Carbohydrate Metabolism in Horses (18-Aug-2003)

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The horse evolved primarily as a grazing and browsing, hindgut fermenting herbivore, with a range of hydrolyzable and fermentable forage carbohydrates as its main source of energy. Pastures provide the primary habitat and nutrition for most horses, and the remaining stall-confined horses have approximately one-half of their nutrition supplied by conserved pasture, i.e., hay. Pasture composition varies with season and weather, especially in carbohydrate content [1]. Performance horse owners supplement a diet of pasture and hay with grain concentrates in order to meet energy demands of exercise. Most commercial horse feeds supply energy, protein and micronutrients that complement forages but are based on cereal grains containing abundant starch, which is alien to the nutritional heritage of the horse. When fed as two large meals a day, grain supplements may cause starch overload and set up a feeding-fasting cycle of metabolites and hormones. Common experience has been supported by epidemiological and experimental studies that associate grain concentrates with several digestive and metabolic disorders, including colic [2-4], laminitis [5], gastric ulcers [6], developmental orthopedic disease [7,8] and some forms of exertional rhabdomyolysis [9]. As hindgut fermenters, horses have an opportunity for small intestinal metabolism of simple sugars to glucose, which is more metabolically efficient than fermentation to volatile fatty acids, which is obligatory in the ruminant. Understanding the sites of carbohydrate digestion in the equine gastrointestinal tract is useful when considering energetic efficiency and performance, as well as digestive and metabolic disorders associated with carbohydrate sources rich in starch and sugar. This chapter will summarize carbohydrate metabolism, which encompasses the biochemical events associated with the breakdown of carbohydrate in food, from ingestion to energy production to excretion, with a focus on horses and comparisons with other species. Disorders associated with carbohydrate metabolism will be reviewed briefly, as well as methods for evaluating carbohydrates in horse feeds and assessment of glucose dynamics in horses.

Carbohydrate Digestion

From the perspective of plant physiology, carbohydrates may be divided into three groups: simple sugars, storage molecules (e.g., starch, fructans), and structural polysaccharides (e.g., hemicelluloses, cellulose). From the standpoint of equine digestive physiology, carbohydrates may be divided into two major groups: those hydrolyzed to simple sugars in the small intestine, and those that undergo bacterial fermentation to volatile fatty acids in the hindgut. The primary difference in these groups is the linkage of the sugar molecules of the carbohydrate. Carbohydrates with α-1,4 linked molecules are subject to enzymatic hydrolysis, while β-1,4 linked molecules must be fermented. Hydrolyzable carbohydrates include disaccharides, some oligosaccharides (e.g., maltotriose) and starch. Fermentable carbohydrates include hemicellulose, cellulose and ligno-cellulose, soluble fibers, some oligosaccharides (e.g., fructans, galactans), and starches resistant to enzymatic hydrolysis [1].
Hydrolytic Digestion - Enzymes secreted in the small intestine specific to carbohydrate hydrolysis include α-amylase, α-glucosidases (sucrase, glucoamylase, maltase), and β-galactosidase (lactase). Unlike humans, relatively little α-amylase is present in saliva, so only limited hydrolysis occurs prior to arrival of carbohydrates in the stomach. Carbohydrates are hydrolyzed to an extent by the gastric acid in the stomach, independently of any enzymes.
Digestion of hydrolyzable carbohydrates is initiated primarily by pancreatic α-amylase in the small intestine. In the lumenal phase, α-amylase cleaves α-1,4 linkages in starch molecules but does not cleave α-1,6 linkages or terminal α-1,4 linkages. Amylopectinase cleaves α-1,6 linkages. The end products at this stage are disaccharides and oligosaccharides; no free sugars are yielded. Disaccharidases sucrase, lactase and maltase are expressed along the length of the equine small intestine [10]. Sucrase activity was higher in the duodenum and jejunum than the ileum, while maltase activity was similar in duodenum, jejunum and ileum [10]. Functional lactase was present in all portions of the small intestine of mature horses, higher in the duodenum and jejunum than the ileum. Although lactase activity was lower than that expressed in weaned horses less than four years of age, it appears that mature horses can digest lactose [10]. The action of these enzymes at the brush border mucosal cell completes hydrolysis to yield free sugars, glucose, galactose and fructose.

Fermentation - Fermentation occurs predominantly in the large bowel of horses but may occur in any portion of the digestive tract where conditions favor proliferation of microorganisms, including adequate retention time and pH greater than 5, although pH less than 6 favors production of lactic acid [11]. The presence of viable anaerobic bacteria, acetate, propionate, butyrate and lactate, suggests that limited fermentation occurs in the equine stomach, particularly in the fundic region [12], and favors lactic acid [13]. However, the brief retention time in the stomach and the dorsal to ventral pH gradient (5.46 in the squamous fundus to 2.7 in the pyloric region) of the gastric mucuosa [14] supports nominal fermentation. It appears that some fermentation occurs in the distal small intestine of the horse [15,16]. It is not well known whether small intestinal fermentation occurs independent of large bowel fermentation, or if fermentation in the small intestine is due to reflux of large bowel contents.

Carbohydrates not hydrolyzed in the small intestine pass to the cecum and large colon and are fermented by intestinal microflora to yield volatile fatty acids, mainly acetate, propionate, butyrate, and to a lesser extent, lactate and valerate. Microflora produce cellulase, which hydrolyzes β-1,4 glucose linkages in hemicellulose and cellulose. Ligno-cellulose may be degraded to cellulose by fungi present in the large bowel, while lignin remains undigested and is excreted in feces.

The relative proportions of volatile fatty acids produced are dependent on substrates, fundamentally the proportions of dietary forage and concentrate (Table 1) [17]. Increasing proportions of grain favored production of propionate as well as lactate [18] at the expense of acetate, suggesting support of rapid over slow fermentation and an overload of hydrolyzable carbohydrate (see Overload, below). Feeding higher percentages of grain depressed the efficiency of fiber utilization by altering the equine microbial ecosystem in the cecum and colon [19].

Table 1. Molar percentage of volatile fatty acids produced in the cecum, as affected by dietary forage-grain ratio [17]				
Diet, Forage-Grain Ratio	**Acetate**	**Propionate**	**Butyrate**	**Valerate**
Alfalfa meal: Grain mix				
1:0	76[a]	15[c]	8	0.5
3:2	70[b]	21[b]	7	0.8
1:4	61[c]	26[a]	10	1.2
Timothy hay: Corn				
1:0	71[a]	19[b]	9	0.3
1:1	69[a]	18[b]	11	0.4
1:4	58[b]	24[a]	13	1.8

[a,b,c] Values with different superscripts differ ($P < 0.05$)

Effects of Processing on Carbohydrate Digestion - Starch digestion is impeded when the physical form of the food limits contact with pancreatic amylase. This occurs if the starch is contained within whole grain or waxy seed coats, such as rice or corn, entrapped within rigid cell walls that hinder swelling and dispersion of the starch, such as soybeans, or if the starch is densely packed, which is more typical in human foods, such as pasta, than horse feeds. Milling and grinding increased susceptibility to hydrolysis in vitro by breaking the seed coats and cell walls, as well as abrading the surface of starch granules at the microscopic level, which is smooth in its natural state [20]. Preileal digestibility of starch was improved in horses when oats or corn were ground, while rolling and breaking did not improve digestibility over that of whole grain [21], so it appears that significant milling is required to increase digestibility *in vivo*. Preileal digestibility of whole or crushed corn was 30%; grinding increased preileal digestibility to 51%, and popping increased preileal digestibility of corn to 90% [22]. Similarly, the glycemic response, hence assumed preileal digesitibilty, was greatest to least, respectively, after equivalent meals of steam flaked, ground and cracked corn [23]. Effects of cooking on starch for horses should be considered carefully: steam flaking appears to be beneficial, but heating a wet mash or gruel using high starch feeds may be contraindicated. When cooked with an excess of water, the crystalline structures of starch lose order, gelatinization occurs, and the starch becomes susceptible to hydrolysis by pancreatic amylase. When cooled, however, the amylose chains are recrystallized, or retrograded, into a configuration that is highly resistant to hydrolysis *in vitro* [24]. Starch resistant to hydrolysis in the small intestine is rapidly fermented in the large intestine and may contribute to carbohydrate overload.

Overload - A carbohydrate overload component has been implicated in the etiology of laminitis and digestive disorders, notably colic [2,25,26]. In horses, the hydrolyzable fraction is digested in the small intestine up to a point at which the enzymatic capacity becomes overloaded, and the excess hydrolyzable carbohydrate is then fermented in the large bowel with the remaining unhydrolyzable carbohydrate [2,26,27]. The critical capacity for hydrolyzable carbohydrate overload in the horse appears to be approximately 0.4% of body weight per meal [28], but may be as little as 0.2%, depending on source of starch [21]. Rapid fermentation of hydrolyzable carbohydrate favors proliferation of *Lactobacilli* spp. and production of lactate, which is poorly absorbed [13,29]. Accumulation of lactic acid may overpower the buffering mechanism of the cecum and colon and lower pH. In grazing horses, hindgut pH remains consistently around 6.4 - 6.7. A cecal pH of 6 was considered to represent sub-clinical acidosis [30], and a pH below 6 was associated with clinical conditions such as osmotic diarrhea, overgrowth of undesired bacterial populations and lysis of desired bacterial populations, increasing the risk of endotoxemia and laminitis [31,32].

Carbohydrate Absorption

Absorption of Sugars - Two classes of glucose carrier proteins have been identified in mammalian cells [33]: the high affinity, low capacity, Na^+/glucose cotransporter type 1 (SGLT1) and facilitative glucose transporters (GLUT). The SGLT1 is present on the intestinal luminal membrane and in kidney proximal tubule absorptive epithelial cells. The SGLT1 transports primarily D-glucose and D-galactose across the brush-border membrane against the concentration gradient by active transport of Na^+ and the Na^+/K^+-ATPase. The sugars accumulate within the enterocytes and are transported down gradient into the systemic circulation via GLUT, primarily GLUT2 in the intestine.

Genes encoding the GLUT proteins have been identified as GLUT 1-12, and are divided into three classes, based on structure and sequence similarities [34,35]. The class I transporters, GLUT 1-4 isoforms, facilitate glucose transport across the plasma membrane down gradient, either into or out of cells throughout the body. GLUT1 is expressed in endothelial cells within the brain, placenta, eye and testis; GLUT2 is found in liver, small intestine, kidney and pancreatic β-cells; GLUT3 is the primary glucose transporter in brain parenchymal cells, and GLUT4 is expressed primarily in tissues dependent on insulin signaling, including adipose tissue, skeletal and cardiac muscle. The class II transporter GLUT5 transports fructose and is expressed mainly in the small intestine but also in kidney, brain, muscle, adipose tissue, testes and sperm. Other class II transporters, GLUT7, 9, 11, and class III transporters, GLUT6, 8, 10, 12, appear to have tissue and cell-specific expression similar to class I transporters [35]. The function and expression of GLUT1-5 are fairly well understood, but the function and structure-to-activity relationship of the more novel GLUT6-12 needs further examination. Future research regarding their molecular function should improve understanding of the mechanisms of glucose transport regulation and further elucidate etiologies of diseases in carbohydrate metabolism.

In horses, D-glucose and galactose are transported across the intestinal brush-border membrane by the SGLT1 carrier protein [10]. The major site of glucose absorption in horses is in the proximal small intestine, with glucose transport highest in the duodenum, followed by jejunum and ileum [10].

The lag time from an abrupt change in dietary hydrolyzable carbohydrate and the appearance of enhanced SGLT1 was between 12 to 24 h in mice [36]. To the best knowledge of this author, comparative lag times have not been studied in horses. Equine SGLT1 has 85% homology with mouse SGLT1 and 92% similarity at the amino acid level [10]. In mice, dietary regulation of glucose transport involves increased transcription of SGLT1, mainly in crypt cells [36]; comparatively in horses, expression of SGLT1 is regulated at the level of mRNA abundance [10]. The differences in length and function of horse and mouse digestive tracts may also play a role in the appearance of SGLT1 after abrupt changes in dietary hydrolyzable carbohydrate. If a similar lag time for SGLT1 exists in horses, then in the event of an abrupt change in diet, sugar transport would be inadequate, thus exacerbating hydrolyzable carbohydrate overload to the hindgut. Whether the differences in homology and expression of SGLT1 between mouse and horse influence the function of equine SGLT1, carbohydrate absorption and perhaps, overload, remains to be seen in future research.

Absorption of Volatile Fatty Acids - Volatile fatty acids are absorbed down the transmucosal pH gradient across the large intestinal wall by passive diffusion, primarily in the form of free acids. The rate of absorption is inversely proportional to molecular weight, with the absorption of acetate > propionate > butyrate > lactate [13]. The absorption of volatile fatty acids is integral to maintaining the pH of the colon above 6, which is required for optimal balance of fiber-digesting bacterial populations [11]. Absorbed volatile fatty acids pass into hepatic portal blood to circulate as neutral anions at blood pH.

Carbohydrate Oxidation

The oxidation of carbohydrates to yield ATP through glycolysis, the citric acid cycle, oxidative phosphorylation and the electron transport chain have been reviewed extensively [37-39]. Briefly, glucose, galactose and fructose undergo a series of glycolytic reactions to form glucose phosphates, which are then catabolized through the tricarboxylic acid cycle to form ATP, CO_2 and water. Alternately, these sugars are converted to and stored as liver or muscle glycogen, or returned to circulation as free glucose. Of the volatile fatty acids, propionate contributes to liver and muscle glycogen storage, while

acetate and butyrate provide carbon for fat synthesis.

Compared to other products of carbohydrate digestion, glucose provides the most efficient ATP generation, especially when directly oxidized (Table 2) [38]. Storage of carbohydrates prior to oxidation via tristearin comes at a cost in terms of ATP required for the synthesis of glycogen and triglycerides.

Table 2. Comparison of adenosine triphosphate (ATP) yield in complete oxidation of carbohydrates, either directly or indirectly [38]				
Substrate	**Enthalpy - kJ/mol**	**ATP Yield**	**ATP kJ** [a]	**ATP Efficiency**
Direct Oxidation				
Glucose	2803	35.5	1100	0.3925
Propionate	1527	18	558	0.3654
Acetate	874	10	310	0.3547
Indirect Oxidation				
Glucose via Lactate	2803	33.9	1052	0.3753
Glucose via Glycogen	2803	33.4	1023	0.3650
Glucose via Tristearin	2803	28.1	871	0.2290
Acetate via Tristearin	874	5.5	170	0.1951

[a] 31 kJ/mol ATP

Carbohydrates and Exercise

Energy for muscle contraction is acquired from creatine phosphate and ATP. During performance, horses utilize primarily glucose and fatty acids to fuel work. The chemical source of the energy generated depends on the duration and intensity of exercise, as well as dietary adaptation. During short duration, high intensity anaerobic work, ATP is generated as glucose is converted to pyruvate in the muscle through glycolysis. Under aerobic conditions, pyruvate is converted in the mitochondria to acetyl CoA and enters the citric acid cycle; however, under anaerobic conditions when pyruvate accumulates more rapidly than the oxygen-dependent citric acid cycle and electron transport chain can process, pyruvate is converted to lactate. Lactate accumulates in the muscle and blood until sufficient oxygen is available for conversion to pyruvate. Muscle accumulation of lactic acid has been generally associated with metabolic fatigue.

During long duration, low intensity aerobic work, both glucose and fatty acids may be oxidized to fuel performance. Feeding a meal rich in hydrolyzable carbohydrate appeared to enhance glucose oxidation during submaximal exercise in horses [40]. Glycogen utilization during submaximal exercise decreased with increasing concentrations of dietary sugar [41]. The high availability of glucose from meals rich in sugar and starch may have contributed to a delay in muscle glycogen utilization during submaximal exercise. However, it is unclear if this feeding practice is warranted for endurance exercise, when conservation of muscle glycogen may be beneficial.

Feeding a fat-supplemented diet may cause a shift from carbohydrate oxidation to fat oxidation during exercise. The result of this shift is the sparing of muscle glycogen [42,43]. Glucose-6-phosphate accumulates when phosphofructokinase is inhibited by citrate [44]. Citrate is produced by fatty acid oxidation, and the accumulation of glucose-6-phosphate is associated with the supression of glucose and glycogen utilization by the inhibition of hexokinase and phosphorylase.

Comparing the energy yield between glucose and fatty acids, glucose nets 35.5 moles of ATP formed from ADP and inorganic phosphate during glycolysis and the tricarboxylic acid cycle (Table 2). Beta-oxidation of fatty acids yields one Acetyl-CoA and 40 ATP from $FADH_2$ and NADH per cycle. Twelve ATP are formed from each Acetyl-CoA in the tricarboxylic acid cycle, and 2 ATP are required to transport the fatty acid into the mitochondria for β-oxidation. In this manner, 129, 142, 144 and 146 moles of ATP are formed from palmitic, linoleic, oleic and stearic acids, respectively. Net ATP yield from the oxidation of other fatty acids may be calculated similarly [38].

Compared to direct oxidation of glucose, the direct oxidation of fat yields not only more ATP but generates approximately 3% less heat. The reduction of 3% heat may be a small but significant advantage to the performance horse working under heat-stressed conditions [45]. This difference in heat production is about 50% when comparing the indirect oxidation of fatty acids via triglycerides to the indirect oxidation of glucose via lactate, glycogen or tristearin.

Glucose Regulation

Glucose homeostasis is the maintenance of a regulated state, rather than a steady state and displays a circadian rhythm [46]. Hormones of the pancreas, anterior pituitary, adrenal cortex and adrenal medulla, including insulin, somatostatin, glucagon, adrenocorticotropic hormone, cortisol and catecholamines, are associated with the metabolism of carbohydrate and the regulation of blood glucose [39]. Perhaps of most fundamental importance to the regulation of glucose is the anabolic action of insulin.

Insulin - Insulin is synthesized as preproinsulin on the rough endoplasmic reticulum in the pancreatic β cells. Preproinsulin is cleaved to proinsulin by microsomal enzymes, and the proinsulin is later cleaved to insulin and C peptide. Blood glucose concentration is the primary regulator of insulin synthesis and release; however, insulin release is also stimulated by amino acids leucine and arginine, vagal stimulation, sulfonylureas, and enteric hormones, including glucagon-like peptide, gastric inhibitory peptide (GIP), cholecystokinin (CCK), secretin and gastrin [39,47]. Insulin release is inhibited by hypoglycemia, somatostatin, and the α-adrenergic effect of catecholamines [39].

Insulin acts to enhance the transport of glucose into adipose and muscle through a redistribution of GLUT-4 from intracellular vesicles to the cell surface [48], resulting in a 10- to 30-fold increase in glucose transport to these tissues [49]. Insulin also enhances nutrient storage by promoting protein synthesis in muscle and the storage of triglycerides in adipose tissue, stimulating hepatic and muscle glycogen synthesis and storage, and inhibiting hepatic glycogenolysis, ketogenesis and gluconeogenesis.

Effects of Pregnancy - Glucose regulation is altered during pregnancy and lactation in many species, including humans [50], laboratory animals [51], sheep [52], cattle [53], pigs [54], and horses [55-57]. The changes in glucose regulation with pregnancy includes progressive development of insulin resistance, which allows for improved placental transfer of glucose in order to meet increasing demands of the fetus. Insulin resistance during late gestation facilitates the supply of glucose to the fetus at the expense of maternal tissues, through a shift in substrate utilization—from carbohydrates to fatty acids, and decreased glucose utilization in peripheral tissues [50,53]. In horses, changes in carbohydrate metabolism and pancreatic β cell function during pregnancy may contribute to insulin resistance [56]. In humans, progressive insulin resistance may trigger gestational diabetes, which increases risks of perinatal complications and subsequent development of maternal non-insulin-dependent diabetes mellitus.

Effects of Exercise - Exercise training elicits a shift in substrate utilization during submaximal exercise in many species. Six weeks of moderate-intensity training decreased glucose flux, muscle glycogen breakdown and whole-body carbohydrate oxidation [58].

Long-term exercise training increased insulin sensitivity in insulin resistant obese ponies [59]. Short-term, low intensity exercise improved insulin sensitivity in obese mares [60]. Exercise training for 12 weeks improved insulin sensitivity in old horses, but did not appear to affect normal insulin sensitivity in young horses [61].

Disorders Associated with Carbohydrate Metabolism

The Feeding-Fasting Cycle - Metabolic and hormonal changes associated with the feeding-fasting cycle are noted primarily in meal feeding animals. During the fed state, glucose, insulin and somatostatin increase, while free fatty acids, growth hormone and glucagon, and perhaps thyroid hormone, decrease. During fasting, free fatty acids, growth hormone, glucagon and thyroid hormone increase, while glucose, insulin and somatostatin decrease.

In the continuously grazing horse, the changes associated with the feeding-fasting cycle may not be largely evident. To provide additional energy in order to meet performance demands, humans introduced starch-rich cereal grains into horses' diets, commonly supplied as two meals per day. These meals have initiated metabolic and hormonal changes associated with the feeding-fasting cycle, which may contribute to disorders linked to grain feeding.

Insulin Resistance (Sensitivity). - Insulin resistance has been generally defined as a state when normal concentrations of insulin fail to elicit a normal physiologic response [62]. The terms insulin resistance and insulin sensitivity have been used rather loosely in equine literature, with higher resistance or lower sensitivity used as comparative terms related to elevated glucose tolerance or the failure of exogenous insulin to suppress blood glucose [59,63,64]. For the purposes of this review, insulin sensitivity and insulin resistance will be used interchangeably, with lower insulin sensitivity or greater insulin resistance generally indicating a state in which normal concentrations of insulin fail to elicit a physiologic response.

Insulin resistance is fundamental in the pathology of type II diabetes and has been identified as a risk factor in obesity [65], cardiovascular disease and hypertension [66], polycystic ovaries [67,68], pregnancy loss [69] and cancer [70] in humans. Diets rich in simple sugars have been associated with insulin resistance in several animal and human studies [71,72], and the common management practice of feeding starch-rich cereal grains in two meals a day may promote insulin resistance in horses [73]. Insulin resistance in horses has been associated with obesity [64,73] and laminitis [5], and may play a role in colic [4], exertional rhabdomyolysis [9] and osteochondrosis [8]. Insulin sensitivity was improved in horses by exercise and controlled feed intake [59,60]. Although insulin resistance has been reported in equine studies, the incidence of non-insulin dependent diabetes appears to be rare or nonexistent [74].

Developmental Orthopedic Disease - Developmental orthopedic disease is a collective term than encompasses all limb problems associated with bone growth disorders in the foal. It includes physitis, angular and flexural limb deformities, cervical vertebral malformation, dyschondroplasia, osteochondrosis and osteochondritis dissecans. The risk of developmental orthopedic disease may be increased by high energy intake [75], especially hydrolyzable carbohydrates [7]. Young horses with radiographic evidence of osteochondrosis lesions had greater responses of glucose and insulin after a starch-rich grain meal, compared with non-lesioned horses [8]. The glucose and insulin responses to the meal may be confounded by intake time, gastric emptying, digestion and rate of absorption; however, since the horses were raised in the same environment and fed similarly, the influences on meal responses are likely genetic or metabolic.

Feeding-fasting cycle changes associated with starch-rich grain meals provoke growth hormone secretion [76]. Growth hormone action is mediated by insulin-like growth factor I (IGF-I), which plays a role in the normal growth and maturation of articular cartilage [77]. Higher circulating concentrations of IGF-I were found in foals consuming starch-rich grain meals [78]. Perturbations in IGF-I concentrations may affect chondrocyte maturation and increase the risk of developmental orthopedic disease.

Evaluating Carbohydrates in Feeds for Horses

Carbohydrate Fractions - Current standardized methods of proximate analysis [79,80] relate to plant anatomy and fit reasonably well with the digestive physiology of ruminants, but not hind-gut fermenters such as the horse. A comprehensive scheme was considered in order to separate carbohydrates into groups for analysis appropriate for horses (Fig. 1) [1]. This scheme compares carbohydrate fractions obtained by two current systems of analysis with fractions as digested by the horse. The system of analysis for ruminant nutrition separates carbohydrates largely on the basis of plant anatomy into neutral detergent fiber (NDF), from plant cell walls, or nonstructural carbohydrate (NSC), mainly cell contents [79,80]. The NSC has been traditionally calculated by difference, 100 - water - protein - fat - NDF. There has been a movement within academia and the industry to improve the definition of terms associated with the non-structural or non-fiber carbohydrate portion of feeds. Thus, some laboratories have recently revised their procedures to analyze NSC directly by hydrolytic methods, and the calculated by difference fraction is now termed "non-fiber carbohydrate" (NFC) [81]. The similarities in terms and varied meaning of NSC (old 'by difference' analysis vs new "hydrolyzable" analysis) depending on methods used in individual laboratories invites confusion, so care should be taken when interpreting laboratory results. Figure 1 reflects the new terminology. Hereafter, this paper will refer to NSC as equivalent to hydrolyzable carbohydrate and NFC as the fraction calculated by difference.
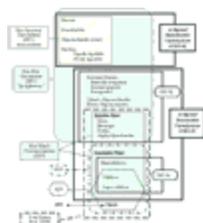

Figure 1. The scheme of carbohydrate fractions for the horse, as a comparison of proximate analysis fractions (left) with fractions as digested (right). Abbreviations include ADL, acid detergent lignin; ADF, acid detergent fiber; NDF, neutral detergent fiber; CHO-FS, slowly fermentable carbohydrate; CHO-FR, rapidly fermentable carbohydrate. (Adapted from Hoffman et al., 2001. Reprinted with permission of the Journal of Animal Science.) - To view this image in full size go to the IVIS website at www.ivis.org . -

A system of carbohydrate analysis for human nutrition places greater emphasis on plant chemistry, so includes polysaccharides resistant to digestion by mammalian enzymes but excludes lignins and ligno-cellulose [82]. Lignin and ligno-cellulose retard the rate of fermentation [83] and are present in much larger proportions in horse feeds than in human diets, so excluding these fractions may limit dietary assessment.

Neither the ruminant or human system fits well with the digestive physiology and intermediary metabolism of horses; however, the revised ruminant system that includes both NSC and NFC is an improvement. Optimally, an analysis based on digestive, metabolic, and energetic efficiency [2,45,84] of the animal, rather than plant properties, would include three main fractions useful in assessing diets for horses:

1. a hydrolyzed group that yields sugars, mainly glucose for metabolism;
2. a rapidly fermented group that yields primarily lactate and propionate, which are metabolized largely as 3-carbon or 6-carbon units, mainly via glucose;
3. a slowly fermented group that yields primarily acetate and butyrate, which are metabolized as 2-carbon and 4-carbon units, largely via acetyl-CoA.

Until such a scheme is available, it may be approximated in terms of hydrolyzable (NSC), rapidly fermented (the difference between NFC and NSC), and slowly fermented (approximated by NDF), as indicated in (Fig. 1). This approximation is

based on limited data reported from *in vivo* studies [16,28,85].

The NFC fraction contains both hydrolyzable and rapidly fermentable portions, including gums, mucilages, β-glucans, and pectins, which are not recovered by the NDF method. The hydrolyzable NSC fraction accounts for about one-fifth of the NFC in hay, one-third of the NFC in pasture, one-half to two-thirds of the NFC in fiber-rich concentrates, and all of the NFC in typical "sweet feed" grain-mixes for horses [1].

Rapidly fermented carbohydrates consist of resistant starches and oligosaccharides, especially fructans, which may comprise 5 to 50% of the dry matter in grasses [86]. Starch may be resistant by physical entrapment, chemical structure, or by heating and degradation. Corn contains physically resistant starch, so its digestibility is improved with processing. Potato and manioc contain chemically resistant starch, with preileal digestibility of less than 10% [22]. The β-2,6 glycocidic bonds in fructans are not broken by mammalian enzymes, so fructans are rapidly fermented in the hind gut. Abrupt increases in fructans were observed from day to day in rapidly growing pastures, and from hour to hour as plant composition changed from night to day or from shade to sunlight [87]. An association between an abrupt increase in fructans and the incidence of laminitis has been suggested [32,87].

Glycemic Index - The glycemic index is a reflection of plasma glucose and insulin responses to a meal, an *in vivo* estimate of the hydrolyzable carbohydrates in a feed. It provides information about the food but not necessarily the animal. The glycemic index has been applied primarily in human nutrition for diabetics in order to formulate diets with a low glycemic impact, with glycemic index calculated as a percentage of the response to a standard food, white bread [88,89]. In horse nutrition, meal-related responses of blood glucose and insulin to different diets have been quantified in several reports [90-92]. Studies in the same laboratory assigned a glycemic index using the glucose area under the curve (AUC) in response to a moderate meal (1.5 kg/horse; [93]), or as 2 g feed/kg body weight (BW) [23], with either whole oats or cracked corn as the reference feed. The glycemic indices from these studies are summarized in Table 3, using whole oats as the reference feed.

Table 3. Area under the curve (AUC) of glucose response and glycemic index relative to whole oats in different feeds and varying degrees of processing		
Feed	**Glucose Response (AUC, mg*min*dL^{-1})**	**Glycemic Index**
From Pagan JD, Harris PA, Kennedy MAP, et al., 1999 [93]		
Sweet feed [a]	2,073	129
Whole oats	1,602	**100**
Cracked corn	1,438	90
Fiber mix [b]	1,378	86
Sweet feed + 10% corn oil	898	56
Alfalfa	733	46
From Hoekstra KE, Newman K, Kennedy MAP, et al., 1999 [23]		
Cracked corn	1,734	108
Ground corn	1,887	118
Steam flaked corn	2,500	156

[a] The sweet feed contained 45% each of cracked corn and whole oats, and 10% molasses.
[b] The fiber mix contained 25% each of rice bran, soybean hulls, wheat bran, soaked beet pulp.

The slight discrepancies between the two studies above may be any of several factors affecting glycemic response including meal size, concentrations of hydrolyzable carbohydrates, fiber and fat in the feed, processing, intake time, gastric emptying, digestibility and rate of absorption. Horses appear to be sensitive to small changes in dietary starch and sugar content, to the point at which starch and sugar overload escapes hydrolysis and is fermented in the hind gut [28]. Previous work demonstrated the glycemic response of a 50% corn: 50% alfalfa diet (estimated 350 g starch/kg feed) was equal to that of a 100% corn (estimated 650 g starch/kg corn) diet, and as expected, higher than a 100% alfalfa (estimated 80 g starch/kg

alfalfa) diet [90]. Research in our laboratory [76] indicated that the glycemic response of a feed rich in fiber and fat (166 g starch/kg feed; 266 g/meal) was equivalent to that of a feed rich in starch and sugar (530 - 645 g starch/kg feed; 850 - 1200 g/meal). Another report indicated a minimal glycemic response at 120 g starch/kg feed or 220 g/meal [92].

Evaluating Glucose Dynamics in Horses

Evaluation of glucose dynamics includes tests that provide information about a horse's glucose homeostasis and metabolism. Information of interest includes glucose tolerance and clearance, indicating the effectiveness of plasma glucose concentrations to mediate its own disposal, as well as insulin sensitivity, or insulin resistance.

Factors affecting equine glucose dynamics include diet adaptation [94], fed or fasted state [95-97], age [61,98], pregnancy and lactation [55,57], obesity [64,73], laminitis [5], pituitary adenoma [99] and polysaccharide storage myopathy [100].

Glucose Tolerance Test - The responses of plasma glucose and insulin to a glucose challenge is known as the glucose tolerance test [101], which provides information about the animal's glucose metabolism and homeostasis. The glucose tolerance test may be administered either orally via nasogastric tube or intravenously.

Oral Glucose Tolerance Test - In an oral glucose tolerance test the initial rise in glucose reflects the sum of absorption and clearance: blood glucose concentration increases while the rate of glucose entry exceeds the rate of glucose removal. Peak blood glucose concentrations in horses occur approximately 60 min after the oral glucose dose [64,94,101]. The subsequent fall in blood glucose is a reflection of clearance exceeding that of absorption. Eventually blood glucose falls below basal concentrations in a hypoglycemic phase characterized by inertia of glucose regulatory mechanisms; then cortisol stimulates hepatic gluconeogenesis, and blood glucose concentrations oscillate and return to baseline. The entire oral glucose tolerance test, including the intial rise, fall, hypoglycemic phase and final return to basal concentrations, is complete in 3 to 5 h in the horse, depending on dose and status of the animal.

The oral glucose tolerance test in the horse was typically used to aid in diagnosis of pancreatic and small intestinal dysfunction [101]. When an increased risk of laminitis appeared to be associated with hyperglycemia and hyperinsulinemia [59,64], oral glucose tolerance tests were applied as a functional index of the metabolic response of horses to certain feeds [57,94]. Adaptation to a diet with high concentrations of sugar and starch favors a minimum rise in the glucose response curve, while adaptation to a low or hydrolyzable carbohydrate-free diet elicits a high glucose response curve.

Horses adapted to pasture only, compared to a stable diet of hay and commercial feed, had a higher response to an oral glucose dose and 1.8 times as much glucose AUC [94]. Compared with pasture fed horses, the horses fed the stable diet had approximately 3.5 times as much hydrolyzable carbohydrate, so their diet likely had a higher glycemic index, which influenced their response to oral glucose. Adaptation to meals having a high glycemic index may have enhanced the ability of these grain-fed stabled horses to clear glucose at a rate faster than horses accustomed to grazing pasture.

Plasma glucose in ponies fasted for 24 h had a delayed peak and delayed return to baseline during an oral glucose tolerance test [96]. Plasma glucose concentrations followed a higher tolerance curve in pony foals, compared to adults [98], but had lower tolerance curves for young adult (7 yrs) than aged (27 yrs) horses [61].

Oral Glucose Tolerance versus Glycemic Response to a Meal - Some reports have used a grain meal to which the horse is adapted as the stimulus to elicit glucose and insulin response, in effect a glycemic index, but assuming equivalence of the meal stimulus to that of oral glucose. The glycemic response to oral glucose compared to a grain meal are not equivalent, as the response to a grain meal will be influenced by the amount of hydrolyzable carbohydrate in the meal, but confounded by mastication, intake time and digestibility of the feed. A pilot study in our laboratory intended to validate the use of a meal of heavy "racehorse" oat grain, provided at 200 mg NSC/kg BW, as a standard for equine oral glucose tolerance tests. However, the individual variation in incremental area under the curve of glucose was 88% for horses consuming heavy oats, compared to 7% for oral glucose. This lack of precision in response to the oat meal was considered unacceptable for a standardized dose. The oral glucose tolerance test removes variability associated with a grain meal stimulus because the specific glucose dose is known, and variation associated with intake is not a factor.

The interpretation of glycemic response to a meal versus oral glucose tolerance is quite different, so reports in the literature should be considered carefully. Feeds with a high glycemic index should elicit a larger glycemic response. However, a larger glycemic response to an oral glucose tolerance test would be expected in horses adapted to diets with a low glycemic index, such as hay or pasture. A lower glycemic response to an oral glucose tolerance test would be expected in horses whose glucose-insulin response is chronically stimulated by twice daily meals with a high glycemic index, such as oats, corn or traditional sweet feeds.

Intravenous Glucose Tolerance Test - The intravenous glucose tolerance test provides information that reflects glucose clearance relative to endogenous insulin secretion. Compared with an oral glucose tolerance test, the interpretation of the intravenous glucose tolerance test is less complicated, because it provides a direct measure of glucose clearance and is not affected by malabsorption.

The rate of glucose clearance was diminished in ponies fasted for 72 h, with fasted ponies having a half time of approximately 140 min, compared with 36 min in fed animals [95]. Horses with polysaccharide storage myopathy (PSSM)

had an increased rate of glucose clearance in response to insulin secretion, compared to normal horses [100]. The enhanced glucose clearance may contribute to abnormally excessive glycogen and polysaccharide in muscle of horses with PSSM. In horses, endogenous insulin secretion in response to intravenous glucose increases rapidly to a plateau after glucose infusion [73,102]. The rapid arrival and persistence of endogenous insulin secretion to a plateau implies that equine insulin secretion is matched by disposal during the first phase of the intravenous glucose tolerance test. The pattern of equine endogenous insulin response to intravenous glucose was different from that in humans [103], which exhibit an insulin peak within minutes after intravenous glucose infusion, followed by a decline as plasma glucose decreases.

Hyperinsulinemic Euglycemic Clamp - The glucose clamp technique allows for the evaluation of insulin sensitivity by stabilizing blood glucose concentration after an insulin injection using variable glucose infusion [104,105]. The glucose clamp technique has been applied successfully in the horse [60,106] but is technically difficult. Application of the glucose clamp indicated that insulin sensitivity was increased in horses subjected to short-term low intensity exercise [60].

Minimal Model of Glucose Dynamics - Glucose dynamics are described by the minimal model [107,108], which applies a modified glucose tolerance test, beginning with an intravenous glucose dose followed by an exogenous insulin bolus. The minimal model of glucose dynamics is a mathematical construct that partitions glucose disposal into "glucose effectiveness", the capacity of glucose to mediate its own disposal independent of plasma insulin, and insulin sensitivity, the capacity of insulin to promote glucose disposal. The minimal model also provides estimates of acute endogenous insulin response to i.v. glucose, and the disposition index, which describes pancreatic β cell responsiveness and accounts for the influence of both insulin sensitivity and endogenous insulin secretion.

The minimal model analysis has been used primarily to elucidate etiologies of diabetes in humans and other species [109]. However, insulin sensitivity estimated using the minimal model was positively correlated with maximal aerobic capacity (VO_{2max}) and proportion of type I muscle fibers in humans [110]. Hence, application of the minimal model may have use as a tool to evaluate athletic potential in horses. The minimal model effectively estimated glucose effectiveness and insulin sensitivity in horses [73], indicating less insulin sensitivity (greater resistance) in obese horses and in horses adapted to a diet rich in hydrolyzable carbohydrate.

Conclusions

Horses evolved consuming primarily fermentable forage carbohydrates, but forage diets have been traditionally supplemented with grain meals rich in hydrolyzable carbohydrates. Grain meals provide convenience for the owner and additional fuel for performance that is metabolically more efficient than fermentable carbohydrates. However, the consumption starch-rich meals may exacerbate equine digestive and metabolic disorders associated with carbohydrate metabolism. The limited preileal digestibility of starch contributes to the risk of hydrolyzable carbohydrate overload to the hindgut followed by rapid fermentation and perturbations in the microbial and pH balance of the cecum and colon. Absorption is limited by the activity and expression of two classes of glucose carrier proteins, which are affected by chronic intake of hydrolyzable carbohydrate but may be sluggish to respond to abrupt changes in diet. Feeding grain concentrates rich in hydrolyzable carbohydrate may increase the risk of insulin resistance, which has been associated with obesity, colic, laminitis, developmental orthopedic disease and some forms of exertional rhabdomyolysis in horses. The evaluation of feed carbohydrates should consider fractions as digested as well as effects on glucose regulation and metabolism.

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