

Effect of Dietary Supplementation of Compound Enzymes on Endogenous Digestive Enzymes in Cherry Valley Ducks Fed Miscellaneous Meal Diet

Yan Rui,¹ Su,¹ Z.H., Yan-min,¹ A. Wen-jun,¹ Nasir Rajput^{1,2} and Wang Tian^{1*}

¹College of Animal Science and Technology, Nanjing Agricultural University, Nanjing, China, 210095.

²Department of Poultry Husbandry, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tando Jam, 20060, Pakistan

Abstract. The study was conducted to investigate the effect of dietary supplementation of compound enzymes on the endogenous digestive enzymes activity in cherry valley ducks fed miscellaneous meal diet. The compound enzymes consist of xylanase (10000 U/g), mannanase (18000 U/g) and glucanase (3000 U/g). One hundred and seventy-six 1-day-old healthy ducklings were randomly divided in a control and treatment group with four replicates of 22 birds each. The dietary treatments consisted the basal diet (control group) and 100 mg/kg diet of compound enzymes supplemented to the basal diet (treatment group). The levels of protease and trypsinase, amylopsin and pancrelipase were determined during starter period (7, 14 and 21 d) and growing period (28 and 42 d). The results showed that protease levels were initially increased and reached at peak level on day 21st, and then gradually decreased with age. The protease level of enzyme supplemented group was higher during starter period, but significantly lowered at growing period as compared to control group. The peak level of amylopsin activity was recorded in enzyme supplemented ducks at starter period and in control group ducks at growing period. Pancrelipase activity of enzyme supplemented ducks was initially higher but later comparatively lower than control group. Interestingly, only the trypsinase level of enzyme-supplemented ducks was significantly higher during growing period compared to control group. The obtained results suggested that dietary supplementation of compound enzymes stimulate some endogenous digestive enzymes at starter period, in cherry valley ducks fed miscellaneous meal diet.

Key words: Cherry Valley ducks, Miscellaneous meal diet, compound enzymes, digestive enzymes.

INTRODUCTION

Miscellaneous cake is a mixture of by-production of oil grains, such as rapeseed, cottonseed and peanut kernel. Miscellaneous cake contains simple protein about 35.7- 42.5% which is equivalent to soybean cake or less a little, but there are some toxins and anti-nutrition factors due to which it is used in limited quantity in the feed as source of protein (Luo *et al.*, 2006). These toxins can be reduced or even can also be eliminated by physical, chemical and microbiotic methods (Ji *et al.*, 1995). Therefore, it is generally perceived that the high contents of soluble non-starch polysaccharides (SNSP), one of anti-nutrition factor which hamper the process of digestion and absorption of nutrients, is the main problem (Li *et al.*, 1995; Chen and Peng, 2001). The feed which

uses rapeseed cake, cotton seed cake, peanut cake and sesame cake etc is called a miscellaneous meal diet (Hu *et al.*, 2005). Exogenous enzymes can partially hydrolyze these factors and release the nutrients (Meng *et al.*, 2005). There are rich and cheap miscellaneous cake sources in China. If miscellaneous cake is used effectively it can be an efficient and reasonably a sound method of solving problem of shortage of protein sources in China. The Cherry Valley duck is one of the famous meat ducks breed worldwide which has number of good characteristics such as growing fast, high percentage of meat and better feed conversion efficiency. In recent years, most researches focused on the effect of enzymes supplement on growth performance, metabolic level and on immunity of animal (Ai and Han, 2002; Alam *et al.*, 2003; Engberg *et al.*, 2004; Hahn, 2010). But only a few numbers of studies have determined the effect of enzymes supplement on endogenous digestive enzymes activities. This study was aimed to determine the effect of compound enzymes supplement on the digestive enzymes activity of proventriculus and pancreas in

* Corresponding author: tianwangnjau@163.com
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cherry valley ducks fed with miscellaneous meal diet to provide a reasonably effective scientific way to supplement with enzyme preparations into corn - miscellaneous meal diet.

MATERIALS AND METHODS

Animals and diets

One hundred and seventy six, healthy 1- day old cherry valley ducklings were randomly divided into control and treatment groups with four replicates of 22 birds in each, under similar environmental conditions. Feed and water were allowed ad libitum to the birds. A corn-miscellaneous meal basal diet (Table I) with or without supplementation with a commercial compound enzyme (0.01%) were used for 6-week study. The enzyme supplement supplied 1000 U of xylanase, 1800 U of mannanase, 300 U of glucanase per kilogram of diet.

Enzymes activity assay

During the starter period (7, 14 and 21 day of age) and grower period (28 and 42 days of age), eight ducks from each group (two ducks per replicate) were killed at 9:00 am after 2h feed deprivation. Proventriculus and pancreas were quickly excised. Proventriculus was cleaned by 0.9% physiological saline and pancreas was frozen in liquid nitrogen, stored at -20°C for enzyme activity assay. After thawing, the proventriculus and pancreas samples were homogenized (1:9 wt/vol) with ice-cold physiological solution, and then centrifuged at 4000 r/min for 20 min at 4°C. Aliquots of the supernatant were collected for enzyme activity assay. The activities of proventriculus protease, trypsinase, amylopsin, pancrelipase as well as pancreatic protein concentration were determined with kits (Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China).

Statistical analysis

All the data was analyzed statistically using independent-samples 't' test (SPSS, 2008) to determine the effects of compound enzyme supplements. All results are presented as means±SEM. The differences were considered to be significant at $P<0.05$.

Table I. Composition and nutrient levels of basal diet (dry matter basis) %.

Ingredients	1-14 d	15- 42 d
Maize	44.1	50
Wheat	15	15
Soybean meal	22.5	11
Solvent rice bran meal	7	8.1
CP40%, Rapeseed meal	3.5	6
Peanut meal	3.5	6
65% Lysine	0.35	0.1
Methionine	0.11	0.05
Limestone powder	1.14	1.5
Calcium phosphate	1.4	0.85
Premix	1	1
Sodium chloride	0.25	0.25
Antimold agent	0.1	0.1
Antioxdant	0.05	0.05
Nutrient level ¹		
ME□MJ/kg□ME (MJ/Kg)	11.30	11.40
Crude protein (%)	19.00	16.40
Total phosphorus (%)	0.76	0.67
Available phosphorus (%)	0.40	0.30
Calcium (%)	0.91	0.91
Lysine (%)	1.12	0.74
Methionine (%)	0.40	0.31

¹ Nutrient level calculated values.

RESULTS

The effect of compound enzymes preparations on proventriculus protease activity in ducks is presented in Table II. The proventriculus protease activities first decreased and then increased with age, after reaching to a peak on day 21 it was gradually decreased to a minimum on day 42. The activity was increased by 11.06% ($P<0.05$) by compound enzymes supplementation but no difference was observed between groups on other samples time. The effect of compound enzymes preparation on trypsinase activity in ducks is shown in Table III. Trypsinase activity was initially increased and then decreased with the age. The highest activity of trypsinase was observed at 14 d and 21-28 d in control and enzyme group respectively. The activity of trypsinase of enzyme supplemented ducks was significantly higher by

189.3, 117.01 and 83.59 % (P<0.05) at day of 21, 28 and 42, respectively. The effect of compound enzymes preparations on amylopsin activity in ducks is presented in Table IV. The activities of amylopsin first increased at 21 d, then decreased at 28 d and then increased smoothly at 42 d during the experiment period in both groups. The peak was observed at 21 d in control and at 42 d in enzymes supplemented ducks. The activity in control group ducks was significantly higher by 44.56% and 26.90% (P<0.05) as compared with enzyme treated ducks. But, the compound enzymes preparation enhanced amylopsin activity 4.41%, though the effect was not statistically significant. The effect of compound enzymes preparations on lipase activity in pancreas of ducks is shown in Table V. The pancreatic lipase activities of ducks were lowest at 7 d in both groups, and then increased at 14 d. It was stable at 14-21 d and reached to a peak level at 28 d in control group while the highest lipase activity in pancreas was observed at 14 d, decreased slightly at 21 d, steadily increased at 28 d then again decreased at 42 d in treated group. Enzymes supplementation enhanced the activities by 30.52% (P<0.05) at 14 d. The increase in activity was also observed on other samples time but the effect was not statistically significant.

Table II.- Effect of compound enzymes preparations on activity of pepsin in glandular stomach.

Age (Days)	Control	Treatment
7	104.75±2.13	104.92±3.06
14	84.09±3.67	86.38±4.32
21	111.54±9.18	116.98±4.43
28	92.06±2.03	81.88±2.32*
42	37.86±3.05	42.44±1.70

* Means significantly difference P<0.05

Table III.- Effect of compound enzymes preparations on activity of trypsinase in pancreas.

Age (Days)	Control	Treatment
7	411.46±54.47	567.21±79.92
14	2443.29±680.54	1920.57±185.55
21	1176.54±163.04	3404.80±346.45*
28	1689.95±448.38	3667.35±209.67*
42	513.50±50.79	942.72±148.58*

* Means significantly difference P<0.05.

Table IV.- Effect of compound enzymes preparations on activity of amylopsin in pancreas.

Age (Days)	Control	Treatment
7	155.73±7.67	143.44±10.59
14	283.27±19.49	195.95±4.78*
21	268.04±28.95	279.85±18.64
28	269.38±21.89	229.43±8.58
42	344.07±17.33	271.14±18.26*

* Means significantly difference P<0.05.

Table V.- Effect of compound enzymes preparations on activity of lipase in pancreas.

Age (Days)	Control	Treatment
7	210.39±35.30	232.22±12.02
14	486.10±32.74	634.48±17.71*
21	449.14±38.13	512.90±61.71
28	663.06±39.02	597.09±30.99
42	437.62±46.51	480.55±17.12

* Means significantly difference P<0.05.

DISCUSSION

Pepsinogen is the original form of pepsin, which is secreted by adolomorphous cell of proventriculus and activated when the pH value is less than 5.0, the lower the pH value the greater will be the activation, but pH value when increases to 6.0 it inactivates the secretion of pepsin completely. Since the digesta passes through proventriculus of poultry very quickly and the pH value of intestine is not favorable for the activation of protease, so trypsinase has to play main role in degradation of protein. Only few numbers of researches have been conducted to find out the effect of exogenous enzymes on glandular stomach activity. Yao (2001) reported that the pepsin activity of 18-22 week old breeding cocks were enhanced by enzymes supplementation, though the effect was not significant, which is also in complete agreement with the results of our research. In current experiment, pepsin activity was higher at the 7th day, which showed that proventriculus adolomorphous cells of young ducklings have already been matured at the time of their hatching.

The peak of pepsin activity was observed at

the 21st day, which might be due to the HCl secretion by gastric glands and later due to pepsinogen secretion by adelomorphous cell of proventriculus, and eventually the activity of pepsin depends on the change of pH value.

The pancreas is an exocrine gland and an important digestive organ of animal which secretes pancreatic juice into duodenum through pancreatic duct. The pancreatic juice consists of magnificent series of digestive enzymes that are vital for nutrient digestion, such as trypsinase, amylopsin and pancrelipase, etc., which plays an important role in digestion and absorption of feed nutrient. Thus it is possible that the enzyme activity in pancreatic tissue can reflect the level of endogenous enzyme secretion. In the present study it was found that, the exogenous compound enzymes has enhanced trypsinase activity in ducks, which was in accordance with previous reports of (Xi and Xu, 1999; Ai and Han, 2001a). In this study the results demonstrated that the activity of trypsinase in ducks control group was higher as compared to that of enzymes treated group, apparently due to reduction of compensatory secretion of trypsinase. Young ducks whose pancreas are in developing phase, their pancreatic secretions are not enough for the digestion of corn-miscellaneous meal. This meal contains many anti-nutritional factors results in low protein availability, leading to a certain compensatory secretion of trypsinase. Enzymes supplementation can improve protein utilization of feed and eventually can also reduce the compensatory secretion of trypsinase.

In present research results showed that compound enzymes significantly reduced amylopsin activity of ducks aging 14 and 21ds, but the effect was noteworthy at other sample time, which was in harmony with previous reports (Ikegami *et al.*, 1990; Mahagna *et al.*, 1995; Xu *et al.*, 2002). These results may be due to the association between these two facts. On one hand, the increase in concentration of amylase substrate in intestine is attributed to number of carbohydrates released by enzymes supplementation, which stimulates amylopsin synthesis to a great extent but the rate of amylopsin synthesis is slower than that of its secretion into intestine. Ai and Han (2001b) reported that by adding enzymes, activity of amylase in young goose

duodenum chymus increased when amylopsin activity decreased or when remained unchanged. On the other hand, a corn-miscellaneous meal may cause compensatory secretion of trypsinase. However, various studies showed that this result might be related to inhibition of exogenous digestive enzymes secretion by endogenous enzymes (Kermanshahi *et al.*, 1998).

A number of research reports indicated that poultry pancrelipase activity is low at hatching time, which goes on gradually increasing with age after hatching (Krogdahl, 1985; Noy and Sklan, 1995; Jin *et al.*, 1998; Yan *et al.*, 2009). The results of our study shows that whether enzymes are added to the ducks diet or not, pancrelipase activity in Young Cherry Valley duck was initially low, which then increased to highest level, and then kept on declining slightly from the peak level till it finally gets stable. If pancrelipase activity in young animal is low, those animals are very much vulnerable to the disease of digestive diarrhea because of fat malabsorption. Through this experiment, it was observed that compound enzymes supplementation can significantly enhance pancrelipase activity in ducks at 21 d and was not numerically noteworthy at other sampling period. Zhang (2008) reported that compound enzymes supplement can increase considerably ($P < 0.05$) pancrelipase activity of 21 d ducks which are fed corn-miscellaneous meal diet. Zhang *et al.* (2007) reported that pancrelipase activity of 21 d chickens was also enhanced significantly ($P < 0.05$) by adding high-dose (25g / t) xylanase in corn-soybean meal diet. This enhancement could be due to destruction of plant cell walls (composed of xylanase and cellulose *etc.*) by compound enzymes. Thus, providing endogenous digestive enzymes, is much better way of to the encapsulated nutrients (Saleh *et al.*, 2003). Additionally, the results may also be related to the deprivation of anti-nutritional factors that restrain enzyme activity (Ikeda and Kusano, 1983; Liu *et al.*, 2008). Almirall *et al.* (1995) observed decreased activities of amylase and lipase in intestinal contents of broiler chicks which were fed high-viscosity barley as compared to those fed with corn, and β -glucanase increased both activities.

This study has established the fact that the dietary supplementation of compound enzymes

containing xylanase, mannanase, and glucanase were able to enhance activities of some endogenous digestive enzymes at starter period, in cherry valley ducks fed miscellaneous meal diet.

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