

The Development of Gastrointestinal Tract and Pancreatic Enzymes in White Roman Geese

B. L. Shih^{1,2}, B. Yu² and J. C. Hsu^{2,*}

¹Nutrition Division, Livestock Research Institute, Council of Agriculture, Hsinhua, Tainan, 712, Taiwan, ROC

ABSTRACT : The objective of this experiment was to investigate the development of gastrointestinal tract and activities of pancreatic enzymes in White Roman geese. Thirty developing embryos at the 22th, 24th and 26th day of incubation and at hatching, and sixteen or eight goslings, half males and half females, at the 1, 3, 7 or 11, 14, 21 and 28 days of age were sampled, respectively. The weights of the yolk, gastrointestinal tract and intestinal length, and the activities of pancreatic enzymes were measured. Residual yolk weight decreased rapidly during late incubation and was nearly depleted at 3 days of age. The protein and energy contents in the residual yolk of goslings at 3 days of age were significantly ($p < 0.05$) less than those at the late incubation. From 6 days before hatching to 28 days of age, the absolute weights of gizzard, proventriculus, liver, pancreas, small intestine and large intestine in goslings increased by 48, 457, 94, 2334, 89 and 76 times, respectively. The relative weights of proventriculus, gizzard, liver, pancreas, small intestine and large intestine reached peaks at 3, 3, 14, 14, 11 and 11 days of age, respectively, and then decreased gradually. However, the relative lengths of small intestine and large intestine reached peaks at 3 days of age and at hatching, respectively. The activities of pancreatic trypsin and chymotrypsin increased sharply from hatching to 14 day of age, and then decreased gradually until 21 days of age. The activity and specific activity of pancreatic amylase were increased following by age and peaked at 7 to 11 and 21 days of age, respectively. The activity and specific activity of pancreatic lipase reached a plateau from 11 to 28 days of age. These results indicate that the gastrointestinal tract and activities of pancreatic enzymes developed more rapidly than body weight through the early growing period of goslings. (*Asian-Aust. J. Anim. Sci.* 2005, Vol 18, No. 6 : 841-847)

Key Words : Geese, Gastrointestinal Tract, Pancreatic Enzymes, Development

INTRODUCTION

During early post-hatching life the poultry undergo a rapid transition in digestive function. The nutrients in young poultry are supplied initially from the yolk, and development to exogenous nutrients uptake occur during the first few days after hatching. The nutrients provided by the yolk emphasize the importance of the early growth period, and the transition from embryonic yolk absorption to food utilization is accompanied by many changes in the developmental process of the goslings (Lilja, 1983). Several studies have indicated that gastrointestinal tract of newly hatched birds is not fully developed (Nitsan et al., 1991a, b; Noy and Sklan, 1998), and both quantitative and qualitative changes in the digestive organs and enzyme secretions have been reported for the chicks (Nitsan et al., 1991b), the turkey poults (Sell et al., 1991) and the ducklings (Lu, 1999). Development of the gastrointestinal tract is an important aspect of growth, especially the development of digestive functional organs (e.g., pancreas and intestine) during the early post-hatching period of chicks (Katanbaf et al., 1988; Nitsan et al., 1991a). Nitsan et al. (1991b) has indicated that the volume of gastrointestinal tract was a limiting factor in feed intake and subsequent growth, more

so for young meat-type chicks than egg-type chicks. These digestive organs grow more rapidly in weight than the whole body mass, so that the relative weights of these digestive organs are maximal from 6 to 8 days of age in the turkey poults (Sell et al., 1991; Noy and Sklan et al., 1998), and 6 to 10 days of age in the chicks (Katanbaf et al., 1988). An increase in the weight of gastrointestinal tract is essential to the secretory activities of enzymes in the pancreas in order to achieve maximal growth in ducklings during the early growth stage (Lu, 1999). A previous study has documented that changes in the activities of pancreatic enzymes are closely correlated both with body weight and intestinal weight in chicks (Noy and Sklan, 2000). Geese, which are herbivorous, can be successfully raised with various forage diets, milling by-products and concentrates (Hsu et al., 2000). They can digest plant structural substance better that result in increased their feed intake and fed higher fiber content in diet than other avian species (Janroz et al., 1992). Moreover, geese had a pair greater digestive capability of caecum and it appeared to digest fiber in diet more efficiently (Chen et al., 2002). Therefore, the development of gastrointestinal tract in geese influenced to differ with the other avian species. However, the changes in development of gastrointestinal tract and pancreatic enzymes with age have not been extensively examined in geese. The aim of this experiment was to investigate the development of gastrointestinal tract and digestive enzymes in the pancreas of geese during the embryonic period to first 28 days after hatching.

* Corresponding Author: J. C. Hsu. Tel: +886-4-22850315, Fax: +886-4-22870781, E-mail: jchsu@dragon.nchu.edu.tw

² Department of Animal Science, National Chung-Hsing University, 250 Kao-Kung Road, Taichung, 402, Taiwan, ROC.

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Table 1. The composition of experimental diet

Ingredients	%
Yellow corn, ground	51.60
Wheat bran	3.00
Soybean meal	29.10
Fish meal, 60%	3.00
Alfalfa, 17%	7.00
Tallow	3.50
Dicalcium phosphate	1.32
Limestone, pulverized	0.50
Salt	0.40
DL-methionine	0.20
Choline chloride, 50%	0.08
Vitamin-mineral premix ¹	0.30
Total	100
Calculated value	
Crude protein (%)	20.60
ME (kcal/kg)	2,826
Calcium (%)	0.83
Available phosphorus (%)	0.45
Analyzed value	
Crude protein (%)	20.25
Calcium (%)	0.85
Total phosphorus (%)	0.68

¹ Supplied per kg of diet: Vitamin A, 10,000 IU; Vitamin D₃, 1,000 IU; Vitamin E, 25 IU; Vitamin K, 3 mg; Thiamin, 3 mg; Riboflavin, 5 mg; Pyridoxine, 3 mg; Vitamin B₁₂, 0.03 mg; Ca-pantothenate, 10 mg; Niacin, 50 mg; Biotin, 0.1 mg; Folic acid, 3 mg; Mn (MnSO₄·H₂O), 60 mg; Zn (ZnO), 60 mg; Cu (Cu₂SO₄·5H₂O), 5 mg; Fe (FeSO₄·7H₂O), 70 mg; Se (Na₂SeO₃), 0.1 mg.

MATERIALS AND METHODS

Animals and treatments

One hundred and twenty breeding White Roman geese eggs on day 22 of incubation got from Chang-Hua animal breeding station, Taiwan Livestock Research Institute, Council of Agriculture (COA-TLRI) were used. The eggs were continuously hatched in our laboratory, and thirty eggs were sampled randomly at days 22, 24 and 26 of incubation and at hatching, respectively. The embryos were removed from the eggs and weighed. Thereafter, the residual yolks were removed and weighed immediately, and frozen at -20°C for further chemical composition analysis. The gastrointestinal tract, then was removed and the proventriculus, gizzard, liver, pancreas, small intestine (duodenum, jejunum and ileum) and large intestine (caecum and colon-rectum) were emptied and weighed, and their lengths were measured.

Eighty day-old goslings, half males and half females, were used for determining gastrointestinal tract developmental and pancreatic enzymes activities after hatching. The birds were fed with starter diet (containing 200 g protein/kg and 2,900 kcal ME/kg) (Table 1). At 1, 3 and 7 days of age, sixteen goslings, 8 males and 8 females, were selected, respectively. In addition, eight goslings, 4

males and 4 females, at 11, 14, 21 and 28 days of age were selected, respectively. Before being sacrificed, the geese were fasted for 12 h and then fed for 3 h. All goslings were weighted individually and sacrificed by decapitation. Thereafter, GIT samples were collected and excised as mentioned above.

Preparation of crude enzymes and chemical analysis

The entire pancreas of the embryos and goslings were removed immediately for determining the activities of amylase, trypsin, chymotrypsin and lipase during experimental period. The crude pancreatic enzymes were prepared according to the method of Kidder and Manners (1980). Samples were weighed and homogenized with a Potter Elvehjem device (TRI-R, Instruments, Model-k41) at 4°C in a saline solution that was 4 times of the sample weight. Thereafter, the homogenates were centrifuged at 2,000×g for 30 min and the resultant clear supernatant fluid were collected for assaying the activities of digestive enzymes. The feed constituents and nutrient compositions in the residue yolk were analyzed according to the standard procedures (AOAC, 1990). The α -amylase (EC 3.2.1.1) activity was determined according to the method of Onodera et al. (1988) using soluble starch (Wako Pure Chemical Industry Ltd., Japan) as substrate. One unit of α -amylase was expressed as 1 mg glucose released per minute at 37°C. The trypsin and chymotrypsin activities were assayed using the method of Rick (1974) with N- α -Benzoyl-L-arginine ethyl-ester (BAEE, Sigma B-4500) and N-Benzoyl-L-tyrosine ethyl ester (BTTEE, Sigma B-612.5) as substrates, respectively. The activities of one unit of trypsin and chymotrypsin were expressed as 1 μ mole of Benzoyl-arginine and Benzoyl-tyrosine, respectively, released per min at 25°C. The lipase activity was analyzed according to the titrimetric method using tributyrin (Sigma, T-8626) as a substrate, as described by Borgstrom (1975) and Gargouri et al. (1986). The protein content of crude enzyme samples were measured according to the method of Bradford (1976).

Statistical analysis

All data were analyzed using the General Linear Models Procedures of SAS (SAS, 1996). Comparison of treatment means was made using a Least Squares Means test. A significance level of $p < 0.05$ was applied in all cases and orthogonal comparison was used in the multi-regression model to Steel and Torrie (1960).

RESULTS AND DISCUSSION

Changes in the residual yolk

The changes of nutrients compositions and weight of

Table 2. Nutrient compositions of residual yolk of goslings at different ages

Items	Days of age					Contrast, P ¹			
	-6	-4	-2	HA	1	3	SEM	L	Q
Relative weight (g/100 g BW)	74.25 ^a	56.90 ^b	22.29 ^c	9.36 ^d	9.93 ^d	1.49 ^d	2.67	***	***
Absolute weight (g)	47.35 ^a	43.00 ^a	21.88 ^b	11.94 ^c	11.78 ^c	2.32 ^d	1.54	***	***
Dry matter (%)	58.25 ^b	57.42 ^b	64.36 ^a	60.45 ^{ab}	59.45 ^{ab}	53.68 ^c	1.96	*	***
Moisture (%)	41.74 ^b	42.57 ^b	35.63 ^c	42.51 ^{ab}	40.55 ^b	46.32 ^a	1.26	*	***
Gross energy (kcal/kg)	7,098 ^a	7,024 ^a	6,996 ^a	7,025 ^a	6,998 ^a	6,757 ^b	58.90	***	*
Protein (% DM)	37.40 ^a	38.76 ^a	38.99 ^a	39.78 ^d	40.05 ^d	31.83 ^b	1.40	***	NS
Fat (% DM)	50.24	48.33	47.97	48.13	47.39	50.93	2.09	NS	NS

^{a,b,c,d} Means within the same row without the same superscripts are significantly different ($p < 0.05$).

¹ Orthogonal comparison of various merits on days of age in goslings. L: linear; Q: quadratic effect.

HA = Day of hatching. NS = Not Significant. * $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$.

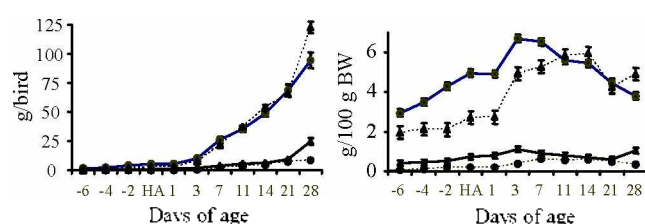


Figure 1. The absolute (left) and relative (right) weights of digestive organs of goslings at different ages. (-▲-) gizzard, (-●-) proventriculus, (-△-) liver, (-□-) pancreas. Value are given as mean \pm SE. HA = day of hatching. The absolute and relative weights of digestive organs of goslings had significant linear ($p < 0.001$) and quadratic ($p < 0.05$) effects with ages, respectively. The linear equations for absolute weights (g/bird) of digestive organs as follows: Y (gizzard) = $9.121 + 2.768X$, $R^2 = 0.93$; Y (liver) = $6.894 + 3.287X$, $R^2 = 0.89$; Y (pancreas) = $0.624 + 0.289X$, $R^2 = 0.92$, and X is days of age; The quadratic equations for relative weights (g/100 g BW) of digestive organs as follows: Y (gizzard) = $43954 + 0.250X - 0.011X^2$, $R^2 = 0.53$; Y (proventriculus) = $0.689 + 0.031X - 0.001X^2$, $R^2 = 0.45$; Y (liver) = $3.337 + 0.276X - 0.008X^2$, $R^2 = 0.73$; Y (pancreas) = $0.273 + 0.042X - 0.001X^2$, $R^2 = 0.68$, and X is days of age.

residual yolk of goslings at different ages are shown in Table 2. The yolk weight of goslings rapidly decreased throughout the experimental period. The present experiment shows that the residual yolk weight was 11.94 g (approximately 9.36% body weight of the goslings) at hatching and was 2.32 g at day 3 (about 1.49% of body weight). These results are similar to previous reports that the residual yolk weight was 10% in body weight of chicks (Nitsan et al., 1991b) and 10% to 12% in turkey poults (Sell et al., 1991) at hatching. In this work, the 90% reduction in the weight of residual yolk at 3 days of age is consistent with the reports on broiler chicks by Romanoff (1960) and Nitsan et al. (1991a, b). The percentage of dry matter (DM) was significantly higher ($p < 0.05$), and the moisture was significantly lower ($p < 0.05$) in the residual yolk at 2 days before hatching. The gross energy (GE) and protein contents of the residual yolk decreased significantly ($p < 0.05$) at 3 days of age compared with the incubation period. Fat content in yolk did not vary significantly

($p > 0.05$) during the experimental period. There were significant ($p < 0.05$) linear and quadratic responses of weight, GE, DM and moisture of residual yolk among age. The residual yolk (DM) contained approximately 50% lipids and 38-40% crude protein at hatching, which is similar to the results reported by Murakami et al. (1988). The yolk is sole nutrient source, containing protein and lipids for the maintenance of the young bird during the embryonic development and after hatching (Noy and Sklan, 1997). The presence of nutrients in the intestine did appear to accelerate the utilization of yolk, however, this acceleration occurred via an increase in peristalsis of the intestine, which increases in proportion with the amount of yolk absorbed (Noy and Sklan, 1998). After hatching, yolk is transported both to the circulation through the vascular system and to the intestine through the yolk stalk (Noy and Sklan, 1997). The result for goslings is consistent with the general belief that these nutrients are used to develop the gastrointestinal tract during the embryonic period. However, the fastest growing line of chicken have the least weight of yolk, and thus suggest that considerable complexity of the need and use of this material existed during the embryonic stage (Nitsan et al., 1991b).

Development of the gastrointestinal tract

Figure 1 shows the absolute and relative weights (g/100 g BW) of the digestive organs in goslings, respectively. Before hatching, organs growth were relatively slow, then rapidly increased through 28 days of age. The development of absolute and relative weights of digestive organs had significant linear ($p < 0.001$, $R^2 = 0.89-0.93$) (except for absolute weight of proventriculus) and quadratic increased ($p < 0.05$, $R^2 = 0.45-0.73$) from 6 days before hatching to 28 days of age, respectively. The absolute weights of gizzard, proventriculus, liver and pancreas increased by 48, 457, 94 and 2,334 times, respectively, from 6 days before hatching to 28 days of age. In all instances, weights of digestive organs increased more rapidly than body weight in goslings during first four weeks. The relative weights of the proventriculus, gizzard, liver and pancreas increased and reached peaks at 3, 3, 14 and 14 days of age, respectively.

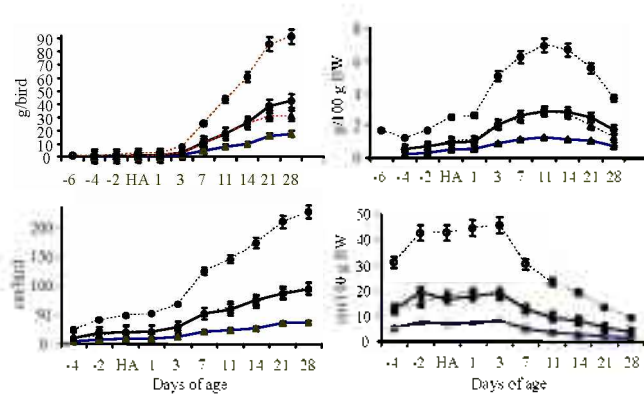


Figure 2. The absolute (left) and relative (right) weights and lengths of small intestines of goslings at different ages. (—▲—) duodenum, (—●—) jejunum, (—△—) ileum, (—○—) small intestine. Value are given as mean±SE. HA = day of hatching. The absolute and relative weights of small intestines of goslings had significant linear ($p<0.001$) and quadratic ($p<0.01$) effects with ages, respectively. The linear equations for absolute weights (g/bird) as follows: Y (duodenum) = $0.92+0.62X$, $R^2=0.94$; Y (small intestine) = $6.274+3.252X$, $R^2 = 0.92$; The quadratic equations for relative weights (g/100 g BW) as follows: Y (duodenum) = $0.564+0.093X-0.003X^2$, $R^2 = 0.81$; Y (small intestine) = $3.245+0.458X-0.016X^2$, $R^2 = 0.82$, and X is days of age. Not enough samples from the each segment of small intestine were collected for weights and lengths determination at 6 days hatching before.

On the other hand, relative weights of the gizzard and proventriculus were heavier as compared with the liver and pancreas through first 7 days of age. These results are consistent with the findings of Lu (1999) who indicated that the weights of proventriculus and gizzard increased more rapidly than the body weight and other digestive organs from 5 to 7 days of age in ducklings. Nitsan et al. (1991b) found that the weight of liver had increased consistently with a maximal weight in chicks on day 11. Moreover, Krogdahl and Sell (1989) and Oakberg (1949) observed that the development of pancreas size with age and reached a peak at 14 days of age in the turkey poults and the chicks. These results were similar to those in the goslings in the present study. Data of the current experiment indicated that the size and weight of gastrointestinal tract increased more rapidly relative to body weight than other organs or tissues of geese at post-hatching. Apparently, these data are similar to those in chicks (Katanbaf et al., 1988; Nitsan et al., 1991b) and in ducklings (Lu, 1999).

The weights of small and large intestines in goslings significantly increased ($p<0.05$) from the later incubation to 28 days post-hatching, as shown in Figure 2 and 3, respectively. The relative weights of the small and large intestine reached peaks at 11 to 14 days of age and at hatching in goslings, respectively. Furthermore, the relative lengths of the small and large intestine in goslings were longest at hatching persistently until 3 days of age. However, the absolute lengths of small and large intestines

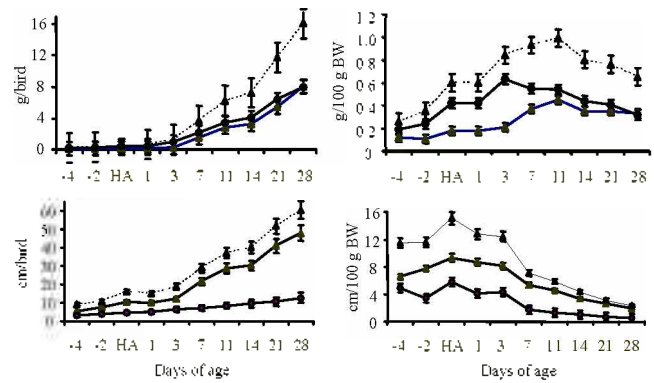


Figure 3. The absolute (left) and relative (right) weights and lengths of large intestines of goslings at different ages. (—▲—) caecum, (—●—) colon-rectum, (—△—) large intestine. Value are given as mean±SE. HA = day of hatching. The absolute and relative weights of large intestines of goslings had significant linear ($p<0.001$) and quadratic ($p<0.01$) effects with ages, respectively. The linear equations for absolute weights (g/bird) as follows: Y (caecum) = $-0.062+0.267X$, $R^2 = 0.92$; Y (large intestine) = $0.678+0.515X$, $R^2 = 0.94$; The quadratic equations for relative weights (g/100 g BW) as follows: Y (caecum) = $0.168+0.029X-0.001X^2$, $R^2 = 0.60$; Y (large intestine) = $0.577+0.058X-0.002X^2$, $R^2 = 0.62$, and X is days of age. Not enough samples from the each segment of large intestine were collected for weights and lengths determination at 6 days hatching before.

increased about four-fold from hatching to 28 days of age, and the development of lengths of jejunum and ileum were similar during the first four weeks. These data agree with the observations reported in several of other avian species (Lilja, 1983; Katanbaf et al., 1988; Lu, 1999). The accelerated GIT growth rate in chickens immediately after hatching demonstrates the developmental changes in these organs (e.g., gastrointestinal tract, liver) (Katanbaf et al., 1988; Jin et al., 1998). However, the results from this study indicated that the goslings had significant linear ($p<0.001$) effects and closely positive correlation ($R^2 = 0.92-0.94$) for the absolute weights with ages. Moreover, there were quadratic effects and positive correlation ($p<0.01$, $R^2 = 0.62-0.82$) for the relative weights of small and large intestines with ages, respectively (Figures 2 and 3). The results was similar to that reported by Nitsan et al. (1991b), who reported closely significant positive correlation has been found between development of GIT and ages in chicks.

Comparing day 4 before hatching with 28 days of age, it showed that the absolute weights of the duodenum, jejunum, ileum, caecum and rectum-colon increased by 95, 97, 78, 78 and 49 times, respectively. The changes in weights of observation were similar within each small intestinal segment (duodenum, jejunum, and ileum) during the experimental period. Previous study emphasized the importance of digestive capacity for mucosal growth and function of duodenum in chicks during the early growth period (Nitsan et al., 1991a). In the goslings, the duodenum showed a more rapid increase in weight (about 7.3 times)

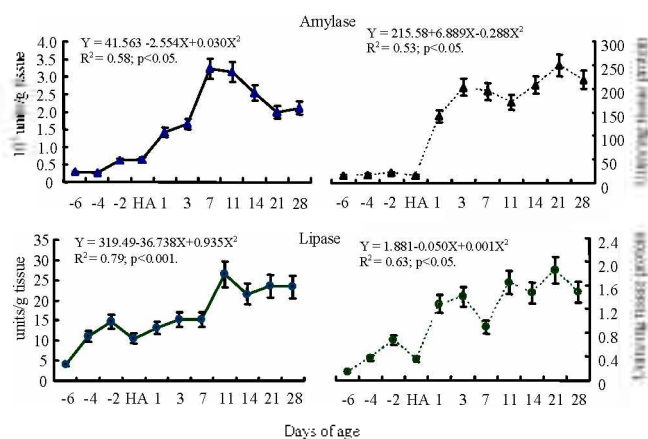


Figure 4. The changes of activity (left) and specific activity (right) of amylase and lipase in pancreas of goslings at different ages. (—▲—) amylase activity, (---△---) specific amylase activity, (—●—) lipase activity, (---▽---) specific lipase activity. Value are given as mean±SE. HA = day of hatching.

than body weight gain (about 5.5 times, data not shown) from hatching to 11 days of age, possibly because of increased intestinal absorptive area, enzyme activity and passage rate of feed (Noy and Sklan, 1998). In the study, duodenal absolute weight had a significant linear correlation ($p < 0.001$, $R^2 = 0.94$), thereafter, there was a significant quadratic correlation ($p < 0.01$, $R^2 = 0.81$) for the relative weight of duodenum in goslings with ages (Figure 2). The results of goslings are in agreement with Jin et al. (1998), who indicated that small intestine especially duodenum had a significant closely positive correlation in chicks. There was a significant positive linear correlation ($p < 0.001$, $R^2 = 0.92$) for the absolute weight of caecum with ages. Furthermore, the goslings had a significant quadratic correlation ($p < 0.01$, $R^2 = 0.60$) for relative weights of caecum (Figure 3) in the present experiment. The caecum is the main digestive segment for degrading cellulose in the alimentary canal of geese (Clemens et al., 1975). In contrast to other avian species, the goslings exhibited accelerated caecal weight and length during the early growth stage (Bjornhang, and Seperber, 1977). The caecal length accounted for 61.5% and 79.2% of the length of the large intestine at hatching and at 28 days of age, respectively. These results are consistent with the report of Hsu et al. (2000), who demonstrated that geese were herbivorous and had a pair of well-developed caecum for degrading cellulose and diets with high fiber content. These results of development of GIT in the present study confirm previous works indicating that gastrointestinal tract growth and digestive function are not fully developed in newly hatched chicks (Nitsan et al., 1991a, b), turkey turkey poults (Krogdahl and Sell, 1989; Sell et al., 1991), and ducklings (Lu, 1999).

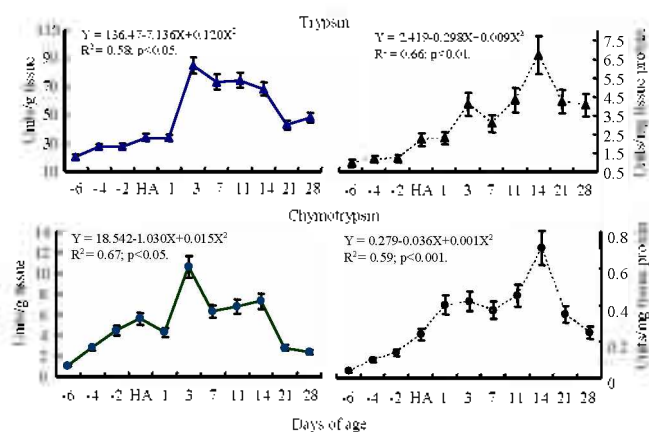


Figure 5. The changes of activities of trypsin and chymotrypsin in pancreas of goslings at different ages. (—▲—) trypsin activity, (---△---) specific trypsin activity, (—●—) chymotrypsin activity, (---▽---) specific chymotrypsin activity. Value are given as mean±SE. HA = day of hatching.

Development of pancreatic enzymes

The activities of pancreatic enzymes containing amylase, lipase, trypsin and chymotrypsin are shown in Figure 4 and 5. In this study, there were significant quadratic increases ($p < 0.05-0.001$) and positive correlation, with $R^2 = 0.53-0.58$ and $0.63-0.79$ for the activities and specific activities (SA) of amylase and lipase in the pancreas of goslings with ages, respectively (Figure 4). The activity and SA of amylase in the pancreas had significant increases with ages, peaking at 7 to 11 and 21 days of age, respectively, while the pancreatic lipase activity and SA reached a plateau during 11 to 28 days of age. These results are consistent with the findings of Krogdahl and Sell (1989), who observed a rapid increase in amylase activity of pancreas in turkey poults from hatching to 14 days of age, then persisted until 21 days of age. Nitsan et al. (1991b) reported that the SA of lipase in chicks was low during the first 6 days after hatching, and then increased up to 21 days of age. Krogdahl and Sell (1989) reported that the SA of lipase in turkey poults was also lower during the first week after hatching, and then increased gradually to reach a plateau at 32 days of age. Those results were consistent with a peak activity of lipase observed in the current research during 11 to 28 days of age.

The absolute activity of trypsin in pancreas increased gradually and reached maximum at 3 days of age, and then remained at a plateau until 14 days of age. The SA of trypsin was highest at 14 days of age, followed by a lag period. The development of chymotrypsin activity increased and was similar to that of the activity of trypsin in pancreas of goslings during this experimental period. The activities and SA of both pancreatic trypsin and chymotrypsin of goslings had a significant quadratic effect ($p < 0.001-0.05$) and positive correlation ($R^2 = 0.53-0.63$) from hatching

before to 28 days of age (Figure 5). These results are in agreement with those reported by Nitsan et al. (1991b) and Nir et al. (1993), that the SA of trypsin in the pancreas of chicks increased from 14 to 20 days of age, reaching a maximum relative activity of chymotrypsin at 11 days of age, and Lu (1999) who suggested that the pancreatic protease activity was significantly higher in ducklings during first two weeks.

CONCLUSION

The data from this experiment showed that the rate of physical development in goslings, including the absolute and relative weights of GIT and activities of pancreatic enzymes were different, and there were significant linear or quadratic effects and closely positive correlation, with a rapid increase during the experimental period. The relative weights of proventriculus, gizzard, liver, pancreas, small intestine and large intestine reached peaks at 3, 3, 14, 14, 11 and 11 days of age, respectively. However, the relative lengths of small intestine and large intestine reached peaks at 3 days of age and at hatching, respectively. The activities of pancreatic trypsin and chymotrypsin increased sharply from hatching to 14 day of age. The activity and specific activity of pancreatic amylase were increased following by age and peaked at 7 to 11 and 21 days of age, respectively. The activity and specific activity of pancreatic lipase reached a plateau from 11 to 28 days of age.

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