



Study on the Characteristics of Intestinal Digestive Enzymes Activities of Three Local Chicken Breeds in South China

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ABSTRACT

Background: This experiment was aimed to research the intestinal α -amylase and lipase activities of three local chicken breeds from Jiangxi Province in South China and to investigate the highest enzyme activity sites of digestive enzymes.

Methods: Six each of Taihe silky fowls, Ningdu three-yellow chickens and Chongren partridge chickens with similar body weights were selected and slaughtered. For each chicken, six portions of chyme were respectively taken from duodenum and jejunum at equal distances and then α -amylase and lipase activities were determined in these 12 portions of chyme.

Result: The results indicated that the α -amylase and lipase activities of the three local chicken breeds did not change significantly in the duodenum, while in the jejunum they gradually increased and then declined. In the meantime, the highest α -amylase activities were found in the 2nd section of the jejunum in Ningdu three-yellow chicken and Chongren partridge chicken and α -amylase activity in Taihe silky fowl was highest in the 3rd section of the jejunum. The highest lipase activity was observed in the 3rd section of the jejunum for both Taihe silky fowl and Chongren partridge chicken, whereas Ningdu three-yellow chicken exhibited the highest lipase activity in the 2nd section of the jejunum. Both α -amylase and lipase activities reached their maximum in the mid-anterior jejunum of the three chickens, but the location of their highest enzyme activities and digestive enzyme activities were different, with the highest α -amylase activity in Ningdu three-yellow chicken and the highest lipase content in Taihe silky fowl.

Key words: Chongren partridge chicken, Digestive enzyme activity, Ningdu three-yellow chicken, Taihe silky fowl.

INTRODUCTION

Nowadays, consumers have higher requirements for chicken meat quality and local chicken breeds grow slowly but have better meat quality are receiving more attention (Ng'ambi *et al.*, 2017; Sehrawat *et al.*, 2021; Motsepe1 H *et al.*, 2016). Taihe silky fowls (TSF), Ningdu three-yellow chickens (NTC) and Chongren partridge chickens (CPC) are all high-quality local chicken breeds originating from Jiangxi Province in South China. These three chickens boast tender meat, delicious flavor and are rich in nutrients, *etc.* Among them, TSF has unique appearance and is utilized for both medicinal and food purposes. This chicken meat is low in total lipids and high in phospholipids and also has active peptides with antioxidant and hydroxyl radical scavenging ability, which can be used as medicine for the whole body (Lin *et al.*, 2007; Tian *et al.*, 2011; Zhang H *et al.*, 2016). However, the three local chicken breeds have high rearing costs and long rearing cycles, requiring 3-4 months to reach slaughter weight. Therefore, it is important to improve the growth performance of these three local chicken breeds from Jiangxi Province in South China.

Starch is the main source of carbohydrates in animal feed and it is extremely important as a source of energy in feed (Zhang *et al.*, 2007), which can help to maintain the normal development of functional organs and contribute to the growth and development of poultry and maintain their health. Lipids are one of the three major nutrients required for body health (Berná *et al.*, 2020), in which fats can provide essential fatty acids and are essential energy storage substances and nutrients for the animal body (Frontini *et al.*,

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2010). In poultry farming, intestinal digestive enzymes play an crucial role in nutrient metabolism (Ramírez-otárola *et al.*, 2011), of which α -amylase and lipase secreted by the pancreas are the most dominant enzyme system for digesting food in animals, which are directly related to their digestive function and the level of their enzyme activity reflects the magnitude of digestive capacity (Wang *et al.*, 2022), which affects the production performance of poultry (Qian *et al.*, 2016). Currently, more studies have been conducted on the effects of feed type (Norozi *et al.*, 2022 and Xu *et al.*, 2023), feed additives (Long *et al.*, 2020) and feeding management practices (León *et al.*, 2014) on the activity of digestive enzymes in the digestive tract of poultry, a few studies have been conducted on the trend of changes in the activity of digestive enzymes in the poultry digestive

tract. Zhang *et al.* (2018) determined the activities of digestive enzymes in various parts of the digestive tract of TSF and found that the activities of both amylase and lipase in TSF were strongest in the jejunum and that the duodenum and the jejunum were the most important parts of feed digestion (Zhang *et al.*, 2018). However, studies on the activities of digestive enzymes at various sites in the avian duodenum and jejunum have not been reported. Therefore, this experiment researched the activities of α -amylase and lipase in the duodenum and jejunum of three local chicken breeds from Jiangxi Province in South China, aiming to investigate the trend of the activities of these two digestive enzymes and the highest enzyme activity, in order to provide a basis for the improvement of the feeding mode of local chicken breeds and the enhancement of their growth performance.

MATERIALS AND METHODS

The experiment was conducted from December 2022 to March 2024 at the research and instructional facility of the College of Life Sciences, Jiangxi Science and Technology Normal University, Jiangxi Province.

Animals and experimental design

The same batch of chickens were fed with the same corn-soybean meal type commercial feed, free-feeding and drinking water and the detailed composition and nutritional profiles of the basal diet are presented in Table 1. In the same batch, six each of TSF, NTC and CPC with similar body weight (BW, 1.25 ± 0.10 kg) and with a good growth rate were selected to be used as the test animals.

Sample collection

All test animals were bled to death at the neck in the morning, they were subsequently dissected and the duodenum from the pylorus to the entrance of the bile duct and the jejunum from the entrance of the bile duct to the Mai's cecum were separated and divided into 6 segments in proportion to the proportion of the total of 12 segments, numbered 1-12 in sequence. Two samples of chyme were collected from each of the 12 intestinal sites for measurement of α -amylase and lipase activities, which were respectively placed in centrifuge tubes. These collected samples were frozen at a low temperature of -20°C in the refrigerator to preserve. These samples would be taken out and rewarmed for use when determining digestive enzyme activity.

Determination of digestive enzyme activities

Indicators of digestive enzyme activity in chyme were measured mainly for α -amylase and lipase by using corresponding kits (Nanjing Jiancheng Institute of Bioengineering, Nanjing, China) and determined according to the instructions. α -amylase activity was determined by the amylose-iodine colorimetric method with absorbance recorded at 660 nm. Each gram of chyme was subjected to the substrate at 37°C for 30 min. Hydrolysis of 10 mg of

starch was defined as 1 unit of amylase activity (1 U/g chyme). Lipase activity was determined by turbidimetric method with absorbance recorded at 420 nm. Each gram of chyme reacted with substrate in this reaction system for 1 min at 37°C and each consumption of 1 μmol of substrate was defined as one unit of lipase activity (1 U/g chyme).

Statistical analysis

Data were analyzed using SPSS27 software (SPSS 27.0 version, SPSS Inc., Chicago, IL, USA) and making individual chickens as the experimental unit for one-way ANOVA and LSD multiple comparisons which is used for analyzing the significance of differences between groups. The results of descriptive statistics were expressed as Mean $\pm$ SD to respectively indicate the centralized trend and dispersion. The level of significance was $P < 0.05$.

RESULTS AND DISCUSSION

Amylase activity

The effect of the α -amylase activities in various parts of duodenum and jejunum of the three local chicken breeds is shown in Table 2. In all three chicken species, α -amylase activity was low and non-significant in the duodenum, increased sharply in the jejunum and reached a maximum in its mid-anterior section, followed by a decrease which was still higher than that in the duodenum. Moreover, the highest α -amylase activity was observed in the 8th segment in NTC and CPC which corresponds to the 2nd section of the jejunum and in the 9th segment of TSF which

Table 1: Ingredient and calculated nutrient composition of the basal diet (as fed basis, %).

Items	Composition
Ingredients	
Corn	69.00
Soybean meal	20.00
Wheat bran	5.90
Soybean oil	1.00
Limestone	1.20
CaHPO ₄	1.40
Salt	0.30
Premix ¹	1.00
Nutrient levels	
ME (MJ/kg)	12.20
Crude protein	15.60
Calcium	0.80
Phosphorus	0.65
Lys	0.68
Met	0.27

¹The premix provides the following per kilogram of diet: VA 1500 IU; VD₃ 200 IU; VE 10.00 IU; VK₃ 3.75 mg; VB 11.80 mg; VB₂ 3.6 mg; VB₆ 3.0 mg; VB₁₂ 0.01 mg; Pantothenic acid 10.00 mg; Niacin 30 mg; Biotin 0.15 mg; Folic acid 0.55 mg; Cu 8 mg; Fe 80 mg; Zn 60 mg; Mn 60 mg; I 0.35 mg; Se 0.3 mg.

Table 2: α -amylase activity (Mean $\pm$ SD) in various parts of duodenum and jejunum of three local chicken breeds.

Item	Intestinal area											
	1	2	3	4	5	6	7	8	9	10	11	12
TSF	49 $\pm$ 39 ^{a,xy}	63 $\pm$ 31 ^{cde}	59 $\pm$ 14 ^{de}	69 $\pm$ 25 ^{cde}	69 $\pm$ 34 ^{cde}	56 $\pm$ 24 ^{de}	163 $\pm$ 90 ^{bc,y}	230 $\pm$ 63 ^{ab}	300 $\pm$ 123 ^a	278 $\pm$ 221 ^a	157 $\pm$ 44 ^{bcd,y}	127 $\pm$ 93 ^{bode}
NTC	83 $\pm$ 36 ^{c,x}	71 $\pm$ 21 ^c	85 $\pm$ 37 ^c	85 $\pm$ 13 ^c	65 $\pm$ 18 ^c	81 $\pm$ 59 ^c	296 $\pm$ 90 ^{b,x}	444 $\pm$ 259 ^a	218 $\pm$ 70 ^b	194 $\pm$ 54 ^b	220 $\pm$ 66 ^{b,x}	197 $\pm$ 61 ^b
CPC	42 $\pm$ 13 ^{d,y}	48 $\pm$ 5 ^d	85 $\pm$ 18 ^{cd}	88 $\pm$ 28 ^{cd}	88 $\pm$ 22 ^{cd}	75 $\pm$ 18 ^{cd}	198 $\pm$ 28 ^{b,y}	348 $\pm$ 161 ^a	293 $\pm$ 96 ^a	201 $\pm$ 58 ^b	183 $\pm$ 35 ^{b,xy}	140 $\pm$ 58 ^{bc}

TSF: Taihe silky fowl; NTC: Ningdu three-yellow chicken; CPC: Chongren partridge chicken.

^{a,b}Means listed in the same row with different superscripts are significantly different (P<0.05); ^{x,y}Means listed in the same list with different superscripts are significantly different (P<0.05).

corresponds to the 3rd section of the jejunum, with statistically significant differences (P<0.05). In addition, by comparing the α -amylase activity at each intestinal segment of the three chickens, it was found that the α -amylase activity of NTC at the 7th segment which corresponds to the 1st section of the jejunum, was significantly higher than that of TSF and CPC (P<0.05). At the 1st and 11th segments which correspond to the 1st section of duodenum and the 5th section of jejunum, the α -amylase activity of NTC was the highest among the three local chickens. In the meantime, the amylase activity of TSF was lowest in above two segments (7th and 11th segments).

Lipase activity

The effect of the lipase activities in various parts of duodenum and jejunum of the three local chicken breeds is shown in Table 3. In all three chicken breeds, lipase activity was low in the duodenum, increased in the jejunum and reached a maximum in its midsection, followed by a decrease. The lipase activity of TSF and CPC were highest in the 9th segment, which is the 3rd section of the jejunum. And at the 8th segment which corresponds to the 2nd section of the jejunum, the Lipase activity of NTC was the highest (P<0.05). In addition, by comparing the lipase activities at each intestinal site of the three chickens, it was found that at the 1st and 2nd segments which correspond to the 1st and 2nd sections of the duodenum, the amylase activities of TSF and NTC were relatively higher. At the 5th and 6th segments which correspond to the last 2 sections of the duodenum, the lipase activities of TSF were significantly higher than those of NTC and CPC (P<0.05). Besides, the lipase activity of CPC was lowest in above four segments (1st, 2nd, 5th and 6th segments).

The main part of avian digestion and absorption of nutrients is the small intestine. The chyme in the small intestine is broken down by digestive enzymes into monosaccharides from starch and into glycerol and fatty acids from fat, which are absorbed by the intestinal wall. Amylase and lipase are important for the digestion and absorption of nutrients, growth and development of avian. The results of this experiment indicated that the α -amylase and lipase activities of NTC, CPC and TSF in the jejunum were significantly higher than those in the duodenum and showed a tendency to increase firstly and then decrease. The end of the duodenum of poultry is connected with the pancreatic duct and biliary duct and the α -amylase and lipase secreted by the pancreas and the bile secreted by the gallbladder thus enter into the digestive tract to participate in the digestion and absorption of nutrients. The bile has the effect of neutralizing the gastric acid. Moreover, when bile enters the digestive tract from the duodenum, the digestive enzymes are sufficiently activated by bile and other substance to promote the digestion and absorption of starch and fat. Zhang SQ *et al.* (2016) found that the activity of digestive enzymes in the jejunum of partridge shank chickens was higher than that in the duodenum (Zhang *et al.*, 2016). Chen (2017a) and Chen

Table 3: Lipase activity (Mean $\pm$ SD) in various parts of duodenum and jejunum of three local chicken breeds.

Item	Intestinal area											
	1	2	3	4	5	6	7	8	9	10	11	12
TSF	0.40 $\pm$ 0.02 ^{b,xy}	0.53 $\pm$ 0.17 ^{b,x}	0.57 $\pm$ 0.38 ^b	0.79 $\pm$ 0.55 ^b	0.63 $\pm$ 0.07 ^{b,x}	0.60 $\pm$ 0.30 ^{b,x}	0.66 $\pm$ 0.61 ^b	0.68 $\pm$ 0.16 ^b	1.35 $\pm$ 0.26 ^a	0.66 $\pm$ 0.07 ^b	0.56 $\pm$ 0.07 ^b	0.53 $\pm$ 0.23 ^b
NTC	0.44 $\pm$ 0.21 ^{bc,x}	0.45 $\pm$ 0.22 ^{bc,xy}	0.48 $\pm$ 0.27 ^{bc}	0.44 $\pm$ 0.27 ^{bc}	0.29 $\pm$ 0.16 ^{c,y}	0.26 $\pm$ 0.15 ^{c,y}	0.76 $\pm$ 0.25 ^{ab}	1.03 $\pm$ 0.78 ^a	0.63 $\pm$ 0.30 ^{abc}	0.55 $\pm$ 0.35 ^{bc}	0.57 $\pm$ 0.45 ^{bc}	0.50 $\pm$ 0.24 ^{bc}
CPC	0.18 $\pm$ 0.14 ^{d,y}	0.24 $\pm$ 0.05 ^{cd,y}	0.39 $\pm$ 0.35 ^{cd}	0.39 $\pm$ 0.12 ^{cd}	0.21 $\pm$ 0.14 ^{cd,y}	0.23 $\pm$ 0.13 ^{cd,y}	0.55 $\pm$ 0.23 ^{bc}	0.80 $\pm$ 0.29 ^{ab}	0.92 $\pm$ 0.62 ^a	0.49 $\pm$ 0.21 ^{cd}	0.42 $\pm$ 0.24 ^{cd}	0.40 $\pm$ 0.29 ^{cd}

TSF: Taihe silky fowl; NTC: Ningdu three-yellow chicken; CPC: Chongren partridge chicken.

^{a,b}Means listed in the same row with different superscripts are significantly different (P<0.05); ^{x,y}Means listed in the same list with different superscripts are significantly different (P<0.05).

(2017b) reported that the activities of amylase and lipase in the intestinal tract of Fengyang big-boned chickens were both greatest in the jejunum (Chen, 2017a; Chen, 2017b and Fan, 2021) showed that lipid digestion and amino acid absorption were more vigorous in the jejunum of chickens (Fan, 2021), which is consistent with the results in our experiment. The jejunum is the longest part of the intestinal tract of chicken, where the chyme remains for a long time and the digestive reaction is more complete. Thus, the research result that both amylase and lipase activities are strongest in the jejunum may also be related to this.

The results of this experiment indicated that the highest point of α -amylase activity was observed in the 8th segment of NTC and CPC and in the 9th segment of TSF. Meanwhile, the highest point of lipase activity was discovered in the 8th segment of NTC and in the 9th segment of TSF and CPC. From this, we can conclude that the highest point of digestive enzyme activities in all three chicken breeds was in the middle-anterior part of the jejunum. Thus, it can be seen that the trend of changes in intestinal digestive enzyme activities was similar in these three chicken breeds. In addition to the secretion of endogenous digestive enzymes, digestive enzyme activity is also affected by intestinal microorganisms (Stanley *et al.*, 2014) and some substances secreted by intestinal flora can assist in the absorption of nutrients (Cai, 2016 and Biswas *et al.*, 2018). Huang *et al.* (2018) showed that chicken intestinal microorganisms gradually increase in diversity and complexity from the duodenum to the cecum (Huang *et al.*, 2018). Duodenal peristalsis is fast, resulting in a short retention time for the chyme and a low number and diversity of intestinal microorganisms. However, the jejunum is the longest part of the avian intestinal segments, contributing to a long retention time for chyme and a higher number of intestinal micro-organisms, of which the dominant bacteria are lactobacilli and the digestive and absorption of nutrients is more adequate (Xiao *et al.*, 2021). In our experiment, the lipase activity of NTC, CPC and TSF was relatively low but fluctuated in the duodenum and the fluctuating segment was located in the middle of the duodenum. The duodenum of poultry is in the shape of U and is connected by the pancreatic duct and bile duct, the fluctuation of lipase activity in the duodenum may be related to the structure of the duodenum and the specific mechanism needs to be further studied.

The results of this experiment indicated that, among the three local chickens, the α -amylase activity in the anterior end of the duodenum and jejunum as well as at the terminal end of the jejunum was highest in the NTC and lowest in the TSF. In contrast, lipase activity in the anterior and terminal ends of the duodenum was greatest in the TSF and least in the CPC. Those may be related to the different nutrients required by chicken of each species as well as their own characteristics. As a good local chicken breed, NTC has the characteristics of fast reproduction, early growth and egg production and prefers to eat cereals, so the high amylase activity in the digestive tract of NTC

may be related to its high demand for starch. The body of TSF is rich in melanin and these melanin particles exhibit a strong adsorption effect on fat molecules (Zhang *et al.*, 2014), which may facilitate intestinal absorption of fat. The high lipase activity of TSF may be related to this and the specific mechanism of this phenomenon needs to be further studied.

CONCLUSION

In conclusion, the results in this experiment indicated that α -amylase and lipase activities in the duodenum and jejunum of TSF, NTC and CPC had similar trends, showing the trend of first increasing and then decreasing and the α -amylase and lipase activities reached the maximum in the mid-anterior part of the jejunum, but there were some differences in the specific enzyme activities of the highest site and digestive enzyme activities. The highest α -amylase activity of NTC and the highest lipase activity of TSF may be related to its nutritional requirements and the specific mechanism needs to be further studied.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish or preparation of the manuscript.

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