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Digestive Enzymes of Cattle

John W. Sissons

National Institute for Research in Dairying, Shinfield, Reading, Berkshire RG2 9AT

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The nature of endogenous enzymes in the alimentary tract of cattle is examined and their functions in digestive processes discussed. The main digestive enzymes in cattle are salivary and pancreatic lipase, rennin, pepsin, trypsin, lactase and amylase. Maltase and isomaltase, celliobiase, trehalase, elastase and carboxypeptidase are also present. There is no evidence that cattle secrete sucrase. Most of the enzymes secreted by the gut of cattle are present at birth, although peak activities are not always reached until animals reach 2–3 weeks of age. These activities often persist through to maturity and are generally unaffected by rumen development. The amounts of enzymes secreted are affected by several factors including the nature of the diet, the method of feeding and age. There is also appreciable variation in the secretion of individual enzymes between animals of similar age and receiving the same diet.

1. Introduction

Demands for inexpensive feedstuffs for rearing cattle have led to an increasing interest in novel sources of dietary fat, protein and carbohydrate. However, satisfactory use of such nutrients by the animal will depend on the capability of its digestive system to secrete suitable enzymes in sufficient quantities and at the appropriate time after feeding. The purpose of this review is to provide an account of existing knowledge of endogenous enzymes found in the alimentary tract of cattle and to focus attention on some limitations in the digestive abilities of these animals.

In the alimentary tract of cattle endogenous digestive enzymes are present in secretions of the mouth, abomasum, pancreas and small intestine. Such enzymes are not secreted by the reticulum, rumen or omasum or the large intestine.[1] There are many exogenous enzymes of microbial origin in the forestomachs of ruminating cattle, but these are not discussed here as they have been described in detail elsewhere.[2,3]

2. Lipases

Major lipases in cattle are present in salivary and pancreatic juice and are responsible for the high digestibility of fat in calves fed on whole milk.[4–6] In ruminating cattle the majority of dietary lipid entering the small intestine is in the form of free fatty acids released within the rumen as a result of hydrolytic activity of rumen microorganisms,[7] and any role that pancreatic juice may have in the further digestion of lipid is obscure.

2.1. Salivary lipase

Oral lipase is secreted by tissues of the tongue; mainly in the region of the vallate papillae, the glosso-epiglottis area and the pharyngeal end of the oesophagus, and the submaxillary and sublingual glands.[8] There is no secretion of lipolytic enzymes by the fundic mucosa.[9]

The action of the enzyme releases short-chain fatty acids from water-insoluble triglycerides of dietary fat.[7] It preferentially releases butyric and caproic acid[10] and has little or no action on triglycerides containing fatty acids longer than 12:0.[11]

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The character of salivary lipase (E.C. 3.1.1.3) is not well defined and it is possible that calf salivary juice may contain a multiple lipase system. Electrophoresis of a preparation of calf tongue tissue has detected six fractions possessing lipolytic activity.[12] Also salivary lipase activity in buffered tributyrin and milk fat substrates exhibits several pH optima between pH 4.8–7.5.[13–15]

Lipase activity in the bovine is present at birth and persists well into maturity.[8] It is uncertain whether or not secretion of the enzyme declines with age. Ramsey *et al.*[8] found that salivary lipase activities in tissues of the tongue were greater in young calves than in adult cattle, although activities in the salivary glands increased slightly with age. Grosskopf[16] detected lipolytic activity in salivary secretions collected from a calf aged 2 months, but found no activity in older calves or in a cow. In contrast to these findings, Young *et al.*[17] observed no appreciable change in the activity of the enzyme in digesta that was diverted through a fistula from the cardial end of the oesophagus, i.e. 'sham feeding' of calves which were allowed to drink milk through a nipple during the first year of life.

The maintenance of lipase potency in saliva of calves seems to be little affected by the nature of the diet. Young *et al.*[17] found that in ruminating calves secretion of the enzyme during ingestion of solid food was somewhat less than that stimulated by the drinking of milk. But, when these animals were given milk by 'sham feeding', the activity of salivary lipase was similar to that in milk collected by the same method from preruminant calves.

Evidence on the effect of dietary fat on the secretion of salivary lipase is conflicting. Grosskopf[16] observed that the lipolytic activity in the saliva of a calf receiving whole milk and hay was about 200 times greater than in samples from calves given skimmed milk and hay. This is in contrast to the findings of Leidy *et al.*[18] who reported that salivary lipase secretion in a steer fed whole milk was similar to that of another animal given reconstituted non-fat milk.

Fats containing a high proportion of short-chain fatty acids are readily hydrolysed by salivary lipase.[11] Studies of the relative activities of the enzyme on various fats and oils showed that the lipase hydrolyses butter oil, colostrum fat and coconut oil to similar extents, whereas lard, tallow, soya bean oil and corn oil are poorly lipolysed.[19] These findings reflect the significant quantities of 6:0, 8:0 and 10:0 fatty acids present in milk fat and coconut oil compared with the absence of short-chain fatty acids from lard, tallow, soya bean oil and corn oil.

Secretion of salivary lipase is stimulated by the calf either sucking or drinking milk,[17,20] although a slow intake of milk, such as occurs by sucking through a nipple, appears to cause greater stimulation.[4,16,21,22] Calves allowed to suckle consume milk more slowly and uniformly, and secrete more saliva than when drinking from an open pail.[21] However, it is uncertain whether or not the physical act of suckling *per se* affects the secretion of salivary lipase.

Considerable variation has been found in the secretion of salivary lipase of individual calves.[4,22–24] Wise *et al.*[23] suggested that several factors such as the rate and pattern of drinking, appetite and health of calves may contribute to differences between animals. Young *et al.*[17] observed that some calves aged between 20–180 days showed cyclic trends in their patterns of salivary lipase secretion which could not be correlated with any detectable changes in health or environment.

Inhibition of salivary lipase activity, which occurs at a pH of below 2.4 and above 7.8[16] is thought to be delayed by the formation of the milk clot. The acidity of fluid contained within the casein matrix is apparently closer to the range of pH optima of 4.8–7.5 for the hydrolysis of triglycerides than that of the surrounding whey fraction.[15,16,25] Using calves fitted with rumen cannulas, Siewert and Otterby[26] observed that during the first 2 h after feeding whole milk, when the pH of abomasal digesta fell from pH 6.4 to 3.4, salivary lipase activity was relatively high. They suggested that when the acidity of digesta leaving the abomasum is above pH 3.0, the enzyme may remain active both within the stomach and in the small intestine. In the milk fed calf this condition persists for about 3–4 h after feeding.[27,28]

2.2. Pancreatic lipase

Pancreatic lipase (E.C. 3.1.1.3) of cattle has been isolated in pure form.[29] During purification of the enzyme, Julien *et al.*[30] separated a cofactor (colipase) which was effective in restoring full enzyme activity with emulsified tributyrin. However, the significance of this colipase in the digestion of fat is not understood.

The activity of pancreatic lipase with butteroil exhibits a pH optimum of 8.8. Lipolysis is inhibited below pH 5.5 and above pH 11.5.[29]

Hydrolysis of triglycerides by pancreatic lipase appears to be less specific than salivary lipase. The pancreatic enzyme is capable of hydrolysing several natural oils including butter oil, coconut oil, safflower oil, corn oil and cotton seed oil, and also various synthetic mono-, di- and triglycerides.[31] Sampugna *et al.*[32] reported that pancreatic lipase releases short- and long-chain fatty acids from triglycerides containing both types of fatty acids at similar rates, and that this fatty acid release was more rapid than from triglycerides containing exclusively long-chain fatty acids. Since the overall extent of lipolysis by salivary lipase seems to be limited by its inability to release long-chain acids,[11,16,33] the release of these acids in the milk fed calf is likely to be dependent on the subsequent action of pancreatic lipase.

During the first few weeks of the calf's life, lipase activity in the pancreatic juice is relatively much less than that in the saliva.[16,19,34] Gooden[35] found that the lipase activity per unit volume of pancreatic juice in 2-day-old calves was similar to that from animals aged 2 weeks. There was, however, a substantial increase in the flow of pancreatic juice in older calves resulting in a three-fold increase in the output of pancreatic lipase activity per kg of body weight. These findings led to the view that in the young calf the output of pancreatic lipase may not be adequate to hydrolyse sufficient milk triglyceride for efficient absorption of lipid. And that at this time salivary lipase serves to supplement pancreatic lipase by playing a major role in lipid digestion in the abomasum of the newborn calf. However, in other studies, Gooden and Lascelles[6] observed that the efficiency of lipid absorption in calves, even at 1–2 weeks of age, was substantially diminished by the absence of pancreatic juice. Furthermore, in calves maintained on milk diets for several months the activities of salivary lipase and pancreatic lipase remain fairly constant.[5,17]

Although only small amounts of lipid entering the duodenum of adult cattle are in an unhydrolysed form,[36] the ruminating animal appears to retain the capability of lipolysing dietary triglycerides in the small intestine. Gooden[35] observed a marked increase in the output of pancreatic lipase of ruminating calves compared with animals receiving a milk diet. Furthermore, several workers have shown increases in yield and fat content of milk of cows receiving diets containing lipid supplements protected against degradation by microorganisms in the rumen.[37–40]

There is evidence that lipase activity in pancreatic juice is affected by the nature of the dietary protein. Ternouth *et al.*[41] compared the effect of diets containing either milk, soya bean or fish protein on pancreatic enzyme secretion in calves fitted with duodenal re-entrant cannulas and pancreatic sac cannulas. They found that pancreatic lipase activity during a 12 h period after giving liquid feeds containing non milk protein was significantly less than for diets containing skimmed milk.

2.3. Pancreatic phospholipase

Lysolecithin formed from biliary lecithin in the presence of pancreatic phospholipase (E.C. 3.1.1.4) may replace the function of monoglyceride in micelle formation in the ruminant.[7,42,43] Gooden[35] found that the conversion of lecithin to lysolecithin occurred much more rapidly in intestinal contents of ruminating animals compared with 1–2-week-old calves, although in-vitro studies showed that there was a rapid conversion using pancreatic juice and bile from both groups of calves at pH 6.2 and 7.5. It was suggested that the presence of triglycerides in the upper small intestine of milk fed calves may inhibit the conversion of lecithin to lysolecithin.[35]

3. Proteases

In cattle, proteases secreted by the abomasum and pancreas hydrolyse dietary protein into small peptides. Amino acids are then released from these early digestion products by peptidases within cells of the small intestine wall.

3.1. Gastric proteases

The calf differs from most other young animals that have been studied in having a relatively high activity of pepsin (E.C. 3.4.23.1) in the abomasum from birth,[44,45] and it is the only animal in which rennin (E.C.3.4.23.4) has been identified in the stomach;[46] although Henschel[47] found evidence of a rennin-like enzyme present in the stomach of the newborn rabbit.

There seems to be wide variation amongst individual calves in their secretion of gastric proteases. In some calves rennin, and in others pepsin, predominates during the first few weeks of life, but pepsin production always becomes more important as the calf gets older.[47-49]

Experiments using fistulated calves receiving a milk diet have shown that the secretion of rennin and pepsin increases immediately after feeding and then falls rapidly, reaching prefeeding levels after about 2–3 h.[50-52] Limited observations indicated that the output of these enzymes was associated with the volume of milk consumed.[50] Hagyard and Davey[53] found that in calves fasted for several days there was a marked decrease in the rennin content of abomasal tissue. Rennin and pepsin activity are also affected by the method of feeding; protease activity in abomasal secretions of calves given milk by a nipple feeder was higher than when the animals were fed from an open bucket.[54]

Recent studies have shown that rennin secretion is influenced by the nature of the dietary protein.[49,52,55] Substituting skimmed milk with fish, soya bean or whey protein in liquid feeds given to calves with abomasal or duodenal cannulas led to a decrease in the output of rennin without modifying pepsin secretion. Reducing the intake of milk, as occurs at weaning, was also found to result in a fall in rennin secretion. Garnot *et al.*[55] have also shown that increasing the proportion of casein to whey in the diets of calves induced an increase in rennin activity, whilst pepsin activity was unaffected. They concluded that casein is responsible for stimulating rennin secretion.

It is not known if the efficiency of protein digestion is affected by the nature of the enzymes secreted. The products of digestion of whole milk *in vitro* appear to be the same whether pepsin or rennin is used, although the rate of hydrolysis is slower with rennin.[56] Pepsin was also more proteolytic than rennin on casein substrate between pH 2.0–6.0.[57]

Actions of pepsin and rennin lead to milk clotting as well as proteolysis. They behave similarly in both of these processes,[58,59] but their optimal pH values are different. For coagulation of milk the optimum pH is 6.5 for rennin and 5.3 for pepsin[60] whereas for proteolysis the optimum pH is 3.5 for rennin and 2.1 for pepsin.[47] This may allow protein hydrolysis to occur over a wider range of acidity in the abomasum of the calf than in the stomachs of other animals.

3.2. Pancreatic proteases

Proteolytic enzymes in the pancreatic juice of cattle are comprised of several endopeptidases including trypsin (E.C. 3.4.21.4), chymotrypsin (E.C. 3.4.21.1) and pancreatopeptidase E (E.C. 3.4.21.11) and two exopeptidases; carboxypeptidase A and B (E.C. 3.4.12.2. 3.4.12.3).[61,62] The endopeptidases hydrolyse centrally located peptide bonds involving carboxyl groups of L-amino acids, whereas carboxypeptidases attack terminal bonds of protein and peptides. The optimum pH of these enzyme activities in the small intestine is between pH 7.5–8.0 for trypsin chymotrypsin and carboxypeptidase and pH 8.8 for pancreatopeptidase E.[62,63]

Except for some studies of trypsin and chymotrypsin, there is little direct information on the importance of pancreatic proteases in cattle. In the newborn calf pancreatic enzymes appear to be present in low concentrations.[5] During the first month of life protease activity of pancreatic tissue and fluid increases markedly and thereafter remains fairly constant.[5,64,65]

Studies in liquid fed calves cannulated in the pancreatic duct showed that the amounts of pancreatic enzymes in pancreatic secretions increase during the first hour after feeding, but the time at which maximum output occurs, and the rate at which secretion decreases, vary markedly in different calves.[34,64,66] These workers also found no correlation between the activities of pancreatic proteases and the volume of juice secreted. For example, Ternouth *et al.*[66] reported that output of pancreatic juice was highest during a period 5–9 h after feeding, whereas secretion of enzyme activity was highest during the first 2 h after feeding.

Secretion of proteolytic enzymes by the pancreas was reduced when calves were given liquid feeds containing milk protein that had been 'severely' heated (74°C for 30 min) or non-milk protein derived from soya bean flour or fish concentrate, rather than feeds containing 'mildly' heated skimmed milk (77 °C for 15 s) or whole milk.[41,64,65] The way in which constituents of dietary protein modify the secretion of pancreatic enzymes in the calf is not understood. Ternouth *et al.*[41] reported that replacing milk protein by fish or soya bean protein in liquid feeds given to calves led to greater proportions of dietary protein escaping into the duodenum in an undigested form. There was, however, no direct relationship between the amounts of pancreatic proteases secreted during a period of 12 h after feeding and the quantities of undigested protein entering the small intestine. It is possible that interactions with humoral mechanisms may be involved. Experiments with other animals have established that the stimulation of pancreatic enzyme secretion is a major physiological action of cholecystokinin,[67,68] and that digestion products of protein are a potent stimulus for the release of this hormone.[69,70]

Investigations of individual proteases in the pancreas and pancreatic juice of milk fed calves has shown that trypsin and chymotrypsin are present at birth. And that there is no marked change in the activities of these enzymes with age.[64,71,72]

It is uncertain whether or not the activities of trypsin and chymotrypsin are affected by rumen development. Gorrill *et al.*[73] found that concentrations of trypsin and chymotrypsin in intestinal contents per unit body weight of 5-month-old ruminating calves were ten-fold higher than those of milk fed animals aged 10 days. In contrast to this finding, Schingoethe *et al.*[71] reported that the activities of these proteases in pancreatic tissue per unit body weight were not affected by rumination. They suggested, however, that the enzyme activity of the pancreas *per se* may not be indicative of the synthesising capacity of the organ.

Several workers have shown that feeding non-milk protein to calves leads to a reduction in the activities of trypsin and chymotrypsin in the pancreatic juice and intestinal contents. This inhibition in the secretion of pancreatic protease affects trypsin and chymotrypsin to the same extent; ratios of chymotrypsin : trypsin activity in pancreatic tissue and fluid of calves fed whole milk were similar to ratios reported for animals given liquid feeds containing fish or soya bean protein.[41,64,74]

4. Carbohydrases

Enzymes in cattle responsible for hydrolysis of carbohydrates in the gut are pancreatic amylase, which hydrolyses starch mainly to maltose, and disaccharidases present in mucosal cells of the small intestine. In contrast to non-ruminants, cattle do not secrete salivary amylase.[75]

4.1. Pancreatic amylase

Amylase activity in calf pancreatic tissue and pancreatic juice is low at birth, increases during the first few weeks of life,[5,65,76,77] and reaches a maximum only in adult cattle.[78–80] Low pancreatic amylase (E.C. 3.2.1.1) secretion is probably a limiting factor in the use of starch by the very young calf. The inclusion of starch as a major energy source in milk replacer diets for young calves has resulted in poor growth and in digestive disturbances,[81,82] although the results of an investigation by Mathieu and Thivend[83] showed that calves up to 13 weeks of age use small amounts of raw starch without any ill effects. There is also some indication that the growth of calves on high starch diets improves after the first few weeks of life.[82]

Feeding milk replacers containing soya bean flour to young calves results in a decrease in the output of pancreatic amylase.[41,80] Several biologically active substances, mostly heat-labile and capable of causing nutritional disturbances in other animals, have been found in soya beans.[84,85] But, whether or not they interfere with the secretion of enzymes is unclear.

In adult cattle dietary starch is broken down mainly by microbial action in the rumen and it is generally assumed that relatively little of this carbohydrate reaches the small intestine. However, some experiments have shown that large amounts of starch may escape fermentation in the rumen when high concentrate rations are fed.[86,87] There is also evidence that the ruminant pancreas is capable of adapting the output of amylase to increased amounts of grain or concentrates in the diet.[88,89]

4.2. Intestinal disaccharidases

Disaccharidase activity located in the small intestine of cattle has a non-uniform distribution; lactase (E.C. 3.2.1.23), cellobiase (E.C. 3.2.1.21) and trehalase (E.C. 3.2.1.28) activity being highest in the proximal jejunum, somewhat lower in the duodenum and decreasing distally with a sharp decline at the ileum.[78,90] Maltase (E.C. 3.2.1.20) and isomaltase (E.C. 3.2.1.10) activities are also possessed by the small intestine and they show similar patterns of distribution along the small intestine; activity was found to be higher in the jejunum than the ileum and lowest in the duodenum.[78,91]

Several workers have reported an absence of sucrase activity from the intestine of the calf.[5,76,92,93] Studies by Siddons[78] confirm their findings and also show that the adult cow possesses no intestinal sucrase activity. Fructose, one of the hydrolysis products of sucrose, is not used by the calf and when present in the diet causes severe diarrhoea.[94]

Intestinal disaccharides of cattle exhibit maximum activity between pH 5.4–6.2.[78,91,95] This pH condition is present in the lumen of the proximal small intestine where the acidity of the digesta, which is influenced by gastric, pancreatic and other secretions, varies from about pH 2–6 in the most proximal duodenum and decreases progressively towards the distal ileum where the normal pH is between 7 and 8.[27,96,97]

Disaccharidase activity has been detected in the mucosal samples of the small intestine of bovine fetuses at 90 day gestation. By 180 days gestation, lactase activity increased five-fold and remained at a high level until birth, whilst the activity of maltase decreased with advancing gestation.[98] In non-ruminating calves intestinal lactase and cellobiase activities are highest at birth and decrease with age, although lactase activity is still relatively high in 4–6-month-old calves.[5,78,93] Similar changes in the patterns of these enzymes with age is consistent with results of heat inactivation studies that suggest lactose and cellobiose to be hydrolysed by the same enzyme.[91]

Intestinal maltase, isomaltase and trehalase activities, which are low compared with lactase activity, show little or no change with age.[5,78,93] However, evidence that intestinal maltase activity is low and does not increase markedly with age is not consistent with other studies[99,100] in which significant use of maltose was observed by milk fed and ruminating calves aged between 6 and 9 months. Siddons[78] showed that the length of the small intestine increases as the animal matures and suggested that this may lead to a greater output of maltase in older calves.

Intestinal lactase appears to be maintained either when milk is supplemented with additional lactose or when calves are kept as preruminants rather than being weaned.[90,101,102] Lactase activity in ruminating animals declines presumably because of either the lack of exposure of the small intestine to lactose or some other change resulting from rumen development. Cellobiase and trehalase activities in the intestine also decline at weaning, whereas maltase does not seem to be affected by rumination.[90,93]

5. Alkaline phosphatase

Preparations of alkaline phosphatase (E.C. 3.1.3.1) from mucosa of the small intestine of young cattle have been characterised.[103,104] The enzyme is believed to be involved in the uptake of phosphates and in fat absorption.[105,106] Apart from a study of the distribution of alkaline phosphatase in the intestine of ruminating cattle there appears to be no direct information on this enzyme. Benz and Ernst[107] found that the activity of alkaline phosphatase in ruminating cattle was highest in the duodenum, decreased throughout the jejunum and lowest in the ileum.

6. Nucleases

There are few detailed studies of endogenous nucleases in cattle. Ribonuclease (E.C. 3.1.4.22) and deoxyribonuclease (E.C. 3.1.4.5) which degrade nucleic acids to mononucleotides are present in pancreatic secretions.[41,108,109] A nucleotidase activity which hydrolyses phosphate esters has been found in the small intestine mucosa of the calf.[110] However, the importance of these enzymes in the digestion of nitrogenous compounds in cattle is poorly understood.

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