NEONATAL DEVELOPMENT OF PROTEIN DIGESTION

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Summary

Protein digestion is one of the major functions contributing to neonatal growth and development, especially during the early postnatal period when adaptive changes in the gut are most rapid. The early postnatal period is the first critical period of the most significant adaptive changes of the gut, which undergoes the transition from endogenous nutrient supply to exogenous feeding. This period is characterised by the accelerated growth and development of the alimentary canal, compared to whole body growth. In mammals it is also associated with maternal colostrum, then milk as the only nutrient source. The second critical period of gut development in mammals is characterised by the major transition from a liquid diet to solid food and is described as post-weaning adaptation. During this period a similar increase in gut relative growth rate is observed. In birds, major events in functional development and maturation of digestive tract occur during the first week after hatch. However, the complete development of the digestive function occurs only after the final resorption of the yolk sac, which happens between two and three weeks of age, depending on the bird's breed.

The activities of various hydrolytic enzymes involved in protein digestion change at different rates during this adaptation period, regulated not only by the time post partum but also by the time of the first meal, the feeding regime, the amount of food consumed and the type of diet. Alimentary regulation of gut development might play a key role in the manipulation of gut maturation, animal growth and productivity of farm animals. However, the extent of this manipulation is limited by the genetic potential of the animal.

I. INTRODUCTION

The development and adaptation of protein digestion in prenatal and postnatal periods have been intensively investigated in animals of different species (Austic 1985; Hennings 1985; Kelly et al. 1992; Le Huerou-Luron et al. 1992; Mellor 1992; Reisenauer et al. 1992). These studies were carried out mainly on mammals and cover all major stages of protein digestion. During the last decade developmental studies in digestive physiology attracted an interest in pig and poultry research (Cranwell 1995; Tarvid 1995). These studies provide not only a broader knowledge about the evolutionary and comparative aspects of digestion, but also information about farm animal nutrition which could help to create new diets and feeding regimes for better productivity. However, current knowledge in protein digestion does not provide complete understanding of both the mechanisms involved in the growth and maturation of the alimentary canal and the role of regulatory factors of different origins. The aim of this paper is not to present the whole picture on neonatal development of protein digestion, that can be found in the latest reviews (Lee 1989; Menard 1989; Foltmann 1992; Cranwell 1995; Tarvid 1995), but to concentrate on some contradictory aspects of protein digestion, to emphasise still unclear mechanisms of protein digestion and to point out different promising directions in digestive physiology and nutrition. The topics to be discussed are: postnatal growth and maturation of the digestive tract, critical periods in gut development, enzymes involved in protein digestion, their classification and non-parallel developmental patterns, regulation of gut maturation, and the role of the first intake of feed as a trigger to postnatal development and adaptation. Special attention will be given to the

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functional development of the entire gut capacities, by characterising the total activities of proteolytic enzymes in different digestive organs. Information about the other aspects of protein digestion can be found in numerous original papers and reviews: mechanisms of intestinal adaptation (Alpers 1990); morphology of the gut development (Smith 1985; Pauer et al. 1991; Yamauchi and Isshiki 1991; Isshiki et al. 1992; Kelly et al. 1992); neonatal development of amino acid and peptide transport (Lerner 1984; Smith 1988; Buddington and Diamond 1989; Buddington 1992); role of genetic factors (McCarthy and Siegel 1983; Leenstra 1986; Lebenthal 1989; Lund et al. 1990; Smith et al. 1990); hormonal regulation and nervous control (Hennings 1981; Harada et al. 1982; Mallon and Betz 1982; Moazam et al. 1982; McNab et al. 1989; Le Hueron-Luron et al. 1992); role of the diet and various feeding regimes (Lindemann et al. 1986; Owsley et al. 1986; Kelly et al. 1991a, 1991b; Tarvid 1992; Makkink et al. 1994).

II. POSTNATAL GROWTH AND DEVELOPMENT OF THE DIGESTIVE SYSTEM

Adaptation to exogenous food is associated with a dramatic increase in the weight of the gastro-intestinal tract and the activity of its digestive enzymes. Adaptive changes of the alimentary canal to the digestion of exogenous food are essential for the maximisation of early growth, and involve differential growth rates of internal organs, including the gastro-intestinal tract. Segments of the gastro-intestinal tract increased in size and weight more rapidly in relation to body weight than did other organs and tissues during the first two days in pigs (Xu et al. 1992a, 1992b; Tarvid et al. 1994a, 1994b), rabbits (Hall and Widdowson 1979), lambs (Trahair and Robinson 1986), rats (Reeds et al. 1993) and during the early post hatching period in chickens and other poultry species (Lilja 1983; Katanfaf 1988) . In pigs, allometric growth of the digestive system as a whole, and its specific organs in particular, was positive during the late gestation period, and continued in the same pattern until the end of the first week post-partum (Marrable 1971). Allometric growth of the small intestine in chickens studied during the first 23 days after hatch, showed a gradual increase until eight days of age, when it attained a maximum approximately four-fold that of body growth and was later followed by a significant decrease (Nitzan et al. 1991a).

Immediately after birth the digestive tract undergoes a period of accelerated growth. This was found for the stomach, pancreatic gland and the small intestine in both mammalian and avian species (Lilja 1983; Lindemann et al. 1986; Xu et al. 1992a, 1992b; Tarvid et al. 1994a, 1994b). However there were species specific patterns for the relative growth of the digestive system, related to the existence of the weaning period in mammals. Depending on the type of feeding changes after birth or hatching one or two adaptation periods might be described. The first change of the environment occurs at birth or hatch and includes the shift from intrauterine/yolk sac nutrition to exogenous feeding. This adaptation occurs in all species and is characterised by the first period of relatively fast growth rate of the digestive system, compared to the whole body. In different poultry species the major changes of the digestive tract, such as an increase of the small intestine weight and a decrease of the weight of the large intestine, occurred during the first 10 days after hatching. It was suggested that morphological and physiological development of the intestine may be completed by the age of 10 days (Crompton and Walters 1979). For mammals the second adaptive period is connected with the shift from milk to solid food. This change in the diet is followed by another period of accelerated growth of the digestive organs. Thus, all principal changes in feeding cause an adaptive reaction of the digestive system. These periods of fast response by the gut might be called 'critical periods' in the development and maturation of the digestive system.

There are general similarities in the development of the gut, but there are also species and even breed specific differences. A comparative study of the growth of the digestive organs in meat (broiler) and egg-type (White Leghorn) chickens showed that in broiler chicks the weights of the pancreas and small intestine increased throughout the 14 days after hatch (Nir et al. 1993), while in White Leghorn chickens there was a reduction in the growth rate of these organs between five and eight days of age. It was shown that specific parameters of intestinal development in different avian species such as intestinal weight, length and surface area were more determined by growth rate than by species and breed (Isshiki et al. 1992). Several studies (Nir et al. 1978; McCarthy and Siegel 1983; Leenstra 1986), have suggested that the differences in the gut development in broiler and layer type chickens were influenced by differences in food intake regulation: in broiler chickens the capacity of the gastrointestinal tract seems to be the limiting factor for food intake, while in layer type birds hypothalamic regulation of food intake is more important. The results of the study of continuous nutrient supply in early-weaned pigs (Kelly et al. 1991a) suggested a certain degree of interaction between nutrient intake in gut development. Two components, nutrient dependent and nutrient independent, were established.

The accelerated growth and functional development of the alimentary canal are critical for optimal somatic growth and survival. Tissues of the gastrointestinal tract represent only 5-7% of body mass, but consume up to 20% of the neonate's oxygen needs (Edelstone and Holzmann 1981). This demonstrates the importance of organs and tissues that fulfil a 'supply' function for achieving early body development.

III. THE DEVELOPMENT OF PROTEIN DIGESTION

(a) Stomach enzymes

The stomach produces several enzymes to break down protein: pepsins (EC 3.4.23.1-2), gastricsin (EC 3.4.23.3) and chymosin (EC 3.4.23.4) which differ in their general proteolytic and milk-clotting activities (Foltmann 1992). All gastric proteases are secreted as zymogens which undergo activation in the presence of hydrogen ions.

In mammals, the most important protease during the early neonatal period is chymosin, which exhibits primarily milk clotting activity with a minor peak of general proteolytic activity. The weak proteolytic activity of chymosin allows all biologically active components of protein and peptide origin from the colostrum and milk to be absorbed in the small intestine. In pigs chymosin is present in the gastric mucosa at birth. During the first week of postnatal development prochymosin concentration in the gastric mucosa declines, and after three to four weeks' chymosin is gradually replaced by pepsinogen, the major gastric protease in adult pigs (Sangild et al. 1991). Traces of pepsinogen A could be found at and immediately after the time of birth in the gastric mucosa. Its concentration increases gradually until three to four weeks' of age, and more rapidly thereafter. Progastricsin, the third gastric protease, has a developmental pattern similar to pepsinogen A.

In poultry, developmental studies of gastric enzymes showed that avian pepsinogens undergo great changes in molecular structure and activities during development both before and after hatch. The specific acid protease activity of the enzymes in the chicken and quail proventriculus showed a peak a few days before hatching and decreased towards the end of the incubation period. The electrophoretic patterns of acid proteases changed considerably during these stages. Within 24 hours after hatching the activity increased to about 30 times that of the embrionic form of the enzyme. Concomitant with this rapid increase was a shift of electrophoretic patterns to the adult type of the enzyme (Yasugi and Mizuno 1981a; 1981b; Yasugi et al. 1979). These changes may reflect the preparation of the fowl for post-hatching life. As these changes were not affected by the complete starvation of hatched fowls it was suggested that they are regulated not by feeding stimuli, but by internally controlled mechanisms.

(b) Pancreatic enzymes

The pancreatic gland produces several enzymes involved in protein digestion. Serine proteinases trypsin (E.C. 3.4.21.4.), chymotrypsin (E.C. 3.4.21.1.) and elastase (E.C. 3.4.21.11.) hydrolyse specific peptide bonds inside the protein molecule, while carboxypeptidase A (E.C. 3.4.17.1.) and carboxypeptidase B (E.C. 3.4.17.2.) participate in luminal peptide hydrolysis releasing free amino acids from the C-terminus of oligopeptides (Ohlsson et al. 1987). Not all of these enzymes have been studied equally. More often the developmental changes of trypsin and chymotrypsin have been reported. There are only a few papers devoted to carboxypeptidases; while the role of elastase in protein digestion has never been studied. Thus, the relative role of each pancreatic enzyme in protein digestion is still unclear.

Biochemical maturation of the pancreas is characterised by the rapid accumulation of digestive enzymes, together with the dramatic morphological maturation of the gland during the early postnatal period. The rate of development of different pancreatic enzymes varies. Direct comparison of the data from the studies of functional development of the pancreas is difficult because different experimental designs were applied in these studies, including unstandardised status of the alimentary canal, different feeding strategies and various methods to activate pancreatic zymogens (Corring et al. 1978; Lindemann et al. 1986; Owsley et al. 1986; Westrom et al. 1987; Pierzynowski et al. 1993). However all studies demonstrated the lack of parallelism in the patterns of accumulation of the enzymes in the pancreas. Total activities of trypsin, chymotrypsin, carboxypeptidase A and general proteolytic activity increased respectively 13, eight, 15 and nine times in the sucking pigs from birth till 30 days' of age (Tarvid et al. 1994b). The highest levels of trypsin and chymotrypsin activities were generally observed in newborn, nunsuckled pigs with a steady decrease in chymotrypsin activities during the entire sucking period and a similar decrease of trypsin activity up to 20 days, followed by an increase at 30 days. The observed decrease in protein concentration at the age of two days suggested that the secretion of pancreatic enzymes in response to feeding, and the growth of the structural components in the pancreas, exceeds digestive enzyme synthesis at this early stage.

In broiler chickens the specific activity of trypsin in the pancreas decreased during the first three to six days after hatching and increased later to between 10 to 20% higher than that at hatching on day 14 (Nitzan et al. 1991b). Activity of chymotrypsin increased gradually from shortly after hatch to day 14, and remained constant until day 23. When expressed as units of activity per kg body weight the activity of both enzymes increased with age reaching a maximum on day 11. The limited synthesis of digestive enzymes in the pancreas during early growth and their increase to maximum values around day 10, when relative growth rate was maximal, indicated a possible association between these two traits. The insufficiency in the synthesis of digestive enzymes during the first few days after hatching was further emphasised when the activities were expressed as units per g of tissue weight. Carboxypeptidase A (CPA) activity peaks were observed in the first day after hatching in Highsex White cross chickens. The enzyme activity significantly declined during the first week and thereafter remained at a constant level (Tarvid 1991).

The role of the first feeding in functional development of the pancreas was studied in chickens (Tarvid 1992). It was shown that the most significant decrease of pancreatic CPA activity, observed on the second day in fasted and fed chicks, was related to the first secretion of enzymes with the pancreatic juice into the lumen of the digestive tract. This first secretion was determined by the time of the hatch and occurred immediately after it, and only slightly depended on the presence of food in the lumen. In contrast, further changes in CPA activity level in the pancreas were closely connected with the functional status of the alimentary tract.

Many experimental data, notwithstanding their heterogeneous and, in some aspects, contradictory character, on the whole show that during the course of the embryonic development of different mammalian and avian species the activity of pancreatic enzymes increases in parallel with the maturation of the gastrointestinal tract. The patterns of digestive enzymes from the exocrine pancreas do not develop in parallel for individual enzymes, indicating independent regulation of each pancreatic protein during the early postnatal period (LeHuerou et al. 1990). Proteolytic enzyme activities in pancreas are characterised by low but increasing levels during the late fetal period, unchanged or even decreased levels during the first postnatal week, and gradual increase to the adult level. This trend was shown for humans (Track et al. 1975), rabbits (Corring et al. 1972), pigs (Westrom et al. 1987; Harada et al. 1988) and rats (Robberecht et al. 1971). Poults also hatch with some reserves of pancreatic enzymes that are produced during embryonic growth. These reserves decrease rapidly, because synthesis during this period is less than is required for secretion to the intestine and for maintaining the initial concentrations.

Two types of regulatory mechanisms in the functional development of the pancreatic gland may be observed. The first mechanism is connected with the realisation of the gene program and depends only on the age post partum. The second — the alimentary mechanism — is essentially determined by the processes of adaptation to the diet, including the protein level in the ration and the feeding regime. Post-weaning feed intake and the type of diet appeared to be an important factor in digestive development of newly-weaned piglets with the age of the pig being of minor importance (Makkink et al. 1994).

The study of age at weaning in pigs suggested that three major factors contribute to the mechanism of induction of pancreas development at weaning (Pierzynowski et al. 1993). Maturation could be initiated by the solid food itself, by triggering factors originating from the diet or through gastrointestinal hormones. The removal of milk from the alimentary canal could be the second critical factor for the induction of maturation. Milk contains several regulatory hormones which might delay the process of maturation. The third factor is associated with the removal of piglets from the sow which is a stressful situation with hormonal changes as an initiating factor. However, the mechanisms of postnatal pancreatic development need further clarification. Special attention must be paid to the time of the first feeding, because if it takes place a long time after birth or hatch, the pattern of development and functional maturation is disturbed.

(c) Small intestinal enzymes

Peptide hydrolysis is the final step in protein digestion in the gut, and is catalysed by pancreatic and intestinal peptidases. The developmental changes in pancreatic exopeptidases were discussed with the other pancreatic enzymes, because of the similarity of the processes of their synthesis and secretion. Two major groups of small intestinal exopeptidases are involved in peptide hydrolysis in the small intestine: aminopeptidases (E.C. 3.4.11.) and dipeptidases (E.C. 3.4.13). Both aminopeptidases and dipeptidases are located in the brush-border and cytosol fractions of enterocyte. The distribution of their activities between the brush-border and cytosol fractions is different for these two classes of peptidases and the specific roles of the brush-border and the soluble forms of the enzymes are to be considered.

Postnatal development of intestinal exopeptidases has been studied mainly for mammalian dipeptidases (Lindberg et al. 1975; Kim 1977). In the study of the development of dipeptidase activity in fetal and neonatal pigs the highest level of activity in new born, unsuckled piglets was observed in the proximal part of the small intestine (Lindberg and Karlsson 1970). In six to eight week old pigs the level of dipeptidase activity was approximately three times lower than in newborns and maximum activity was found in the middle segment of the small intestine. The pattern of development for aminopeptidase activity showed a general decrease with age mainly in proximal and medial regions and the development of a proximo-distal gradient with maximum activity in the distal segments (Tarvid et al. 1994a). The pattern of development for dipeptidase activity was similar to that of aminopeptidase except that the gradient in activity developed at a later age and was more pronounced in the distal region. In general, specific dipeptidase activity decreased with age and by the age of five weeks reached the level of adult animals. Morphological and cytochemical study of two brush-border enzymes, aminopeptidase N and dipeptidylpeptidase IV in newborn and 28-day old pigs (Tivey and Smith 1989), showed that in 28-day old pigs villi were half as long and twice as wide at their base as those from the newborn animals. The changes in shape did not affect the total surface area of the villus. Intact villi contained 30% more aminopeptidase N and 400% more dipeptidylpeptidase IV than villi from newborn pig intestine. Two types of changes in enzyme activity were demonstrated; 90% of the total increase in peptidase activity occurring during early postnatal development involved enterocyte reprogramming of enzyme production, and only a small part of the activity changes was influenced by the changing geometry of villi.

Similar results were obtained for chicks, where the highest level of peptidase activity was found immediately after hatching with maximal decrease of activity during the first week (Tarvid 1991). Significant differences depending on enzyme localisation in the cell and along the surface of the small intestine were observed. A number of investigations provided the evidence that brush-border and intracellular dipeptidases in various species differ from each other. These differences were connected with substrate specificity, physicochemical properties, enzyme thermostability and sensitivity to inhibitors, and their electrophoretic characteristics (Kim et al. 1973; 1974; 1979; Nicholson et al. 1974). In the rat small intestine different patterns were demonstrated for the membrane and cytosolic forms of aminooligopeptidase during neonatal development (Reisenauer et al. 1992). In the jejunum the membrane form of the enzyme had a major increase in activity during the period from 18 to 22 days, while the increase for the cytosolic form of the enzyme was not so expressed. More significant differences were observed between the brush-border and the cytosolic forms of the enzyme in the ileum. The cytosol enzyme decreased in activity from the age of 14 days, while the activity of the membrane form showed practically no change. The results demonstrated that during postnatal growth the type of developmental change in aminooligopeptidase activity was influenced by the form of the enzyme and its location along the small intestine. These two groups of peptidases may play different roles in the digestive and metabolic processes. Several authors have suggested that the basic role of brush-border enzymes is membrane hydrolysis of dipeptides; intracellular peptidases participate mainly in the cell protein turnover (Kim et al. 1973; Ugolev 1974; Das and Radhakrishnan 1975; Kim 1977; Corring 1980).

Studies on the distribution of peptidase activity along the small intestine showed that in adult animals the distal part of the small intestine was characterised by a high level of dipeptidase activity (Harrison and Webster 1971; Laval et al. 1978; Skovbjerg 1981; Laganierre et al. 1984). However, the distribution of enzyme activities underwent specific changes during the neonatal period. In human and pig embryos maximal dipeptidase activity was located in the proximal part of the intestine. During postnatal development the distribution of dipeptidase activity changed and its maximum was found in the distal parts (Lindberg and Karlsson 1970). Distribution of soluble and particulate dipeptidases in four mammalian species (monkey, rabbit, guinea pig and rat) showed that the soluble enzyme demonstrated its peak activity either in the proximal or in the middle region of the intestine, whereas the particulate enzyme showed higher activity in the distal parts of the intestine (Das and Radhakrishnan 1974). In chickens, the gradient of surface dipeptide hydrolysis appeared by the end of the first week. It was characterised by two peaks, the larger in the proximal part and a smaller one in the distal part of the small intestine (Tarvid 1991). These observations emphasised the significant role of the distal ileum in membrane peptide hydrolysis. Several authors explained the high level of dipeptidase activity in the distal regions of the small intestine in adult animals by the dominating role of this part of the intestine in oligomer hydrolysis (Das and Radhakrishnan 1975; Corring 1980), while the others related this fact to the necessity for endogenous protein assimilation (Skovbjerg 1981).

The main conclusion is that the small intestine grows very rapidly during the early postnatal period, especially during the first week post partum. The function of peptide hydrolysis develops at a slower rate than the growth of the small intestine. Different regions of the small intestine have their own distinctive patterns for the development of enzyme activity that provide the specific gradient of distribution of peptidase activities in the small intestine with its maximum in the distal segments. The intensity of peptide hydrolysis during the early postnatal period is practically constant in homogenates and, significantly, decreases on the intestinal surface. Evaluation of the functional capacity of peptide hydrolysis during animal growth and development indicated that total peptidase activity in the whole small intestine increased intracellularly and on the cell surface of the small intestine with age (Tarvid 1991; Tarvid et al. 1994a). From these studies it became clear that the rate of body mass and organ growth was higher than the rate of increase of enzyme activity.

IV. CONCLUSIONS

In new-born mammals and newly hatched birds the digestive processes are not fully developed, though the activity of different enzymes in the stomach, in the pancreatic gland and in the small intestine might be very high just before birth or the time of hatch. Shortly post partum some enzyme activities decline. Most reported age-related studies in protein digestion have been based on measurements performed in a single tissue portion or in a single region of the small intestine, generally the jejunum. As a result, there is relatively little information about age-related shifts in the functional capacity of protein digestion along the entire small intestine. However, it is obvious from the above data that characteristics of certain digestive functions when expressed per unit of the organ weight, protein content in the organ, or even per unit of body weight, are not enough to demonstrate all the developmental changes. As developing animals grow, their intestines must provide ever increasing quantities of nutrients and energy. The conflict between the requirements of animals for protein and the decrease of the function providing its assimilation is obvious. Despite age-related declines in activities of some pancreatic and small intestinal enzymes expressed per unit of tissue weight or protein concentration, total functional capacities to break down dietary protein increase because of intestinal growth and generally parallel increases in whole body metabolic requirements. Only the characterisation of the function along the entire length or total weight of the organ may reflect real developmental patterns in young animals.

In general, the activity of different hydrolytic enzymes and the major transport mechanisms for amino acids in the digestive system do not develop synchronously during the growth of the alimentary canal. The genetic background of the breed has a profound effect on the maturation of the gastrointestinal tract and on the developmental patterns for the digestive enzymes. The rate of the maturation of the gut in fast growing animal breeds is much higher that in slow growing ones. Alimentary factors such as the time of the first feed post partum, diet composition, feeding regime, and supplementation with different growth promoters have secondary effects on the final maturation of the digestive system. Several specific features characterise species-related differences in the final development and maturation of the digestive system. In mammals the gut underdoes two stages of gut adaptation, after birth and post-weaning, until complete maturation. The development of the gut in the fowl has another specific feature. The chick is less dependent on external food while the yolk sac is present, and only after its total resorption during the third week does digestive function develop fully.

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