

*Full Length Research Paper*

# Activities of amylase, trypsin and chymotrypsin of pancreas and small intestinal contents in the red jungle fowl and broiler breed

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The digestive enzyme activities of the pancreas and small intestinal segments were examined in two breeds of chickens that differ in growth rate over the period of 1-day (1-d) to 4-months (120-d) of age. The total body weight (BW) of the red jungle fowl (RJF) increased slowly during the experiment, in contrast to the commercial broiler chicken (CBC) which showed markedly increase ( $P < 0.05$ ) over the same experiment. The pancreas weight (g/100 g BW) was greater ( $P < 0.05$ ) in the RJF during the experiment, with the exception of 1-day old group. There was significantly higher ( $P < 0.05$ ) pancreatic enzyme activity (unit/g) and (unit/100 g) BW for the CBC, particularly at 10-days. However, there were no differences between the breeds at (20 to 56 days) for amylase and (120 days) for chymotrypsin. The enzyme activity (unit/g) for all intestinal segments increased with age. The CBC attained a maximal value on 10 days for jejunal and ileal trypsin, and duodenal, jejunal and ileal chymotrypsin. Activities (unit/100 g BW) showed decrease with age for both breeds. The CBC had a relatively greater value ( $P < 0.05$ ) at 1 day, except for the jejunal trypsin and the chymotrypsin. Thereafter, the relative activities were higher for the RJF until the end of the experiment. Although significant differences in the digestive enzyme activities were obtained between the two breeds, these differences were generally associated with differences in body weight. The effects of selection affected the development rate of the synthesis of these enzymes according to the body requirements and biological function and this may affect the digestion and finally the growth rate.

**Key words:** Pancreatic enzyme, small intestine, amylase, trypsin, chymotrypsin.

## INTRODUCTION

The wild red jungle fowl (RJF), *Gallus gallus* of Southeast Asia, is a tropical member of the pheasant family, the *G. gallus spadiceus* and is the only subspecies occurring in Peninsular Malaysia (Nishida et al., 1992; Lee and Amin-Babjee, 1993). Many comparisons of animal domesticated with their wild ancestors have shown the effects of

artificial selection on anatomical traits in fowl (Jackson and Diamond, 1996; Watkins et al., 2004) and duck (Gille et al., 1999).

Starch is a major component of poultry feed and therefore, its efficient utilization would require the presence of a capable system of appropriate enzymes in the gastrointestinal tracts of the birds (Osman, 1982). The development of supply organs (e.g., pancreas and small intestine) accelerate shortly after hatching in breeds selected for high body weight for which it is essential for the secretory activity of the pancreas to achieve maximal growth at an early age. Thus, the functional maturation of these organs is important in the assimilation of feed (Nitsan et al., 1991a,b). The lack of pancreatic enzymic

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**Abbreviations:** CBC, Commercial broiler chicken; RJF, red jungle fowl.

hydrolysis in the intestinal lumen decreases the apparent digestibility of the dietary components and reduces growth (Corring and Ourdon, 1977). Many digestive enzymes are synthesized by the exocrine pancreas, stored in the zymogen granules and released into the duodenum (Pubols, 1990). Levels of digestive enzymes in organs and contents of gastrointestinal tract are influenced by genetic stock (O'Sullivan et al., 1992).

Proteolytic enzyme assays involving the pancreas are dependent on complete extraction and solubilization of zymogen granules prior to activation (Gorrill and Thomas, 1967). The ratios of digestive enzymes produced by the pancreas of turkeys (Krogdahl and Sell, 1989) and chickens (Pubols, 1991) have been profiled by several variable methods, and age was determined as a factor (Nitsan et al., 1991a; Sell et al., 1991). Enzyme activity levels in the pancreas increased with age for relative amylase, total trypsin, total and relative chymotrypsin (O'Sullivan et al., 1992).

In the present study, we used the RJF, which is characterized by a slow growth rate to compare with commercial broiler chicken (CBC) as a breed selected for high growth rate. The experiment was undertaken to determine enzymes (Amylase, Trypsin and Chymotrypsin) activities within both the pancreas and the small intestine (duodenum, jejunum, and ileum). The comparisons were made at 1 day, 10-d, 20-d, two (60-d) and four months (120-d) after hatching.

## MATERIALS AND METHODS

### Experimental animals

The pancreas and intestinal contents were collected from RJF which was procured from the University Putra Malaysia poultry farm. The eggs of the RJF descended from a stock which conforms to the wild RJF and differs from domestic chickens in all eight specific characters that differentiate most sensitively, the two subspecies (Jackson and Diamond, 1996). The birds were reared in small cages with a commercial diet and water was provided *ad libitum*. Five groups, five males in each, aged one day, 10 days, 20 days, 2 months and 4 months were sacrificed by intravenous injection of sodium pentobarbitone (80 mg/kg BW) (Mitchell and Smith, 1991). The broiler breed chicks (Ross) were procured from Linggi poultry farm (C.P.Com).

### Sample collection

Body and the pancreas weights of each bird were recorded. The small intestine was divided into duodenum, jejunum and ileum, following the demarcation set by Mitchell and Smith (1990). The intestinal contents of each segment were emptied into tubes by gentle pressure between thumb and fore-finger. All samples were placed in liquid nitrogen and stored at -80°C.

### Measurement of enzyme activity

The activity of amylase was measured as described by Howard and Yudkin (1963). The method is based on the decrease in the

intensity of blue colour given by the reaction of starch and iodine as the starch is hydrolysed. The homogenate was made by 1/20 wt/vol ice-cold distilled water for pancreatic tissue and 1/4 wt/vol for intestinal chyme, and homogenized in a metal mortar. The homogenate was centrifuged at 70,000 g for 20 min at 4°C; the supernatant was stored at -70°C.

Comparisons of colour were made in absorptiometer with filter maximum absorption at 620 nm and cells of 1 cm width (Howard and Yudkin, 1963). Enzyme activity was expressed as units of activity where one unit was defined for amylase as an increase in  $10^{-5}$  extinction at 620 nm/10 min at 37°C and  $10^{-2}$  for trypsin and chymotrypsin, at 410 nm/20 min at 37°C.

The activities of pancreatic trypsin and chymotrypsin were determined after activation of the pancreatic homogenate according to Gertler and Nitsan (1970). The activation was carried out by mixing equal volumes of pancreas supernatant and 1% enterokinase and incubating for 1 h at 37°C. The purified enterokinase (Sigma-Aldrich) was prepared in 0.1 M-Tris-HCl buffer (tris-hydroxy-methyl amino methane), pH 7.2, containing 0.1 M CaCl<sub>2</sub>. It was pre-incubated at 37°C for 30 min and centrifuged at 2000 g to remove solid material.

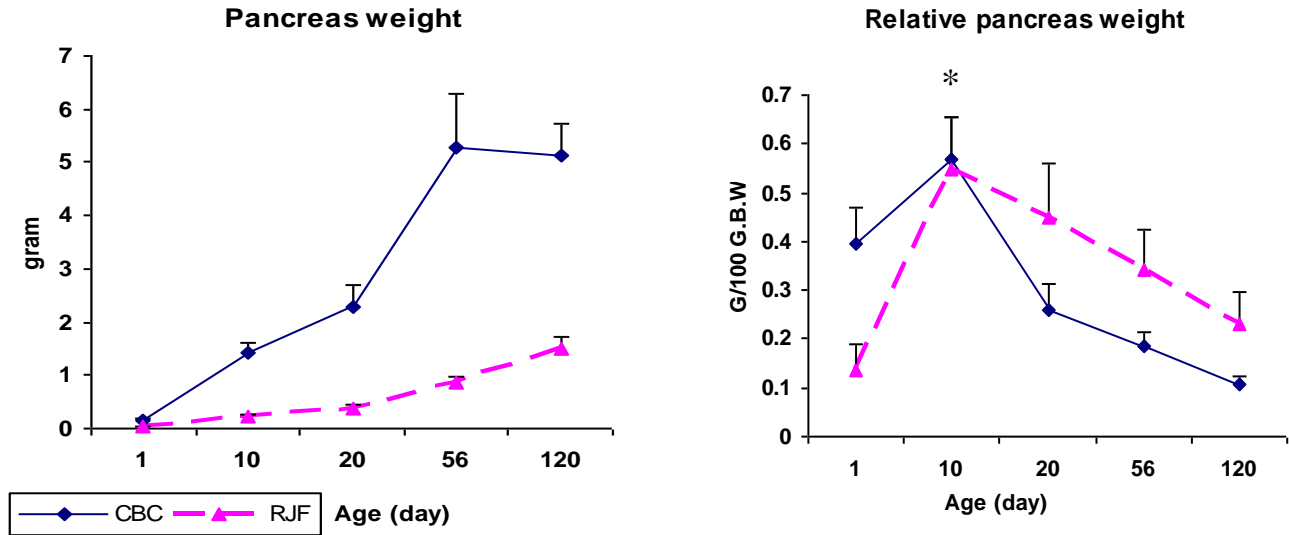
Trypsin and chymotrypsin activities were estimated with N-benzoyl-L-arginine ethyl ester HCl (BAEE) and N-benzoyl-L-tyrosine ethyl ester (BTEE) as substrates for trypsin and chymotrypsin, respectively (Gertler and Nitsan, 1970), the BAEE and BTEE were obtained from Sigma-Aldrich. The final concentration being 1.25 mM in 3.7 mM-Tris buffer, pH 7.8, 0.6 mM-CaCl<sub>2</sub> and 25 mg/ml dimethyl sulfoxide. The reaction proceeded at 30°C for 30 min and was stopped with acetic acid (300 ml/l). The colour developed was measured by using the spectrophotometer at 410 nm, and cells of 1 cm width. Activity units were defined as one  $\mu$ mole substrate hydrolysed /min at 25°C.

Data of the enzyme activity for pancreatic and intestinal contents were expressed in units per gram of pancreatic tissue or intestinal contents, respectively, and units per 100 g body weight in relative bases. The variance between breeds at each age was analyzed by independent t tests.

## RESULTS

The pattern of total body weight for CBC showed a marked increase ( $P < 0.05$ ). It increased approximately 6 folds and 23 folds at 10 and 20 days, respectively, and more than this rate thereafter. However, for the RJF, the body weight increased very slowly and not at the same rate to the end of the experiment. It only reached about a 4 fold increase at 20 days. The absolute weight of the pancreas for the CBC was significantly greater than for the RJF during the period of the experiment. The pancreas weight for CBC, increased about 10- to 15-fold at 10 and 20 days, respectively, as compared to the first day, while it was not more than 6- and 13-fold for the RJF during the same period. The pancreas weight seemed to be constant for CBC after 56 days (Figure 1). The weight of pancreas per 100 g body weight, showed significantly higher values for the CBC, while both breeds showed no variance at 10 days, the relative weight of the pancreas declined for the both breeds after that. However, the RJF showed a significantly greater value at 20 day, and thereafter (Figure 1).

The specific activities of pancreatic amylase, trypsin and chymotrypsin increased with age. There was a marked



**Figure 1.** Absolute (gram) and relative (g/100 g BW) pancreas weight for CBC and RJF, from 1day post-hatch to 4 months of age. Vertical bars represent the SD; when not shown, the SD is smaller than the symbol. (\*) not significantly different (P > 0.05).

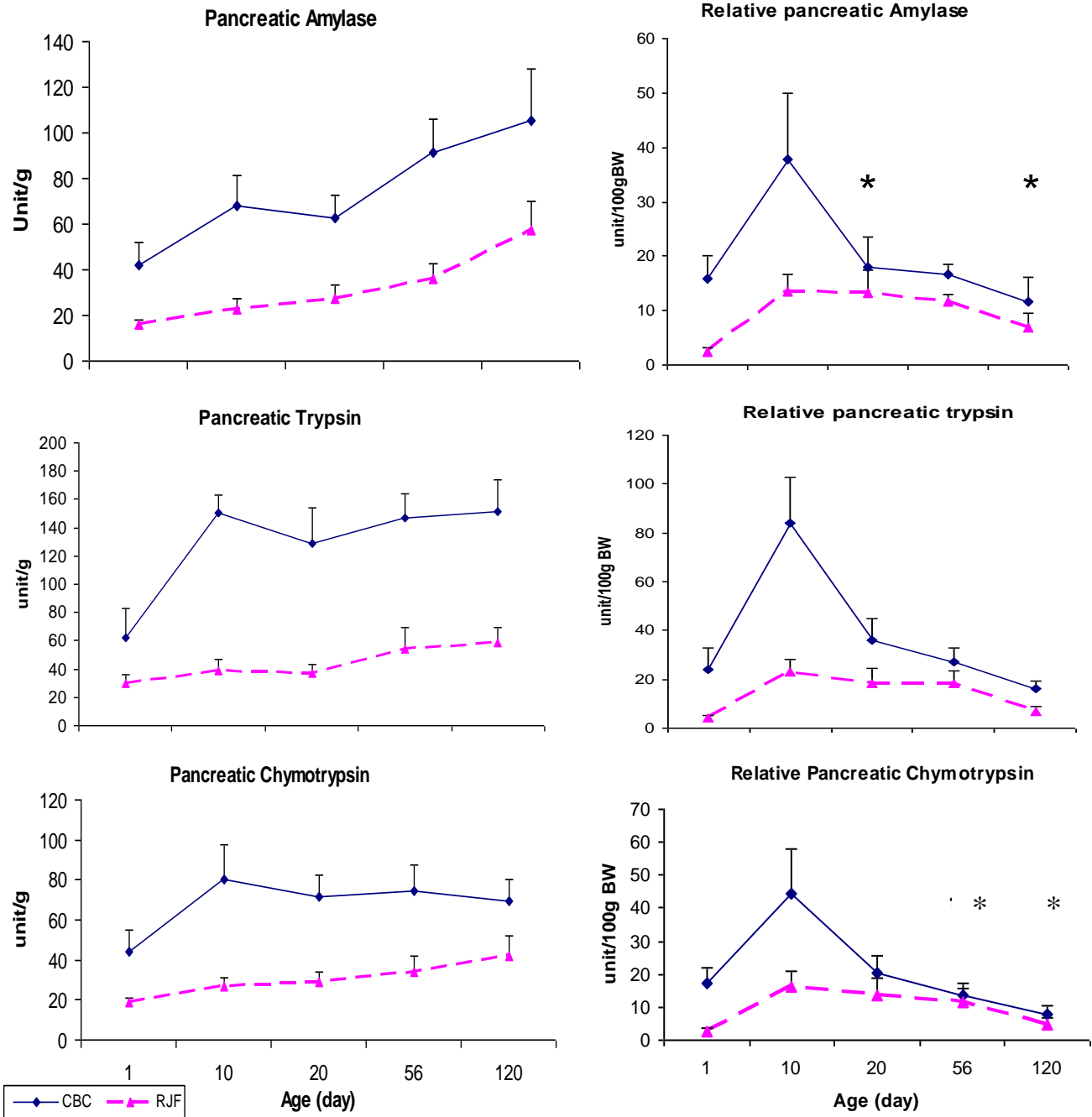
increase at 10 days post-hatching, for CBC. However, there were minor decreases of all these enzymes at 20 days for the CBC, which increased thereafter until the end of the experiment (Figure 2). In contrast, the RJF showed gradual increases for all pancreatic enzymes during the experiment. The pancreatic enzyme activities for the CBC were significantly greater than those for the RJF at all age groups. The pancreatic trypsin and chymotrypsin activities of the CBC reached the maximum value at 10 days, with an increase of about two folds compared to just after hatching. The pancreatic amylase activity persistently increased at 120 days for both breeds, although the result seemed constant for trypsin and chymotrypsin. When expressed as units of activity per 100 g body weight, the activities of all these pancreatic enzymes increased after hatching, reached a maximum at 10 days, when they attained approximately 2-3-2 fold increases for amylase, trypsin and chymotrypsin, respectively, for the CBC, and 5-5-8 fold increases for the RJF (Figure 2). The activity of these enzymes showed a decrease after that for both breeds. The relative activities of pancreatic enzymes for the CBC were significantly higher than those for the RJF, except at 20 and 120 days for amylase, and at 56 and 120 days for chymotrypsin, when there were no differences between the breeds.

For the contents of the small intestine, the absolute enzyme activity for the CBC showed a significantly higher value than the RJF during the experiment. The enzyme activity of the RJF increased gradually during the experiment. On the other hand, the enzyme activity of the jejunum was greater than in the duodenum, the latter showing a lower value than the ileum in both breeds. For the amylase activity, the CBC attained a marked increase at 10 days, for all intestinal segments (Figure 3). The

increasing rate for both breeds was approximately 2 folds compared to that at 1 day post-hatch, and 3 to 5 folds at 120 days, for the CBC and RJF, respectively.

When expressed in units of activity per 100 g body weight, the activity of amylase at 1 day post-hatch, represent a significant higher value for the CBC in all intestinal segments. After that, the activity curve declined with the age. The relative activity of amylase, remains constant to 10 days in RJF, but after that point, it decreased until the end of the experiment. Although, the relative activity of amylase for RJF was significantly higher, both breeds showed no variances at 10 days for the duodenum, 10 and 56 days for the jejunum, and at 120 days for the ileum (Figure 3). The pattern of the duodenal trypsin activity was similar to amylase for both breeds (Figure 4). At 10 day, there was a marked increase in jejunum and ileum by approximately 2 folds over that at 1 day. However, the CBC showed a decrease in the duodenal trypsin activity at 20 days, and at 56 days for the jejunum and ileum, and increased thereafter. For the RJF, the enzyme activity progressed very slowly during the experiment. The relative activity of trypsin was markedly decreased for both breeds during the experiment (Figure 4). Except for the RJF, the ileum relative trypsin activity did not change during the first 10 days post-hatch. At 1 days, the relative trypsin activity of the CBC was significantly higher for the duodenum and ileum, although there was no difference for the jejunum, while the RJF had the greater value during the remainder of the experiment.

The chymotrypsin activity increased by about 2 folds for both breeds during the first 10 days in the all intestinal segments, however it reached 3 fold in the jejunum of the CBC. Both breeds showed decreases in enzyme activity after the 56 days (Figure 5). The data for the relative

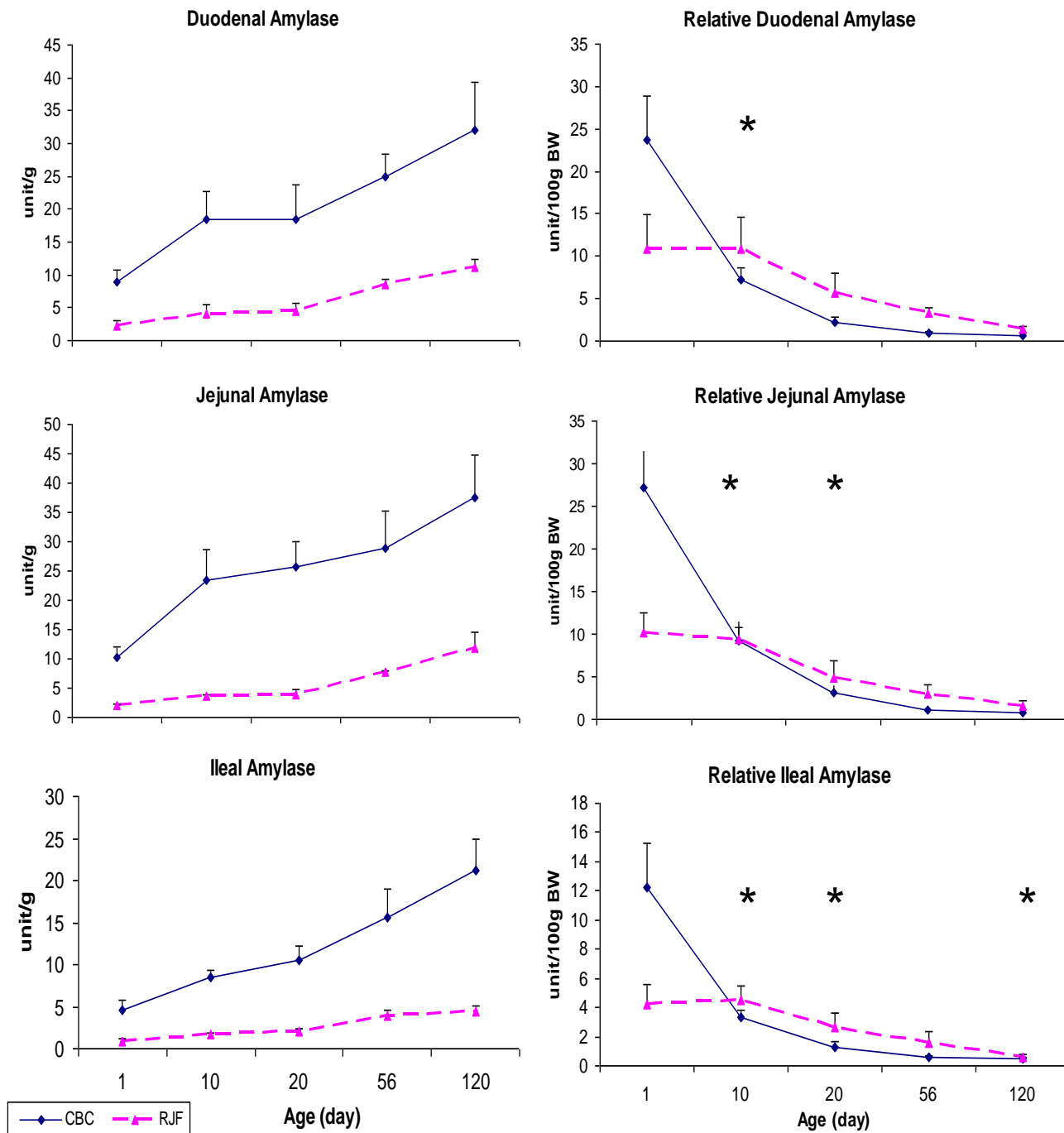


**Figure 2.** Activities of amylase, trypsin and chymotrypsin in the pancreas of CBC and RJF from 1 day post-hatch to 4 months of age, expressed in units /g (left) and units /100 g BW (right). Vertical bars represent the SD; when not shown, the SD is smaller than the symbol. (\*) not significantly different ( $P > .05$ ).

chymotrypsin activities showed a decrease during the experiment for all intestinal segments of both breeds. However, the relative enzyme activity for RJF did not change during the first 10 days for all intestinal segments. In addition, there was no difference between the two breeds at the 1 day, but the RJF showed a significantly higher value during the remaining days of the experiment (Figure 5).

## DISCUSSION

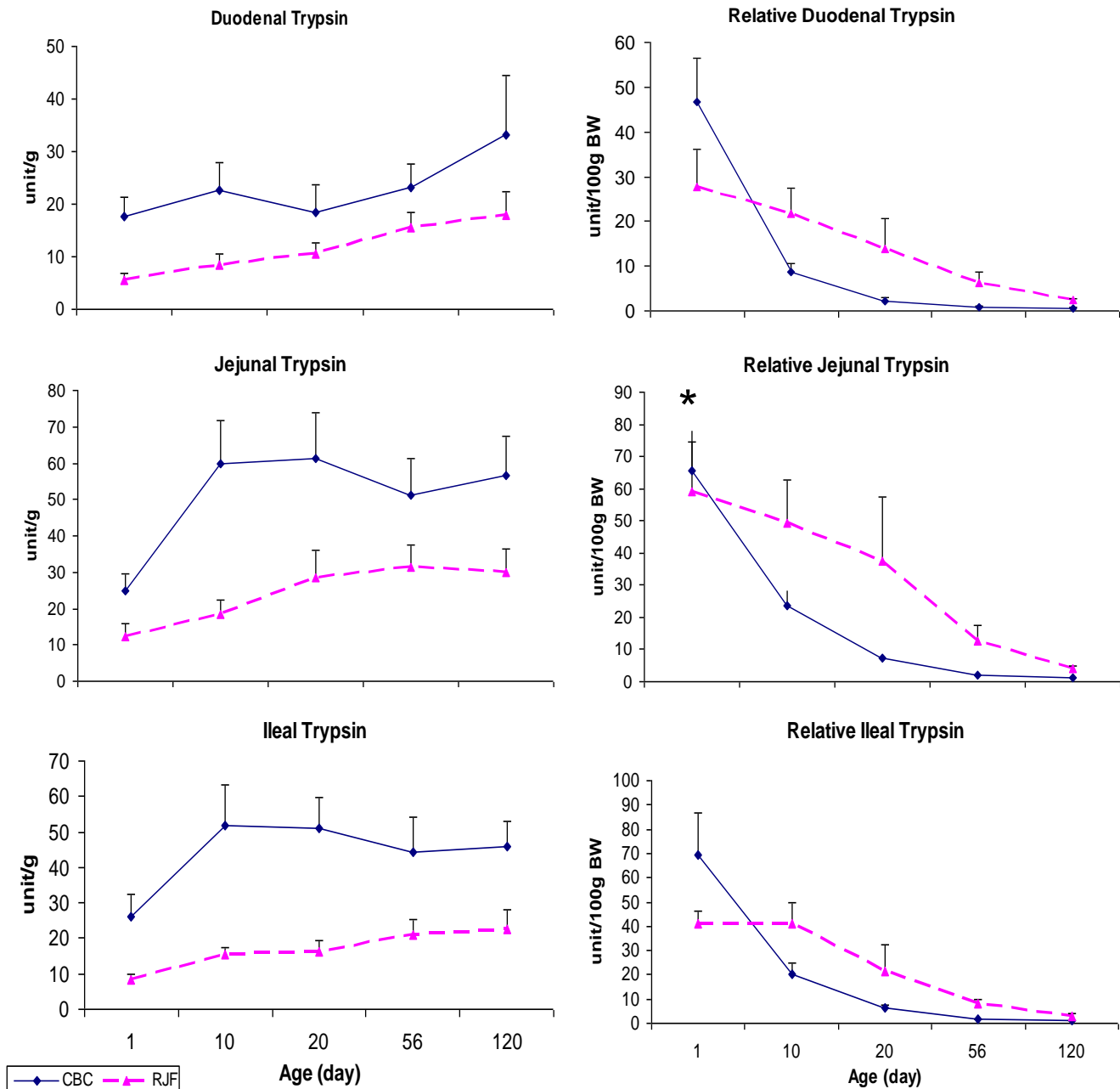
The differences in the pancreatic weight was clearly observed between the two breeds; the relative pancreatic weight constituted 0.4 and 0.1% of body weight on the 1st day of life for the CBC and RJF respectively, increased by less than 2 and 4 folds and peaked at 10 days of age, respectively. These differences in the relative



**Figure 3.** Activities of amylase in the contents of the duodenum, jejunum and ileum of CBC and RJJ from 1 day post-hatch to 4 months of age, expressed in units /g(left) and units /100 g BW (right). Vertical bars represent the SD; when not shown, the SD is smaller than the symbol; (\*) not significantly different (P > .05).

pancreatic weight between both breeds might reflect differences in the body growth rate, the pancreas, or both. These results were consistent with the hypothesis that growth is greater for supply than for demand organs during the early period of post-hatching in chickens (Lilja, 1983; Katanbaf et al., 1988) and turkeys (Sell et al., 1991). Nitsan et al. (1991a, b) suggested that the highest

food conversion efficiency was observed during the first 10 days of age, when the relative growth reached its peak, and after the relative weights of the pancreas and small intestine were maximal. Whereas, a lack of pancreatic enzyme activity decreases the apparent digestibility of the dietary components and reduces growth (Corring and Ourdon, 1977).

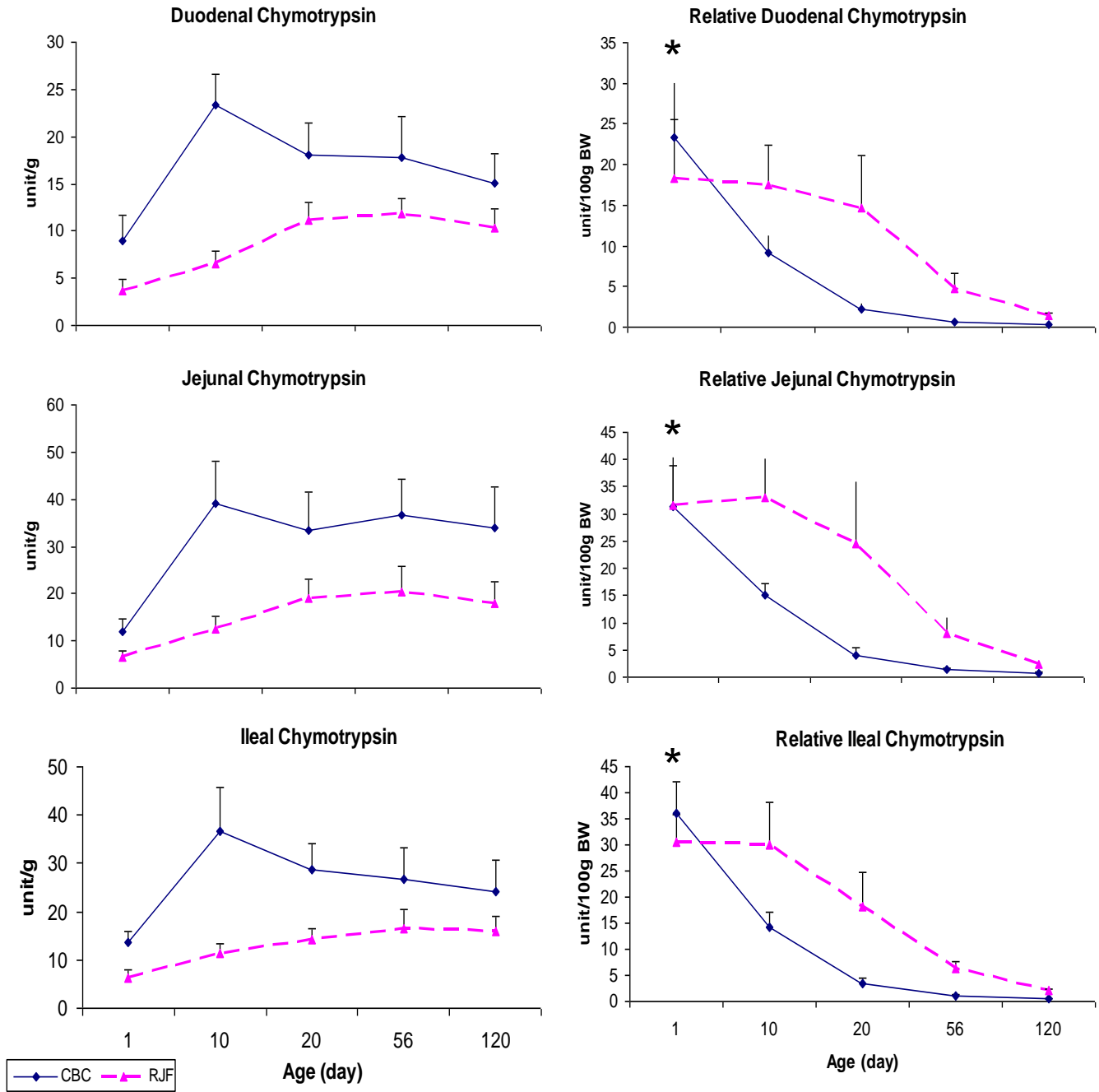


**Figure 4.** Activities of trypsin in the contents of the duodenum, jejunum and ileum of CBC and RJF from 1 day post-hatch to 4 months of age, expressed in units /g(left) and units /100 g BW (right). Vertical bars represent the SD; when not shown, the SD is smaller than the symbol; (\*) not significantly different ( $P > .05$ ).

For our results, all pancreatic enzymes were higher in the CBC than the RJF, in the relative bases. Except for the last days for amylase and chymotrypsin, there was no difference between the breeds. Whereas, according to the finding of Dunnington and Siegel (1995), the relative trypsin was an exception to this fact when compared between the heavy and light breeds. However, their experiment was limited to only 4 weeks. O'Sullivan et al. (1992), reported that the enzyme activity levels in the

pancreas increased with age for relative amylase, total trypsin, and total and relative chymotrypsin, for the heavy breed at 3 weeks old. Krogdahl and Sell (1989), suggested that the activity of pancreatic amylase increased rapidly at the first 10 days after hatching, while the trypsin increased after that.

It was obvious from our results that the absolute activities of the intestinal enzymes were greater in CBC than the RJF, but this relationship was reversed in relative



**Figure 5.** Activities of chymotrypsin in the contents of the duodenum, jejunum and ileum of CBC and RJF from 1 day post-hatch to 4 months of age, expressed in units /g(left) and units /100 g BW (right). Vertical bars represent the SD; when not shown, the SD is smaller than the symbol; (\*) not significantly different ( $P > .05$ ).

bases, except for 1 day after hatching. This is due to decrease in enzyme secretion of the RJF on one hand, and on the other hands, the effects of rapid body growth rate for the CBC. These facts seemed consistent with the previous report for amylase by Leslie et al. (2007) when comparisons were done between the high-and low-weight lines of chicks. However, their experiment was not extended more than 3 weeks of age. Further-more, it

agrees with the report of Cherry et al. (1987) concerning the intestinal trypsin and chymotrypsin activities in heavy and light breed at 61 days old. However, Dunnington and Siegel (1995) reported that among the intestinal enzymes, only trypsin activity decreased for the heavy line when compared with the light lines. In our results, all intestinal enzymes activities in both breeds showed greater value for the jejunum rather than duodenum or

ileum. These findings were expected because the pancreatic juices are emptied into the distal end of the loop of the duodenum in fowl. Thus, all the duodenal enzymes were lower than in the jejunum, and it decreased after that, and reached the ileum in low concentration. Osman (1982) suggested that the level of amylase activity was found to be high in chickens and was present in all parts of the small intestine but mainly confined to the jejunum luminal contents, from which it is assumed that the jejunum is the major site of starch digestion in chickens.

From the pattern distribution of the pancreatic and intestinal enzymes activities of this experiment, we did not find any correlation between the reduction of the enzymes activities in the intestinal contents and any accumulation of these enzymes in the pancreatic tissue, as reported by Pinchasov and Nitsan (1990) who suggested that the synthesis of pancreatic enzymes is regulated by the presence of chyme in the small intestine. However, O'Sullivan et al. (1992) showed that, the levels of digestive enzymes in organs and the contents of gastrointestinal tract are influenced by genetic stock.

Both breeds in our results showed differences in their enzymes activities. When the activities were corrected for the body weight, the data failed to follow the rapid gain of the body weight for the CBC, except for the 1 day post-hatching. However, it was dominantly greater than the RJF for the pancreatic enzymes only, whereas for the CBC, the activities of the intestinal enzymes showed lower values than the RJF. A comparison between these two different breeds showed that genetic variation was an important source of differences, not only in body weight but also in enzymatic activities.

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