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DEVELOPMENT OF THE EQUINE GASTROINTESTINAL TRACT

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Introduction

Foals have a long gestational period (about 335 days) and are precocious at birth, standing and nursing within 20 minutes to 1 hour after birth. The transition from placental nutrition to enteral nutrition results in anatomic growth and differentiation of the gastrointestinal tract, gastric and pancreatic secretory development, and functional absorptive adaptations.

Development and maturation of the gastrointestinal tract begin in utero and continue through adulthood in the horse. The gastrointestinal tract and accessory glands must effectively secrete saliva, gastric acid, proteolytic enzymes, glycolytic enzymes, and bile. The gastric and intestinal mucosa must perform protective and absorptive functions.

Successful feeding of the equine neonate is dependent on optimal nutrition of the mare (Lawrence, 2006) and absorption of colostrum that contains specific nutrients, growth factors, and immunoglobulins. Changes in the gut and pancreas within 24 hours of birth increase the digestion and absorption of the nutrients found in mare's milk (Table 1).

Table 1. Composition of mare's milk.

<i>Time After Foaling</i>	<i>Total Solids (%)</i>	<i>Energy (kcal/100 g)</i>	<i>Protein (%)</i>	<i>Fat (%)</i>	<i>Lactose (%)</i>
1-4 weeks	10.7	58	2.7	1.8	6.2
5-8 weeks	10.5	52	2.2	1.7	6.4
9-21 weeks	10.0	50	1.8	1.4	6.5

Adapted from NRC, 2007.

Early Development of the Gastrointestinal Tract

The rate and extent of maturation of the equine gastrointestinal tract at any point in time is determined by genetics, a developmental biological clock, endogenous



regulatory mechanisms, and environmental effects (Lebenthal and Lebenthal, 1999). As parturition approaches, systemic adrenocortical hormones are responsible for the final stages of tissue and system development. The equine gastrointestinal tract is affected by cortisol. Developmental changes influenced by glucocorticoids include structural, cellular, and functional differentiation. Prenatal development of gastric acid, gastrin secretion, and hydrolase activities including chymosin, pepsin, amylase, lactase, and aminopeptidases are all influenced by cortisol (Trahair and Sangild, 1997). Additionally, growth factors, hormones, and nutrients from swallowed amniotic fluid and colostrum may influence gastrointestinal tract development. Fetal fluid ingestion has been shown to modulate tissue growth, macromolecule and immunoglobulin absorption, enterocyte differentiation, cell turnover, and activity of brush-border hydrolases (Trahair and Sangild, 1997).

The structural and functional adaptations of the gastrointestinal tract in the fetus and neonate that are designed to provide passive transfer of antibodies, including low luminal proteolysis and macromolecule transport in the neonate, are thought to be controlled by growth factors derived from the lining of the gastrointestinal tract. One role of cortisol is to alter partitioning of uterine glucose uptake to favor uteroplacental tissues, which may limit uteroplacental prostaglandin production (Fowden et al., 1991). This alteration enhances the glucogenic capacity of the fetus in late gestation (Fowden et al., 2002). Cortisol is a critical signal in the maturation of the gastrointestinal tract's endocrine system. In the horse, fetal cortisol levels increase later in gestation than other species. The fetal hypothalamic-pituitary-adrenal axis of foals only increases activity during the final few weeks of gestation (Fowden et al., 1991). This may explain the frequency of premature births and neonatal hypoglycemia in horses.

A significant rise in cortisol takes place 2 to 3 days before parturition (Silver and Fowden, 1994). Cortisol is important for maturation of pancreatic β -cells in the fetal horse. Fowden et al. (1991) reported that concentrations of hepatic glycogen and glucose-6-phosphatase, the enzyme responsible for mobilization of glycogen, increase substantially between late gestation and after birth concurrent with the rise in fetal cortisol. Fetal cortisol correlates positively with the prepartum rise in glucose stimulated by a rise in fetal insulin.

Ousey et al. (1991) used respiratory quotients (RQ) to determine what energy sources are used by foals during the first 24 hours of life. They reported that the foal uses endogenous carbohydrate and fat until it establishes mare's milk as the primary source of nutrition. Initially, the newborn foal uses carbohydrate that is stored in the liver and glycogen from skeletal muscle. The authors estimate liver glycogen stores would provide energy for less than 1 hour. By 2 to 4 hours of age, the RQ indicates stored body fat is the primary energy source. In well-nourished foals, this energy could last over 24 hours (Meyer and Ahlswede, 1976). Beyond 2 to 4 hours, the RQ stabilizes at about 0.82, indicating energy is being supplied by a combination of carbohydrate and fat that reflects the nutrient composition of colostrum and milk.

Aside from essential nutrients, mare's milk and colostrum contain growth factors.

The gastrointestinal tract increases in length and diameter in response to mare's milk. Increased villi height, density, and width; crypt density and depth; and enterocyte differentiation are all associated with local luminal factors and systemic signals. Mare's milk and colostrum contain trophic hormones, growth factors, enzymes, and bioactive factors. Cortisol, insulin, thyroid hormones, insulin-like growth factors (IGF), epidermal growth factor (EGF), lysozyme, and lactoferrin are important in maturation of gut enterocytes and provide protective mechanisms for infection, disease, and gastric ulceration (Ousey et al., 1995).

Feeding stimulates gastrin production of the stomach. Gastrin causes the release of pepsinogen and hydrochloric acid (HCl) for protein digestion. Pancreatic polypeptide stimulates gastric emptying and inhibits pancreatic exocrine secretions. Gastrin increases in fed foals from 1 day of age to 30 days of age, resulting in increased gastric acid and lower stomach pH.

Gastrointestinal tract hormone response is different between the first feeding and subsequent meals (Ousey et al., 1995). Gastric pH of newborn foals is higher (4.1) than in older foals (2.6) (Baker and Gerring, 1993; Murray and Grodinsky, 1989). The first colostrum feeding does not stimulate high gastrin production and therefore HCl and pepsinogen secretion are limited. Plasma polypeptide is higher after the first feeding. Rapid gastric emptying reduces gastric acid and pepsinogen, resulting in rapid transfer of immunoglobulins through the stomach where they can be absorbed intact in the small intestine.

Development of Hydrolytic Enzyme Systems

Roberts et al. (1973) reported significant changes in the development of lactase enzymes from fetus to maturity in the horse. Neutral- β -galactosidase can be isolated from the mucosal lining of the gastrointestinal tract in the fetus. This enzyme hydrolyzes lactose into the readily-absorbed monosaccharides glucose and galactose. The enzyme is located in the brush border of the enterocytes of the small intestinal mucosa. Neutral- β -galactosidase has an optimum pH of 5.5 to 6.0 (Asp and Dahlquist, 1968). Acid- β -galactosidase is present at birth in the jejunal and ileal mucosa and has an optimal pH of 3.4. This enzyme has a maximal production (6.29 units) at 2 days postpartum. Neutral- β -galactosidase (14.6 units) and β -glucosidase (cellobiase) (3 units) peak at birth and decrease steadily for 3 years (Figure 1). These enzymes are virtually undetectable in adult horses.

Lactase is the primary hydrolase until 3 months of age, at which time maltase activity that had been relatively low begins to increase. Between 3 and 6 months of age, maltase and amylase reach adult levels in the gastrointestinal tract.

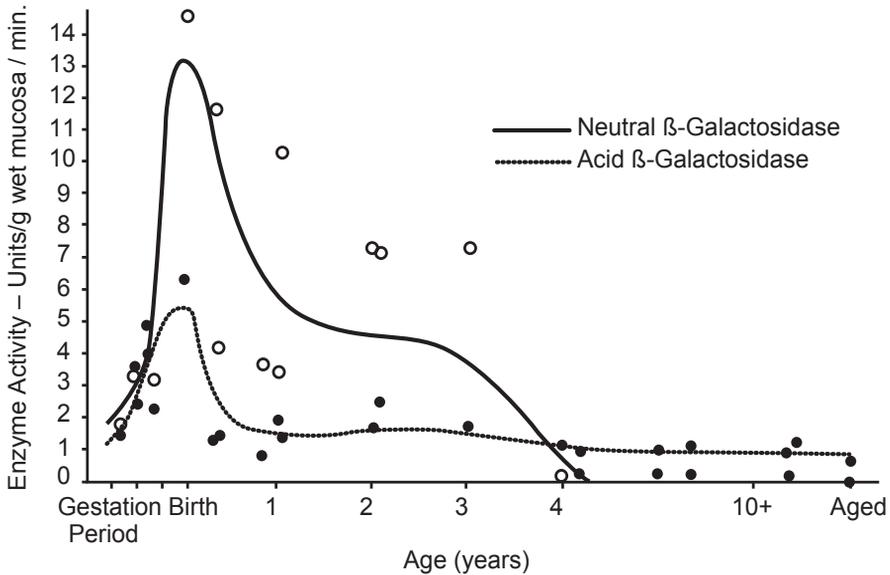


Figure 1. Development of β -galactosidase activities in the horse (Roberts et al., 1973).

Physical Development of the Gastrointestinal Tract

Functional changes in the equine gastrointestinal tract are reflected in increases in length of the various segments including the initiation of hindgut fermentation. Smyth (1988) measured the length of the small intestine, cecum, ascending colon, and descending colon of fetuses, foals, growing horses, and adult horses (Figures 2 and 3). The horses ranged in age from 150 days of gestation to 35 years. Data was collected on 130 horses of multiple breeds. For analysis, he expressed the proportion of the various segments as a percentage of total length.

The total tract length increases from midgestation to one year of age. The total length ranged from 2.5 meters in a 150-day-old fetus to 29.7 meters in a 16-year-old adult horse. The proportional length of the small intestine increases during gestation and the early postnatal period. The greatest increase occurred between 1 and 4 weeks after birth (Smyth, 1988). The proportion of the small intestine was significantly greater from birth to 16 days of age when compared to fetuses and adult horses. Between 2 and 6 months of age, the proportional length of the small intestine decreased in comparison to changes in the large intestine.

The cecum ranged in length from 0.08 meters in a 150-day-old fetus to 1.1 meters in an 18-year-old adult horse. The greatest increase was between 1 and 6 months of age. The cecum continued to increase in length until 1 year of age.

The length of the ascending colon ranged from 0.2 meters in a 160-day-old fetus to 3.8 meters in an 18-year-old horse. The ascending colon was the major contributor

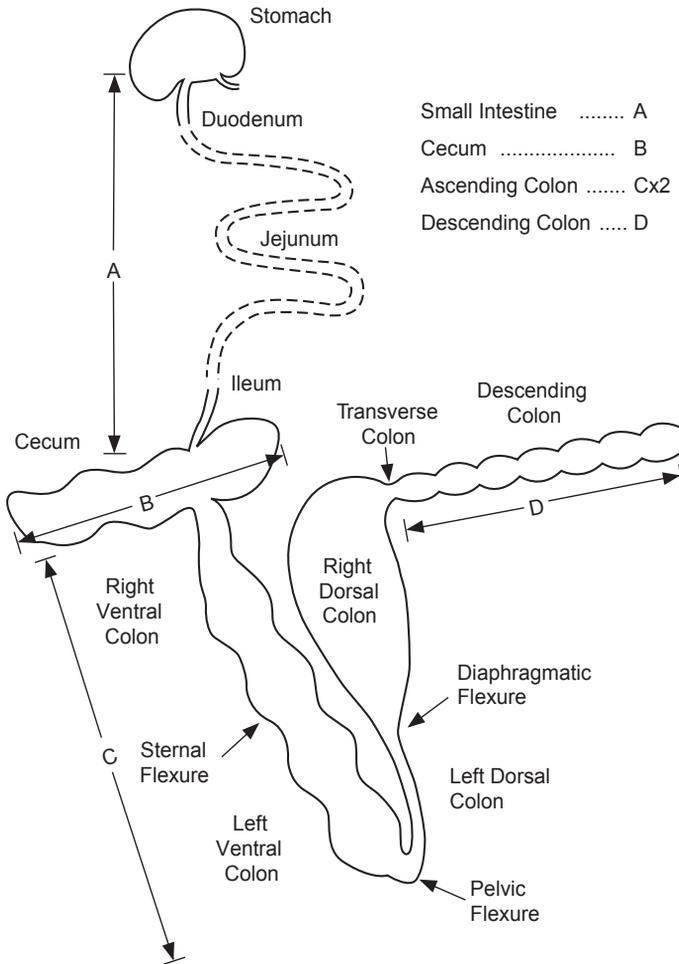


Figure 2. Diagram of the horse intestine showing points of reference used to measure lengths of the small intestine (A), cecum (B), ascending colon (Cx2), and descending colon (D) (Smyth, 1988).

to the increase in total intestinal length between 1 and 6 months after birth. It also was the most significant contributor to the increase in intestinal length between 1 and 2 years, and 10 and 20 years.

The length of the descending colon ranged from 0.32 meters in a 160-day fetus to 3.3 meters in an 11-year-old horse. The two most rapid periods of development of the descending colon were between 1 and 4 weeks of age and from 1 to 6 months of age. The percentage of gut length contributed by the descending colon was significantly greater in foals between 1 and 16 days and between 17 and 175 days. The period of

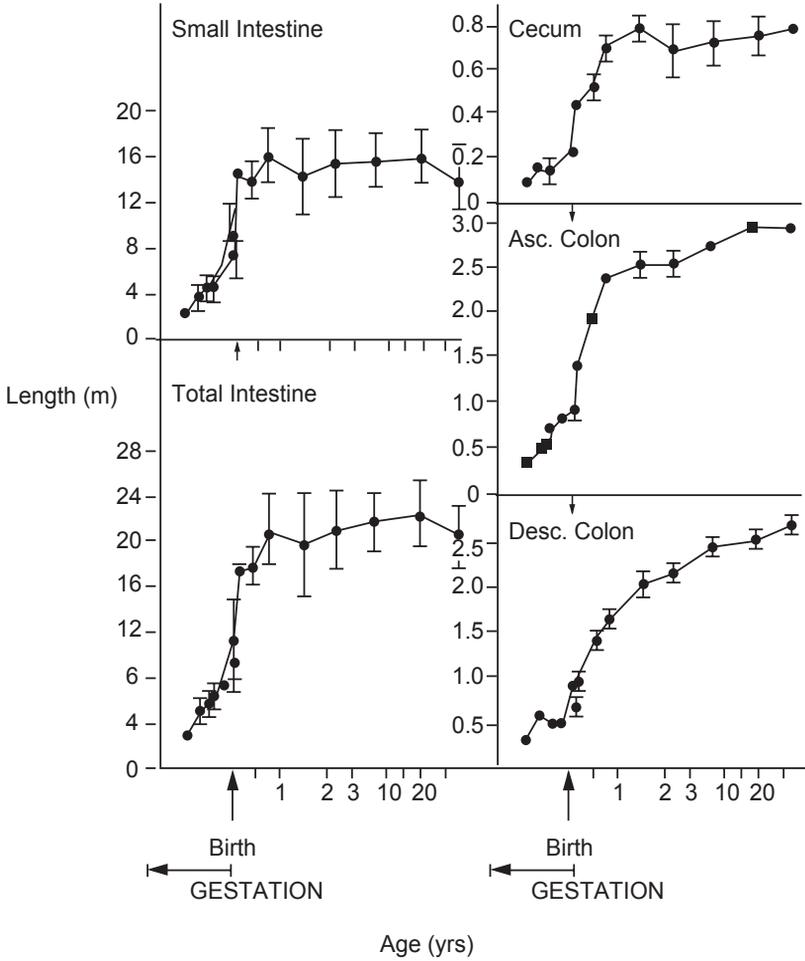


Figure 3. Lengths of the small intestine, cecum, ascending colon (ASC), descending colon (DESC), and total intestine in horses of different ages. Values represent mean \pm SEM (Smyth, 1988).

greatest increase in intestinal length after birth was the increase in the length of the small intestine from 1 to 4 weeks postpartum. This corresponds to the period of most rapid growth in the foal (Hintz et al., 1979; Pagan et al., 1996).

The rapid increase in small intestinal length is in response to increased milk consumption. Intestinal growth in length is coordinated with increases in diameter. These morphological changes are also accompanied with structural changes including development of villi, crypts, blood vessels, and nerves (Trahair and Sangild, 1997). From 2 to 6 months of age, there were greater relative increases in lengths in the cecum, ascending colon, and descending colon. After one year of age, only the ascending and descending colons increase in relative length. Increases in length of the segments of the colon continue to a lesser degree through adulthood.

Foals forage very little when they are less than one month of age. As they mature, the amount of time spent foraging increases from 20 to almost 50% of the day from 1 to 5 months of age. By 10 months of age, growing horses are spending 60% of each day grazing (Boy and Duncan, 1979). Increased length of the hindgut corresponds to developmental age and changing diet (Smyth, 1988). The largest proportional change in length of hindgut segments is from 1 to 6 months of age. Foals increase forage intake dramatically during this time, suggesting that a functional hindgut is important during the dietary transition from milk and the soluble carbohydrates in grain to the structural carbohydrates of forage at weaning.

Development of Microbial Fermentation

The fetus develops in a sterile environment. Consequently, the gastrointestinal tract of the fetus is also devoid of microorganisms until parturition. The physical process of parturition exposes the newborn to environmental bacteria from the dam's vagina, feces, and saliva. In human infants bifidobacteria are the predominant lactic acid bacteria, whereas lactobacilli are the primary lactic acid bacteria colonizing the gastrointestinal tract of newborn farm animals (Eadie and Mann, 1970).

In 2000, Norikatsu et al. found a layer of lactobacilli lining the nonsecretory area of horse stomachs. Such bacterial layers were observed in animals from 1 to 23 years old. Their research indicated that this lactobacillus flora becomes established soon after birth and adheres to the stomach epithelium throughout the life of the horse. This host-microbe interaction demonstrates one mechanism of prevention of pathogenic microorganisms from inhabiting and colonizing the mucosal layers of the stomach as well as the subsequent small and large intestines.

Many organisms enter the gastrointestinal system only transiently and due to either internal or external factors are not able to colonize. Yukikiko and coworkers (1999) determined that the majority of the coliform bacteria identified in young foals at 3, 7, 14, 30, and 60 days of age were *Escherichia coli*. However, the α -toxin-producing bacterium *Clostridium perfringens*, suggested to be related to equine diarrhea, was detected at high levels in all specimens sampled at 3 and 7 days of age but was undetectable in any specimens obtained at 60 days of age, indicating that this organism only colonizes the gut transiently immediately after birth (Yukikiko et al., 1999).

The development of the large intestine (cecum, ascending colon, and descending colon) coincides with the development of the microbial ecology and microbial digestion. The succession of microbial colonization is most marked in the early stages of life. From suckling to weaning, the dramatic shifts in host-produced enzymatic digestion to anaerobic fermentation are directly associated with the changes in the type and quantity of feedstuffs ingested by the growing foal. Dynamic balances exist between the gastrointestinal microbiota, host physiology, and diet that directly influence the initial acquisition, developmental succession, and eventual stability of the gut ecosystem (Mackie et al., 1999).

Microbial succession during the first few weeks of life in the alimentary tracts of humans and farm animal species is remarkably similar even though neonatal animals are exposed to greater numbers of fecal and environmental bacteria than are human neonates (Smith, 1965). Within a few days of birth, coliforms and streptococci dominate the microbial environment in many mammalian species (Mackie et al., 1999). Obligate anaerobes appear later in the developmental process. Clostridia and lactobacilli species may also be present in most hosts within a short period of time.

After birth newborns are continually exposed to new microbes that enter the gastrointestinal tract via food intake. This process begins with the ingestion of milk, which contains up to 10^9 microbes/L (Moughan et al., 1992). The most abundant organisms cultured in mammalian milk secretions include staphylococci, streptococci, corynebacteria, lactobacilli, propionibacteria, and bifidobacteria. These commensal organisms originate from the nipple and surrounding skin as well as the milk duct within the mammary gland (Mackie et al., 1999).

Both adults and newborns are continually exposed to microorganisms that enter the alimentary system with food, but the two groups are affected differently. The microorganisms entering newborns via milk consumption are more likely to colonize than are those entering healthy adults with stabilized populations. Opportunities arise, both internal and external, in which colonization of ingested microbes is favored in newborn and developing foals.

External factors that affect microbial colonization and succession in the gastrointestinal tract include the microbial load of the immediate environment, food and feeding habits, and composition of the maternal microbiota. As previously described, the feeding habits of foals change drastically during the first 5 months of life as they spend less time nursing and more time grazing (Boy and Duncan, 1979). As this shift in feeding occurs, so too do the profile and population of the gastrointestinal microorganisms.

The increased intake of feeds high in complex carbohydrates such as cellulose and hemicellulose requires the establishment of colonies of bacteria that can ferment such compounds in an anaerobic environment. Examples of anaerobic, cellulolytic organisms include *Ruminococcus albus*, *Fibrobacter succinogenes*, and *Butyrivibrio fibrisolvens*. Young foals are frequently observed consuming their dam's feces, and this serves as a direct inoculation of these vital gastrointestinal organisms. Due to the increased time required for bacterial digestion, the rate of passage through the large intestine greatly increases, particularly after weaning. In the young suckling foal, the stomach empties more slowly after a meal allowing more time for milk digestion by the acids and enzymes in the stomach and small intestine. After weaning, however, the stomach empties rapidly and the ingesta spends the majority of its time in the large intestine, where the relatively slow process of microbial digestion occurs, along with reabsorption of the large volumes of digestive secretions (Findlay, 1998). Internal or host-related factors affecting microbial succession include intestinal pH, microbial interactions, environmental temperature (within the host animal), physiologic factors,

peristalsis, bile acids, host secretions, immune responses, drug therapy, and bacterial mucosal receptors (Conway, 1997).

As the foal matures and ingests a greater variety of feedstuffs, its microbiota also multiplies and develops in much the same way an organ would. The function and development of the equine hindgut and its microbial inhabitants are markedly similar to that of the rumen of the growing calf (Hungate, 1966). For this reason, much of the research performed on calf rumens is highly correlated with the processes that occur in the hindgut of the foal. Researchers in the mid-1950s evaluated the functionality of the rumen and its dynamic microflora. The scientists determined that *in vitro* cellulose digestion by rumen contents was 25 to 40% by 1 week of age and had essentially doubled by 15 weeks (McCarthy and Kesler, 1956). Ruminant volatile fatty acid (VFA) production, the end products of fermentation transformed to energy sources in the liver of the bovine and equine, peaked at 7 weeks and then leveled off. The researchers concluded that although the rumen continues to increase in size and volume, rumen function in the calf can be considered qualitatively similar to that of an adult by six weeks of age. Several other reports have confirmed that rumen concentration of VFA from calves on solid feeds reaches adult proportions between the sixth and eighth week of life (Huber, 1968). Cellulolytic activity of rumen microorganisms approached adult levels by six weeks of age (Huber, 1968).

It is important to clarify that efficiency, activity, and VFA concentrations that are similar to adult levels do not imply that volumes or population sizes are comparable. The proportional growth of the large intestine and the distention of the cecum due to significant quantities of forage ingestion are some necessary components for increasing the quantity of the microbial population and volumes of VFA. The cyclic and dynamic processes that stimulate cecal growth and function are all directly related to the ingestion of diverse nutrients, plant constituents, and environmental inoculum, and help in establishing each horse's hindgut ecology. A consistent diet including quality forages will help develop the neonate's hindgut microbial population into an efficient organ of plant and forage digestion that will play an essential role in the nutrition of the horse throughout its lifetime.

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