



The Enzyme Activities of Pancreas and Small Intestinal Contents in the Malaysian Village Chicken and Broiler Strains

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ABSTRACT

The digestive enzyme activities of the pancreas and small intestinal contents were examined in two strains of chickens which differ in growth rates from 1 day to 4 months of age. The total body weight (BW) of Commercial Broiler Chicken (CBC) showed marked increase ($P < .05$) during the experiment, in contrast to Malaysian village chicken (MVC) which only increased slowly over the same period. The pancreas weight of MVC (g/100g BW) was greater ($P < .05$) than CBC throughout the experiment except for 1 day of age. The pancreatic enzyme activity (unit/g and unit/100g BW) was significantly higher ($P < .05$) in the CBC, particularly at 10 days old. However, there was no difference between the strains at 20 to 56 days of age for amylase and 120 days for chymotrypsin. The enzyme activity (unit/g) for all intestinal segments increased with age in both strains. In particular, CBC attained a maximal value at 10 days of age for jejunal and ileal trypsin, as well as for duodenal, jejunal and ileal chymotrypsin. Meanwhile, the enzyme activities (unit/100g BW) decreased with age for both the strains. In specific, CBC had a relatively greater enzyme activities ($P < .05$) at day 1, except for the jejunal trypsin and the chymotrypsin. Thereafter, the relative activities were higher for MVC until the end of the experiment. Although significant differences in the digestive enzyme activities were obtained between

the two strains, these differences were generally associated with the differences in body weight. The selection affected the development rate of the synthesis of these enzymes according to the body requirements and biological function and this might affect the digestion and finally the growth rate.

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INTRODUCTION

In Malaysia, the original Malayan fowls were widespread in villages before the arrival of Europeans in the late 1800s. The present Malaysian native chickens, commonly known as *ayam kampung* (village chickens), are the results of cross-breeding of the Red jungle fowl with mixed exotic domestic breeds brought in by Europeans, mainly the British (Azahan & Zahari, 1983). In general, the indigenous chickens are of small body size, slow growth rate, with plumage of different colours, and of dual-purpose type, as well as variable body conformation and physical characteristics. The chicken's market weight of 1-1.5 kg is reached within four to five months. During the late 1940s, the broiler breeds required 12 weeks to reach the live body weight of 1,800g. Four decades later, this period was reduced by half, with a reduction of essentially 1 day/year to reach this weight, showing no evidence of abatement (Gyles, 1989). Tremendous progress has been made in the selection of broilers for increased growth, feed conversion and carcass quality (Schreiweis *et al.*, 2005).

Starch is a major component of poultry feed and its efficient utilization requires 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) accelerates shortly after hatching in breeds selected for high body weight

for which it is essential for 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 hydrolysis in the intestinal lumen decreases the apparent digestibility of the dietary components and reduces growth (Corring & 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). The ratios of digestive enzymes produced by the pancreas of turkeys (Krogdahl & Sell, 1989) and chickens (Pubols, 1991) have been profiled through several variable methods, while age has been determined as a factor (Nitsan *et al.*, 1991a; Sell *et al.*, 1991). The enzyme activity levels in the pancreas increase with age for relative amylase, total trypsin, total and relative chymotrypsin (O'Sullivan *et al.*, 1992).

In the present study, MVC, which is characterized by a slow growth rate, was used to compare with 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 days 1, 10, 20, 60 and 120 (4 months) after hatching.

MATERIALS AND METHODS

Experimental animals

The pancreas and intestinal contents were collected from MVC procured from a poultry farm at Universiti Putra Malaysia. The eggs of MVC were obtained from Jenderam Hulu, in Sepang. The villager stocks comprise the local unimproved poultry breeds, including mixed (unspecified) breeds resulting from uncontrolled breeding. The day one old chicks of commercial line (Ross) selected for high body weight were supplied by a private hatchery (Linggi Poultry Farm Sdn. Bhd. C-P lot 1354, 33010, Kuala Kangsar, Perak, Malaysia). The birds were reared in separate cages with a commercial diet and water provided *ad libitum*. Five groups, six 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 & Smith, 1991).

Sample Collection

Body weight and the pancreas weight 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 the samples were then placed in liquid nitrogen and stored at -80°C .

Measurement of the 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, homogenized in a metal mortar. The homogenate was centrifuged at 70.000g for 20 min at 4°C and the supernatant was stored at -70°C .

Comparison of colour was made using an absorptiometer with filter maximum absorption at 620 m μ and cells of 1 cm width (Howard & Yudkin, 1963). The enzyme activity was expressed as units of activity, where one unit is 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 .

According to Gertler and Nitsan (1970), the activities of pancreatic trypsin and chymotrypsin were determined after the activation of the pancreatic homogenate. 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 0.1M- CaCl_2 . It was pre-incubated at 37°C for 30 min and centrifuged at 2000g to remove solid materials.

Trypsin and chymotrypsin activities were estimated with N α -benzoyl-L-arginine ethyl ester HCL (BAEE) and N-benzoyl-L-tyrosine ethyl ester (BTEE) as the substrates for trypsin and chymotrypsin, respectively

(Gertler & Nitsan, 1970), with BAEE and BTEE obtained from Sigma-Aldrich. The final concentration was 1.25 mM in 3.7 mM-Tris buffer, pH 7.8, 0.6 mM-CaCl² and 25/mg/ml dimethyl sulfoxide. The reaction proceeded at 30°C for 30 min and was stopped with (300 ml/l) acetic acid. The colour developed was measured by using the spectrophotometer at 410 nm and the cells of 1 cm width. Activity units were defined as one μ 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 difference between strains at each age was analyzed by independent t tests.

RESULTS

The pattern of the total body weight for CBC showed a marked increase ($P < .05$) compared to MVC. It increased approximately 4 folds and 5 folds at days 10 and 20 post

hatch, respectively, and more than this rate thereafter. For MVC, however, the body weight increased very slowly to the end of the experiment (Fig.1). The absolute weight of the pancreas for the CBC was significantly greater than for MVC throughout the period of the experiment. The pancreas weight for CBC increased approximately 10 folds and 15 folds at days 10 and 20 post hatch, respectively, as compared to the first day, while it was not more than 4 folds and 6 folds for MVC during the same period. The pancreas weight seemed to be constant for CBC after 56 days of age. The weight of pancreas per 100 g body weight showed significantly higher for the CBC at first day and no difference at the remaining days between both strains, while the relative weight of the pancreas declined for both the strains after that. However, MVC showed a significantly greater value at 120 days of age (Fig.2).

In general, the specific activities of pancreatic amylase, trypsin and chymotrypsin increased with age. There

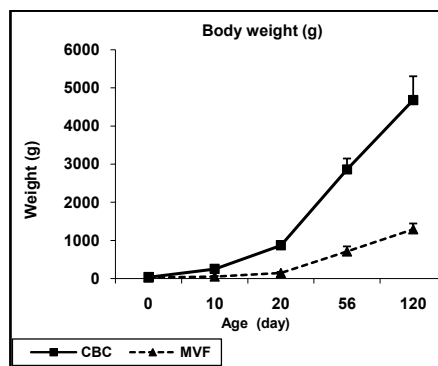


Fig.1: The total body weights of CBC and MVC from 1 day to 4 months of age. Values are means \pm SD, N= 6, the difference between breeds at the same age were significant ($P \leq .05$), where, CBC > MVC

was a marked increase at 10 days post hatch for CBC. However, there were minor decreases detected in all these enzymes at 20 days post hatch, particularly for CBC, which increased thereafter until the end of the experiment (Fig.3). Meanwhile, MVC showed gradual increases for all the pancreatic enzymes during the experiment. The pancreatic enzyme activities for CBC were significantly greater than those for MVC at all age groups. The pancreatic amylase activity persistently increased at 120 days for both the strains but showed no difference between them. Meanwhile, 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 peak at 10 days, when they attained approximately 2 folds, 3 folds and 2 folds increases for amylase, trypsin and chymotrypsin, respectively, for CBC, and 3 folds and 4 folds increases for MVC (Fig.2). The activity of these enzymes showed a decrease after that for both the

strains. The relative activities of pancreatic enzymes for CBC were significantly higher than those for MVC, except after 10 day for amylase, when no differences were found between the strains.

As for the contents of the small intestine, the absolute enzyme activity for CBC showed a significantly higher value than MVC throughout the experiment. The enzyme activity of MVC increased gradually throughout the experiment.

For the amylase activity, CBC attained a marked increase at 10 days for all intestinal segments (Fig.4). 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 CBC and MVC, respectively.

When expressed in units of activity per 100g body weight, the activity of amylase at 1 day post hatch represents a significant higher value for CBC in all intestinal segments. After that, the activity curve declines with the age. The relative activity of amylase remains constant to 10 days in

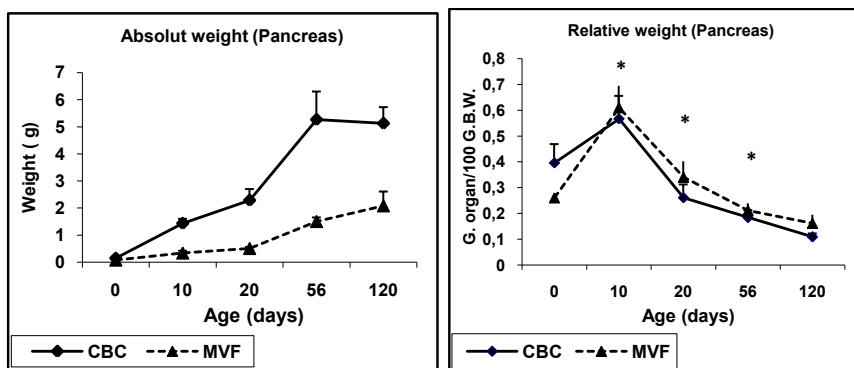


Fig.2: Absolute (a) and relative (b) pancreas weights (g/100 g BW) for CBC and MVC, from 1 day to 4 months of age. Vertical bars represent SD; when not shown, SD is smaller than the symbol. (*) Star superscript indicating not significantly different ($P > .05$).

MVC, but after that point it decreases until the end of the experiment. Although the relative activity of amylase for MVC was found to be significantly higher, both the strains showed no variances at 10 days for the jejunum (Fig.3).

The pattern of the duodenal trypsin activity was somewhat different than that for amylase in both the strains (Fig.5). At 10 days, there was a marked increase in jejunum and ileum by approximately 2 folds over that at 1 day. However, CBC showed

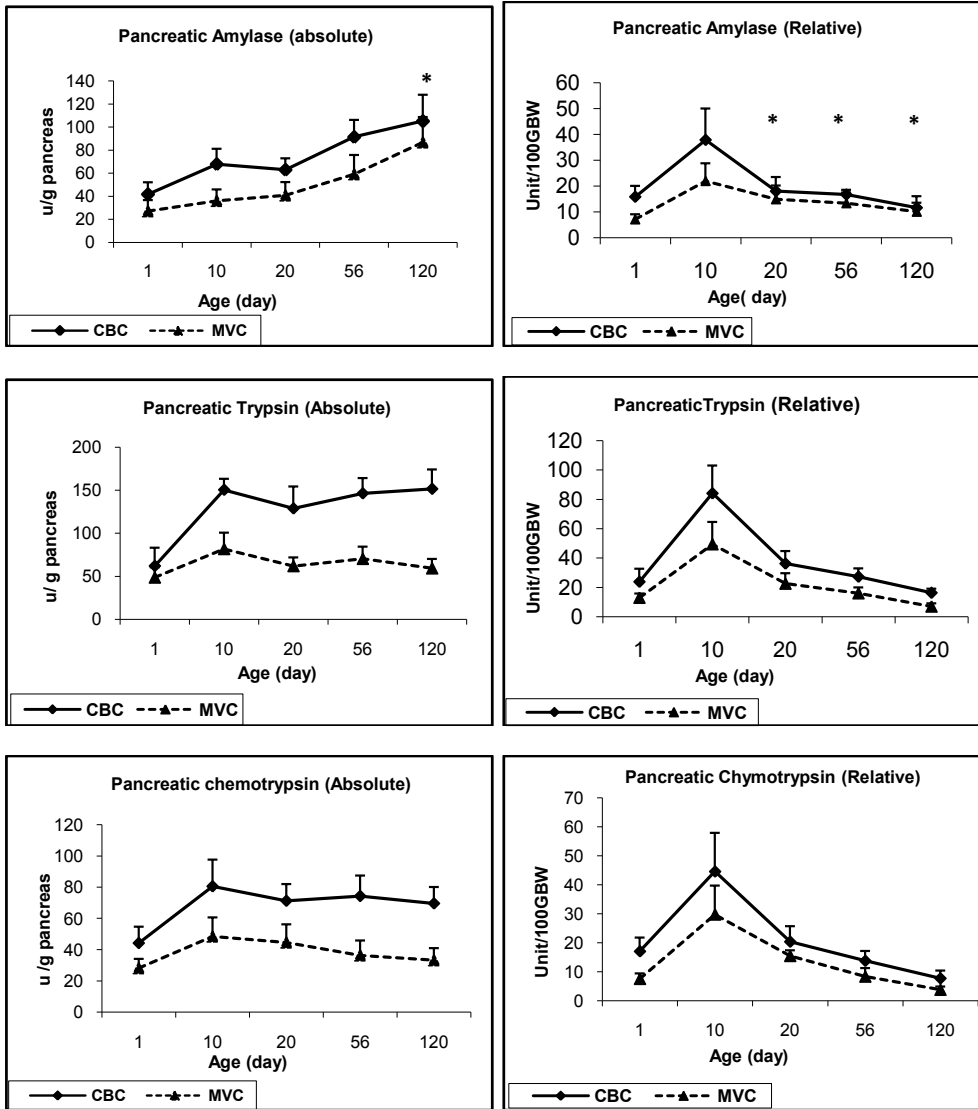


Fig.3: Pancreatic activities of amylase (a, a'), trypsin (b, b') and chymotrypsin (c, c') from 1 day to 4 months of age for CBC and MVC, expressed in units/g organ (left) and units/100g BW (right). Comparisons of the mean are made between lines at each age. Vertical bars represent the SD. N=6. (*) Star superscript indicating not significantly different ($P > .05$).

a decrease in the duodenal trypsin activity at 20 days and at 56 days for the jejunum and ileum, but it increased thereafter. For MVC, the enzyme activity progressed very slowly and declined after 20 days in both jejunum and ileum. The relative activity of

trypsin was markedly decreased for both the strains during the experiment (Fig.4). Except for MVC, the jejunum and ileum relative trypsin activity did not change during the first 10 day post-hatch. At 1 day, the relative trypsin activity of CBC was

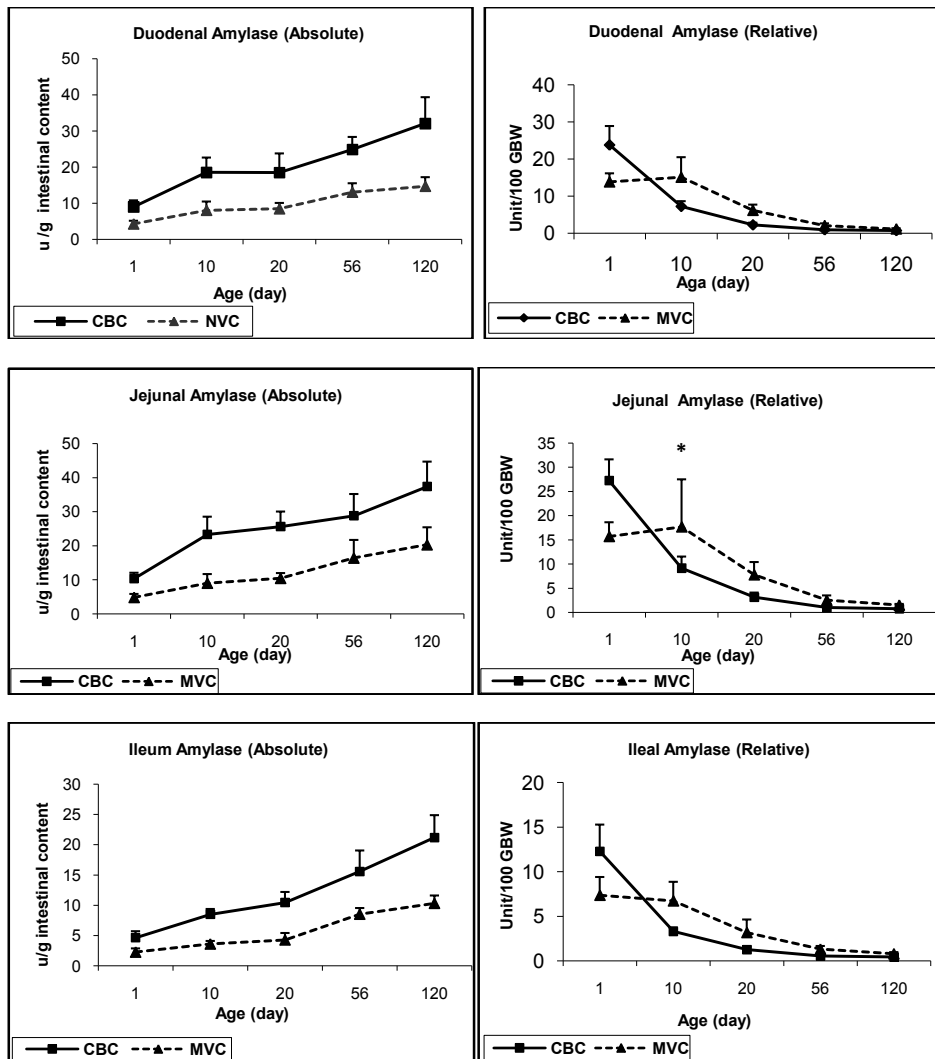


Fig.4: Activities of Amylase in the contents of the duodenum (a, a'), jejunum (b, b') and ileum (c, c') of CBC and MVC from 1 day to 4 months of age, expressed in units /g (left) and units /100g BW (right). Vertical bars represent SD; when not shown, SD is smaller than the symbol. (*) Star superscript indicating not significantly different ($P > .05$).

significantly higher for the duodenum and ileum, although there was no difference for the jejunum and no difference at 56 day for duodenum and jejunum, while MVC had the greater value during the remaining of the experiment.

The chymotrypsin activity was found to increase by approximately 2 folds for both the strains in all the intestinal segments during the first 10 days. However, it reached 3 folds in the jejunum of CBC. Both the strains showed gradual decreases in the

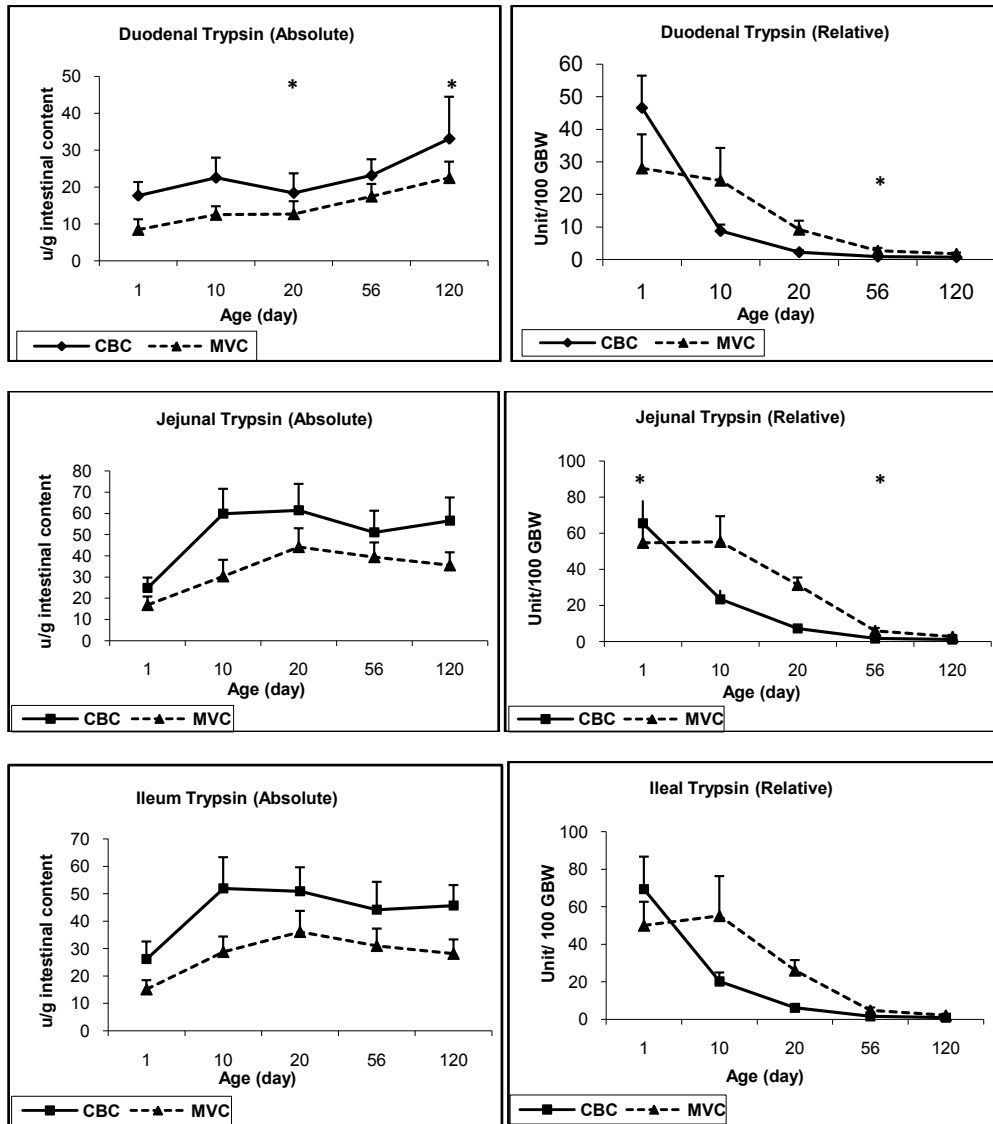


Fig.5: Activities of trypsin in the contents of the duodenum (a, a'), jejunum (b, b') and ileum (c, c') of CBC and MVC from 1 day to 4 months of age, expressed in units /g (left) and units /100g BW (right). Vertical bars represent SD; when not shown, SD is smaller than the symbol. (*) Star superscript indicating not significantly different ($P > .05$).

enzyme activity with age (Fig.6). The data for the relative chymotrypsin activities showed a decrease during the experiment for all the intestinal segments of both strains, except the relative enzyme activity for MVC showed increases during the first 10 days for all the intestinal segments. In addition,

there was no difference between the two strains at the 1 day in jejunum, and at 56 days in duodenum and from this time to the end of experiment in the jejunum. However, MVC showed a significantly higher value during the remaining days of the experiment (Fig.6).

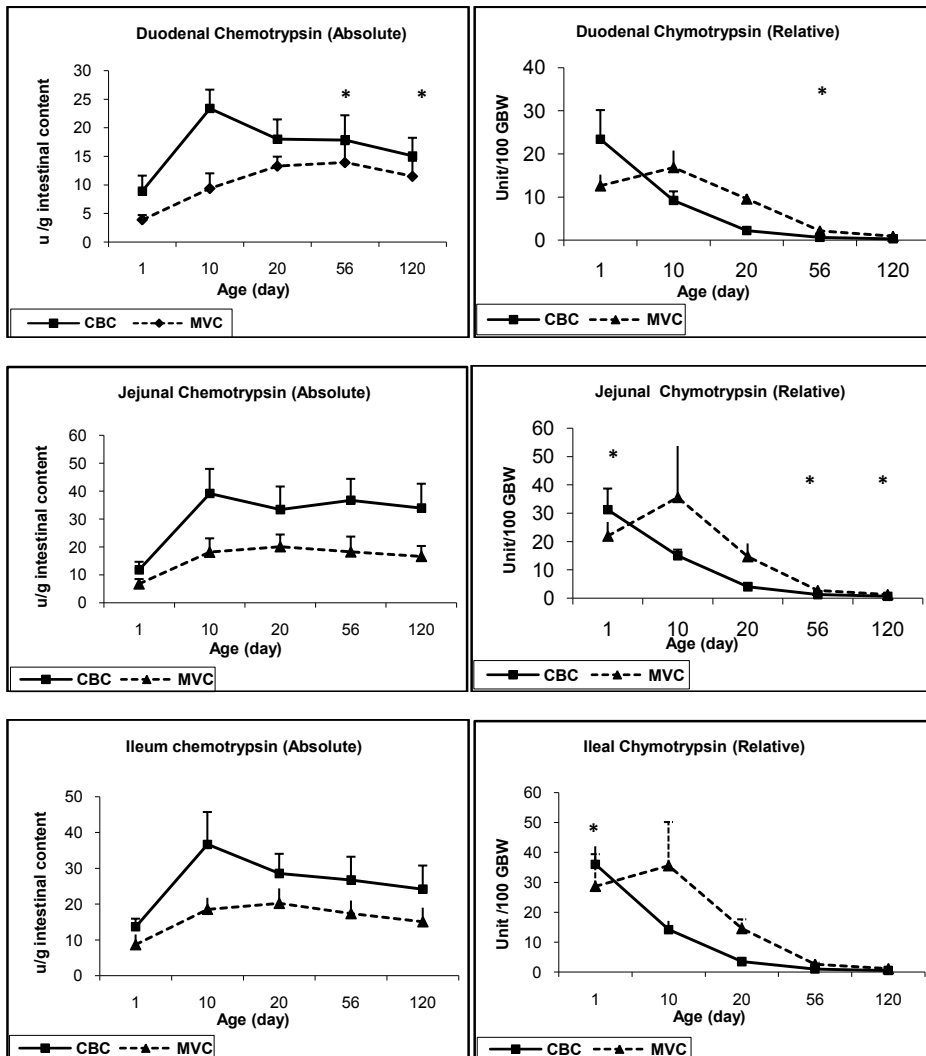


Fig.6: Activities of chymotrypsin, in the contents of the duodenum (a, a'), jejunum (b, b') and ileum (c, c') of CBC and MVC from 1 day to 4 months of age, expressed in units /g (left) and units /100g BW (right). Vertical bars represent SD; when not shown, SD is smaller than the symbol. (*) Star superscript indicating not significantly different ($P > .05$).

DISCUSSION

The difference in the pancreatic weight was clearly observed between the two strains. The relative pancreatic weight constituted 0.4% and 0.2% of body weight on the 1st day of life for CBC and MVC, respectively, increased and peaked at 10 days of age. These differences in the relative pancreatic weight between the two strains might reflect differences in the body growth rate, the pancreas, or both. These results are consistent with the hypothesis that growth is greater for supply than for demand organs during the early period 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 are maximal. Meanwhile, a lack of the pancreatic enzyme activity decreases the apparent digestibility of the dietary components and reduces growth (Corring & Ourdon, 1977).

The results of the current study revealed that all the pancreatic enzymes were higher in CBC than MVC in the relative bases, except for amylase where there was no difference between the breeds after 10 days of age. According to 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

increase with age for relative amylase, total trypsin, total and relative chymotrypsin for the heavy breed at 3 weeks of age. Krogdahl and Sell (1989) reported that the activity of pancreatic amylase increases rapidly at the first 10 day after hatching while the trypsin increases after that.

It was obvious from the results of the current study that the absolute activities of the intestinal enzymes were greater in CBC than MVC. However, this relationship is reversed in relative bases, except for 1-d after hatching. This is due to lower enzyme secretion of MVC on one hand, and on the other hand, the effects of rapid body growth rate for CBC. These facts seemed consistent with the previous report for amylase by Leslie *et al.* (2007) who made comparisons of the high-and low-weight lines of chicks. However, their experiment was for 3 weeks of age. Furthermore, the current result agreed with the report of Cherry *et al.* (1987) concerning the intestinal trypsin and chymotrypsin activities in heavy and light breed at 61 day age. However, Dunnington and Siegel (1995) reported that among the intestinal enzymes, only the trypsin activity decreased for the heavy line compared with the light lines.

In this study, all intestinal enzyme activities in the two strains 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 when reaching the

ileum in low concentration. Osman (1982) reported 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 enzyme activities in this experiment, the authors did not find any correlation between the reduction of the enzyme 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 the strains in this study showed differences in their enzyme activities. When the activities were corrected for the body weight, the data failed to follow the rapid gain of the body weight for CBC, except for the 1 day post hatch. However, it was dominantly greater than MVC for the pancreatic enzymes only, whereas for CBC, the activities of the intestinal enzymes showed lower values than MVC. A comparison between these two different strains showed that genetic variation was an important source of differences, not only in body weight but also in enzymatic activities.

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