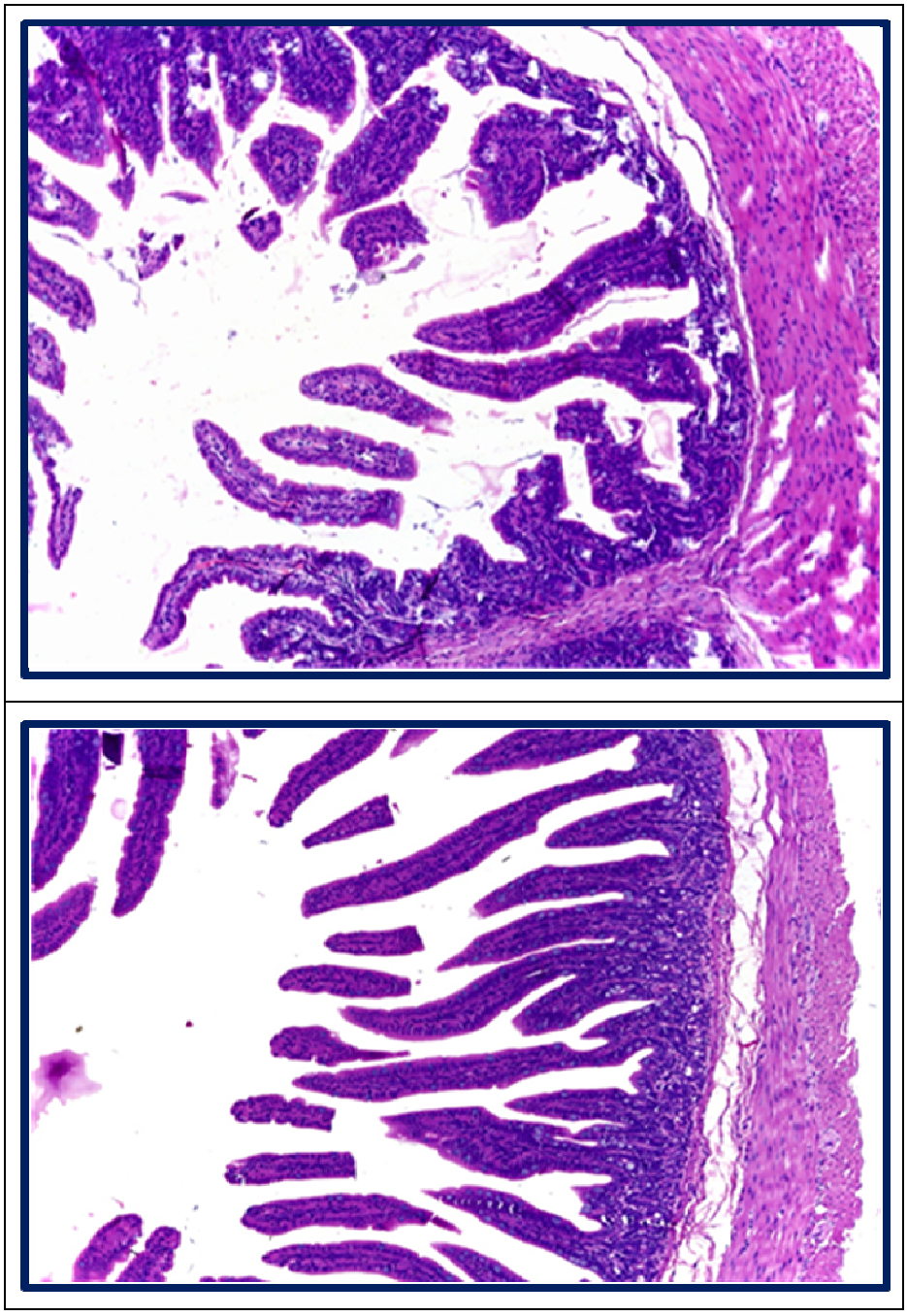

Figure 4. Villus and crypt depth of ileum wall (Upper) control and (Lower) kemzyme supplemented rabbits (x40)

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