Research Articles

Development of Specific Activity of Amylolytic and Proteolytic Enzymes in Duodenum and Jejunum of the Small Intestinal Tract of Meat-Type Ducks during 1-42 Days of Age

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Abstract

This experiment was conducted to elucidate the pattern of amylolytic and proteolytic digestive enzyme activities in meat-type ducks during 1-42 days of age. Twenty-eight male meat type ducks (Cherry Valley strain) were used, and diets based on corn-soybean meal were offered ad lib throughout the experimental period. At duodenal and jejunal segments, the specific activity (SA) of amylolytic (amylase) and proteolytic (trypsin, chymotrypsin and total proteases) enzymes were determined at 1, 7, 14, 21, 28, 35 and 42 days of age. Maximal or nearly maximal SA of all enzymes at first day after hatch (1 day of age) and the activity declined on 7 day of age was found ($P<0.05$). After 7 days of age, all enzymes tended to reach a nadir at day 14 or day 21, then the SA were significantly increased from 21 to 42 days of age ($P<0.05$). Therefore, the pattern of SA of all enzymes at duodenal and jejunal segments were changed in a cubic trend ($P< 0.01$) with age. In ducks, it is indicated that the embryonic enzyme reserve may be a reason of high SA of amylolytic and proteolytic enzymes at 1 day of age, then the SA are activated again after 21 days of age by intestinal development processes and/or increasing amount of feed intake.

Keywords: Meat-Type Duck, Amylolytic enzyme, Proteolytic enzyme, Specific activity, Age

การเปลี่ยนแปลงของการทำงานของเอนไซม์ย่อยแป้งและเอนไซม์ย่อยโปรตีนในลำไส้เล็กดูโอดีนัมและเจจูนัมของเป็ดเนื้อ

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บทคัดย่อ

เพื่อศึกษาการเปลี่ยนแปลงของการทำงานของเอนไซม์ย่อยแป้งและเอนไซม์ย่อยโปรตีนในเป็ดเนื้อสายพันธุ์เชอร์รี่วัลเลย์ อายุ 1-42 วัน จำนวน 28 ตัว โดยให้เป็ดกินอาหารซึ่งมีส่วนประกอบหลักเป็นข้าวโพดและกากถั่วเหลืองแบบไม่จำกัด วิเคราะห์ค่าการทำงานของเอนไซม์ในดูโอดีนัมและเจจูนัมของลำไส้เล็ก ในวันที่ 1, 7, 14, 21, 28, 35 และ 42 พบว่า มีปริมาณสูงสุดหรือเกือบสูงสุดในวันที่เป็ดมีอายุ 1 วัน และลดลงในวันที่เป็ดมีอายุ 7 วัน อย่างมีนัยสำคัญ ($P<0.05$) การทำงานของเอนไซม์ส่วนใหญ่มีแนวโน้มลดลงจนถึงจุดต่ำสุดในวันที่ 14 หรือ 21 จากนั้นมีค่าเพิ่มสูงขึ้นจนถึงอายุ 42 วัน พบว่ารูปแบบการเปลี่ยนแปลงของการทำงานเอนไซม์ในดูโอดีนัมและเจจูนัมของลำไส้เล็กเป็นแบบคิวบิคเมื่อเป็ดมีอายุมากขึ้น ($P<0.01$) การที่เอนไซม์ยังคงหลงเหลือภายหลังการฟักนั้นอาจเกิดจากเอนไซม์ของลูกเป็ดที่ผลิตในช่วงฟักไข่ และเอนไซม์เหล่านี้ถูกกระตุ้นจากการพัฒนาของระบบทางเดินอาหารอีกเมื่ออายุเพิ่มมากขึ้นที่ 21 วัน และ/หรือ อาจร่วมกับการกระตุ้นจากการกินอาหารที่เพิ่มขึ้น

คำสำคัญ : เป็ดเนื้อ เอนไซม์ย่อยแป้ง เอนไซม์ย่อยโปรตีน การทำงานของเอนไซม์ อายุ

Introduction

Duck production continues to be one of promising food and agriculture businesses, while feeds and feeding are the foundation for profitable duck farming. It is known that feed digestion is of paramount importance and shown to be a vital mechanism for growth, energy, and cell repairs among animal species. It is duodenum in which hydrolysis of α-(1,4) bonds in starch with pancreatic α-amylase takes place and thus liberate glucose, oligosaccharides and dextrin (Zelenka and Ceresnakova 2005). Trypsin activity was reported to present in jejunum (Borges et al., 1995). Therefore, the segments of duodenum and jejunum of the small intestine are the important sites of the digestion of carbohydrate and protein.

It is known that dietary carbohydrate and protein are major fractions and cost of the animal feeds. In commercial, corn or broken rice (as a carbohydrate source) and soybean meal (as a protein source) have been used in feed formulation of poultry including ducks and other waterfowls. Dietary carbohydrate manipulations could improve livability or growth of hatching (Christensen 2009), it is initially digested by salivary amylase, and more digestion of starch continues in the duodenum by the action of pancreatic amylase. For digestion of dietary protein, the digestion processes are more complicated than that of the carbohydrate due to many pancreatic proactive enzymes such as pepsinogen, trypsinogen, chymotrypsinogen or carboxypeptidase are activated in duodenum.

The small intestine is considered a major site for the digestion of macronutrients, nearly all of which need hydrolysis by endogenous digestive enzymes for further processes of nutrient absorption and assimilation (Adibi 1976; Noy and Sklan 1995). Many studies reported that the small intestine expresses higher rate of protein synthesis than the stomach and large intestine do (Attaix and Arnal 1987; Attaix et al., 1992; Burrin et al., 1999). Moreover, (Krogdahl and Sell 1989) suggested that development of enzyme activities in the intestinal contents might be a better indicator of digestive processes and development than that of pancreatic tissue activities. In hatching birds, sufficient quantities of digestive enzymes have to meet for hydrolysis of exogenous feed before uptake to the enterocytes can occur (Uni et al., 1998). Although the intestinal growth and health of poultry have been over the last decades increasing interest, research information on ducks' endogenous digestive enzymes remains scarce.

Therefore, the current experiment was conducted to elucidate the pattern of post-hatch development of amylolytic and proteolytic digestive enzymes activity in meat-type ducks fed diets based on corn-soy meal in meat-type ducks from 1-42 days of age.

Materials and Methods

Animals and diets

A total of twenty-eight 1-day-old male broiler ducks of the commercial Cherry Valley strain were used from 1-42 days of age. The ducks were fed ad lib pelleted feeds and had free access to water under standard controlled evaporative cooling system throughout the experimental period. The nutrients in diets were formulated to meet the requirement according to strain recommendation. The experimental diet composition analysis is shown in Table 1.

Table 1. Composition and nutrient concentration of basal diets (as fed)

Items	Starter (1-9 days)	Grower (10-16 days)	Finisher (17-42 days)
Ingredients (%)			
	52.46	57.17	62.40
Palm oil	3.04	3.09	2.11
Soybean meal	39.63	34.84	30.49
Calcium carbonate	1.36	1.39	1.41
Monodicalcium phosphate	2.17	2.20	2.22
Salt	0.20	0.20	0.20
Choline chloride	0.08	0.14	0.18
Mycotoxin binder	0.05	0.05	0.05
DL-methionine	0.24	0.23	0.17
L-lysine HCL	0.26	0.23	0.17
L-threonine	0.05	0.07	0.03
BHA	0.01	0.01	0.01
PremixA	0.50	0.50	0.50
Nutrients by calculation			
Metabolizable energy (kcal/kg)	2850	2900	2900
Crude protein (%)	22.00	20.00	18.50
Crude fat (%)	5.26	5.43	4.62
Crude fiber (%)	4.09	3.87	3.70
Lysine (%)	1.35	1.17	1.11
Methionine (%)	0.57	0.52	0.45
TSAA (Met+Cys) (%)	0.90	0.84	0.75
Threonine (%)	0.90	0.85	0.75
Calcium (%)	1.00	1.00	1.00
Available phosphorus (%)	0.50	0.50	0.50

A Vitamin and mineral premix content (per kg of total diet): vitamin A 12,000 IU, vitamin D 3,000 IU, vitamin E 20 IU, vitamin K 2 mg, thiamin 2 mg, riboflavin 8 mg, pyridoxine 4 mg, niacin 25 mg, cobalamin 0.02 mg, panthothenic 20 mg, nicothenic 20 mg, folic acid 3 mg, biotin 0.2 mg, choline chloride 1,000 mg, iron 60 mg, manganese 80 mg, zinc 60 mg, selenium 0.2 mg, iodine 0.5 mg, copper 8 mg, iodine 0.5 mg.

Housing and managements

The ducklings were kept in floored pens (7 pens with 4 ducks each; 0.24 m^2/bird) under an evaporative cooling housing system. The floor of each pens was littered with rice hull. According to strain recommendation, the lighting program was provided as follows: 1-3 days using 24 hours of lighting, 4-9 days stepping down to 18 hours of lighting day by day; and 10 days until killed using 18 hours of lighting. The ducklings were kept, maintained, and treated in compliance with the standards of animal welfare. Cares were taken to minimize the number of animals used. The experimental design and procedures were approved and conducted at Kasetsart University's Luang Suwanvajokkasikij Poultry Farm, Bangkok, Thailand.

Experimental Procedures

Each duck was deeply anesthetized by ether and killed by cervical dislocation. The abdominal and thoracic cavities were open, then the small intestinal tract was excised. Freshly dissected digestive tracts were collected from each of 4 ducks on day 1, 7, 14, 21, 28, 35, and 42 of the experiment. They were immediately placed in iced-chilled containers and transported to the laboratory. According to the location of enzyme activities, the digestive tract of each duck was individually divided into duodenal and jejunal segments.

Sampling and enzyme activity assay

All procedures were conducted at cold temperatures (0-4°C). Each segment was individually iced-cold homogenized with a waring blender. The homogenate was centrifuged at 4°C with 10,000 x g for 60 min. Supernatant was decant and stored in small portions of 1.5 ml test tubes at -80°C until used for the determination of digestive enzymes. The collected supernatant was referred as "crude enzyme extract". All assays were performed with slice modification for a microtiter plate assay in duplicate at 40°C (Lainé et al., 1993), which is considered as a body temperature of ducks and other avian species, using a microplate reader (Synergy HT, BIOTEK). Protein determination was determined using bovine serum albumin (BSA, Sigma) as a standard and reported as mg protein equivalent to BSA (Bradford 1976) using a 96- well microplate reader.

The activity of α-amylase was determined by measuring the increase in reducing sugar from the starch solution, using the 3,5-dinitrosalicylic acid (DNS, Sigma) (Areekijseree et al., 2006). The α-amylase specific activity was expressed as mU of maltose produced h-1 mg protein-1.

Specific activities of trypsin and chymotrypsin were determined spectrophotometrically in 96-well microplates using Bensoyl-L-arginine-p-nitoanilide (BAPNA, Sigma B 4875) and N-succinyl-ala-ala-pro-phe-p-nitroanilide (SAPNA, Sigma S 7388) as specific substrates respectively (Kakade et al., 1969). Both trypsin and chymotrypsin specific activities was expressed as mU of *p*-nitroaniline produced h^{-1} mg $protein^{-1}$.

The activity of total proteases of the crude extracts (100 µl) was determined using azocasein as substrate according to (García-Carreño 1992).Total protease specific activity was expressed as the increase in absorbance at 440nm h^{-1} mg $protein^{-1}$.

Statistical analysis

All data were analyzed using the SAS software (SAS institute, 2016). Least Square Means test was used for comparison of treatment means. A significance level of $P<0.05$ was criteria for all cases. Orthogonal comparison was used in the regression model (Steel and Torrie 1960).

Results

Growth performance

Table 2 shows the parameters on growth performance (body weight, body weight gain, feed intake and growth rate per day) of the experimental ducks at different growth stages. At day 9, 16 and 42, the body weight of ducks was 320.01, 815.91 and 3,398.62 g, respectively. Feed intake (g/day) during 1-9 days, 10-16 days and 17-42 days were 43.96, 100.24 and 201.35, and FCR was 1.48, 1.42 and 2.03, respectively.

Table 3 shows the changes in α-amylase specific activity (SA) of duodenum and jejunum segments of the small intestine in different ages of the ducks. In duodenum, the SA of α-amylase was highest at day1 of age, followed by the reduction of the activity at day 7 and 14 of age ($P<0.05$). Inversely, the SA was gradually increased from 21 days of age. Therefore, the SA at 42 day of age was significantly higher than the SA at day 14 of age ($P<0.05$). By orthogonal comparison, the α-amylase SA of the duodenum changes in a cubic trend ($P<0.01$) with age.

In jejunum, the SA of α-amylase was high at day 1 and day 42 of age ($P< 0.05$). At day 21, the SA was lower than the SA at day1, 7, 28, 35 and 42 ($P< 0.05$). At day 14, the SA was lower than that of day 1 and 42 ($P<0.05$). By orthogonal comparison, the α-amylase SA of the jejunum was changed in a cubic trend ($P< 0.01$) with age similarly changed in the duodenum segment.

Table 4 shows the changes in trypsin SA of duodenum and jejunum segments of the small intestine in different ages of the ducks. In the duodenum and jejunum, the SA of trypsin was highest at day 42 ($P<0.05$). The SA of trypsin at the other days of age in the duodenum and jejunum were indifferent. By orthogonal comparison, the trypsin SA of the duodenum and jejunum of the small intestine were changed in a cubic trend ($P<0.05$).

Table 5 shows the changes in chymotrypsin SA of duodenum and jejunum segments in different ages of the ducks. In duodenum, the SA of chymotrypsin was the highest at 1 day of age, followed by the reduction of the activity from 7 to 35 days of age ($P<0.05$). At day 42 of age, the SA was conversely increased and significantly higher than the SA during 7-35 days of age ($P<0.05$).

In jejunum, the SA of chymotrypsin was also highest at 1 day of age ($P<0.05$). The SA of chymotrypsin from day 21 was gradually increased, then the SA at day 35 and 42 was significantly higher than that of the day 21 ($P<0.05$). By orthogonal comparison, the chymotrypsin SA of two segments of the small intestine was changed in a cubic trend ($P<0.01$) with age.

Table 6 shows the changes in total proteases SA of duodenum and jejunum segments of the small intestine in different ages of the ducks. In duodenum, the SA of total proteases was highest at 1 day of age ($P<0.05$), and significantly declined to 21 day of age. However, the SA of total proteases from day 21 to 41 was inversely increased, so the SA at day 42 was significantly higher than at day 14 and 21 ($P<0.05$).

In jejunum, the SA of total proteases at day 42 showed the highest value ($P< 0.05$), while the SA at 42 day of age was not differed from 1 day of age. The SA was significantly decreased from 1 to 21 days of age, then dramatically increased after 21 days of age and achieved the highest level at 42 days of age ($P<0.05$). By orthogonal comparison, the total protease SA of the two segments of the small intestine was changed in a cubic trend ($P<0.01$) with age.

Table 2. Growth performance of meat type ducks (n=4) age from 1- 42 days.

Items	Starter (1-9 days)	Grower (10-16 days)	Finisher (17-42 days)
Initial weight (g/duck)	53.56	-	-
Body weight (g)	320.01	815.91	3398.62
Body weight gain (g)	267.87	495.88	2583.43
Feed intake (g/day)	43.96	100.24	201.35
Growth rate per day (g)	29.76	70.84	99.36
FCR	1.48	1.42	2.03

Table 3. Changes in α-amylase specific activity (mU/ mg protein) of duodenum and jejunum of the small intestine in different age of the ducks (n=4)

Day of age	Intestinal segments	
	Duodenum	Jejunum
1	913.70^{A}	545.46^{AB}
7	283.70^{BC}	249.97^{C}
14	81.20^{C}	229.20^{CD}
21	$117.70B^{C}$	152.55^{D}
28	$256.90B^{C}$	281.34^{C}
35	$307.50B^{C}$	360.86^{BC}
42	339.10^{B}	579.88^{A}
SEM	54.94	39.20
Contrast, P^{1}		
C	**	**

^{ABCD} Means within the same column not sharing a common superscript are significantly different (P<0.05)

[1] Orthogonal comparison of variates at one day old in ducks. C = cubic effect; NS = not significant; *P<0.05; **P<0.01

Table 4. Changes in trypsin specific activity (mU/ mg protein) of duodenum and jejunum segments of the small intestine in different age of the ducks (n=4)

Day of age	Intestinal segments	
	Duodenum	Jejunum
1	208.69^{AB}	486.80^{B}
7	89.39^{B}	133.20^{B}
14	48.13^{B}	100.20^{B}
21	49.34^{B}	94.30^{B}
28	57.76^{B}	343.10^{B}
35	51.49^{B}	185.40^{B}
42	260.68^{A}	958.80^{A}
SEM	23.55	74.44
Contrast, P^{1}		
C	**	**

^{AB} Means within the same column not sharing a common superscript are significantly different (P<0.05)

[1] Orthogonal comparison of variates at one day old in ducks. C = cubic effect; NS = not significant; *P<0.05; **P<0.01

Table 5. Changes in chymotrypsin specific activity (mU/mg protein) of duodenum and jejunum segments of the small intestine in different age of the ducks (n=4).

Day of age	Intestinal segments	
	Duodenum	Jejunum
1	689.29^{A}	467.10^{A}
7	121.90^{C}	149.80^{CD}
14	55.73^{C}	138.10^{CD}
21	63.25^{C}	100.20^{D}
28	74.38^{C}	198.30B^{CD}
35	99.86^{C}	345.70AB^{C}
42	306.89^{B}	420.50A^{B}
SEM	45.81	74.44
Contrast, P^{1}		
C	*	**

^{ABCD} Means within the same column not sharing a common superscript are significantly different (P<0.05)

[1] Orthogonal comparison of variates at one day old in ducks. C = cubic effect; NS = not significant; *P<0.05; **P<0.01

Table 6. Changes in total protease specific activity (mU/mg protein) of different segments of the small intestine of the ducks (n=4).

Day of age	Intestinal segments	
	Duodenum	Jejunum
1	8.75^{A}	6.71^{AB}
7	1.33^{BC}	1.65^{CD}
14	0.42^{C}	1.51^{CD}
21	0.39^{C}	0.67^{D}
28	1.45^{BC}	2.87^{CD}
35	1.06^{BC}	4.04^{BC}
42	3.17^{B}	8.06^{A}
SEM	0.57	0.59
Contrast, P^{1}		
C	**	**

^{ABCD} Means within the same column not sharing a common superscript are significantly different (P<0.05)

[1] Orthogonal comparison of variates at one day old in ducks. C = cubic effect; NS = not significant; *P<0.05; **P<0.01

Discussion

Although the development of digestive enzymes is affected by age, it can also be altered by feed intake and composition (Tarvid 1992), external environment, genetic and hormonal regulation (Farmer et al., 1993). Since feeds and management were provided according to commercial or the strain's requirements, and the ducks were apparently healthy, and no mortalities or sick ducks were observed. Moreover, the ducks during starter, grower and finisher periods showed similar body weight gain, feed intake, and FCR are in accordance with the strain's recommendation (Cherry Valley 2004; Kaewtapee et al., 2011). Therefore, it is assumed that the SA value of each enzyme in current study should be mainly influenced by the age.

Optimal endogenous enzymatic hydrolysis (in duodenum and jejunum) is indispensable for digestion and absorption of nutrients for growth of animals. Development of activities of α-amylase and proteases in the intestinal contents might be a better indicator of development of digestive processes than activities of enzyme in the pancreatic tissue (Krogdahl and Sell 1989). In the current study, it is obvious that all the amylolytic and proteolytic enzymes of duodenal and jejunal parts of the small intestine showed very high specific activity at the first day after hatch, and the activity declined on 7 days of age. After 7 days of age, all enzymes tended to reach a nadir at 14 or 20 days after hatch, then enzymes started to increase and showed a second peak on 42 days of age. Hence, fluctuations in the activity of enzymes were clearly expressed in cubic manner. The explanation of this phenomenon may be given as birds hatch with some reserves of pancreatic enzymes that are produced during embryonic growth, where the highest level of total

protease activity was found immediately after hatching with a maximal decrease of activity during the first week (Tarvid 1991). It was also reported that high capacity to digest and absorb carbohydrates develops during incubation give the newly hatched chick with a relatively mature system for starch utilization (Moran 1985). Therefore, it is suggested that 1-day-old ducks may be able to digest starch well via the high SA of α-amylase.

The SA of trypsin which is important for protein digestion was also high at day 1, but the value was slightly lower than at 42 days of age. The phenomenon of high SA of α-amylase and slightly low SA of trypsin at day 1 compared to at 42 days of age may be explained that newly hatched birds need glucose from dietary starch (less carbohydrate in yolk), while protein is directly used from yolk sac. Therefore, high SA of α-amylase in newly hatched duck is required.

From 7 to 21 days of age, low SA of all the enzymes (α-amylase, trypsin, chymotrypsin and total proteases) of ducks were found. This is agreed with serval investigators who reported that gastrointestinal tract growth and digestive function are not fully developed in young chicks (Nitsan et al., 1991), turkeys (Krogdahl and Sell 1989; Sell et al., 1991), and ducklings (Lu 1999). Conversely, the SA of the enzymes were increased from day 21, and achieved high level again at 42 days of age. The increase of SA of all enzymes may be caused by the development of digestive tract and increase amount of feed intake during 21-42 days of age. Accordingly, (Kenyon et al., 2004) reported that increased relative digestive activity could be a reflection of a higher absolute mass and growth rate of the digestive system in domesticated ducks. Additionally, adaptation to exogenous food is associated with a dramatic increase in the weight of the gastro-intestinal tract and the activity of its digestive enzymes (Nitsan, et al. 1991; Tarvid 1995). When comparing to that of other poultry species, on the other hand, the SA of α-amylase in the pancreas of goose had significant increases with ages, peaking at 7 to 11 and 21 days of age (Krogdahl and Sell 1989). The SA of trypsin and proteases in the pancreas of chicks increased from 14 to 20 days of age (Nir et al., 1993; Nitsan, et al. 1991). Thus, in ducks, it seems that maximal SA of enzymes are activated later than those of chicken and goose.

In conclusions, the amylolytic and proteolytic enzymes at duodenal and jejunal parts of the small intestine expressed different levels in the ducks aged from 1-42 days. The maximal or nearly maximal specific activity after hatch (1 day of age) maybe come from the reserve of pancreatic enzymes during embryonic growth, and then the activities are low during 7-21 days of age due to the immaturity of the digestive tract. During 21-42 days of age, well development of the digestive tract and feed intake increase the SA of all enzymes.

For further implications based on the results of current study, the early postnatal period is likely the first critical period of most significant adaptive changes of the gut. Introducing the early feeding strategy into meat-type duck production could be promising and plausible to be applicable in the meat-type duck industry.

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