QUANTIFICATION OF AMYLASE, LIPASE AND PROTEASE IN THE DIGESTIVE TRACT OF JAPANESE QUAIL

BY

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THESIS

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DECLARATION

I hereby declare that the thesis entitled "**QUANTIFICATION OF AMYLASE, LIPASE AND PROTEASE IN THE DIGESTIVE TRACT OF JAPANESE QUAIL**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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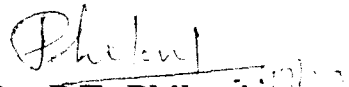
CERTIFICATE

Certified that the thesis, entitled "**QUANTIFICATION OF AMYLASE, LIPASE AND PROTEASE IN THE DIGESTIVE TRACT OF JAPANESE QUAIL**" is a record of research work done independently by **Miss Beena, V.**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.



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Mannuthy ***BEENA, V.***

Dedicated To My

Loving Parents and

To My Sister

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Introduction

Chapter 1

INTRODUCTION

Japanese quails, a native of Japanese islands have been introduced in India in early seventies. Since then large scale quail farming has gathered noticeable momentum in all parts of the country. Their small body size, lesser feed consumption, smaller space requirements and the ease of handling have made them an acceptable animal model for research in poultry. The rapid growth rate, early maturity, short generation interval, high production rate and the medicinal properties attributed to the quail eggs and meat added much to their acceptability.

For economic rearing of any livestock a thorough knowledge of the basic tenets of their digestive process is essential. So an understanding of the digestive physiology of Japanese quail is of fundamental importance for successful quail farming. Progress in the understanding of the digestive physiology of poultry in general and Japanese quail in particular has been less rapid.

In almost all animals and birds, the carbohydrates, fats and proteins as they exist in food are in most cases not in a state ready to be used by the body tissues in the form in which they are taken, but they must undergo thorough

alteration in the digestive tract to make them fit for easy absorption and assimilation. In so far as the changes which the food undergoes in the alimentary canal are concerned, they are brought about mainly by the action of digestive enzymes. Eventhough fundamentally the chemical changes undergone by the food in both animals and birds are the same, considerable species differences exist because of the variations in the anatomical structure and physiological functions. The distinctive developmental functions of the avian digestive system is primarily oriented towards its high level of performance to meet the energy requirements of homeothermy and flight.

Plimer and Rosedale (1922) studied the distribution of digestive enzymes in the alimentary canal of chicken. The occurrence of an amylase in the extracts of salivary glands and saliva of turkeys, geese, domestic fowl and pigeon has been reported by Farner (1960). According to him the salivary amylase could be of functional importance only in the crop as the food remains in the oral cavity of the bird only for a short duration. Informations regarding the salivary amylase activity in Japanese quails are sparse in the available literature.

The major source of amylase in poultry was considered to be the pancreas (Moran, 1982) and therefore starch digestion

is considered to be completed in the intestine where the pancreatic duct empties its secretion. Conflicting reports exist regarding the occurrence of amylase in the avian bile. Sturkie (1954) considered that the amylase of avian bile favoured the carbohydrate digestion in the intestine. From the foregoing it was considered relevant to study the existence of amylase in crop, pancreas, small intestine and bile in Japanese quail and to quantify the enzymes so as to locate the site of greater activity of this enzyme.

As far as the protein digestion of the bird is concerned, it begins in the glandular stomach (proventriculus). The only enzyme of proventriculus is supposed to be a protease which resembles the mammalian pepsin in its activity (Farner, 1960). Sadanandan (1968) could detect the protease activity in the gastric juice of ducks. Therefore, it was considered appropriate to estimate the protease (pepsin) activity in the glandular stomach (proventriculus), which is considered to be the major source of protease in Japanese quail.

No information could be gathered from the available literature on the presence of lipase in the upper gastro intestinal tract of birds corresponding to the lingual lipase of rats or gastric lipase of rabbits (Krogdahl, 1985). In case of birds pancreas is considered to be the major source of

lipase (Hill, 1965) and the lipolytic activity takes place in the intestine. The presence of an intestinal lipase in birds has not been established yet. Hence it was thought worthwhile to investigate and quantify the lipase activity in the pancreas and intestine of Japanese quail.

Variation in the hydrogen ion concentration (pH) in different parts of the digestive tract is an indication of its relative importance in different regions creating the necessary environment for ideal chemical digestion by different enzymes. Hence an assessment of the pH in the different regions of the gastro intestinal tract can throw better light on the fundamental pre-requisition for the proper action of digestive enzymes. Furthermore the determination of the rate at which the feed is digested and passed through the digestive tract ie. the feed passage rate (FPR) can be used to adjudge the efficacy of digestive process in Japanese quails.

From the foregoing, it is clear that many lacunae exist in the understanding of physiology of digestion in birds, especially in Japanese quail. Hence it was thought worthwhile to study some aspects of digestive physiology of Japanese quailswith special reference to the detection and estimation of the activity of some of the more important digestive enzymes like amylase, protease (pepsin) and lipase in their alimentary canal. The estimation of amylase was carried out

in the mucous membranes of crop and small intestine, pancreas and bile from the gall bladder. The quantification was carried out for protease in the proventricular mucosa and for lipolytic enzyme, lipase in the pancreas and mucous membrane of intestine. An exploratory assessment of the pH of the different regions of the tubular digestive tract and the rate of passage of ingesta in both the sexes of adult Japanese quail birds were also envisaged.

Review of Literature

Chapter 2

REVIEW OF LITERATURE

Anatomically and physiologically birds differ notably from mammals. Hence it is evident that eventhough fundamentally there is unity in the digestive process of all monogastric mammals, considerable variation exists in the manner in which it is achieved in mammals and birds. The present concepts of digestion in birds are largely based on scattered investigations on birds interpreted in the light of informations available in mammals. The informations available on the avian digestive physiology are sparse and that on Japanese quails (*Coturnix coturnix japonica*) in particular are extremely meagre. So no informations on the digestive importance of the different regions of the digestive tract in Japanese quail could be gathered from the available literature excepting stray references in regard to the activities of amylase, trypsin and lipase in the pancreas and small intestine of adult female quail during starvation by Majumdar *et al.* (1994a). The literature reviewed, therefore, relates to other species of poultry.

2.1 Amylase activity

2.1.1 Crop

As early as in 1940, Leasure and Link could detect the presence of amylase and an inactive lipase in the chicken saliva. The presence of enzymes in the crop and their role in digestion have been subjects of controversy. Hans Fisher and Weiss (1957) were of the opinion that entirpation of crop did not interfere with normal development or production in birds. According to Pisano *et al.* (1959) no digestion took place in the crop of chicken. However, Farner (1960) reported that amylase was present in the saliva of turkey, goose, pigeon and domestic fowl, but the enzyme could be of functional importance only in the crop. Bolton (1965) believed that the significant amount of starch digestion which occurred in the crop of chicken was due to the bacterial action. Hill (1965) was of the opinion that no enzyme was secreted in the crop of chicken and that crop favoured digestion by amylase derived from plants. Sadanandan (1968) demonstrated the presence of enzyme, maltase in the mucosal extracts of oesophagus and crop of ducks. He observed that pH of crop contents was 5.9 which did not favour the action of salivary amylase in the crop. Pritchard (1972) demonstrated the non bacterial digestion of carbohydrate like sucrose in the chicken crop. However, Ziswiler and Farner (1972) reported that within the crop of

domestic fowl the pH range was 4.5 to 6.7 which favoured the activity of plant and microbial amylases. According to them any chemical digestion in the crop would have to precede the important physical digestive processes of the gizzard and that would certainly restrict chemical digestion in the crop to a relatively minor extent. At the same time they also reported the presence of serous and mucous elements in the pigeon crop glands and pH in the crop that could be reasonably favourable for the amylase activity.

Bayer *et al.* (1975) observed the presence of bacterial population on the crop mucosa of chicken in a scanning electron microscopic study. Duke (1977) was of the opinion that even if amylase was present in the saliva of birds, little digestion could occur in the mouth due to the lack of sufficient maceration and the short duration of time the food remaining in the mouth. Nitsan and Madar (1978) detected minor amylase activity in the crop contents of chicks. Turk (1982) believed that avian crop primarily functions as a storage organ where little or no digestion occurred except for some bacterial fermentation or minor amylase activity. Rodeheaver and Wyatt (1986) reported that the fluid from the oral cavity of broilers had only very low level of alpha amylase but was not a primary source of the enzyme activity. Maya (1995) could not detect any serous cells on the crop mucous membrane of Japanese quails. Ritz *et al.* (1995a)

observed the presence of amylase in the crop of turkeys which indicated the beginning of starch digestion in the crop and the activity of the enzyme was not influenced by the diet. They were also of the opinion that amylase activity in the crop was due to regurgitation of intestinal contents.

2.1.2 Pancreas

Hill (1971) reported that the pancreas of newly hatched chick had a high amylase content which declined gradually over the first three weeks of life and then remained constant. Hulan and Bird (1972) observed that in birds the activity of amylase in the pancreatic juice was influenced by the intake of carbohydrates. Neiss *et al.* (1972) reported that pancreozymin had no significant role to play on the amylase activity of chicken pancreas. Nitsan *et al.* (1974) reported that the synthesis of digestive enzymes of pancreas and small intestine in chicks was not predominantly controlled by the amount and concentration of substrates or products of digestion in the gastro intestinal tract (GIT) but was under the influence of mechanical or humoral stimulation caused by the passage of a greater amount of chyme through the digestive tract. They also observed that although the absolute weights of pancreas and intestinal chyme were increased in force-fed chicks, specific activities of the digestive enzymes were the same except for a greater activity of the intestinal amylase.

The amylolytic property of pancreatic juice in fowl had been pointed out by Sturkie (1976). Johnson *et al.* (1977) observed that synthesis of pancreatic amylase was increased two to three fold times in rats fed on a high carbohydrate diet containing 64 per cent sucrose. According to them amylase synthesis was considerably depressed and was unresponsive to the carbohydrate rich diet in hypophysectomised rats. This effect could be partially modified by the administration of hydrocortisone, cortisone or thyroxine but not with growth hormone.

A 24 h study conducted by Girald-Globa *et al.* (1980) in rats evidenced a clear rhythmicity of both synthesis and storage of pancreatic hydrolases. Amylase and chymotrypsin contents of pancreas of rats were two-fold higher during the day (resting period) than at night (feeding period) while trypsinogen did not vary significantly. They also reported that during periods of active feeding, stimulated synthesis of enzymes and balanced secretion occurred while during the periods of spontaneous fasting, basal synthesis was greater than basal secretion resulting in a preprandial accumulation of the hydrolases. According to Moran (1982) pancreatic alpha amylase was the only enzyme elaborated by fowl that digested starch. Osman (1982) found that in chicken amylase activity was high in the pancreas than in the intestine and the pancreatic amylase differed from that of intestine, in pH optimum, as well as chloride ion effect of substrate concentration.

Lee and Lebenthal (1983) reported that within 24 h, early weaned rats increased their pancreatic enzyme activities with amylase increased to twice that of the continuous suckling non-weaned littermates. Serum cortisone level was increased three to four times in early weaned and fasted rats than that found in continuously suckling littermates. Moran (1985) reported that in birds neural impulses as well as cholecystokinin and pancreozymin stimulations were distinctly different stimuli that influenced expulsion of stored granules which contained the full array of zymogens. He also found that the activity of amylase was increased with neural stimulation and as the amount of dietary starch changed the concentration of amylase in the pancreatic juice was altered accordingly.

Rodeheaver and Wyatt (1986) revealed that in broiler chicken pancreas exhibited the highest degree of alpha amylase activity and that sonication of the pancreatic homogenates was found to increase significantly the apparent activity of alpha amylase 35-fold over the unsonicated homogenates. So they suggested that enzyme was synthesized and stored intracellularly in the pancreas and subsequently secreted into the duodenum for digestion. They also noticed that electrophoretic zymograms of alpha amylase from serum, liver and pancreatic homogenate were identical and that the level of pancreatic amylase activity increased from zero to seven weeks of age.

Ikeno and Ikeno (1991) suggested that amylase appeared in the pancreas of chicken embryo at the sixth day of incubation. Activity increased roughly in four stages (6 to 12 days, 13 to 16 days, 17 to 19 days and 20 days to hatching) with the greatest increase occurring after 19 days of incubation. Amylase in the yolk was electrophoretically identical to that in the pancreas of the embryo and hen and that amylase activity in the pancreas of hen was higher than that of embryo. Nitsan *et al.* (1991), in a genetic study observed line differences in the amylase level of the pancreas and contents of small intestine of cockerels throughout the first 15 days post hatch period. Pancreatic amylase decreased with age in all the lines, with patterns appearing line dependent.

The ratio of exocrine enzymes in the extracts of pancreas from broiler chicken was determined by Pubols (1991) and reported that amylase was about 28.9 per cent, the three chymotrypsinogens together about 20 per cent and a single anionic form of trypsinogen about 10 per cent of the total protein of the pancreatic extract. Pro-carboxypeptidase A and B, pro-elastase, lipase and a secretory inhibitor were also present in traces. According to Sell *et al.* (1991) a moderate increase in the specific activity of amylase occurred during the period of late embryonic development in turkeys. Relatively large increase in the specific activity of amylase was observed between the day of hatching and one day of age, which continued to increase even after the second day.

Majumdar *et al.* (1994a) reported that starvation caused marked decrease in amylase activity of pancreas in Japanese quail. Pinchasov (1995) demonstrated that forced oral administration of a nutrient mixture of glucose, starch and oil immediately after hatching induced rapid growth of pancreas in turkey poults and broiler chicks, but the activity of alpha amylase in the pancreas decreased significantly by second day indicating that the secretion of amylase immediately after hatching was only slightly dependent on the presence of food in the lumen. Ritz *et al.* (1995a) reported that the pancreatic amylase activity in male turkeys was not consistently affected by the diet, but the activity was increased in a linear fashion till the eighth week.

2.1.3 Bile

Unlike in mammals, bile of domestic fowl showed appreciable quantities of amylase activity (Sturkie, 1954; Farner, 1960; Li, 1963 and Hill, 1965). According to Ariyoshi *et al.* (1964) the relatively high digestibility in the depancreatized chickens might be attributed to the presence of amylase in the bile reaching the intestine. Sadanandan (1968) detected amylolytic activity in the gall bladder bile of ducks. Sturkie (1976) observed in chicken, a greater amylolytic activity of gall bladder bile compared to that of the bile from the liver. Rodeheaver and Wyatt (1986) analysed

alpha amylase activity in various body fluids and tissues of broiler chicks and revealed that bile exhibited the lowest mean levels of amylase activity, with no difference between samples from 11 day old and seven-week old chicks in comparison to pancreatic amylase activity. According to them biliary amylase did not contribute much to the starch digestion in intestine.

2.1.4 Small intestine

According to Farner (1960), studies involving extracts of intestinal mucosa lead to possibly incorrect conclusions because of contamination with enzymes of pancreatic, hepatic or bacterial origin and also by tissue enzymes which were not secreted into the lumen of intestine and therefore did not function as digestive enzymes. He was also of the opinion that, if at all an intestinal amylase existed, it played only a small role in the digestion of starch. Ariyoshi *et al.* (1964) pointed out that depancreatized chicken could utilize only about 72 per cent of ingested starch compared to 97 per cent in normal chicken. Sadanandan (1968) could detect amylase activity in the small intestine of ducks.

Pisharody (1970) quantified the activity of amylase of small intestinal mucosa of both chicks and ducklings on normal ration and found that ducklings exhibited higher enzyme activity. According to him the optimum temperature for

intestinal amylase activity for both the species was 41°C and the optimum pH for chicken and duckling intestinal amylase were 7.2 and 7.0 respectively. He also observed that a high protein diet was not influencing the intestinal amylase activity in both chicks and ducklings. But on a fat diet decrease in amylase activity was noticed in ducklings, whereas in chicks the activity was unchanged. He also observed that the activity of amylase decreased towards the lower segments of intestine in both chicks and ducklings. The presence of amylase in the intestinal juice of chicken had been reported by Hill (1971).

Forman and Schneeman (1980) detected lowered intestinal amylase level in rats reared on high level of corn oil. Schneeman and Gallaher (1980) reported that in rats a high fibre diet lowered amylase activity of the intestinal contents. Osman (1982) observed that in Fayoumi chickens amylase activity was present in all the parts of intestine with the greatest activity in the jejunum. Rideau *et al.* (1983) were of the opinion that specific activity of amylase was maximum in the distal part of the small intestine of the laying hen during egg formation. They also found that the activity of intestinal amylase decreased from two hours after oviposition.

Moran (1985) reported that the enzymes finalising digestion of carbohydrates to yield absorbable products were localised at the mucosal surface. Krogdahl and Sell (1989) observed that the amylase activity in intestinal contents increased from the date of hatch to 21 days of age.

Majumdar *et al.* (1994a) reported a reduction in the amylase activity of intestine on starvation. Noy and Sklan (1995) reported that net duodenal secretion of amylase was low on the fourth day of age which increased with age upto the 21st day.

2.2 Protease activity

2.2.1 Proventriculus

Balloun and Baker (1957) reported that day old chicks had a well developed proteolytic enzyme system and feeding of enzymes did not improve their growth or feed efficiency. Farner (1960) believed that the cells of the secretory elements of the compound glands of proventiculus were functionally homologous with both chief and parietal cellsof mammals and the properties of proteolytic activity indicated that the enzyme was pepsin. According to him both neural and hormonal mechanisms were involved in the regulation of the secretary activity of proventriculus. Histamine had a stimulatory effect on the gastric juice secretion and the juice secreted was rich in hydrochloric acid and poor in pepsin and it was not clear whether this was a normal regulatory mechanism. He also reported that the secretary function of the proventriculus was adjustable with respect to the amount and kind of food present in the anterior portion of

the digestive system. However, he could not demonstrate a secretary response to the sight of food in bird.

Toner (1963) reported that oxyntico-peptic cells of chicken proventriculus secrete both hydrochloric acid and pepsin and these cells resembled amphibian's gastric gland cell. It was also observed that the onset of secretion by proventricular glands in the chicken embryo occurred between 11th and 13th day of incubation in response to ingestion of albumin by the embryo and peptic activity was detected in the proventricular glands from 12th to 16th day of incubation (Toner, 1965). Long (1967) indicated that chicken proventriculus secreted 8.8 ml of gastric juice per kg body weight per hour, which was considerably higher than that for man, dog, rat and monkey. He also proved that the proventricular secretion was under the vagal control and the secretion was continuous in conformity with the continuous feeding behaviour of birds. Eventhough a single cell secreted both acid and pepsin he could notice that this cell could respond differently to various forms of stimulation like histamine or acetyl choline. According to him, the inhibition of gastric secretion in birds following insulin hypoglycemia, rather than stimulation that occurred in mammals, was due to the difference in their glucose metabolism. He also reported that chicken did not have enterogastrone since there was no

gastric secretary inhibition after the infusion of fat into the duodenum.

Sadanandan (1968) detected the presence of high proteolytic activity of the proventricular mucosa of ducks at acidic pH. Pisharody (1970) quantified the protease present in the mucosal extracts of proventriculus of both chicks and ducklings. He also observed that the optimum pH for pepsin activity of chicks and ducklings were 2 and 2.4 respectively and the optimum temperature for pepsin activity in both these species was 41°C. According to him there was no appreciable increase in protease activity of pepsin both in chicks and ducklings maintained on a high protein diet compared to that of birds on a normal diet.

Hill (1971) reported that the volume of gastric juice produced in fowl at starvation could vary from 6 to 21 ml/h and pH of the proventricular contents became markedly acidic at about the 20th day of incubation indicating the fact that considerable secretion of hydrochloric acid occurred in the proventriculus at this age. He was doubtful about the presence of a conditioned cephalic gastric secretory response in fowl, but was of the opinion that during *ad libitum* feeding the reaction of the contents of proventriculus and gizzard was always acidic indicating a continuous secretion of gastric juice. Hill also explained the inhibition of gastric

secretion following induced insulin hypoglycemia due to the lack of sensitivity of the vagal centres of birds to hypoglycemia. Turk (1982) was of the opinion that the glands of proventriculus lack goblet cells and contain only secretary cells with the properties of both chief and parietal cells of mammals. According to Austic (1985) the post-natal developmental pattern of secretions of proteolytic enzyme of stomach was more clearly established for rats than for any other species. The pepsinogen content of rat stomach tissue was low after birth until approximately 15 days after which time pepsinogen increased to adult level by 30th day of age. He also observed a low pepsin activity in the newborn pigs. Majumdar *et al.* (1994b) observed an increase in pepsin activity from day of hatch to sixth week of age in broilers.

2.3 Lipase activity

2.3.1 Pancreas

Hill (1971) pointed out that the lipase activity of pancreas was high in the newly hatched chick and that remained at a more or less constant level during growth, while traces of esterase activity, that was present at the time of hatching showed an increase in concentration during the first 10 days after hatching. He also suggested that secretin and pancreozymin were the two major humoral factors involved in

the stimulatory mechanism of the avian pancreatic secretion. Hulan and Bird (1972) reported an increase in the activity of lipase in the pancreatic juice of birds by the intake of a high fat diet. Kokue and Hayama (1972) in a comparative study observed that the volume of pancreatic secretion per one kg body weight of 24 h starved chicken was same as that of rat and more than those of sheep and dog. They also reported that unlike in mammals, chicken pancreatic secretion was less influenced by the length of fasting time. According to them in chicken basal secretion was continuous and not influenced by nervous factors or the presence of diet in the gastro intestinal tract. Dror *et al.* (1976) found that the pancreatic lipase activity of birds fed a diet containing 15 per cent soybean oil was 30 per cent higher than that in birds fed a diet without added fat. The proteolytic and lipolytic properties of pancreatic juice of birds had been reported by Sturkie (1976). Dimaline and Dockray (1979) found that in turkey, both chicken and porcine vaso active intestinal peptide (VIP) strongly stimulated the flow of pancreatic juice indicating the possibility that VIP had a physiological role in the control of pancreas in birds which was analogous to that of secretin in mammals. They also observed that secretin was a poor stimulant for avian pancreas.

Krogdahl (1985) suggested that the importance of pancreatic and biliary secretions for yolk utilisation in

birds seemed negligible. According to him the role of liver and pancreas in digestion did not become important until the bird started eating. Escribano *et al.* (1988) conducted studies on the development of lipase activity in the pancreas of young turkeys and reported that the specific lipase activity as lipase activity per milligram of dry weight of pancreas was increased from 3.1 μeq/min at 27th day of incubation to 26.2 μeq/min by 16th day after hatching. Krogdahl and Sell (1989) reported that in pancreatic tissue of young turkeys lipase activity increased with age after a lag period of about 14 days.

Sell *et al.* (1991) reported that the specific activity of pancreatic lipase moderately increased during the late embryonic development in turkeys. Between the day of hatching and one day of age relatively large increase in the specific activity of lipase was observed and this lipase activity did not change noticably after the second day. Majumdar *et al.* (1994a) observed an increase in pancreatic lipase activity with the increase in the length of starvation in adult female Japanese quails. Dunnington and Siegel (1995) observed higher levels of pancreatic total and relative lipase activities in cockerels fed a diet containing more crude protein and more metabolisable energy. Palo *et al.* (1995) observed a significant decrease in relative and specific activities of pancreatic enzymes namely trypsin, amylase and lipase in feed

restricted broiler chickens. Satoh *et al.* (1995) observed the complete suppression of both pancreatic flow and protein output by atropin and suggested that the avian intestinal phase of pancreatic secretion was mainly controlled by cholinergic action through hydrochloric acid (HCl) stimulation. They also noticed that intestinal infusion of neither peptone solution nor fat emulsion affected the pancreatic secretion. HCl enhanced the flow rate of pancreatic juice, but not its protein output.

2.3.2 Small intestine

Ariyoshi *et al.* (1964) reported that depancreatized chicken digested only about 25 per cent of ingested proteins and fats. They also found that protease and lipase disappeared from the faeces of depancreatized chicken. Sadanandan (1968) could not detect lipase activity in the small intestinal mucosa of ducks. Pisharody (1970) quantified the lipase activity of small intestinal mucosa of both chicks and ducklings on normal ration and found that ducklings exhibited higher values for the enzyme activity. He also observed a decrease in intestinal lipase activity in both chicks and ducklings on a high protein diet. However, on a high fat diet there was a decrease in the activity of lipase in ducklings, whereas the activity of the enzyme remained unchanged in chicks. He also observed that the activity of

intestinal lipase decreased towards the lower segments of intestine in both chicks and ducklings. Hill (1971) stated that the intestinal juice collected from the duodenal loop of chicken from which the proventricular, biliary and pancreatic secretions were excluded, contained mucus, protease, amylase and lipase.

Forman and Schneeman (1980) could detect higher intestinal lipase levels in rats reared on a high level of corn oil. Schneeman and Gallaher (1980) observed that the activity of lipase of the intestinal contents was lower in rats reared on a high fibre diet. Rideau *et al.* (1983) were of the opinion that specific activity of lipase was maximum in the proximal part of the small intestine of laying hen during egg formation. They also found that intestinal lipase activity tended to decrease during the light period (2 to 10 h after oviposition) and to increase during the dark period (14 to 24 h after oviposition). In non-layers intestinal contents and enzyme activities were lower than that of the layers. According to Krogdahl and Sell (1989) the increase in intestinal lipase activity was dependent on the dietary fat level, low activity was observed with low fat diet. But with a high fat diet a lag period of three weeks was followed by a five-fold increase in the lipase activity. Majumdar *et al.* (1994a) reported that in adult female Japanese quails, the intestinal lipase remained unchanged even after 24 h of

starvation and that a drastic reduction in lipase activity was noticed at 48 and 72 h of starvation. Noy and Sklan (1995) observed that net duodenal secretion of lipase was low on the fourth day of age and which increased gradually till the bird attained 21st day of age.

2.4 pH

Farner (1960) stated that pure gastric juice in birds must have a hydrogen ion concentration ([H^+]) of the order of pH 1 to 2 and the pH of gizzard contents of domestic fowl, turkey, pigeon and duck were 2.77, 2.19, 2.00 and 2.33 respectively. According to him the nature of the diet could influence the pH of gastric contents in the domestic fowl. He also reported that the pH of the succus entericus was on alkaline side with a substantially higher acid neutralising capacity than either pancreatic juice or bile. Laws and Moore (1963) pointed out that the pancreatic and intestinal enzymes whose digestive functions occur in the small intestine, had their maximum activity at the optimum pH range of 6 to 8.

Hurwitz and Bar (1968) were of the opinion that the pH of the avian intestinal tract increased from the oral to the aboral end and pH of each portion of the tract was maintained by secretary activity within that portion. Sadanandan (1968) detected [H^+] of the contents of the different regions of the

gastro intestinal tract such as crop, proventriculus, gizzard, duodenum, jejunum and ileum of ducks and the mean pH values were 5.9, 3.3, 4.6, 6.3, 7.3 and 7.3 respectively. Mongin (1976) on evaluation of the effect of egg formation on pH of the gizzard contents observed that the pH changed from 4.87 to 3.64 at 2 h to 14 h after oviposition which gradually increased to 4.37 at about the 22nd h after oviposition. According to Duke (1977) the optimum secretary activity of the intestine occurred at the pH range of 6 to 8.

2.5 Feed passage rate (FPR)

Kaupp and Ivey (1923) conducted a study on feed passage rate (FPR) using lamp black as the marker in starved fowl for 24 hours. They recorded food passage time in layers, non layers, broody hen and pullets and the values were 3 h and 46 min., 8 h, 11 h and 44 min. and 3 h and 52 min. respectively. In most of the studies on the rate of passage of ingesta (Hillerman *et al.*, 1953; Tuckey *et al.*, 1958; Sadanandan, 1968; Pisharody, 1970; Sturkie, 1976; Wilson *et al.*, 1980; Matoes and Sell, 1981 and Matoes *et al.*, 1982) a marker was administered and the time of first appearance of marker in the faeces was used as an index of the food passage rate (FPR) through the gastro intestinal tract. Hillerman *et al.* (1953) recorded the time taken for food passage through the alimentary canal of laying turkey hen as 3 h and 13 min. and

that for non-layers as 4 h and 16 min. They also studied the influence of age on FPR through the gastrointestinal tract and found that in younger turkey hens the time taken was less as 2 h and 27 min, whereas in older turkey it was more, as 3 h and 52 min. Laying and non-laying hens showed similar FPR values of 3 h and 42 min and 3 h and 50 min. respectively. They could not find any influence of environmental temperature on the FPR of chicken when the birds were maintained at 15.6°C and 32.2°C.

Tuckey *et al.* (1958) noticed that fats were utilised very efficiently by the fowl and a level upto 12 per cent in the diet of growing chickens had little effect on the rate of passage of food or its digestibility. According to Sturkie (1965) differences in experimental conditions or methods could bring about variations in the results on FPR in chicken. Sadanandan (1968) determined FPR in ducks on a standard ration using carmine dye as the marker, and the mean value obtained for FPR was 2 h and 17 min. He could also observe a decreased FPR in ducks on a diet high in fibre and fat contents. Pisharody (1970) reported the rate of passage of ingesta in chicks and ducklings as 163 ± 10.34 min. and 108 ± 8.36 min. respectively. He could not observe any variation in the FPR of chicks and ducklings when different levels of fibre were incorporated in their ration.

Cherry and Siegel (1978) observed that the FPR was significantly higher in male chicks of a high body weight line than in male chicks of the low body weight line. Sibbald (1979) found that under a force-feeding system, the rate of drymatter excretion of adult White Leghorn males was increased when larger amounts of feed was force-fed. Wilson *et al.* (1980) noticed an increase in FPR of Peking ducks at a high environmental temperature. They also observed that restriction of feed intake of Peking ducks for six hours resulted in reduction in FPR in comparison to the unrestricted ducks. According to him sex had no influence on the feed passage time. Matoes and Sell (1981) reported that addition of fat in the diet slowed FPR in chickens. Duffy *et al.* (1985) evaluated two methods of measuring food transit rate of sea birds (Jackass Penguins) using $Cerium^{141}$ and Carmine red dye as indicators and observed that mean excretion time were 11.65 h for Ce^{141} and 10.15 h for carmine. Both markers started to appear in the droppings within 2 h of ingestion and continued to be excreted even after 25 h of feeding.

Vergara *et al.* (1989) observed that the transit time for a liquid substance was less than that for a solid one because of longer retention time of the latter. However from the second week onwards, transit of soluble marker took longer time because only liquids or soluble compounds of feed enter the chicken caeca which became functional only after one week

of age. They also studied the influence of age on digestive transit time and found that the rate of food passage increased in the first three weeks of age probably due to the increased feed intake. Washburn (1991) observed some evidence for increased FPR in chicken raised at higher temperatures eventhough the difference was not consistent. He could not observe any difference in the rate of passage of ingesta between male and female birds. According to him dietary factors that influenced efficiency of feed utilisation also influenced FPR and no relationship existed between feed restriction or amount of feed consumed and FPR.

Dunnington and Siegel (1995) reported that chicks from high weight lines consumed more feed, utilised feed more efficiently and had a faster FPR than the low weight line chicks. Noy and Sklan (1995) revealed that feed consumption of chicks increased from 7 g/day at 4th day of age to 45 g/day at 21st day of age. In the same period, feed passage time through the small intestine decreased from 161 min. on day four to 110 min. on day 14, which remained constant upto 21st day. The decrease in transit time observed was especially marked in the duodenum, indicating that the duodenum was the major site of activity of digestive enzyme. Ritz *et al.* (1995b) reported that neither the difference in protein level nor enzyme supplementation influenced the FPR within the male poult at any time during the growing period.

Materials and Methods

Chapter 3

MATERIALS AND METHODS

One hundred and ninety two, six week old Japanese quail birds (96 males and 96 females) of the same strain (egg type) were selected, at random, from the Kerala Agricultural University, Poultry Farm, Mannuthy. At a time, a batch of 24, six-week old birds (12 males and 12 females) of the same strain and hatch were taken and reared in separate compartments of the cage for a period of two weeks in order to make the birds familiarised to the experimental conditions. They were reared on standard adult quail ration, as given in Table 1.1 and 1.2 (Panda, 1990 and Philomina, 1994). Feed and water were provided *ad libitum*.

Table 1.1 The percentage composition of the experimental (layer) ration

Ingredients	Quantity in kg/100 kg feed
Yellow maize	38.0
Groundnut cake	38.0
Gingelly oil cake	5.0
Fish meal	5.0
Rice polish	5.0
Salt	0.5
Shell meal	3.5
Mineral mixture 1	3.0
Fat (Gingelly oil)	2.0
Total	100.0

Mineral mixture[1]

Poultry min (Aries Agro-Vet Industries Private Ltd.) - contained calcium (min)-32.00%, Phosphorus (min)-6.00%, Copper (min)-100 ppm, Cobalt (min)-60 ppm, Manganese (min)-2700 ppm, Iodine-100 ppm, Zinc-2600 ppm, Iron-0.1% and Magnesium-1000 ppm.

For every 100 kg feed, added the following vitamin supplements.

Rovibe[2]: 75 g; Rovimix[3]: 25 g

Choline chloride 50 g

Rovibe[2] (Roche Products Ltd.) Guaranteed potency per gram. Vit. B_1-4mg, B_6-8 mg, B_{12}-40 mg, Niacin-60 mg, Calcium pantothenate-40 mg and Vit.E-40 IU.

Rovimix[3] A, B_2, D_3 (Roche Products Ltd.) Guaranteed potency per gram. Vit.A-40,000 IU, B_2-20 mg, D_3-5000 IU.

Table 1.2 Chemical composition of feed

Metabolisable energy (Kcal/kg)*	2751.00
Crude protein (%)**	22.00
Crude fibre (%)**	3.84
Ether extract (%)**	9.79
Calcium (%)**	3.00
Phosphorus (%)**	0.65
Lysine (%)*	0.91
Methionine (%)*	0.46

* Calculated value
** Analysed value

The different batches of 24 quails (12 males and 12 females) of the same strain and hatch were procured from the poultry farm at weekly intervals and maintained on identical feed and managemental conditions for a period of two weeks. The birds were sacrificed when they attained eight weeks of age for the collection of the different regions of the digestive tract.

The enzymes studied in the various regions of the digestive tract were as per the Table 2.

Table 2. Details about the assay of enzymes in the digestive tract of Japanese quails

Group	Number of birds (equal numbers of males and females)	Enzymes studied	Areas from where tissue samples were collected	Methods of estimation
1.	24	Amylase	Crop mucous membrane	King and Wootton (1959)
2.	24	Amylase	Pancreas	King and Wootton (1959)
3.	24	Amylase	Intestinal mucous membrane	King and Wootton (1959)
4.	24	Amylase	Bile	King and Wootton (1959)
5.	24	Protease (pepsin)	Proventri-cular mucous membrane	Volhard and Lohlein (Hawk, *et al.*, 1954)
6.	24	Lipase	Pancreas	Boutwell, Jr. (1962)
7.	24	Lipase	Intestinal mucous membrane	Boutwell, Jr. (1962)

The last batch (eighth batch) of 24 birds were used to study the rate of passage of feed through the gastro intestinal tract. From this last batch 12 birds were randomly selected for the estimation of pH of the contents of different regions (crop, proventriculus, gizzard and small intestine) of the digestive tract.

3.1 Preparation of Tissue Homogenata

At the eighth week of age, the birds were sacrificed by decapitation, the body cavity was exposed and the whole of the gastro intestinal tract was dissected out. The organs such as pancreas and gall bladder with bile were taken and weighed immediately. The different regions of the gastro-intestinal tract (crop, proventriculus and intestine) were exposed, washed out with ice cold normal saline and then mopped off the moisture from the inner side. The narrow membrane was carefully scrapped out from these different regions and weight of these tissue scrappings were taken immediately. Weighed samples of tissue scrappings from crop, proventriculus and intestine, the whole pancreas and gall bladder were homogenised in Potter-Elvehjem glass homogeniser with ice cold physiological saline and the final volume of the homogenate of each sample was made upto 25 ml.

The enzyme activities of the tissue homogenates were estimated as per the methods indicated in Table 2.

3.2 Estimation of amylase (King and Wootton, 1959)

Principle: An aliquot of the tissue homogenate is incubated at 37°C with 0.4 mg of starch and the loss in blue colour which the starch gives with iodine solution is taken as a

measure of the extent to which starch has been digested by the amylase.

The amylase activity is expressed in terms of "units" (somogyi units) of amylase. The "unit" is here defined as the amount of amylase which will destroy 5 mg of starch in 15 minutes.

Reagents

1. Starch solution 0.1%: 100 mg pure, analar, soluble starch was mixed with 5 ml of glass distilled water in a 100 ml volumetric flask and three lots of 30 ml boiling glass distilled water were added, mixed well, cooled, diluted to the mark and preserved in the freezing chamber of a refrigerator.

2. 0.02 M phosphate buffer pH 7.0: 1.736 g of anhydrous disodium hydrogen phosphate and 1.059 g of monopotassium dihydrogen phosphate were dissolved in one litre of glass distilled water and pH was adjusted to 7.0. A few ml of chloroform was added to the solution as a preservative and stored in a refrigerator.

3. Buffered substrate: 5 volume of phosphate buffer was mixed with 4 volumes of starch solution and was prepared fresh.

4. Stock iodine 0.1 N: 13.5 g of pure sublimated iodine was dissolved in a solution of 24 g of potassium iodide in 100 ml of glass distilled water contained in a litre volumetric flask. The solution was diluted to the mark and standardised against O.IN sodium thiosulphate using starch as indicator.

5. Dilute iodine solution: 50 g of potassium fluoride was dissolved in glass distilled water in a litre volumetric flask and 100 ml of the stock iodine solution was added to it. The volume was made upto the mark and the solution stored in a brown bottle in a refrigerator.

Procedure

Test: 0.9 ml of buffered substrate was warmed in a glass stoppered, labelled test tube kept in a water bath at 37°C for 2 minutes. 0.1 ml of the tissue homogenate was then mixed with it and incubated at 37°C for 15 minutes. The tube was then taken out of the water bath and cooled, added 8.6 ml of glass distilled water followed by 0.4 ml of dilute iodine solution and mixed well.

Blank: Prepared in a similar manner as the test, except for the fact that 0.1 ml of the tissue homogenate was added to the substrate after incubation and addition of dilute iodine solution.

The test and blank were read in a spectrophotometer at 680 nm within five minutes after addition of iodine solution, setting the instrument to zero with distilled water.

Calculation

$$\text{Amylase activity (Somogyi units/g of tissue)} = \frac{B-T}{B} \times \frac{0.4 \times 25}{5 \times 0.1 \times W}$$

where,

B = Reading of blank

T = Reading of test

W = Weight of tissue in g

25 = The dilution factor

3.3 Estimation of protease (Volhard and Lohlein-Hawk *et al.*, 1954)

Principle

The tissue homogenate is incubated with casein solution. The unaltered casein is salted out. The filtrate from this contained digestion products of casein which is estimated titrimetrically.

Reagents

1. Casein solution 5%: 50 g of the casein powder was taken in a litre flask, added 500 ml of glass distilled water,

mixed well and allowed to stand for 3 hours. Added 40 ml of 1N sodium hydroxide solution and the volume was made upto 1 litre, warmed gently until it became clear and then heated rapidly to 85 to 90°C to destroy any proteinases. The solution was then transferred to a stoppered bottle, added a few drops of toluol as preservative and stored in a refrigerator.

2. Hydrochloric acid 1 N: 9 ml of concentrated hydrochloric acid was diluted to 100 ml and standardised against IN sodium hydroxide using phenophthalein as indicator.

3. Sodium sulphate solution 20%: 200 g of anhydrous sodium sulphate was dissolved in glass distilled water in a litre volumetric flask and the volume was made up to the mark.

4. Sodium hydroxide solution 0.1 N: 5.9 ml of saturated solution of sodium hydroxide was diluted to 1000 ml with glass distilled water and standardised against O.IN oxalic acid using phenolphthalein as indicator.

5. Phenolphthalein indicator: 0.5 g phenolphthalein was dissolved in 50 ml of absolute alcohol and final volume was made up to 100 ml with glass distilled water.

Procedure

Into each one of two big test tubes introduced 1.1 ml of IN hydrochloric acid and diluted with glass distilled water to 15 ml. With constant shaking added 10 ml of casein solution to each tube. 5 ml of the tissue homogenate was added to one of the tubes labelled "test". The other tube formed the "control". The tubes were kept in a water bath at 40°C for one hour. At the end of this period, they were taken out and 5 ml of the homogenate was added to the control. Added 10 ml of 20 per cent sodium sulphate solution to each tube and filtered through Whatman No.1 filter paper, to a clean dry tube. Titrated 10 ml of the filtrate in each case with 0.1N sodium hydroxide solution using phenolphthalein as indicator.

Calculation

$$\text{Protease activity (Pepsin units/g of tissue)} = \frac{[(T-C)4]^2 \times 5}{W}$$

where,

T = titre value of test

C = titre value of control

W = weight of tissue in g

5 = the dilution factor

3.4 Estimation of lipase (Boutwell, Jr. 1962)

Principle

The tissue homogenate is incubated with an olive oil emulsion substrate. Lipase activity results in splitting off the glyceryl-fatty acid ester bond with the liberation of free fatty acids. The amount split off is determined by titrating the liberated fatty acids with standard alkali using thymolphthalein as the indicator.

Reagents

1. Olive oil emulsion: Dissolved 12.50 g of gum acacia and 0.20 g of sodium benzoate in glass distilled water. Added 50 ml of pure olive oil to it, emulsified the mixture in a homogenizer until a smooth emulsion was obtained and stored in a refrigerator. Shaken well before use.

2. 0.067 M phosphate buffer pH 7.0: Dissolved 5.51 g of anhydrous disodium monohydrogen phosphate and 3.83 g of anhydrous monopotassium dihydrogen phosphate in glass distilled water in a litre volumetric flask and the volume was made upto the mark. Adjusted the pH of the solution to 7.0 using a pH meter. One ml of toluene was added as a preservative and stored in a refrigerator.

3. Sodium hydroxide solution 0.05 N: Diluted 500 ml of 0.1 N sodium hydroxide to a litre with glass distilled water, in a litre volumetric flask. Standardised against 0.1 N oxalic acid using thymolphthalein as indicator.

Procedure

The substrate-buffer mixture was prepared by stirring 5 volumes of phosphate buffer with 1 volume of olive-oil emulsion. Transferred 12 ml portions of the substrate buffer mixture into 2 tubes. Warmed the tubes in a water bath to 37°C. Added 5 ml of tissue homogenate to one of the tubes and mixed thoroughly by gentle inversion. Second tube served as the blank. Incubated both the tubes at 37°C for 24 hours. At the end of incubation, the tubes were taken and 5 ml of tissue homogenate was added to the blank. Titrated the mixture with 0.05 N sodium hydroxide to a "distinct" blue colour using 4 to 6 drops of thymolphthalein indicator.

Calculation

A lipase unit is defined as that quantity of enzyme which releases acid equivalent to 1 ml of N/20 sodium hydroxide in 24 hours from an olive oil substrate (Abderhalden, 1961).

$$\text{Lipase activity (Lipase units/g of tissue)} = \frac{(T-B)\,5}{W}$$

where,

T = titre value of test

B = titre value of blank

W = weight of tissue in g

5 = the dilution factor

3.5 Determination of pH

The pH of the different regions of the gastro-intestinal tract namely crop, proventriculus, gizzard and intestine was determined by using Merck Universal Indicator Paper.

Procedure

The birds were sacrificed by decapitation and after exposing the body cavity, the entire gastro-intestinal tract from oesophagus to cloacal opening was exposed. The different regions were cut open and small pieces of indicator paper were dipped in the contents immediately. The colour developed was compared with the colour chart provided with the Merck Universal Indicator Paper.

3.6 Estimation of feed passage rate (FPR)

Principle

An inert indigestible colouring matter is fed to the bird as a marker at the time of feeding and the time taken for its first appearance in the faeces is recorded.

Procedure

10% solution of carmine (inert indigestible colouring matter) was prepared. 0.5 ml of carmine solution was administered to each bird by opening the beaks and the solution was poured directly at the back of the tongue, care being taken to see that the whole quantity was swallowed by the bird. Time of administration of the dye was recorded. The birds were then returned to their respective cages and allowed to feed the ration *ad libitum*. The birds were closely watched (without disturbing) for the appearance of the dye in the droppings as it was voided and the time of first appearance of the dye in the droppings was recorded.

Statistical analysis of the data collected from the experiment was carried out as outlined by Snedecor and Cochran, 1967.

Results

Chapter 4

RESULTS

Results obtained in the present study on enzyme activity, pH of different regions of the digestive tract and the feed passage rate (FPR) of adult, eight-week old male and female Japanese quails are presented below.

4.1 Amylase activity

4.1.1 Crop

The mean amylase activity of the mucous membrane of crop of male birds was 47.98 ± 10.49 SU/g of tissue and that of female birds was 13.26 ± 4.46 SU/g of tissue (Table 3.1 and Fig.1.3). Analysis of the data by student 't' test revealed that the difference in amylase activity of crop mucous membrane of male and female quails was highly significant ($P \leq 0.01$). The pooled mean value for amylase activity of crop mucous membrane of males and females put together was found to be 30.62 ± 6.65 SU/g of tissue (Table 3.5 and Fig1.3). As a general observation it was noticed that amylase activity of crop mucous membrane was not influenced by the presence of food in the crop.

Table 3.1 Amylase activity of mucous membrane of crop in eight-week old Japanese quails

Sl.No.	Amylase activity (Somogyi units/g of tissue)	
	Males	Females
1.	52.33	0.98
2.	72.00	27.69
3.	40.00	0.94
4.	81.82	7.23
5.	21.33	1.16
6.	17.60	3.24
7.	80.00	0.55
8.	110.00	13.33
9.	5.16	5.28
10.	8.00	7.14
11.	11.20	24.49
12.	3.33	21.05
Mean±S.E.[1]	47.98±10.49	13.26±4.46
Mean±S.E.[2]	30.62±6.65	
t value	3.0464**	

1. Mean of 12 birds
2. Mean of 24 birds (12 males and 12 females)
** P≤0.01

4.1.2 Pancreas

Pancreas of eight week old male birds exhibited a mean amylase activity of 59.89 ± 3.36 SU/g of tissue and in the female birds it was 38.72 ± 2.59 SU/g of tissue (Table 3.2 and Fig.1.3). The enzyme activity was significantly ($P \leq 0.01$) higher in males. The mean weight of pancreas of male quails was 0.325 ± 0.018 g and that of females was 0.474 ± 0.030 g (Table 3.2). When the data were subjected to 't' test, significant difference ($P \leq 0.01$) was observed. In contrast to the amylase activity, the pancreatic weight of females exhibited a significantly higher value ($P \leq 0.01$) than that of males. A significantly negative correlation was observed between the pancreatic weight and amylase activity ($P \leq 0.01$) in male and female quails with a correlation of -0.975 and -0.968 respectively (Table 6, Fig.1.1 and Fig.1.2). The pooled (male and female) mean value for pancreatic amylase activity was found to be 49.30 ± 3.03 SU/g of tissue (Table 3.2, 3.5 and Fig.1.3) and that for weight of pancreas was 0.399 ± 0.023 g (Table 3.2).

4.1.3 Bile

The bile from gall bladder of male Japanese quails exhibited a mean amylase activity of 156.23 ± 27.72 S U/g of tissue and that of female quails was 87.65 ± 14.36 S U/g of

Table 3.2 Amylase activity and weight of pancreas in eight-week old Japanese quails

Sl.No.	Amylase activity (Somogyi units/g of tissue)		Pancreas (weight in g)	
	Males	Females	Males	Females
1.	78.47	52.96	0.241	0.323
2.	72.87	45.95	0.250	0.380
3.	70.67	48.20	0.260	0.383
4.	72.00	47.28	0.271	0.404
5.	65.84	45.24	0.302	0.412
6.	58.02	38.18	0.325	0.442
7.	55.62	34.96	0.337	0.477
8.	53.24	33.62	0.349	0.504
9.	50.39	32.91	0.362	0.519
10.	50.00	33.05	0.364	0.551
11.	49.07	27.51	0.384	0.634
12.	42.44	38.72	0.449	0.658
Mean±S.E.[1]	59.89± 3.36	38.72± 2.59	0.325± 0.018	0.474± 0.030
Mean±S.E.[2]	49.30±3.03		0.339±0.023	
t value	4.9908**		4.2885**	

1. Mean of 12 birds
2. Mean of 24 birds (12 males and 12 females)

** $P \leq 0.01$

Fig.1.1 **CORRELATION BETWEEN AMYLASE ACTIVITY AND PANCREATIC WEIGHT IN MALE JAPANESE QUAILS**

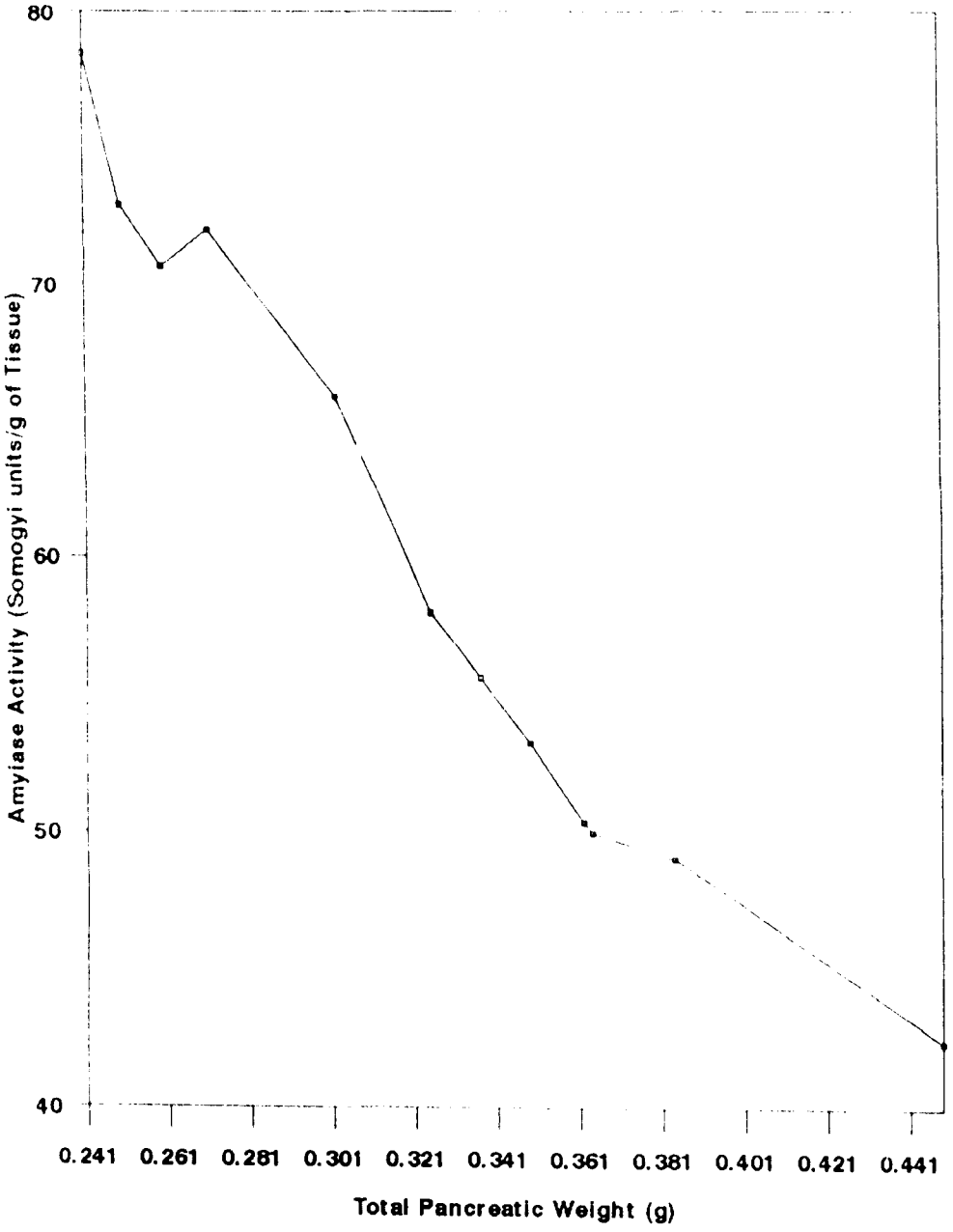
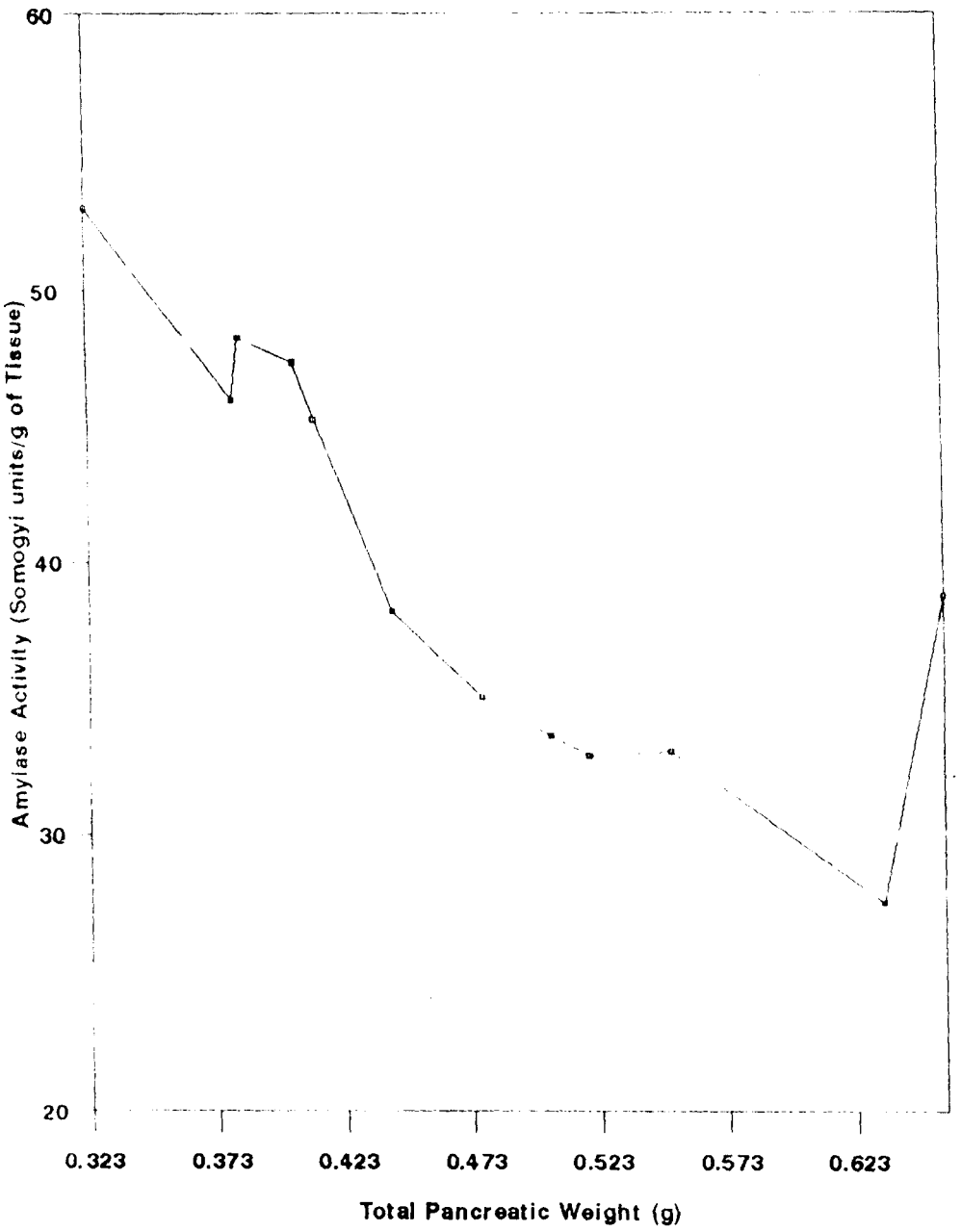


Fig.1.2 **CORRELATION BETWEEN AMYLASE ACTIVITY AND PANCREATIC WEIGHT IN FEMALE JAPANESE QUAILS**



tissue (Table 3.3 and Fig.1.3). The value was significantly higher in males ($P \leq 0.05$). The mean gall bladder weight in males was 0.042 ± 0.006 g and that in females was 0.044 ± 0.009 g (Table 3.3). The difference in gall bladder weight between the two sexes was non significant. The pooled mean value for amylase activity per gram of tissue and the gall bladder weight, when both the sexes were taken together, were 121.93 ± 16.86 S U/g of tissue and 0.043 ± 0.005 g respectively (Table 3.3).

4.1.4 Small intestine

The mean amylase activity of intestinal mucous membrane of adult male Japanese quails was 234.38 ± 38.96 SU/g of tissue and in female quails it was 103.23 ± 8.08 SU/g of tissue (Table 3.4 and Fig.1.3). The influence of sex on amylase activity of small intestinal mucous membrane was highly significant ($P \leq 0.01$). Male birds exhibited higher activity than the female birds. The mean intestinal amylase activity for both male and female adult Japanese quails put together was 168.81 ± 23.78 SU/g of tissue (Table 3.5 and Fig.1.3).

Table 3.3 Amylase activity of bile from gall bladder and weight of gall bladder in eight-week old Japanese quails

Sl.No.	Amylase activity (Somogyi units/g of tissue)		Gall bladder (weight in g)	
	Males	Females	Males	Females
1.	108.89	60.00	0.063	0.020
2.	162.27	74.29	0.044	0.007
3.	297.62	73.69	0.021	0.084
4.	167.59	26.89	0.029	0.045
5.	214.17	35.10	0.024	0.049
6.	76.14	92.30	0.057	0.061
7.	309.60	117.65	0.025	0.017
8.	97.60	140.23	0.050	0.044
9.	40.00	175.26	0.046	0.019
10.	55.81	159.47	0.031	0.019
11.	267.73	53.63	0.022	0.113
12.	77.05	43.33	0.088	0.048
Mean±S.E.[1]	156.23± 27.72	87.65± 14.36	0.042± 0.006	0.044± 0.009
Mean±S.E.[2]	121.93±16.86		0.043±0.005	
t value	2.1965*		0.2054	

1. Mean of 12 birds
2. Mean of 24 birds (12 males and 12 females)
* $P \leq 0.05$

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Table 3.4 Amylase activity of mucous membrane of small intestine of eight-week old Japanese quails

Sl.No.	Amylase activity (Somogyi units/g of tissue)	
	Males	Females
1.	240.45	103.87
2.	89.09	82.78
3.	331.48	157.53
4.	468.00	109.65
5.	157.32	98.97
6.	120.00	72.43
7.	259.09	78.57
8.	167.35	143.96
9.	115.56	87.28
10.	204.26	104.88
11.	160.00	128.57
12.	500.00	70.31
Mean±S.E.[1]	234.38±38.96	103.23±8.08
Mean±S.E.[2]	168.81±23.78	
t value	3.2958**	

1. Mean of 12 birds
2. Mean of 24 birds (12 males and 12 females)

** $P \leq 0.01$

4.1.5 Comparison of amylase activity in different regions of digestive system

The mean amylase activity in different regions of digestive tract like crop, pancreas, bile and intestine of adult Japanese quails were 30.62 ± 6.65 S U/g of tissue, 49.30 ± 3.03 S U/g of tissue, 121.93 ± 16.86 S U/g of tissue and 168.81 ± 23.78 S U/g of tissue respectively (Table 3.5 and Fig.1.3). The highest amylase activity was observed in the intestinal mucous membrane and the lowest in the crop mucosa. It was also observed that the highest amylase activity in different regions of digestive tract was exhibited by the male birds than the female birds (Table 3.5).

4.2 Protease activity in the proventriculus of Japanese quail

The mean protease activity of proventricular mucous membrane of male quails was 185.67 ± 11.64 PU/g of tissue and that of female quails was 223.31 ± 38.91 PU/g of tissue (Table 4). The data on protease activity of proventricular mucous membrane of both male and female birds were subjected to 't' test and found that the difference between sexes was non significant with the 't' value as 1.0675 (Table 4). Though not statistically significant, female birds exhibited a higher proventricular protease activity. The pooled mean

Table 3.5 Amylase activity in different regions of digestive tract of eight-week old Japanese quails

Birds	Amylase activity (Somogyi units/g of tissue)			
	Crop	Pancreas	Small intestine	Gall bladder Bile
	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.
Males[1]	47.98± 10.49	59.89± 3.36	234.38± 38.96	156.23± 27.72
Females[2]	13.26± 4.46	38.72± 2.59	103.23± 8.08	87.65± 14.36
Overall[3]	30.62± 6.65	49.30± 3.03	168.81± 23.78	121.93± 16.86

1&2 Average of 12 birds
3 Average of 24 birds (12 males and 12 females)

Fig.1.3 AMYLASE ACTIVITY OF CROP, PANCREAS, BILE AND INTESTINE OF JAPANESE QUAILS

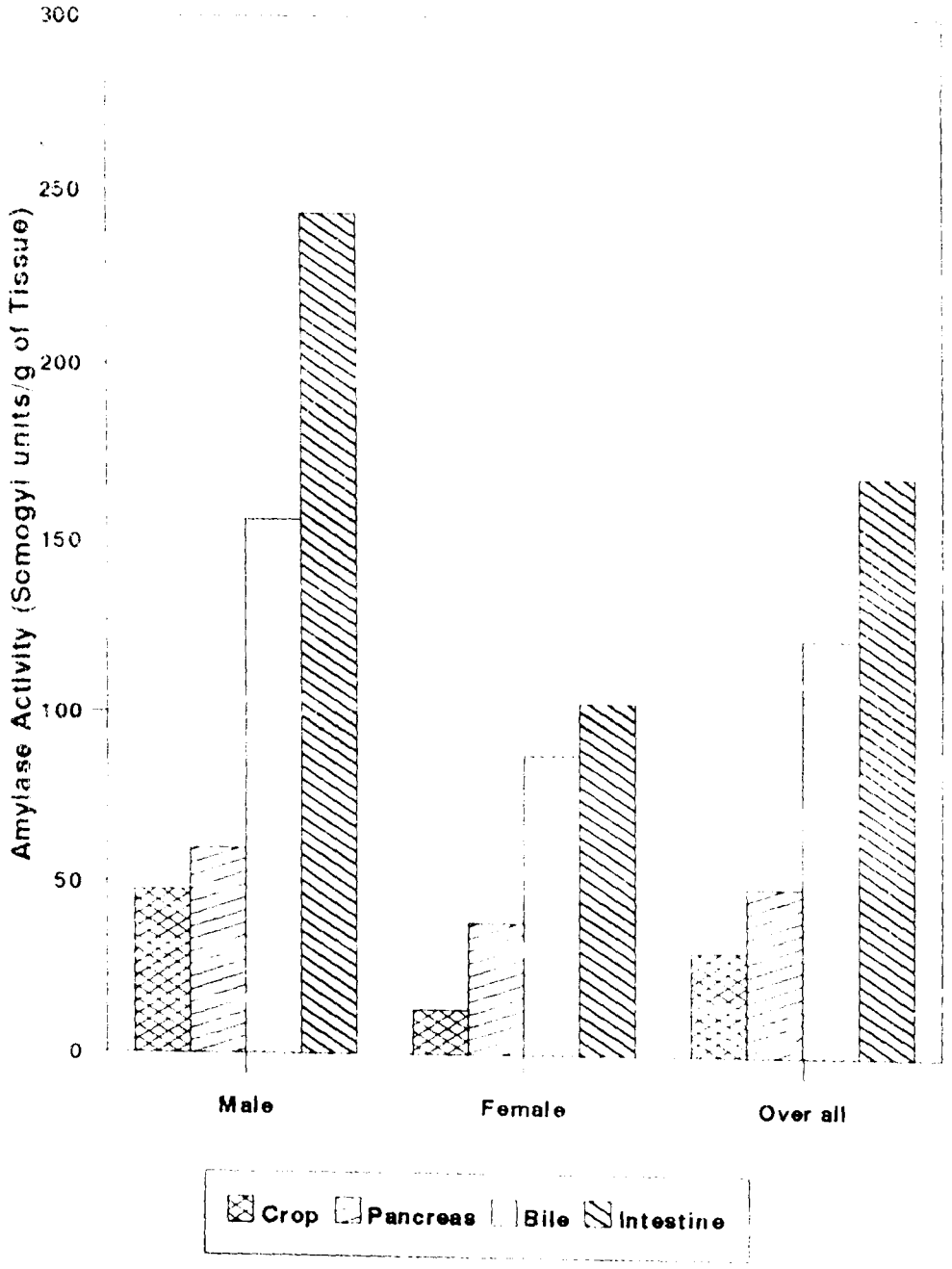


Table 4. Protease activity of proventricular mucous membrane of eight-week old Japanese quails

Sl.No.	Protease activity (Pepsin units/g of tissue)	
	Males	Females
1.	229.67	83.14
2.	144.00	241.04
3.	231.51	223.21
4.	212.70	504.04
5.	122.08	311.81
6.	210.99	249.38
7.	163.40	284.87
8.	114.08	83.72
9.	186.05	120.69
10.	203.53	145.16
11.	207.01	241.81
12.	203.05	185.87
Mean±S.E.[1]	185.67±11.64	223.31±38.91
Mean±S.E.[2]	204.49±17.97	
t value	1.0675	

1. Mean of 12 birds
2. Mean of 24 birds (12 males and 12 females)

value for protease activity of proventricular mucous membrane of adult Japanese quails of both sexes was 204.49 ± 17.97 PU/g of tissue (Table 4).

4.3 Lipase activity

4.3.1 Pancreas

The mean lipase activity of pancreas of adult (eight-week old) males was 73.37 ± 7.78 LU/g of tissue and that of females was 38.40 ± 3.39 LU/g of tissue (Table 5.1 and Fig.2.3). There was significant difference ($P \leq 0.01$) in the lipase activity of pancreas, the males having higher activity than females. The mean pancreatic weight of adult male Japanese quails was 0.315 ± 0.017 g which was significantly lower ($P \leq 0.01$) than that in female quails where the value was 0.428 ± 0.023 g (Table 5.1). In male birds the lipase activity per gram of pancreatic tissue was significantly ($P \leq 0.01$) negatively correlated to the total pancreatic weight (Table 6 and Fig.2.1), with a correlation of -0.882. However, in female birds, though there existed a negative correlation between the pancreatic weight and lipase activity, it was found to be non significant and the value was -0.499 (Table 6 and Fig.2.2). The pooled mean value for pancreatic weight and the lipase activity of adult quail of both sexes were 0.371 ±

Table 5.1 Lipase activity and weight of pancreas in eight-week old Japanese quails

Sl.No.	Lipase activity (Lipase units/g of tissue)		Pancreas (weight in g)	
	Males	Females	Males	Females
1.	54.54	36.61	0.339	0.396
2.	72.43	66.96	0.335	0.347
3.	59.92	43.67	0.342	0.401
4.	108.98	41.29	0.232	0.436
5.	35.00	24.30	0.450	0.535
6.	102.90	37.84	0.279	0.278
7.	53.51	26.06	0.322	0.489
8.	102.00	28.58	0.226	0.560
9.	103.05	40.64	0.272	0.381
10.	34.48	43.97	0.355	0.500
11.	86.85	44.25	0.308	0.418
12.	66.82	26.57	0.318	0.400
Mean±S.E.[1]	73.37± 7.78	38.40± 3.39	0.315± 0.017	0.428± 0.023
Mean±S.E.[2]	55.88±5.82		0.371±0.018	
t value	4.1243**		3.8875**	

1. Mean of 12 birds
2. Mean of 24 birds (12 males and 12 females)

** $P \leq 0.01$

Fig.2.1 CORRELATION BETWEEN LIPASE ACTIVITY AND PANCREATIC WEIGHT IN MALE JAPANESE QUAILS

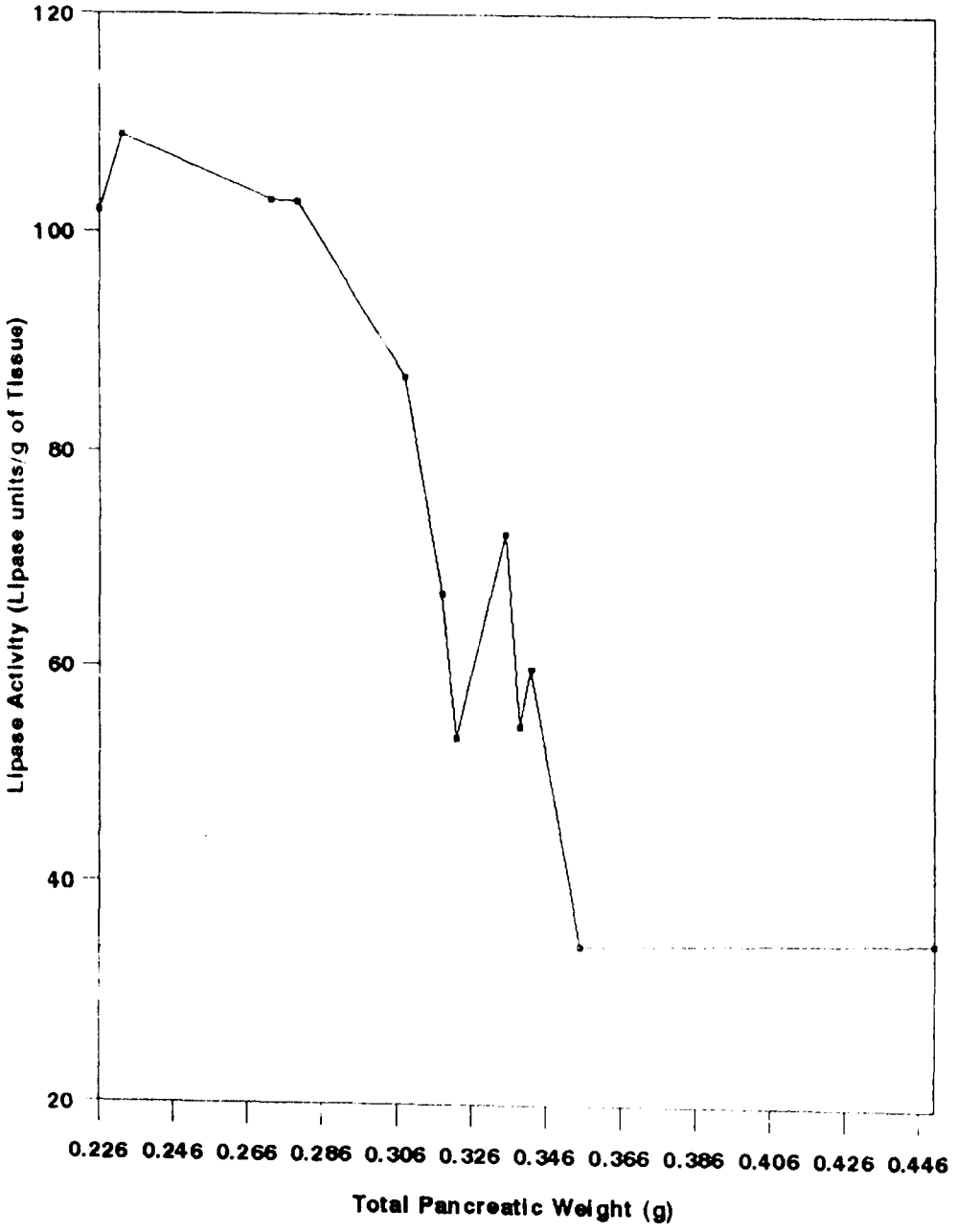
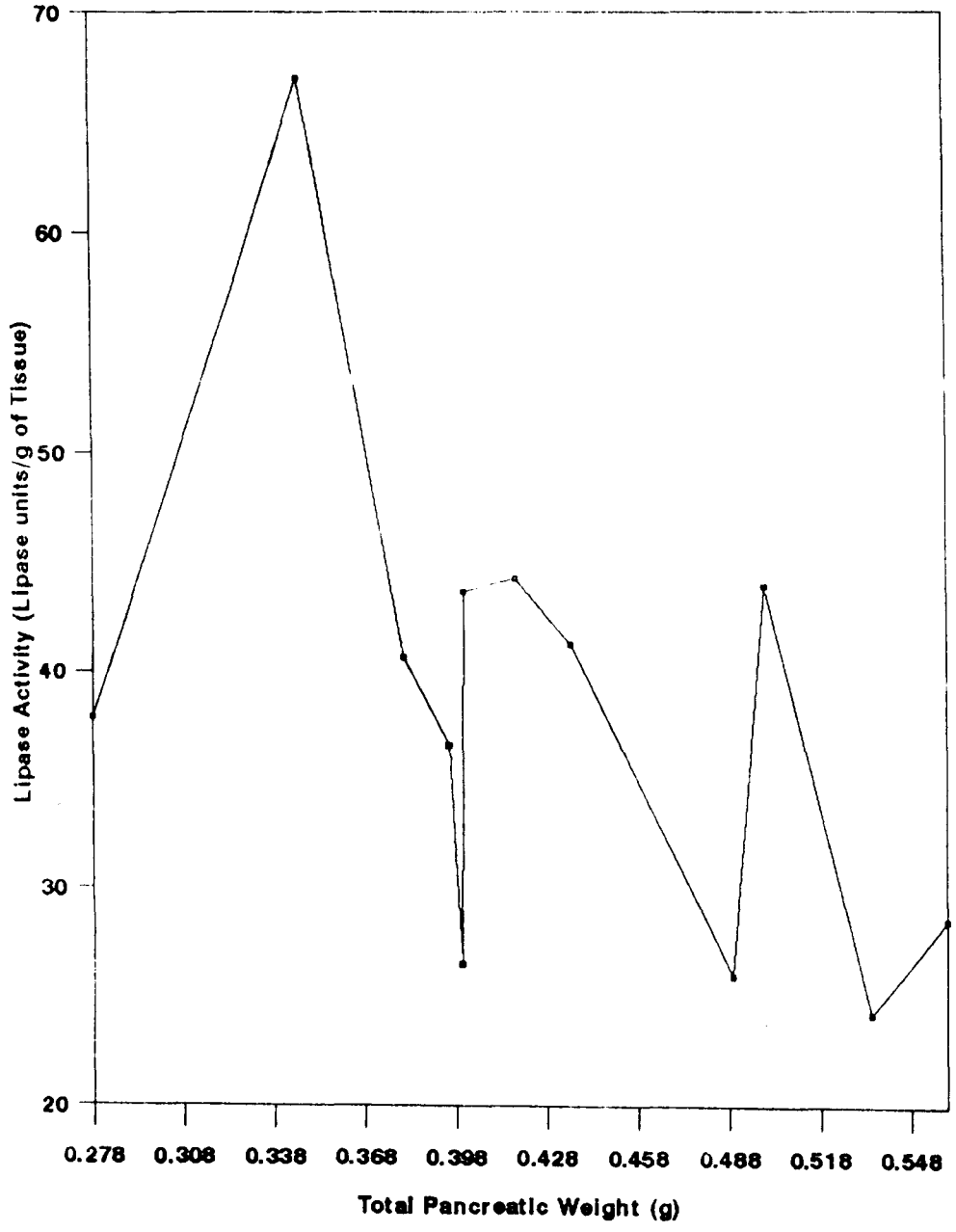


Fig.2.2 CORRELATION BETWEEN LIPASE ACTIVITY AND PANCREATIC WEIGHT IN FEMALE JAPANESE QUAILS



0.018 g and 55.88 ± 5.52 LU/g of tissue respectively (Table 5.1 and Fig.2.3).

4.3.2 Small intestine

Intestinal mucous membrane of adult male Japanese quails showed a mean lipase activity of 38.80 ± 10.93 LU/g of tissue, which was significantly higher ($P \leq 0.05$) than that of female quails, where the value was 12.44 ± 4.15 LU/g of tissue (Table 5.2 and Fig.2.3). The mean lipase activity of intestinal mucous membrane of both male and female birds put together was 25.62 ± 6.34 LU/g of tissue (Table 5.2 and Fig.2.3).

4.3.3 Comparison of lipase activity in different regions of digestive system

The mean lipase activity of pancreas and intestinal mucous membrane of adult (eight-week old) Japanese quails were 55.88 ± 5.52 LU/g of tissue and 25.62 ± 6.34 LU/g of tissue respectively (Table 5.3 and Fig.2.3), indicating a significant ($P \leq 0.01$) higher lipase activity in the pancreatic tissue. Lipase activity of pancreas and intestinal mucous membrane was highest in the male birds than in the female quails (Table 5.3 and Fig.2.3). The highest lipase activity was noticed in the male quails and that too was in the pancreatic tissue.

Table 5.2 Lipase activity of mucous membrane of intestine of eight-week old Japanese quails

Sl.No.	Lipase activity (Lipase units/g of tissue)	
	Males	Females
1.	11.79	3.66
2.	12.22	12.51
3.	12.89	18.56
4.	8.43	54.90
5.	6.74	4.82
6.	89.16	8.35
7.	70.15	14.74
8.	81.97	12.35
9.	68.97	1.37
10.	5.76	9.23
11.	3.34	1.63
12.	94.18	7.13
Mean±S.E.[1]	38.80±10.93	12.44±4.15
Mean±S.E.[2]	25.62±6.34	
t value	2.2549*	

1. Mean of 12 birds
2. Mean of 24 birds (12 males and 12 females)
* $P \leq 0.05$

Table 5.3 Lipase activity in different regions of the digestive tract of eight-week old Japanese quails

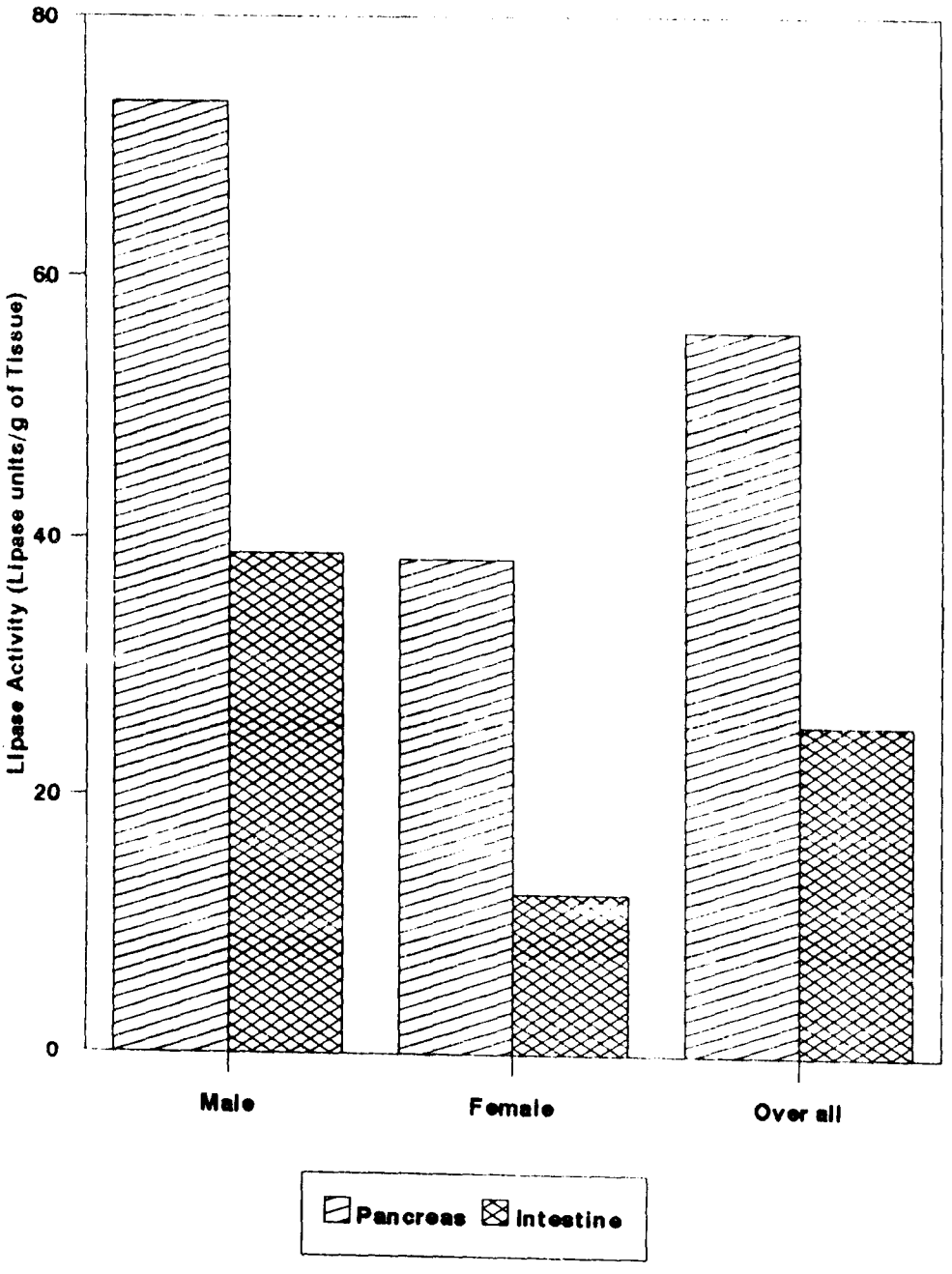
Birds	Lipase activity (Lipase units/g of tissue)	
	Pancreas Mean±S.E.	Small intestine Mean±S.E.
Males	73.37±7.78	38.80±10.93
Females	38.40±3.39	12.44±4.15
Overall	55.88±5.52	25.62±6.34

Table 6. Correlation between pancreatic weight and activity of enzymes (amylase and lipase)

Enzymes	Correlation in males	Correlation in females
Amylase	-0.975**	-0.968**
Lipase	-0.882**	-0.499

** $P \leq 0.01$

Fig.2.3 LIPASE ACTIVITY OF PANCREAS AND INTESTINE OF JAPANESE QUAILS



4.4 pH in the different regions of digestive tract of Japanese quail

The pH in the different regions of the gastro intestinal tract of adult Japanese quails were recorded as, crop: 5.0 ± 0.26, proventriculus: 4.3 ± 0.11, gizzard: 3.5 ± 0.17 and small intestine (duodenum): 6.5 ± 0.00 (Table 7 and Fig.3). Among the recorded values the lowest pH was observed in the gizzard (3.5 ± 0.17) and the highest pH in the small intestine (6.5 ± 0.00).

4.5 Feed passage rate (FPR)

The mean value of feed passage rate (FPR) recorded in adult (eight-week old)male Japanese quails was 120.5 ± 10.88 min. and in females it was 92.4 ± 10.65 min. (Table 8). There was no significant difference in feed passage rate of male and female birds, when the 't' test was conducted (t value being 1.8432). The average value for both males and females put together was 106.5 ± 8.00 min. (Table 8).

In this experiment, it was also observed that the Japanese quails (both males and females) maintained on identical ration as well as managemental conditions, the male quails preferred coarse particles of maize from the feed where as the females preferred the finer particles of oil cakes.

Table 7. pH of different regions of digestive tract of eight-week old Japanese quails

Sl.No.	Crop	Proventriculus	Gizzard	Duodenum
1.	4.5	4.5	3.5	6.5
2.	6.5	3.5	2.5	6.5
3.	6.5	4.5	3.5	6.5
4.	4.5	3.5	2.5	6.5
5.	4.5	4.5	3.5	6.5
6.	6.5	4.5	4.5	6.5
7.	4.5	4.5	3.5	6.5
8.	4.5	4.5	3.5	6.5
9.	4.5	4.5	3.5	6.5
10.	4.5	4.5	3.5	6.5
11.	4.5	4.5	3.5	6.5
12.	4.5	4.5	3.5	6.5
Mean±S.E.	5.0±0.26	4.3±0.11	3.5±0.17	6.5±0.00

Fig.3 pH OF DIFFERENT REGIONS OF G.I.TRACT OF ADULT JAPANESE QUAILS

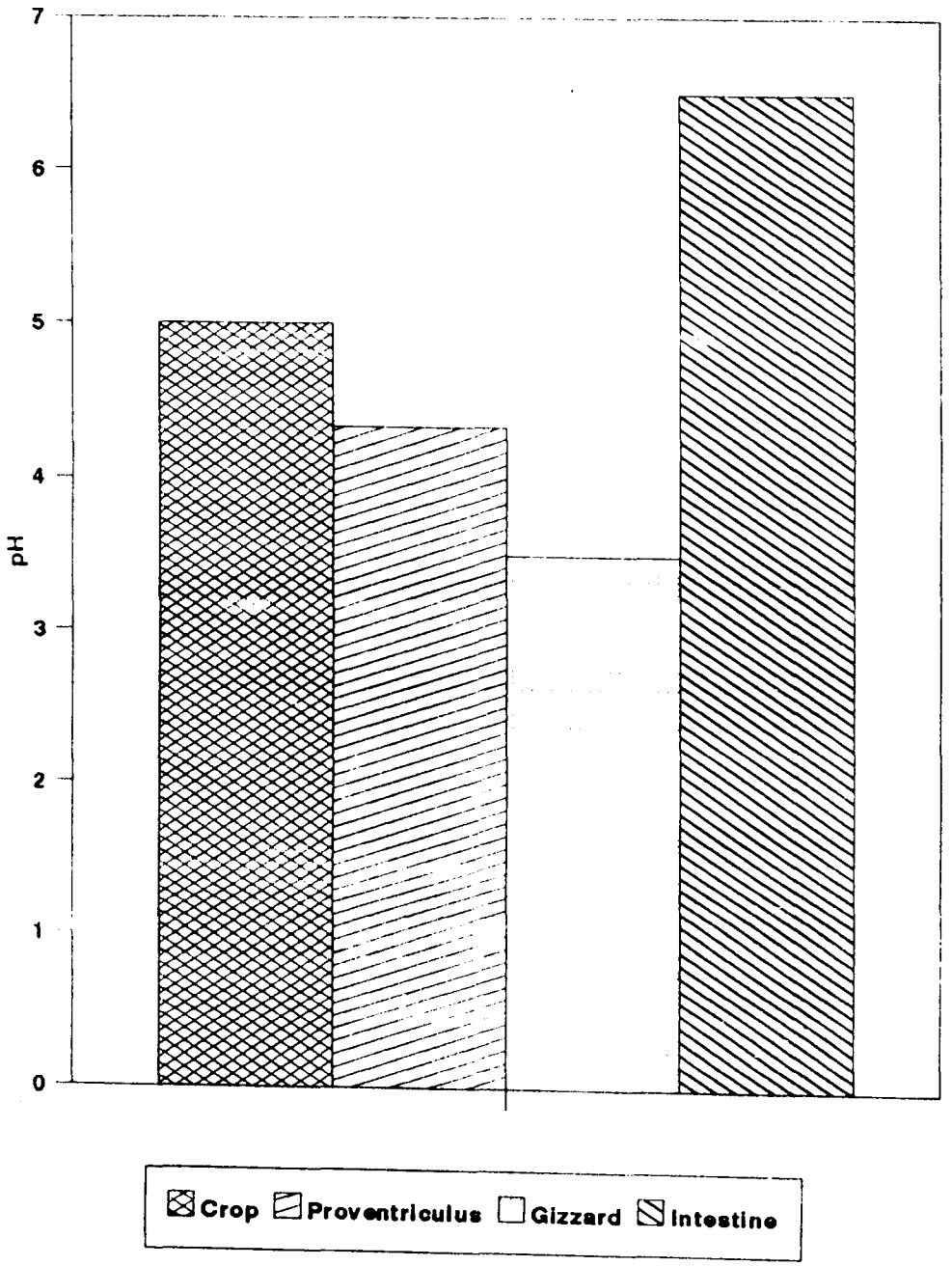


Table 8. Feed passage rate in eight-week old Japanese quails

Sl.No.	Rate of passage in minutes	
	Males	Females
1.	125	110
2.	117	104
3.	177	93
4.	133	77
5.	176	76
6.	135	55
7.	161	89
8.	105	103
9.	65	87
10.	89	68
11.	83	194
12.	80	53
Mean±S.E.[1]	120.5±10.88	92.4±10.65
Mean±S.E.[2]	106.5±8	
t value	1.8432	

1. Mean of 12 birds
2. Mean of 24 birds (12 males and 12 females)

Discussion

Chapter 5

DISCUSSION

5.1 Amylase activity

5.1.1 Crop

The functional significance of crop in birds is poorly understood and is still a controversial subject. The presence of a fairly active amylase in saliva suggests the possibility of amylolytic activity in the crop, as food remains for considerable time in this organ. In the present study, the quantification of amylase activity of the mucous membrane of crop of adult (eight-week old) male and female Japanese quails revealed considerable amylolytic activity in the crop. Male birds exhibited a significantly ($P \leq 0.01$) higher amylase activity (47.98 ± 10.49 S U/g of tissue) than the female birds (13.26 ± 4.46 S U/g of tissue) vide ref Table 3.1. The presence of amylase in the crop of domestic fowl had been reported by Farner (1960), Bolton (1965), Ziswiler and Farner (1972), Nitsan and Madar (1978) and Rodeheaver and Wyatt (1986). Though the presence of serous cells in the crop glands of pigeons had been reported by Ziswiler and Farner (1972), similar reports in any other avian species were not available. Sadanandan (1968) could detect only mucous secreting cells on the crop mucosa of ducks and Maya (1995)

could not detect any serous cells in the crop mucous membrane of Japanese quails. However in the present study, significant activity of amylase (30.62 ± 6.65 S U/g of tissue) was observed in the crop mucosa of Japanese quail (Table 3.5) suggestive of appreciable amount of starch digestion occurring in the crop. Though Bolton (1965) and Hill (1965) pointed out the major role played by bacterial and plant amylases in the amylolytic activity in the crop, the significant difference in the amylase activity of crop mucosa of both sexes reared on identical rations and managemental conditions in the present study indicated that the major source of this amylase was from the bird itself (either from crop or saliva). However, in the absence of serous secreting cells in the crop mucous membrane (Maya, 1995) the major source of amylase could be from saliva only.

As early as in 1940, Leasure and Link reported the occurrence of amylase in the chicken saliva. Farner (1960) also reported the occurrence of salivary amylase in different avian species and opined that this enzyme could be of functional importance only in the crop since food remains in the crop for a considerable duration. Rodeheaver and Wyatt (1986) reported the limited synthesis and secretion of alpha amylase into the oral cavity of broilers. Maya (1995) could detect the presence of serous elements in the salivary glands of Japanese quails. It can be concluded that a major portion

of crop amylase is contributed by the salivary glands and an appreciable amount of starch digestion might be undergoing in the crop.

No reports have been found supporting the influence of sex on the amylase activity of crop mucosa of birds. But in the present study, it was observed that male birds preferred carbohydrate rich maize particles than the female birds and the higher amylase activity of crop mucosa of males may be a natural response to the increased intake of the substrate.

In this study, it was also noticed that the amylase activity within the crop was not at all influenced by the presence of food in the crop. Ritz *et al.* (1995a) were also of the opinion that amylase activity of the crop was not influenced by the presence of food in the crop. These observations also supported the view that the origin of amylase was from a source other than the crop mucous membrane, probably from the salivary glands themselves.

5.1.2 Pancreas

The mean amylase activity of pancreas in adult male Japanese quails was 59.89 ± 3.36 S U/g of tissue and in female quails it was 38.72 ± 2.59 S U/g of tissue (Table 3.2). There existed a statistically significant difference in the amylase activity of male and female birds, the male birds exhibited

higher amylase activity of pancreas as noted in the crop. Available literature indicated that such an experiment had not been previously conducted in poultry. In the present study the intake of carbohydrate rich dietary particles was greater in males than in females. So the pancreas of male birds secreted more amylase for the effective utilisation of the substrate. Pancreas was the primary source of amylase in poultry (Moran, 1982). So the difference in amylase activity between males and females was more significant in this tissue. Hulan and Bird (1972) reported that avian pancreas adapted not only to dietary regimen, but also to the predominance of the particular substrate in the diet on which the enzyme acted. They also found that whenever a greater amount of carbohydrate was ingested, the amylase output per milligram of protein secreted by the pancreas was also increased. Johnson *et al.* (1977) observed a two or three fold increase in the synthesis of pancreatic amylase in rats fed a high carbohydrate diet (i.e. 64% sucrose). Forman and Schneeman (1980) reported that a high level of corn oil in the ration suppressed the amylase activity in the pancreas of rats. According to Moran (1985) the variation in the level of dietary starch in poultry resulted in the concomitant alteration of alpha amylase activity of pancreas. However, Ritz *et al.* (1995a) was of the opinion that pancreatic amylase activity was not consistently affected by the diet.

In the present study, a significant negative correlation ($P \leq 0.01$) was revealed between the amylase activity per gram of pancreatic tissue and total pancreatic weight in both sexes of quails (Table 6). The pancreatic weight of male and female birds were 0.325 ± 0.018 g and 0.474 ± 0.030 g respectively. This difference was related to the difference in their body weight i.e., female birds were heavier than the male birds. Rodeheaver and Wyatt (1986) observed that sonication of the pancreatic homogenates increased the alpha amylase activity of pancreatic tissue 35 fold over unsonicated homogenates. So they suggested that enzyme was synthesized and stored intracellularly in the pancreas and subsequently secreted into the duodenum for digestion. The present study was conducted in unsonicated homogenates. Simple homogenisation might not have liberated the intracellularly accumulated enzyme completely. So it can be inferred that pancreatic weight was mainly contributed by the intracellularly accumulated enzyme.

5.1.3 Bile

The amylase activity of bile from gall bladder of male birds was 156.23 ± 27.72 S U/g of tissue and that of female birds was 87.65 ± 14.36 S U/g of tissue (Table 3.3). The significantly higher value ($P \leq 0.05$) observed in males was due to the increased intake of carbohydrates by the male birds. There was no significant difference in gall bladder weight of

male and female birds. The pooled mean value of amylase activity of gall bladder bile of adult Japanese quails was 121.93 ± 16.86 S U/g of tissue. Though the presence of amylase activity in gall bladder bile of fowl was reported by Sturkie (1954), Farner (1960) and Rodeheaver and Wyatt (1986), no such reports were available in Japanese quail. According to Ariyoshi *et al.* (1964) the relatively high, digestibility of starch in depancreatized chickens might be related to the amylase contributed by the bile to the intestine. Sadanandan (1968) reported the presence of amylase in the gall bladder bile of ducks. However, Rodeheaver and Wyatt (1986) observed that biliary amylase did not represent a significant reservoir of alpha amylase activity for the starch digestion in broilers.

5.1.4 Small intestine

The amylase activity of the mucous membrane of small intestine of Japanese quails was quantified and found to be 234.38 ± 38.96 S U/g of tissue in males and 103.23 ± 8.08 S U/g of tissue in females (Table 3.4). The pooled value for amylase activity of intestinal mucous membrane of adult males and females was 168.81 ± 23.78 S U/g of tissue (Table 3.5). Small intestine recorded the highest degree of amylase activity in both sexes, indicating that small intestine was the major site of amylolytic digestion in quails. Sadanandan

(1968) detected the presence of amylase activity in the small intestinal segments of ducks. In domestic fowl, the occurrence of an intestinal amylase was reported by Hill (1971), Osman (1982) and Rideau *et al.* (1983). According to Ariyoshi *et al.* (1963) even the depancreatized chicken could utilize 72 per cent of ingested starch indicating the significant role of intestinal tract in amylolytic digestion. Pisharody (1970) quantified the amylase activity of small intestine of chicks as 48.75 ± 6.06 S U/g of tissue and in ducklings as 70.27 ± 14.04 S U/g of tissue. In the present study, the pooled mean value of amylase activity of small intestinal mucosa of male and female quails was 168.81 ± 23.78 S U/g of tissue and this was comparatively higher than those reported in chicks and ducklings. This difference may be due to the difference in species.

5.1.5 Comparison of amylase activity in different regions of digestive system

The mean amylase activity in different regions of digestive tract like crop, pancreas, gall bladder bile and intestine of adult japanese quails were 30.62 ± 6.65 S U/g of tissue, 49.30 ± 3.03 S U/g of tissue, 121.93 ± 16.86 S U/g of tissue and 168.81 ± 23.78 S U/g of tissue respectively (Table 3.5). The highest amylase activity was observed in the small intestine (duodenum) and the lowest in the crop

(Table 3.5 and Fig.1.3). Though the occurrence of amylase in the crop had been reported by many workers they were of the opinion that crop had relatively a minor role in the chemical digestion of carbohydrates. Hans Fisher and Weiss (1957) were of the opinion that extirpation of crop in chicken did not interfere with the normal development or production. According to Turk (1982) crop was primarily a storage organ. The comparatively low amylase activity observed in the crop mucosa of Japanese quail was suggestive of a relatively minor role for the crop in starch digestion.

The pancreatic amylase activity in Japanese quail was comparatively lower than that of the small intestine (Fig.1.3). But Osman (1982) and Rodeheaver and Wyatt (1986) have reported that pancreas exhibited the highest degree of amylase activity in chicken. Rodeheaver and Wyatt (1986) observed that sonication of the pancreatic homogenates, significantly increased the apparent activity of alpha amylase 35 fold over unsonicated homogenates. In the present study, homogenisation might not have liberated all the intracellularly accumulated enzymes. The existence of an inverse relationship between the total pancreatic weight and amylase activity per gram of pancreatic tissue also supported this view. Major portion of pancreatic weight might be contributed by the intracellularly accumulated enzyme.

The bile from gall bladder showed a higher amylase activity when compared to pancreas and crop in Japanese quails. Therefore, a major portion of amylase activity in the intestine must be contributed by the gall bladder bile. This finding is in agreement with the suggestions of Ariyoshi *et al.* (1964). According to them, the relatively high digestibility of starch in pancreatectomised chickens was related to the contribution of amylase from the intestinal mucosa and bile.

Perusal of literature available revealed that in birds pancreas was the site of highest concentration of amylase. But in the present investigation, high amylolytic activity was seen in the small intestine which could be due to the combined enzymic activity of pancreatic, biliary and intestinal amylolytic enzymes. However, Farner (1960) was of the opinion that over and above the above enzymatic activity, the amylase produced from microbial and tissue sources may be contributory to a great extent in making the small intestine as the major site of amylolytic activity. Therefore the highest amylase activity of intestine observed in the present study might be mainly due to the enzymes contributed by pancreas, bile and intestine itself.

The increase in the level of amylase activity noticed in different tissues of digestive tract of male quails may be a

normal response, to the increased intake of carbohydrate rich diet as observed in male quails in the present experiment.

5.2 Protease activity

5.2.1 Proventriculus

The protease activity of proventricular mucous membrane of male and female Japanese quails was found to be 185.67 ± 11.64 P U/g of tissue and 223.31 ± 38.91 P U/g of tissue respectively (Table 4). The presence of acid proteolytic enzyme pepsin in the proventricular mucous membrane of birds had been reported by Farner (1960), Toner (1963) and Sadanandan (1968). Pisharody (1970) quantified the protease present in the mucosal extracts of proventriculus and found that chicks had a proteolytic activity of 403.63 ± 75.11 P U/g of tissue and ducklings had 325.17 ± 63.42 P U/g of tissue. In the present study, the mean proteolytic activity of proventriculus of adult, eight-week old birds (when males and females put together) was 204.49 ± 17.97 P U/g of tissue which was considerably lower than that reported for ducklings and chicks by Pisharody (1970). This difference may be due to their species difference. In this study, the difference in protease activity of male and female birds was found to be non significant. Reports available on the factors influencing the protease activity of proventriculus were meagre. Long (1967)

was of the opinion that the proventricular secretion was continuous even in a starved fowl. Hill (1971) also supported this continuous secretary activity of proventricular glands irrespective of the presence or absence of food.

5.3 Lipase activity

5.3.1 Pancreas

Pancreatic lipase activity was found to be 73.37 ± 7.78 L U/g of tissue in the male birds and 38.40 ± 3.39 L U/g of tissue in the female birds (Table 5.1). The lipolytic activity of pancreas in ducks had been reported by Sadanandan (1968) and in domestic fowl by Hill (1971) and Sturkie (1976). But information in the available literature did not reveal any influence of sex on the quantum of pancreatic lipase activity in any of the species in poultry.

A significant ($P \leq 0.01$) negative correlation was observed between the pancreatic weight and lipase activity per gram of pancreatic tissue in males (Table 6 and Fig.2.1). In female quails also, eventhough statistically non-significant, a negative correlation could be observed between the pancreatic weight and lipase activity (Table 6 and Fig.2.2). As Rødeheaver and Wyatt (1986) pointed out in the case of pancreatic amylase, homogenisation might not have completely liberated all the intracellularly accumulated lipase too.

5.3.2 Small intestine

The extracts of intestinal mucous membrane of adult male and female Japanese quails showed a mean lipase activity of 38.80 ± 10.93 L U/g of tissue and 12.44 ± 4.15 L U/g of tissue respectively (Table 5.2). The value in males was significantly ($P \leq 0.05$) higher. No reports were available explaining the influence of sex on lipase activity of digestive system in avian species. But the lipolytic activity of intestine had been reported by Laws and Moore (1963) in chicks. Hill (1965) was of the opinion that lipase activity in the intestine of birds was mainly derived from pancreatic juice. In the present study the pooled mean lipase activity of adult Japanese quails was 25.62 ± 6.34 L U/g of tissue which was low in comparison to the reported values in chicks (31.19 ± 6.28 L U/g of tissue) and in ducklings (105.43 ± 39.18 L U/g of tissue) by Pisharody (1970), who also observed a decrease in lipase activity of intestinal mucosa of ducklings consuming a high fat diet. In the present study also female quails consuming finer particles of feed rich in fat had a lower lipase activity. This may be due to the adjustment of the system to reduce the hydrolysis of excess lipids in the tubular digestive tract as a result of increased consumption. Since fat is the high energy yielding nutrient, the quantity in excess present in the diet is prevented from hydrolysis by the reduced lipase secretion in the females.

5.3.3 Comparison of lipase activity in different regions of digestive system

The mean lipase activity of pancreas and intestinal mucous membrane of adult Japanese quails were 55.88 ± 5.52 L U/g of tissue and 25.62 ± 6.34 L U/g of tissue respectively indicating a higher lipase activity in pancreatic tissue. It can be inferred that pancreas could be the richest source of lipase enzyme in the digestive system in Japanese quail. Ariyoshi *et al.* (1964) reported that depancreatized chicken could digest fat only upto a level of 25 per cent and the lipase content of droppings of a depancreatized chicken was negligible. Hill (1965) also reported that lipase activity in the intestine of bird was mainly derived from pancreatic juice. Sadanandan (1968) could detect lipase activity in the small intestinal mucosa of ducks. It can also be stated from the results of this study that the role of intestinal lipase in the lipid digestion in Japanese quail is relatively low in comparison to that of the lipase derived from pancreas. So pancreas can be considered to be the major source of lipase in quails.

5.4 pH in the different regions of digestive tract

Digestion of the major food stuffs is an orderly process involving the action of a large number of digestive enzymes

which require optimum pH for their action. The recorded mean pH values of contents of different regions of digestive tract such as crop, proventriculus, gizzard and intestine of adult Japanese quails were 5.00 ± 0.26, 4.30 ± 0.11, 3.50 ± 0.17 and 6.50 ± 0.00 respectively (Table 7).

The pH of the crop content was 5.00 ± 0.26, which was slightly lesser than the value reported for ducks (5.90) by Sadanandan (1968). According to him this pH did not preclude the action of salivary amylase in the crop. Ziswiler and Farner (1972) observed the pH range of crop in domestic fowl as 4.5 to 6.7 which favoured the activity of plant and microbial amylases. According to them any chemical digestion in the crop would precede the important physical digestive processes in the gizzard and that would certainly restrict chemical digestion in the crop to a relatively minor extent. So it can be concluded that the pH of crop favoured the amylolytic activity and the crop could be considered as the primary site of starch digestion in birds since little digestion could occur in the buccal cavity of birds due to the lack of sufficient maceration and the short duration for which the food remains in the mouth.

The pH value of the contents of proventriculus in Japanese quail was 4.30 ± 0.11 which was higher than the reported values in other avian species. Farner (1960) stated

that the pure gastric juice in birds must have a hydrogen ion concentration of 1 to 2. Sadanandan (1968) recorded the pH of proventricular contents of ducks as 3.3. Pisharody (1970) reported that in chicks and ducklings the optimum pH for pepsin activity were 2.0 and 2.4 respectively. The higher pH value observed in this study revealed that though proventricular mucosa was the site of secretion of acid proteolytic enzyme pepsin, its action was taking place at some other site, probably in the gizzard (Sturkie, 1976).

The pH of the gizzard contents of Japanese quails was recorded as 3.5 ± 0.17. Farner (1960) recorded the pH of the gizzard contents of domestic fowl, turkey, pigeon and ducks as 2.77, 2.19, 2.00 and 2.33 respectively. However, Sadanandan (1968) recorded a much higher pH (4.6) for gizzard contents in ducks. Mongin (1976) an evaluation of the effect of egg formation on pH of the gizzard contents, observed that the pH ranged from 4.87 to 3.54 at 2h to 14h after oviposition which gradually increased to 4.37 at about 22 h after oviposition. The comparatively lower pH on the gizzard contents than that observed in proventriculus of Japanese quail indicated that though proventriculus was the site of secretion of pepsin, proteolytic activity of pepsin was undergoing in the gizzard as the pH was more acidic and powerful mixing and churning contractions occur in the gizzard.

The pH observed in the duodenal contents of Japanese quails was 6.50. Sadanandan (1968) reported the mean pH values of the contents of duodenum, jejunum and ileum of ducks as 6.3, 7.3 and 7.3 respectively. Hurwitz and Bar (1968) were of the opinion that the pH of avian intestinal tract increased from the oral to the aboral end. According to Duke (1977) the pH range should be 6 to 8 for optimum digestive activity in the intestine. Laws and Moore (1963) pointed out that the digestive functions of pancreatic and intestinal enzymes occur in the small intestine at an optimum pH of 6 to 8. In the present study, comparatively higher pH (6.50) was observed in the duodenum than the crop, proventriculus and gizzard of Japanese quail. So small intestine can be considered as the major site of action of digestive enzymes of pancreas, small intestine and bile in this species.

5.5 Feed passage rate (FPR)

The feed passage rate (FPR) recorded as the first appearance of marker in male Japanese quails was 120.5 ± 10.88 min. and in the female quails was 92.4 ± 10.65 min. (Table 7). No significant difference due to sex was observed in FPR in Japanese quails. It was also observed that the time taken for the first appearance of excreta without marker was found to show wide variation as ranging from 249 min. to even after a day.

The FPR observed in the present study in adult Japanese quails was found to be lower than the reported values in other species of poultry. Kaupp and Ivey (1923) recorded food passage time in layers, non layers, broody hen and pullets as 3 h and 46 min, 8 h, 11 h and 44 min and 3 h and 52 min respectively. Hillerman *et al.* (1953) recorded the time taken for food passage through the alimentary canal of laying turkey hen as 3 h and 13 min and that for non layers as 4 h and 16 min. They also reported that in younger turkey the time taken was comparatively less as 2 h and 27 min, whereas in old turkey hens it was more 3 h and 52 min. According to Sturkie (1965) differences in experimental condition or methods could bring about variations in experimental results on the FPR values in chicken. Sadanandan (1968) determined the FPR in ducks on a standard ration using carmine dye as the indicator and the mean FPR value recorded was 2 h and 17 min. Pisharody (1970) reported the rate of passage of ingesta in chicks and ducklings as 163 ± 10.34 min and 108 ± 8.36 min respectively. The pooled mean value for FPR in male and female Japanese quails was 106.5 ± 8 min., which was more or less similar to the reported values in other species of birds by Sadanandan (1968) in ducks and Pisharody (1970) in chicks and ducklings.

No significant difference was noticed in the FPR between adult male and female Japanese quail. Wilson *et al.* (1980) had reported that sex had no influence on the feed passage

rate in Peking ducks. In this study, it was observed that the time for the first appearance of excreta without marker was found to show wide variation, from 249 min. to even after a day. Duffy *et al.* (1985) have made a similar observation in sea birds (Jackass Penguines) in an experiment using Cerium^{141} and Carmine red dye. Both markers started to appear in the droppings within 2 h of ingestion and were still being excreted after 24 h. Dunnington and Siegel (1995) reported that chicks from high weight line consumed more feed, utilized feed more efficiently and had a faster FPR than the low weight line chicks. Though not significant, the difference in FPR observed in male and female quails could there be due to the difference in their body weight.

Summary

Chapter 6

SUMMARY

Quantification of digestive enzymes, amylase, acid protease and lipase was conducted in the different regions of the digestive tract like crop, proventriculus, pancreas, gall bladder bile and small intestine of adult (eight-week old) male and female Japanese quails (*Coturnix coturnix japonica*). Besides the estimation of the digestive enzymes, pH of the contents of the different regions of digestive tract and the feed passage rate (FPR) were also measured in the birds.

A total number of 192, six-week old Japanese quails (96 males and 96 females) of the same strain (egg type) were selected at random from Kerala Agricultural University Poultry Farm, Mannuthy. The birds were reared under standard managemental conditions in cage system in the department of Physiology and Biochemistry, College of Veterinary and Animal Sciences, Mannuthy. Feed and water were provided *ad libitum*. At a time, 24 birds (12 males and 12 females) of the same strain and hatch were selected at random and reared in separate compartments of the cage for a period of two weeks in order to stabilize the experimental conditions. The subsequent batches of 24 birds were procured from the farm at weekly intervals and reared on identical standard quail layer ration. Each batch of 24 birds (12 males and 12 females) were

sacrificed by decapitation at eight weeks of age and the different regions of the digestive tract like crop, proventriculus, pancreas, gall bladder and small intestine were collected for estimation of different enzymes. Each enzyme in a particular region was quantified from a batch of 24 birds (12 males and 12 females) and a total of seven batches were utilised for studying the three enzymes (amylase, protease and lipase) in specific regions. The last batch of 24 birds was utilised for studying the feed passage rate (FPR). Twelve birds were selected at random from the last batch of 24 birds and utilised for the estimation of pH values in specific areas like crop, proventriculus, gizzard and small intestine.

The enzyme amylase was quantified in the mucous membrane of crop and small intestine, pancreas and bile from the gall bladder. Quantification of acid protease (pepsin) was conducted in the proventricular mucosa and lipase in the pancreas as well as small intestinal mucous membrane. The data obtained were statistically analysed.

The amylase activity of mucous membrane of crop and small intestine, pancreas and gall bladder bile of male Japanese quails showed significantly higher values. Among the various tissues studied in both sexes crop mucosa exhibited the lowest value while the small intestinal mucosa recorded the highest.

The amylase activity of gall bladder bile was greater than that of pancreas. The amylase activity of crop mucous membrane was not influenced by the presence of food in the crop. The pancreatic amylase activity and total pancreatic weight in both males and females were significantly negatively correlated, probably because homogenisation might not have liberated intracellularly accumulated enzyme completely and that pancreatic weight was mainly contributed by the intracellularly accumulated enzyme. The results of this study indicated that the initiation of starch digestion occurred in the crop itself with salivary amylase and digestion continued even in the small intestine and small intestine acted as the major site of amylolytic digestion utilising amylase of small intestine, pancrease and bile. It was also observed that male quails preferred coarser maize particles of the feed than the female quails eventhough both sexes were provided the identical ration. The higher amylase activity observed in the various tissues of male quails might be a normal response to the increased intake of carbohydrate (starch).

Quantification of acid protease (pepsin) from the proventricular mucosa of both sexes indicated that there was no statistically significant difference between the protease activity of male and female birds. The female quails preferred finer particles of protein rich oil cakes even if both sexes of quails were maintained on identical ration and

managemental conditions. Though not significant, slightly higher protease activity observed in female quails might be due to the increased intake of protein rich finer particles of oil cakes.

It was also observed from the experiment that when both sexes of quails were fed the identical ration the males had a higher lipase activity in both pancreas and small intestine than the female. This may be due to the fact that female birds preferred finer particles of oil cakes of the feed than the males. Since fat is the high energy yielding nutrient, the quantity in excess present in the diet was prevented from hydrolysis by the reduced lipase secretion in the females. In both sexes of quails the lipase activity was higher in pancreatic tissue than the small intestinal mucosa, indicating that the pancreas was the major source of lipase enzyme of digestive system of Japanese quail.

When the pH value of different regions of tubular digestive tract of Japanese quail were recorded, it was observed that the pH of crop (5.0 ± 0.26) favoured the hydrolysis of starch by salivary amylase. Though proventriculus was the site of production of pepsin, the comparatively higher pH (4.3 ±0.11) observed in proventriculus than the gizzard suggested that the proteolytic action of acid protease was not taking place in the proventriculus and a

better proteolytic digestion was taking place in the gizzard, where the pH was found to be 3.5 ± 0.17. The highest pH observed in the duodenum (6.5 ± 0.00) indicated that the optimum pH for the action of enzymes of pancreas, bile and small intestine was comparatively higher for eliciting their maximum action in the lumen of small intestine.

The mean values of feed passage rate (FPR) in adult male and female Japanese quails were 120.5 ± 10.88 min and 92.4 ± 10.65 min respectively, when the first appearance of coloured excreta was taken as the index. The time taken for the disappearance of the marker from the excreta was found to vary from 249 min to even more than a day. The difference between sexes in the FPR value was nonsignificant, indicating that sex had no influence on the feed passage rate in Japanese quails.

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QUANTIFICATION OF AMYLASE, LIPASE AND PROTEASE IN THE DIGESTIVE TRACT OF JAPANESE QUAIL

BY

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ABSTRACT OF A THESIS

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ABSTRACT

Japanese quails, being the most recently introduced species of poultry in India, are the least studied among the different domestic species of birds. For economic rearing of these valuable birds a thorough understanding of their basic digestive processes is essential. Keeping this point in view an attempt was made to study the digestive physiology of adult Japanese quails (*Coturnix coturnix japonica*) especially the quantification of digestive enzymes like amylase, protease (pepsin) and lipase of different regions of digestive tract, the determination of pH of the contents of crop, proventriculus, gizzard and small intestine and the feed passage rate (FPR).

One hundred and ninety two, six-week old Japanese quails of the same strain (egg type) were selected, at random, from the Kerala Agricultural University Poultry Farm, Mannuthy and maintained on standard, identical managemental and feeding conditions in cage system for a period of two weeks in order to stabilize the experimental conditions in the Department of Physiology and Biochemistry, College of Veterinary and Animal Sciences, Mannuthy. A batch of 24 birds (12 males and 12 females) of the same hatch were subjected for the quantification of the specific digestive enzyme in a particular region of the digestive system. Seven batches of

24 birds (a total number of 168 birds) were selected at weekly interval from the poultry farm for quantifying the different enzymes in specific regions. The eighth batch of 12 males and 12 females of the same hatch was utilized for studying the feed passage rate (FPR). From this last batch, 12 birds were randomly selected and utilized for estimation of pH of the contents of different regions (crop, proventriculus, gizzard and small intestine) of the digestive tract.

Each batch of 24 Japanese quails (12 males and 12 females) were sacrificed when they attained eight weeks of age for the quantification of the digestive enzymes (amylase, acid protease and lipase) in specific areas of the digestive system. The enzymes were quantified in the tissue homogenate of the respective tissues. Amylase activity was estimated in the mucous membrane of crop and small intestine, pancreas and gall bladder bile. Quantification of acid protease (pepsin) was conducted in the mucous membrane of proventriculus. Lipase was quantified in the pancreas as well as small intestinal mucous membrane. The data obtained were analysed statistically.

From the results of the study it was observed that crop mucosa of both sexes of quails showed an appreciable amount of amylase activity and the activity in males was significantly ($P \leq 0.01$) higher (47.98 ± 10.49 S U/g of tissue) than in females (13.26 ± 4.46 S U/g of tissue). In adult Japanese

quails, when the values for both the sexes were pooled, the activity was found to be 30.62 ± 6.65 S U/g of tissue. The activity of amylase was independent of the presence of food in the crop. In pancreatic tissue also, amylase activity was significantly ($P \leq 0.01$) higher in males (59.89 ± 3.36 S U./g of tissue) than in females (38.72 ± 2.59 S U/g of tissue). When both the sexes were taken together, the overall value of pancreatic amylase was 49.30 ± 3.03 S U/g of tissue. The value was higher than that of the crop. The bile from gall bladder of male Japanese quails exhibited, a higher ($P \leq 0.05$) mean amylase activity (156.23 ± 27.72 S U/g of tissue) compared to that of female quails (87.65 ± 14.36 S U/g of tissue). The pooled mean value of both sexes was found to be 121.93 ± 16.86 S U/g of tissue, indicating an appreciably high amylase activity in the bile of Japanese quail. The amylase activity in the small intestinal mucous membrane of males (234.38 ± 38.96 S U/g of tissue) was significantly higher ($P \leq 0.01$) than that of females (103.23 ± 8.08 S U/g of tissue). The pooled mean value of amylase activity in small intestinal mucous membrane of both the sexes of Japanese quails was 168.81 ± 23.78 S U/g of tissue.

When the amylase activity in different areas of digestive system as crop, pancreas, bile and small intestine was compared in both sexes of birds, it was observed that the highest value was noticed in the male birds. It was also observed that when the birds (both males and females) were

maintained on identical ration and managemental conditions, male birds preferred coarser particles of maize where as females preferred finer particles of oil cakes. So the increased amylase activity in different regions of male quails, might be a normal response to the increased intake of carbohydrates. In adult birds, intestinal mucous membrane exhibited the highest amylase activity and the crop mucosa exhibited the lowest activity indicating that digestion of starch starts in the crop with salivary amylase and completion of starch occurs in the small intestine with the help of amylase derived from pancreas, bile and small intestine. There was a negative correlation between the pancreatic weight and amylase activity with the correlation in males as -0.975 and as -0.968 in females. This indicated that simple homogenisation might not have liberated the intracellularly accumulated enzyme completely and that the pancreatic weight is mainly contributed by the intracellularly accumulated enzyme.

The acid protease (pepsin) activity of proventricular mucous membrane of male quails was found to be 185.67 ± 11.64 PU/g of tissue and that of female quails was 223.31 ± 38.91 P U/g of tissue. Though statistically nonsignificant, females exhibited a slightly higher value than the males. It might be a response to the increased intake of protein rich finer particles of oil cakes of feed by the females. The pooled

mean value for pepsin activity when both the sexes taken together was 204.49 ± 17.97 P U/g of tissue.

The lipase activity of pancreas was quantified to 73.37 ± 7.78 L U/g of tissue in males and 38.40 ± 3.39 L U/g of tissue in females, the males had a significantly ($P \leq 0.01$) higher value. The intestinal mucous membrane exhibited a significantly higher ($P \leq 0.05$) lipase activity in the males (38.80 ± 10.93 L U/g of tissue) than in the females (12.44 ± 4.15 L U/g of tissue). In the present study, it was also observed that the female birds preferred fat rich finer particles of oil cakes than the males. Since fat is the high energy yielding nutrient, the quantity in excess present in the diet is prevented from hydrolysis by the reduced lipase secretion in the females. The overall lipase activity in pancreas and small intestinal mucous membrane (when both the sexes were taken together) were 55.88 ± 5.52 L U/g of tissue and 25.62 ± 6.34 L U/g of tissue respectively, indicating that pancreas was the major site of lipase origin in quails. There was a negative correlation of pancreatic weight to lipase activity in both sexes, with a correlation value of -0.882 in males and -0.499 in females. As in case of amylase, homogenisation might not have liberated the intracellularly accumulated lipase too.

The pH values recorded in the contents of crop, proventiculus, gizzard and small intestine (duodenum) were

5.00 ± 0.26, 4.30 ± 0.11, 3.50 ± 0.17 and 6.50 ± 0.00 respectively. The observed pH in crop is suggestive of the amylolytic digestion in the crop. Though proventriculus is the site of secretion of pepsin, the comparatively lower pH (acidic pH) recorded in the gizzard suggested that acid proteolytic digestion was undergoing in a better way in the gizzard. The intestinal contents exhibited the highest pH than the upper regions of the digestive tract indicating that the enzymes of pancreas, bile and small intestine require a comparatively higher pH for eliciting the optimum action in the small intestine, thereby hydrolysis of starch, protein and lipids are completed.

The feed passage rate (FPR) was recorded in the last batch of 24 adult Japanese quails (12 males and 12 females) by using carmine as the indicator dye and the time taken for the first appearance of coloured excreta was taken as the index. The recorded FPR values for males and females were 120.5 ± 10.88 min and 92.4 ± 10.65 min respectively. The difference in FPR between male and female birds was not significant. The overall FPR (when data from both the sexes were pooled) was found to be 106.5 ± 8.00 min. The time taken for complete disappearance of the dye from the excreta was found to vary from 249 min to even more than a day. Though nonsignificant, the difference in FPR values observed between males and females may be due to the difference in their body weight.

The results of the present study provide informations on the relative functional importance of enzymes such as amylase, protease and lipase at different regions of digestive tract of Japanese quails. The study also throws light on the pH in different regions of the digestive tract as well as transit time of feed in Japanese quails. These informations may be of use in understanding the physiology of digestion and formulation of quail rations. The observation during the course of this experiment of the preferential uptake of feed particle by male and female quails is interesting warranting further studies on feed intake behaviour in Japanese quails.