

**Effects of Diet and Probiotic Supplementation on Stress
during Weaning in Thoroughbred Foals**

by

Carrie A. Swanson

Thesis submitted to the Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

Master of Science
in
Animal and Poultry Sciences

August 23, 2002
Blacksburg, Virginia

David S. Kronfeld – Committee Chair
Rhonda M. Hoffman – Committee Co-chair
Rebecca K. Splan – Committee member

Key Words: Horses, Probiotics, Weaning, Vitamin E, Fat

Abstract

This study investigated effects of diet and probiotic supplement on stress in Thoroughbred foals at weaning. Twenty foals, whose dams were paired by age and breeding date, then randomly assigned to one of two diets prior to parturition, were used. Two groups were maintained on mixed grass pastures and fed supplements, one high in sugar and starch (SS) and one high in fat and fiber (FF) that met or exceeded NRC requirements. Half the foals on each diet were fed a commercial probiotic, (Probios) containing lactic acid bacteria, while the rest were given a placebo. Plasma, fecal samples and behavioral ethograms were collected for four days pre- and post-weaning, and an ACTH response test was administered 48 h post-weaning. Cortisol, lactate, IgG, IgA and α -tocopherol were analyzed in plasma, volatile fatty acids and pH in feces. Foals fed FF had higher concentrations of IgA ($P = 0.006$), IgG ($P = 0.012$) and α -tocopherol ($P = 0.005$). Butyric and valeric acid concentrations were higher in feces of SS but not FF foals ($P = 0.052$), which may reflect better adaptation to forage in FF foals. Foals supplemented with probiotic had higher fecal lactate ($P = 0.002$) and lower fecal acetate ($P = 0.0003$) concentrations, suggesting that the lactic acid bacteria survived to the hindgut. Probiotic supplementation did not appear to benefit foals at weaning. Supplementation with FF may improve immune status and encourage a more diverse intestinal microbial population, enabling foals to better cope with the physiological stresses of weaning.

Table of Contents

Table of Contents	iii
Acknowledgements	v
Introduction	1
Literature Review	3
Probiotics	3
Weaning Stress	10
High Fat, High Fiber Diets	12
Vitamin E	13
Materials and Methods	14
Table 1. Ingredient composition of the SS and FF feeds	16
Table 2. Nutrient content of pasture and feeds.....	16
Results and Discussion.....	23
Conclusions	30
Implications	31
Literature Cited	32
Figures	
Figure 1. Effect of weaning on <i>Lactobacillus</i> and <i>E. Coli</i> in pigs	39
Figure 2. Effect of probiotic LAB supplement on <i>Lactobacillus</i> and <i>E. Coli</i> in pigs at weaning.....	39
Figure 3. Diet Effects on Fecal Lactate	40
Figure 4. Probiotic Effect on Fecal Lactate.....	40
Figure 5. Probiotic Effects on Acetic Acid	41
Figure 6. Diet Effects on Acetic Acid	41
Figure 7. Diet Effects on Propionic Acid.....	42

Figure 8. Lactate : Acetate	43
Figure 9. Propionate : Acetate	43
Figure 10. Diet Effects on Butyric Acid.....	44
Figure 11. Diet Effects on Valeric Acid.....	44
Figure 12. Diet Effects on Isobutyric Acid.....	45
Figure 13. Diet Effects on Isovaleric Acid	45
Figure 14. Diet Effect on Fecal pH	46
Figure 15. Weaning Effect on Fecal pH.....	46
Figure 16. Diet Effect on Immunoglobulins	47
Figure 17. Diet Effects on Plasma Tocopherol.....	47
Figure 18. Weaning Effects on Behavior	48
Figure 19. Weaning Effect on Plasma Cortisol	49
Figure 20. ACTH Response Test.....	49
 Vita	 50

Acknowledgements

First, a special thanks to my family (since they've put up with me the longest): To my mom, who always said I was going to teach...I hate it when you're right. To my dad, who asked only that I not "turn into one of those horse people"...sorry Dad. To my brother, who has literally ALWAYS been there...there aren't enough words...but you know how I feel anyway. To my sister, who can see the beauty in all things...I am a better person for knowing you. To my grandmothers, who, while very different women, both inspired me with their strength and compassion, and who would both be proud if they could see me now. To Susan, Danni, Margie, Roberta, Fred and Cat who listened, gave advice, and stood by me while I went my own way.

I would also like to thank the Animal Science Department of Virginia Tech and the faculty and staff of the Middleburg Agricultural Research and Extension center. Especially Dr. David Kronfeld, who has provided me with endless learning opportunities, and Dr. Rhonda Hoffman whose cool head and willingness to explain things have made all the difference. Thanks to Dr. Rebecca Splan for her help with the sadistics...I mean statistics☺

My fellow graduate students deserve thanks as well...Kari, Rob, Carey, Tanja, Kibby and Tanya...who put up with my shit (samples, of course) and were willing to help where ever they could. Same goes for Dr. Burt Staniar...who put up with more than just the samples, and still managed to teach me a few things.

A HUGE thanks goes out to Bobbi, Alvin and Tim for all their help and friendship. You all made Middleburg seem like home. And even though they don't care about things like this, thanks to Bill and Scott, who sacrificed their time (during bow season no less) to help with my study.

Introduction

The balance of microflora within the gastrointestinal tract of all mammals is important to their digestive process and critical to their overall health. This bacterial population is particularly significant in herbivores, where it is responsible for fermenting dietary fiber and the subsequent production of volatile fatty acids (VFAs). Volatile fatty acids produced in the rumen are responsible for 60-85% of the metabolizable energy provided to sheep and cattle (Bergman, 1990). While this process is less efficient in hindgut fermenters, such as the horse, VFAs produced in the large intestine of these animals still provide an estimated 30-40% of the metabolizable energy derived from feedstuff (Glinsky, 1976).

Probiotics are commonly defined as ‘Live microbial feed supplements that beneficially affect the host animal by improving its intestinal microbial balance’ (Fuller, 1989). The use of probiotics in farm animals is based on the concept that the balance of intestinal microorganisms in healthy animals increases resistance to diseases and is necessary for efficient digestion and maximum absorption of nutrients (Fuller, 1999). Probiotics are most commonly used to combat problems associated with stress. During periods of stress, including some intense management practices of production agriculture, this microbial balance can be disrupted, resulting in increased populations of pathogenic microorganisms, which negatively impact host animal performance (Montes and Pugh, 1993). The purpose of administering probiotics is to re-establish the ideal balance between beneficial and pathogenic microorganisms, or to prevent this balance from being disturbed.

Research at the Virginia Tech Middleburg Agricultural Research and Extension Center (MARE Center) has focused on development of a high fat, high fiber pasture supplement. The pasture supplement currently under development is designed to promote a diverse microbial population that can readily adapt to changes in pasture carbohydrate content. While this pasture supplement has the fiber content of a roughage, the additional fat provides energy concentration (3.0 Mcal DE/kg) comparable to traditional concentrates. It contains a much smaller amount of hydrolyzable sugar and starch than typically found in commercial concentrates, a large spectrum of fibers, and a processed cereal byproduct containing 26.5 % fat and 18% protein. If a high fiber, high fat diet promotes a more diverse microbial population in the horse than traditional diets, it may enable horses to better withstand the adverse affects to gut microbes seen in times of stress.

Domesticated horses are subject to management and feeding practices that vary greatly from their natural routines. Equine athletes experience a variety of physical and mental stresses during intense competition, as well as long hours of transport and frequent changes in available forage. Whether the stress involves physical exertion, extended transport, sudden change in diet or feed intake, weaning, prolonged use of antibiotics, or simply old age, the balance of microbes in the gastrointestinal tract may be disturbed, effecting the animal's health and performance (Montes and Pugh, 1993). Unfortunately, many of these stresses are unavoidable, especially for businesses with heavily campaigned athletes or young bloodstock being prepared for sale. Professionals and hobbyists alike, however, are extremely concerned with improving equine welfare. Therefore, a diet or feed supplement that protects horses from adverse effects of physical and psychological stresses would be a welcome benefit to the horse industry.

Literature Review

Probiotics

The concept of probiotics was first reported by Elie Metchnikoff in 1907. He theorized that the longevity of certain ethnic groups was due to ingestion of fermented milk products, which manipulated the intestinal microflora to maintain the normal balance between pathogenic and non-pathogenic bacteria. The term probiotic was proposed in 1965, by Lilley and Stillwell, to describe substances that favored the growth of microorganisms. Fuller (1989) later revised this definition to specifically include microbes that directly benefit an animal by improving its balance of intestinal microorganisms. The term “probiotic” has since been used to refer to viable bacteria and fungal cultures, enzyme preparations, culture extracts or any combination of the above. Because of the confusion surrounding the term probiotic, the US Food and Drug Administration (FDA) began requiring manufacturers of animal feeds to use the term direct-fed microbial (DFM), instead of probiotic, in 1989 (Yoon and Stern, 1995). The FDA defined the term DFM as “a source of live (viable) naturally-occurring microorganisms”. Within the context of this paper, probiotic and DFM will be used interchangeably to refer to Fuller’s definition.

One of the reasons that use of probiotics has been viewed with skepticism is that the mechanism of action is not fully understood. Theories have included production of anti-microbial substances (Naidu et al., 1999), competition for adhesion receptors (Spring, 2000), competition for nutrients (Montes and Pugh, 1993) and stimulation of immune responses (Perdignon, 1986). Individual probiotics may use unique mechanisms or the same probiotic may inhibit various pathogens by different mechanisms.

Depending on the strain and type of nutrients available, lactic acid bacteria (LAB) are known to produce various anti-microbial substances. Lactic and other volatile fatty acids, which are end products of carbohydrate metabolism, decrease luminal pH, resulting in broad spectrum inhibition of gram-positive and gram-negative bacteria (Naidu et al., 1999). Some strains of LAB, *Lactobacillus acidophilus* for example, produce hydrogen peroxide (H₂O₂), which lowers the oxidation-reduction potential and specifically inhibits the growth of aerobic organisms. Anti-microbial proteins called bacteriocins, such as acidophilin, lactolin and acidolin, have anti-microbial activity against some strains of *Escherichia coli* and *Salmonella typhimurium* and are also produced by microbes in the gut (Naidu et al., 1999). Lactic acid bacteria can produce carbon dioxide (CO₂) via a number of pathways (Naidu et al., 1999). Carbon dioxide has destructive effects on cell membranes and is also able to decrease luminal pH. Two additional products of metabolism by LAB are diacetyl (2,3 butanedione) and acetaldehyde. Both have shown bactericidal effects against *E. coli* and *Salmonella* (Jay, 1982; Kulshrestha and Marsh, 1974).

The needs of pathogenic bacteria are similar to the needs of probiotics in that they must be able to survive and adhere to the intestinal epithelia in order to effect the host animal. Lactobacilli may prevent this by occupying epithelial binding sites or by producing a biofilm that physically protects the cells (Montes and Pugh, 1993). Both specific and nonspecific mechanisms have been identified for *L. acidophilus* (Kleeman, 1982). Some strains may also cause a nutrient depletion effect by exhausting food sources that are essential for the growth of other microorganisms (Montes and Pugh, 1993).

Finally, immune stimulation has been proposed as a mechanism of action for probiotic bacteria. Studies in mice suggest that administration of *L. acidophilus* and *S. thermophilus* significantly enhances the enzymatic and phagocytic activity of peritoneal macrophages (Perdignon, 1986). *L. casei* has demonstrated the ability to increase mucosal immunity via local production of IgA against *Salmonella typhimurium* infection when orally administered to mice (Perdignon, 1990).

In order for microorganisms to have a “probiotic” effect, they must meet specific criteria. They must have the ability to survive transit through acidic portions of the gut, adhere to the intestinal epithelial cells, colonize the intestinal tract and inhibit the growth of pathogenic bacteria. If intended for use in commercial products, they must also withstand processing techniques. Various commercial preparations of probiotics are available for livestock species, including the horse. The primary microorganisms that have been used as DFM for ruminants are fungal cultures including *Aspergillus oryzae* and *Saccharomyces cerevisiae* and lactic acid bacteria (LAB) such as *Lactobacillus* or *Streptococcus* (Yoon and Stern, 1995). Preparations for horses are similar; strains of *Lactobacillus* and *Bifidobacterium* are the most commonly used LAB, while *Saccharomyces cerevisiae* is the most common strain of yeast (Weese, 2001). It is important to know specifically which strain of a species is being used since only certain strains of a species have positive health effects and have the ability to survive processing and storage. Numbers of viable beneficial microorganisms in a product are quantified by listing colony-forming units per g (CFU/g).

Despite both their availability and widespread use in the industry, there are some concerns regarding commercially prepared probiotics. The two most pressing concerns are

quality control and recommended dosing. Probiotic organisms are classified by the FDA as generally regarded as safe (GRAS), a designation that has led to frequent use without standard efficacy or safety trials. Misidentification of bacteria is common in both human and veterinary products (Weese, 2002). A majority of products tested in a recent study did not contain the claimed organisms (or they were not viable), contained additional species, or contained lower concentrations than stated (Weese, 2002). Effective doses for probiotics are often underestimated by commercial producers. The dose of viable organisms required to ensure colonization of the equine intestinal tract is unknown, but researchers extrapolating from human studies report an average (~450 kg) horse would require at least 1×10^{10} to 1×10^{11} CFU/day of viable organisms (Weese, 2001). In most cases probiotic bacteria must be dosed daily. For example, lactobacilli, which attach to the epithelial cells in the intestinal tract, form a coating on the villi, which is eventually shed. Due to this sloughing, high doses of lactobacilli must be administered regularly (Montes and Pugh, 1993).

Reported benefits of probiotic supplementation in farm animals include improved digestion and absorption of nutrients (Abe et al., 1995), increased growth rate (Topliff and Monin, 1990), milk yield and egg production (Hoyos et al., 1987), as well as greater resistance to infectious disease (Lema et al., 2001). Condition and management of the animal seems to affect results. Stressed animals and those in sub-optimal conditions have shown the greatest response (Fuller, 1999). Some studies have shown little or no benefit to supplementation, however researchers seldom verify the strains being tested or assess the bacteria's ability to colonize the gut, and studies most often use different combinations of microorganisms, making it difficult to analyze results (Fuller, 1992). Sound, scientific research involving the use of probiotics in the horse is even scarcer than in production livestock. As in other species, the results for horses have

been mixed. Experimental trials are often in-house experiments or contracted by commercial producers. Still, some reviews conclude that the right probiotic, given at the right time, in the right dose, has the potential to provide significant health benefits to horses and other livestock (Fuller, 1999).

Infection with *Salmonella* can be fatal to horses and can also cause costly outbreaks in veterinary hospitals. One survey reported 14 outbreaks of the disease between 1985-1996 with 6 of these resulting in hospital closure, and costs per outbreak ranged from \$10,000-\$420,000 (Parraga et al, 1997). The microbial population in a horse's gut may play a vital role in the prevention of *Salmonella* infection. Anti-microbial therapy decreases the dose of *Salmonella* organisms required for infection. Among horses with colic, the incidence of *Salmonella* infection is higher in those cases where the hindgut, and presumably the bacterial micro flora, was affected, such as feed and sand impactions (Parraga et al., 1997).

The use of probiotics to prevent *Salmonella* shedding in post-operative colic patients was the focus of two recent studies. The first evaluated two commercial probiotic preparations and found no effect on shedding of *Salmonella*, prevalence of post-operative diarrhea or length of hospitalization in a group of 200 horses (Parraga et al., 1997). Both probiotics were given daily, one claimed to contain *L. plantarum*, *L. casei*, *L. acidophilus*, and *Streptococcus faecium*, and was dosed at 3×10^8 CFU/d, while the other claimed to contain *L. acidophilus*, *S. faecium*, *Bifidobacterium thermophilum* and *B. longum*, and was dosed at 4.1×10^9 CFU/d. The second study (Kim et al., 2001) also used a commercial probiotic, and like the first, saw no effect on fecal *Salmonella* shedding in 96 hospitalized horses with colic. The dose of probiotic used was 5

$\times 10^9$ CFU each of *L. lactis* and *E. Faecium*, and 1×10^8 live yeast cells per d, as based on commercial label claims. Neither study provided evidence that the species purported on the label were actually present, that they were able to colonize in the equine intestinal tract or that the doses given were adequate. While neither study addressed this concern, it is common practice to withhold feed from colic patients, often for two or three days; without nutrients in the gut, the probiotic microorganisms given would be unlikely to survive, much less elicit a beneficial effect. Both studies cited the use of varying levels of antibiotic therapies as well. While undoubtedly necessary in surgical cases, antibiotic treatment would negatively impact existing microbe populations in addition to those trying to establish themselves within the gut.

Effects of probiotic supplementation on equine exercise and performance have been investigated as well. Bioracing®, a commercial probiotic preparation, was claimed to modify certain physiological effects of training (Art et al., 1994). Eleven Thoroughbreds were trained using a treadmill, with six of the horses receiving a probiotic after initial evaluation. Horses treated with the probiotic showed earlier and greater training induced modifications of various cardio-respiratory parameters, including peak oxygen uptake, peak carbon dioxide output, ventilation/min to oxygen-uptake ratio and oxygen-uptake to heart-rate ratio. The researchers hypothesized that Bioracing® modified expected effects of training by enhancing the metabolic capacities for carbohydrate utilization during exercise. In another study, commercially available yeast culture preparation decreased plasma lactate concentrations, slowed the increase of plasma triglyceride concentration and lowered heart rates in young horses during conditioning (Glade and Campbell-Taylor, 1990). The authors interpreted these results to indicate an enhanced state of fitness in the horses treated with probiotic.

It has been claimed that probiotics increase digestibility of feeds in horses; however, two studies by independent researchers show conflicting results. Both studies supplemented with a dried yeast culture. The first showed no differences in apparent digestibility of nutrients in four Quarter Horse geldings fed 40g/d yeast culture (Hall et al., 1990). The second reported increased digestibility of feed in eight Thoroughbred mares and also increased concentrations of nutrients in the mare's milk when the animals were supplemented with 20 g/d of commercial yeast (Glade, 1991). It is possible that the differences in results between the two studies were due primarily to diet, since the horses used by Hall and associates were fed a ration with a high levels (35%) of indigestible fiber (rice hulls). The supplemental yeast may have been utilized as a nutrient source rather than enhancing fermentation.

Knowledge concerning the exact concentration and speciation of the bacteria, protozoa and fungi in the gastrointestinal tract of equines is limited, especially when compared to the extensive amount of information known regarding these aspects of digestion in the rumen. Gross anatomy varies dramatically between hindgut fermenters and ruminants as well. Physiologically however, the cecum and rumen have remarkable similarities. Both possess the physical and chemical environments needed to support anaerobic microbial populations and prolonged retention of digesta (Stevens et al., 1980). While the contribution of the equine cecum to nutrition is less efficient than that of the rumen (Kern et al., 1974), secretion, absorption and microbial digestion are similar (Stevens et al., 1980). Because of these similarities, certain information and procedures developed in ruminants may be directly applied to horses (Julliand, 1992).

Direct-fed microbials (including yeast and lactic acid bacteria) have been reported to enhance milk production in dairy cattle and to increase feed efficiency and body weight gain in growing ruminants (Yoon and Stern, 1995). Probiotics reduced fecal shedding of *Escherichia coli* in lambs (Lema et al., 2001) and in poultry, and prevented antibiotic-associated diarrhea in humans and *Salmonella* colonization in poultry (Fuller, 1999).

Weaning Stress

Stress is defined as the homeostatic, physiological, and behavioral responses that are detectable in an animal as a result of its interactions with environmental stressors. Stimuli can be classified as stressors when the rate at which they are perceived by an animal deviates significantly from normal, or they are unusually prolonged or intense (Stephens, 1980). Weaning has been implicated as a source of psychological and physiological stress in many species (McCall et al., 1987). In addition to a significant change in diet, the emotional strain of social dislocation for a herd animal, such as the horse, can make weaning especially traumatic. Excessive stress due to weaning may also affect appetite, metabolism, and immune competence (Malinowski et al., 1990).

The degree of stress an animal is undergoing may be quantified using both physiological and behavioral responses. A well-known effect of stress is the activation of the hypothalamo-pituitary-adrenocortical system. Corticotrophin-releasing factor is secreted from the hypothalamus, causing the release of adrenocorticotrophic hormone (ACTH), from the anterior pituitary, which triggers the release of corticosteroid hormones, such as cortisol (which accounts

for almost 90% of the circulating corticoids in horses), into the blood (Covalesky et al., 1992). The adrenal cortex quickly becomes depleted following the onset of stress; however, if the stress continues it undergoes hypertrophy and hyperplasia, which allows increased sustained secretion of glucocorticoids. This enhanced level of glucocorticoid production continues until the animal adapts, the stress is removed, or adrenal exhaustion occurs and the animal dies (McCall et al., 1987).

Cortisol response to an ACTH challenge has been used to evaluate stress due to weaning in horses (Apter and Householder, 1996). Plasma cortisol concentrations are significantly higher post-weaning compared to pre-weaning and controls. The post-weaning levels of plasma cortisol are also affected by various management strategies. Abrupt weaning causes a higher response than gradual methods (McCall et al., 1987). Foals weaned in pairs show a greater response than those weaned singly when housed in stalls, but not on pasture (Malinowski et al., 1990; Hoffman et al., 1995). Pre-weaning adaptation to a balanced grain supplement, compared to pasture only, appeared to moderate stress in foals at weaning (Hoffman et al., 1995).

Stress is thought to affect intestinal microflora by reducing the number of anaerobic microorganisms. Increased levels of endogenous corticosteroids, caused by stress, may decrease the secretion of mucin (an energy source for anaerobic bacteria) and result in an overgrowth of coliform bacteria (Montes and Pugh, 1993). This may explain why stressed animals show the most dramatic responses to probiotic administration.

A study by Huis in 't Veld and Havenaar (1993) investigated the composition of gut microflora of piglets from birth through weaning. A critical period was noted immediately after weaning at which the numbers of lactobacilli decreased dramatically (approx. 1000-fold), while the numbers of *E. coli* increased, to the point that the number coliform bacteria far surpassed the LAB (Figure 1). Similar effects of weaning on the gut microflora were observed in subsequent studies (Methew et al., 1996). Researchers attributed the rapid change to the stress of weaning combined with a change in feed composition as well as intake. Feeding a combination of Lactobacillus strains isolated from healthy pigs successfully prevented this change in microflora (Figure 2). Each strain was fed at a dose of 4×10^6 cfu/g from 17 to 35 d of age (weaning was at 26 d of age). Incidence of death and diarrhea was lower in the piglets fed probiotics as compared to the control group.

High Fat and Fiber Diets

Feeding diets high in soluble carbohydrates, which are hydrolyzable and rapidly fermentable, has been associated with certain diseases in the horse (colic, laminitis) and is commonly believed to cause “hot” or excitable behavior as well. Fat and fiber supplementation provides a means by which to reduce the amount of hydrolyzable carbohydrates while still providing enough energy to meet the needs of growing or exercising horses. This feeding practice may have several advantages to weanlings in particular. During the first year of life, a horse obtains the majority of its mature height and weight. This rapid growth requires careful nutritional management to avoid issues such as developmental orthopedic disease, which has been associated with the feeding of high levels of soluble carbohydrates (Kronfeld et al., 1990).

Replacing hydrolyzable carbohydrates in the diet with fat and fiber may also minimize some of the effects of stress seen during weaning. Foals fed a dietary concentrate prior to weaning coped better (based on behavioral observation and ACTH response test) with this change in lifestyle and diet than foals kept on pasture and hay alone (Hoffman et al., 1995). Many farms adhere to this practice in order to begin conditioning young horses for sale, but most rely on traditional grain based supplements, high in soluble carbohydrates. Dietary fats have been shown to reduce the spontaneous activity and reactivity of young horses (Holland et al., 1996). Less excitability, in combination with the increased safety of a feed source high in slowly fermentable carbohydrates, could equal a better feed choice for growing weanlings.

Vitamin E

As an antioxidant, the benefits of vitamin E are well established. Over the last ten years, however, researchers have begun to investigate its potential in immune stimulation. Excessive amounts of reactive oxygen species (particularly hydrogen peroxide) may have detrimental effects on lymphocytes, which play a key role in immune response (McDowell, 2000). Vitamin E reacts with these free radicals to reduce H₂O₂-induced DNA damage on peripheral blood lymphocytes (Brennan et al., 2000). Although the mechanisms are not fully understood, supplementation with vitamin E has been shown to stimulate antibody synthesis in calves (Ready et al., 1986) as well as rats (Gu et al., 1995). In the horse, supplemental vitamin E (327 IU/kg) enhanced concentrations of IgG, IgM, and IgA in mare colostrum, and also improved passive transfer to foals (Hoffman et al., 1999).

Materials and Methods

Animals

This study was performed at the Virginia Tech Agricultural Research and Extension Center in Middleburg, VA. Twenty Thoroughbred foals (10 geldings and 10 fillies), aged 190 ± 37 d, and weighing 279.2 ± 32 kg, were used. Except during sample collection, all horses were kept on pasture in two groups, according to diet, with free choice white salt and fresh water. Two adjacent 30-acre pastures with primarily Kentucky Bluegrass and White Clover were utilized. Although the pastures were virtually identical in both size and nutrient analysis, groups were rotated between pastures during the study as necessary for management of the land and to eliminate a pasture effect.

In order to facilitate sample collection and accustom foals to stalls, mares and foals were kept in stalls during the day (8 h), for four days, beginning two weeks before their weaning date. Each mare and foal pair was assigned an un-bedded (rubber mat flooring), 10.9-m^2 stall, within sight of pasture-mates (in adjacent stalls), and provided free-choice water and orchard grass hay.

Foals were weaned in two groups of five, one week apart. Five foals from each pasture remained with their group while their mares were taken, all at once, out of sight and hearing. Beginning 24 hours after weaning, these foals were brought back into the same stalls as before weaning, for four days, eight hours each day. The second group from each pasture was weaned in the same manner. An additional mare, whose foal was not on study, remained with each group throughout the weaning period as a “babysitter”.

Diets

Two pasture supplements (Table 1), one high in sugar and starch (SS) and one high in fat and fiber (FF) were fed in order to meet or exceed the NRC requirements (NRC, 1989). The SS supplement was designed to mimic traditional commercial sweet feed, while the FF supplement was designed with much less hydrolyzable sugar and starch than typically found in commercial concentrates, a larger spectrum of fibers (amounting to 30% NDF) as well as higher fat content (Table 2). Mares had been paired by age, breeding date, and sire of their foal, and then randomly assigned to one of these two diets prior to parturition. The supplements were formulated to be isocaloric and isonitrogenous, with mineral contents balanced to complement the pasture. Supplements were fed in varying amounts (2 to 3 kg twice a day) with the goal of achieving an approximate supplement to forage ratio of 1:2 (Kronfeld, 1998a) and body condition scores between 5 and 6 using a scale of 1 to 9 (Henneke et al., 1983). Mares were group fed in pans on the ground and foals allowed free access to their dam's feed.

Table 1. Ingredient composition (%) of the SS and FF feeds

Ingredient, %	Diet	
	SS	FF
Dent yellow grain corn	60	3
Soybean meal (48% CP)	15.5	8.25
Oat straw	7	8.25
Alfalfa Hay (early bloom)	0	12
Soybean hulls	4	11
Beet pulp	0	14
Processed cereal by-product ^a	0	30
Molasses (cane)	10	5
Dicalcium phosphate	1.5	0
Limestone	1	0
Corn oil	0	7.5
Mineral premix ^b	0.5	0.5
Vitamin premix ^c	0.5	0.5

^a Processed cereal by-product contained 92.5% DM, 21% EE, 15% CP, 24% NSC, and 30% NDF.

^b Provided the following amounts per kg of feed: Fe, 46.1 mg; Zn, 105.8 mg; Cu, 25.11 mg; Mn, 18.02 mg; Se, 0.55 mg; I, 0.35 mg; NaCl used as a carrier, 4160 mg.

^c Provided the following amounts per kg of feed: vitamin A, 6,900 IU; β -carotene, 17.6; vitamin D3, 1,290 IU; vitamin E, 132 mg; vitamin C, 333 mg; Niacin, 15 mg; Thiamin, 7 mg; Riboflavin, 3.5 mg; Folic acid, 0.33mg; Biotin, 0.21 mg.

Table 2. Nutrient Content of Pasture and Feeds

Component	SS	FF	Pasture (avg. n=2)
% Crude Protein	15.1	14.9	17.7
% Acid Detergent Fiber	10.2	23.4	32.9
% Neutral Detergent Fiber	20.6	39.1	59.6
% Starch	40.6	6.0	1.05
% Sugar	12.9	11.1	10.1
% Crude Fat	3.6	17.6	1.9
% Calcium	1.21	1.72	0.493
% Phosphorus	0.63	0.81	0.298
% Magnesium	0.20	0.51	0.223
% Potassium	1.35	1.38	2.29
% Sodium	0.361	0.356	0.006
DE, Mcal/kg	3.69	4.04	1.09

- All values are on a dry matter basis
- Feed analysis was performed on composite samples

Probiotic Supplement

A commercial probiotic supplement, Probios (CHR Hansen, Milwaukee WI), guaranteed to contain four species of Lactic Acid Bacteria (*Enterococcus faecium*, *Lactobacillus acidophilus*, *Lactobacillus casei* and *Lactobacillus plantarum*) at not less than 10 million CFU/g was used. Foals were fed 10g per day (twice the manufacturer's recommendation), beginning 1 month prior to the first sampling period, and ending after the final collection day post-weaning. Half of the foals in each group were hand-fed the commercial probiotic supplement, mixed into the SS or the FF feed, depending on their assigned diet, while the remaining foals received a placebo (same feed mixture minus the probiotic). Foals were individually fed this supplement at approximately 1330 h each day. For foals on the FF diet, 10 g of probiotic was mixed with 500g of feed and 400 ml of corn oil; for foals on the SS diet, 10 g of probiotic were mixed with 700g of feed and 350ml of water.

Sample collection

Fecal samples were collected, over an 8 h period, during the eight days foals were in stalls (four days pre-weaning and four days post-weaning). Three grab samples were compiled for each day. All samples were placed in airtight plastic containers, at -80°C , within 20 min of defecation. Samples were thawed within ten weeks and analyzed for pH, lactic acid and volatile fatty acids.

Blood samples were drawn on the second and fourth days pre-weaning, between 1000 and 1030 h, and the second day post-weaning between 1000 and 1030 h and again between 1500 and 1530 h. 2-10 ml Vacutainer® vials (Fisher Scientific, Pittsburgh PA) containing sodium heparin (Fisher 2001) were collected at each sample time. Samples were centrifuged within 30 min of collection, at 3000 g for 10 min. Plasma was drawn off into three separate vials and stored at -80°C until analyzed, within 10 weeks, for total lactate, IgG, IgA and cortisol.

An ACTH response test was conducted 48 h after each foal was weaned. Baseline blood samples were drawn immediately prior (between 1000 and 1030 h) to an ACTH challenge of 0.0075 mg/kg BW cosyntropin (Cortrosyn®, Ben Venue Laboratories, New Jersey), injected intramuscularly. A 0.1 mg dose of cosyntropin is equivalent to 1 unit of natural ACTH. Post ACTH challenge samples were drawn 5 h later (between 1500 and 1530 h). These collection times were chosen in order to minimize the effects of diurnal variation of cortisol found in horses. Only the pre ACTH challenge values were used to evaluate total lactate, IgG and IgA; since the administration of cosyntropin may affect other blood variables besides cortisol.

Behavioral ethograms, for each foal, were compiled daily, using a 30-min observation period. All ten foals were observed at once, from an elevated position, where all could be viewed easily without affecting their behavior. Behaviors, including vocalization, standing, walking, eating, urination/defecation and certain vices attributed to stress (such as weaving, pawing, wood chewing and rearing) were recorded. Vocalizations and defecation/urination were recorded as frequencies per 30 min while all other data were recorded as cumulative intervals of time spent in the activity. A behavioral score was assigned to each foal using the following formula:

$$\text{Score} = 10 - 1*(\text{walking}) + 2*(\text{eating}) - 1*(\text{vocalizations}) - 0.5*(\text{defecations/urinations}) - 1*(\text{other})$$

All foals were given an arbitrary baseline of ten to start and scores were adjusted (up/down) depending on the duration or frequency of certain behaviors and whether the behavior indicated more or less stress. Each behavior was given a multiplier, depending on its impact to the stress status of the animal relative to other influences. Negative multipliers were assigned to behaviors indicating distress (e.g. stall walking, vocalizations, defecation, other), and positive multipliers were assigned to behaviors indicating eustress or no stress (e.g. eating).

Sample Analysis

Pasture and Feed Samples

Pasture samples were taken from both fields. Several samples were obtained from each batch (ton) of feed used during the study. All samples for a given diet were then mixed and a composite sample was ground and sent for analysis. Nutrient analysis for both pasture and grain were obtained from Dairy One DHI Forage Testing Laboratory, Ithaca, NY.

Fecal Samples

Fecal samples were thawed for 30 min in a warm water bath. The samples were first diluted in a 1:10 ratio with deionized water and mixed in a commercial blender (Hamilton Beach model 57171) for 1 min. Fecal pH was then measured using a digital pH meter (Corning model

340, Corning NY) calibrated by using a chemical standard (SB107-500 Fisher Scientific, Fairlawn NJ). The mixture was then filtered through a cheese cloth to remove large particulate matter and two aliquots of the extract drawn off, one for determination of total lactic acid and the other for volatile fatty acid analysis.

Total lactic acid concentration was determined using techniques adapted from Barker and Summerson (1941) and modified by Pennington and Sutherland (1956). Fecal extract, 20% CuSO₄, deionized H₂O, and Ca(OH)₂ were mixed, using a mechanical shaker for 10 min and then centrifuged at 2000 rpm for 10 min. Dilute CuSO₄ (4%) and concentrated H₂SO₄ were added to the supernatant and immediately mixed on a vortex. Tubes were heated in a boiling water bath (90°C) for 7 minutes, then cooled in ice water (<20°C). Two drops of alkaline p-hydroxydiphenyl were added and the tubes again vortexed. Tubes were placed in cool water during color development (30 min) and vortexed at 5, 10 and 25 minutes. Tubes were then placed in boiling water for 2 min, in order to stabilize color, and then in ice water until cooled to room temperature. Samples were read on a spectrophotometer at 560 nm.

Volatile fatty acid concentrations were determined by gas chromatography techniques adapted from procedures used in evaluation of rumen fluid (Erwin et al., 1961). Samples were prepared as follows: 5 ml of fecal extract were combined with 1 ml of Metaphosphoric acid and 5 ml of internal standard (4-methyl valeric acid @ 10 µmole/ml) and frozen for later analysis. Once thawed, the samples were centrifuged at 2000 rpm for 10 min, the supernatant drawn off and run through a filter (Gelman 60172). Prepared samples were analyzed using a gas chromatograph (Agilent 6890 with Injector 7683). The column used was 30 m x 0.53 mm I.D., 1

µm thick (J & W Scientific 125-7332). The carrier gas was helium. The inlet temperature was 250°C and the oven temperature was 260°C. A flame ionization detector was used at 250°C.

Plasma Samples

Immunoglobulin (IgG and IgA) concentrations were determined using equine specific single radial immunodiffusion assays (VMRD Inc, Pullman WA). Reference standards included in each kit and statistical analysis software (Statistix7, Analytical Software, Tallahassee FL) were used to define the standard curve and determine actual sample values.

Plasma cortisol levels were obtained by radioimmunoassay (Coat-A-Count® Cortisol, Diagnostics Products Corporation, Los Angeles, CA). Plasma Lactate concentration was determined using a Lactate specific chemistry assay and CX MULTI Calibrator (Beckman Instruments Inc, Brea', CA).

Plasma α -tocopherol levels were obtained using high pressure liquid chromatography (HPLC), using an Agilent 1100 series (Agilent Technologies, Wilmington, DE) HPLC and a reverse phase C18 column, 110 mm long and 4.6 mm wide (Varian Scientific). Conditions for the HPLC were as follows: mobile phase was 99% HPLC grade Methanol and 1% di H₂O, the flow rate was 1 ml/min, with detection at 292 nm. Internal standard (tocol) and standard (alpha-tocopherol) concentrations were checked via spectrophotometer for accuracy, and used to establish the standard curve. Samples were prepared by combining 100 ul of plasma, 100 ul of 2.03 ug/ml of tocol in ethanol, and 600 ul of hexane, vortexing, then centrifuging for 2 min at

700 g. The upper hexane layer was removed, dried under N₂ and resuspended with 100 ul of ethanol.

Statistical Analysis

Plasma variables and fecal data for which there were no interactions by day within weaning period, are reported as pooled values and evaluated using the general linear model of SAS (1999). Diet, weaning, probiotic treatment, and all interactions were included in the model. Statistical analysis of VFA ratios (lactate:actate and propionate:acetate) was performed on the log transforms of these data in order to achieve normal distributions. The following fecal variables were subjected to analysis of variance using the mixed model procedure of SAS, with repeated measures over time (SAS, 1999): isobutyric acid, butyric acid, isovaleric acid and valeric acid. Interactions that were not significant ($P > 0.10$) were dropped from the models. Correlations between behavioral and physiological data were examined using multiple regression procedures (SAS, 1999).

Results and Discussion

Fecal Variables

Fecal volatile fatty acids – lactic acid, acetic acid, propionic acid

Total (d- and l-) lactate concentrations were not affected by diet ($P = 0.841$; Figure 3), but were higher overall in foals supplemented with probiotics ($P = 0.002$), both before ($P = 0.019$) and after ($P = 0.031$) weaning (Figure 4). Foals supplemented with probiotics had lower concentrations of fecal acetic acid (Figure 5), both before ($P = 0.005$) and after ($P = 0.010$) weaning. Diet did not largely affect fecal acetic acid, but there was a trend ($P = 0.072$) towards higher fecal acetic acid concentrations in FF foals (Figure 6). Fecal propionic acid concentrations (Figure 7) were lower ($P = 0.023$) post-weaning than pre-weaning in all foals, regardless of diet or probiotic treatment. Fecal propionic acid concentrations were not affected by probiotic supplementation ($P = 0.185$), but were lower in foals fed FF, both before ($P = 0.026$) and after ($P = 0.052$) weaning. The ratio of fecal lactate:acetate was higher ($P < 0.0001$) in probiotic-supplemented than non-supplemented foals (Figure 8). A diet x probiotic interaction effect ($P = 0.0074$) was noted in the ratio of fecal propionate:acetate, with lower propionate:acetate ratio noted only in FF foals not supplemented with probiotic (Figure 9).

Higher fecal lactate was anticipated in foals supplemented with probiotic, because the product (Probios) contained lactic acid producing bacteria. The higher fecal lactate suggests that one or more species of LAB that were in the probiotic survived gastrointestinal transit to the hindgut and established colonization. In other species, including cattle, sheep and pigs, cultures of intestinal and ruminal contents have been used to establish the ability of supplemental lactic acid bacteria to survive and colonize the gut (Fuller, 1997). Studies in horses have not reported cecal colonization or other evidence of LAB survival (Spring, 2000). The higher fecal lactic acid

concentrations yielded by this probiotic may be a concern. Accumulation of lactic acid in the gut has been implicated in several diseases in the horse, including colic, osmotic diarrhea and laminitis (Kronfeld, 1998b). Rapid fermentation of hydrolyzable carbohydrates can cause a decrease in pH, which if sustained, can adversely affect lactate-consuming bacteria, resulting in accumulation of lactic acid (Clarke et al., 1990). While the probiotic used here did not cause a decrease in pH, the increased lactic acid could predispose them to grain associated carbohydrate problems.

The higher fecal lactic acid and lower acetic acid in foals supplemented with probiotics may have been caused by competitive exclusion by the LAB against acetic acid producing bacteria already present in the hindgut. While sound scientific studies involving the use of lactic acid bacteria are lacking in the horse, competitive exclusion by these types of bacteria have been seen in a variety of species (cattle, sheep, pigs and rats) and are thought to be a universal mechanism of action (Spring, 2000).

The lower propionic acid and a trend towards higher acetic acid in feces of FF compared to SS foals may reflect differences in composition of carbohydrate fractions of the FF and SS supplements. Compared to SS, the FF supplement had two times more NDF, and one-third the amount of sugar and starch, or hydrolyzable carbohydrate. Overload of hydrolyzable carbohydrate in the small intestine may lead to rapid fermentation of sugar and starch in the hindgut (Kronfeld, 1998b). Rapid fermentation produces propionate and lactate as end-products, while slow fermentation favors acetate and butyrate (Spring, 2000). In ponies, a high grain diet resulted in lower concentrations of acetate and higher concentrations of propionate in cecal fluid (Hintz et al., 1971). Fecal propionate increased in pigs when fed diets with higher levels of carbohydrate (Imoto and Namioka, 1978).

Fecal volatile fatty acids – butyric acid, isobutyric acid, valeric acid, isovaleric acid

There was a diet x day interaction effect pre- and post- weaning ($P < 0.002$) for fecal concentrations of butyric and valeric acids (Figures 10 and 11) and a trend ($P < 0.135$) for diet x day interaction post-weaning for isobutyric and isovaleric acids (Figures 12 and 13). Fecal butyric acid concentrations were similar on day 1 of the pre- and post-weaning periods, but on days 2 through 4, higher concentrations of butyric acid were seen in SS than FF foals both before ($P < 0.032$), and after ($P < 0.007$) weaning. Higher concentrations of valeric acid in SS compared to FF foals were also noted on days 2 through 4 both before ($P < 0.057$) and after ($P < 0.020$) weaning. The differences on days 2 to 4 were attributed to higher concentrations of these volatile fatty acids in SS foals, while fecal concentrations in FF foals remained similar to day 1. Previous studies showed fecal concentrations of butyrate, isovalerate and valerate increased in ponies when high grain diets were fed (Hintz et al., 1971). Fecal butyrate increases in pigs with the level of carbohydrate in the diet (Imoto and Namioka, 1978). Our results showed similar differences in these VFAs due to diet, but only after hay replaced a portion of the available forage, which had been entirely pasture. These differences are not seen on day 1 of the collection periods since the foals were not fed hay until they were brought into stalls, nor had enough time elapsed to allow results of the pasture to hay change to be seen in the feces.

Fecal pH

Foals fed FF had higher ($P = 0.0001$) fecal pH throughout the study compared to foals fed SS (Figure 14). Differences between diets were greater post-weaning ($P = 0.002$) than pre-weaning ($P = 0.043$; Figure 15). All foals had higher ($P < 0.0001$) fecal pH post-weaning (Figure

15) compared to pre-weaning. There was no effect seen due to probiotic supplementation on fecal pH ($P = 0.297$).

Changes in fecal lactic acid concentrations were not affected by diet, therefore the diet effects on pH were not attributed to lactic acid. While the differences in fecal pH between the two diets were significant, both pre- and post- weaning, both diets remained in the normal range (Argenzio and Stevens, 1984). In steers, fecal pH was lower (5.4 to 6.3) when animals were fed a corn diet as compared to those on a cottonseed and barley diet (6.6 to 7.5) (Buchko et al., 2000). Fecal pH has been negatively correlated to the amount of starch that reaches the lower digestive tract in cattle (Oliveira et al., 1995).

Plasma Variables

Plasma Lactate

Plasma lactate concentrations were not influenced by weaning ($P = 0.214$), diet ($P = 0.155$), or probiotic supplementation ($P = 0.955$). While there were changes in fecal lactate production seen in foals due to probiotic treatment, the stable concentrations of plasma lactate indicated that the increases in fecal lactate were not great enough to induce physiological problems.

Changes in serum lactic acid have been linked to accumulation of both d- and l- lactate in the gastrointestinal tract, with higher levels correlated to the onset of acidosis in diarrheic calves (Omole et al., 2001).

Immunoglobulins and α -tocopherol

Plasma concentrations of immunoglobulins (IgG and IgA) are shown in Figure 16. Compared to foals fed SS, foals fed FF had higher plasma concentrations of IgA ($P = 0.006$) and IgG ($P = 0.012$). Plasma α -tocopherol concentrations were higher in FF than SS foals post-weaning ($P = 0.014$), but not pre-weaning ($P = 0.126$); (Figure 17).

The higher IgG and IgA concentrations in plasma of FF foals may be attributed to differences in vitamin E content of the diet, or improved absorption of dietary vitamin E as a result of higher fat content of the FF feed. Corn oil, which has a vitamin E content of 1148 ± 113 mg/kg (Lynch, 1991), composed 7.5% of the FF supplement, with an estimated dietary vitamin E concentration of 252.4 mg/kg, compared to 156.1 mg/kg in the SS supplement. Higher vitamin E in a similarly formulated FF supplement was hypothesized to improve IgG concentration in mares' colostrum (Hoffman et al., 1998). Pregnant mares supplemented with vitamin E at 160, compared to 80 IU/kg of intake, had higher ($P < 0.0001$) concentrations of IgG, IgA and IgM in pre-suckled colostrums, and their foals had greater ($P = 0.020$) passive transfer of IgG (Hoffman et al., 1999). Vitamin E is a strong antioxidant, and may reduce the detrimental effects of reactive oxygen species on the immune system (McDowell, 2000). Supplementation with vitamin E has been shown to stimulate antibody synthesis in calves (Ready et al., 1986) as well as rats (Gu et al., 1995). Psychological and physical stress, like that at weaning, can affect immune competence (Malinowski et al., 1990).

Behavior

Compared to pre-weaning, foals spent less time standing ($P = 0.012$) and eating ($P = 0.017$), more time walking ($P = 0.012$), and vocalized more frequently ($P = 0.0001$; Figure 18). Weaning did not influence frequency of defecation ($P = 0.271$) and there was no change in the

frequency of behavioral vices such as pawing, wood chewing, and kicking ($P = 0.550$). None of the behaviors studied were influenced by diet ($P > 0.137$) or probiotic ($P > 0.263$). The overall behavior score was lower post-weaning than pre-weaning ($P = 0.0001$).

While a high fat, high fiber diet has been shown to decrease spontaneous activity and reactivity in adult horses (Holland et al., 1996), this improved behavior was not noted in FF foals during this study. Our results agreed with previous studies, which have associated behavioral and physical responses of foals with social dislocative stress of weaning (Hoffman et al., 1995; Holland et al., 1996a).

Cortisol and cortisol response to ACTH

Plasma cortisol concentrations were higher post-weaning than pre-weaning ($P = 0.0002$); (Figure 19). There was no effect of diet ($P = 0.385$) or probiotic ($P = 0.388$) on plasma cortisol concentrations. No difference between treatment groups was found for change in plasma cortisol in response to exogenous ACTH. There was a trend towards a greater cortisol response in FF as compared to SS foals ($P = 0.109$; Figure 20).

The effect of weaning on plasma cortisol in this study agreed with previous reports in which higher concentrations were reported post-weaning than pre-weaning (Stephens, 1980; Malinowski et al., 1990). While the behavioral scoring system used in this study was adequate for comparing levels of stress before and after weaning, it may not have been sensitive enough to distinguish effects of diet. Foals demonstrated sufficient behavioral evidence to suggest that they were mildly distressed by weaning, but not severely distressed enough to indicate adrenal exhaustion. In this case, a less marked response, indicating partial depletion of the adrenal gland,

would be expected. The trend towards greater cortisol response in FF foals that was observed was interpreted to mean that these foals were slightly less stressed than the SS foals. In previous studies, cortisol response to ACTH in foals has been affected by weaning method and diet (McCall et al., 1987; Hoffman et al., 1995).

Correlation of behavior score and cortisol

Concentrations of plasma cortisol were negatively correlated to behavior score ($R = -0.487$; $P = 0.0014$) however the cortisol response to exogenous ACTH was not correlated to behavior score ($R = -0.139$; $P = 0.557$), nor was it correlated to any other behavior observed.

Previous studies have shown correlations between behavior and cortisol response to an ACTH challenge (Hoffman et al., 1995). Since cortisol concentrations in our study were well correlated to behavior, we would also have expected a positive correlation between behavior and cortisol response. Behaviors observed in our study also did not exhibit the extremes seen in previous reports; therefore our weaning protocol may not have been stressful enough to elicit a measurable response. Restraint of cattle and sheep have been shown to elicit a cortisol release from the adrenal cortex (Wohlt et al., 1994; Fulkerson and Jamieson, 1982). The combination of these two factors (minimal weaning stress and handling/restraint) may have moderated the expected cortisol response. Practical weaning management warrants moderation of weaning stress, and the protocol used in this study was designed to mimic traditional management, rather than eliciting a stress response that may not apply in the field.

Conclusions

This study offers sound evidence that supplemental LAB survive transit in the gastrointestinal tract of the horse and colonize in the hindgut. However, since this type of probiotic bacteria did not alter any of the indicators of stress measured, it would be difficult to give sound reasons for the use of this particular probiotic preparation in weaning situations based in these results. More stressful situations might elicit better results, but would not be typical of industry practice. The higher fecal lactic acid concentrations yielded by this probiotic may be a concern. However, the probiotic used here did not cause a decrease in pH, the increased lactic acid could predispose them to grain associated carbohydrate problems. It should be noted that since different species and strains of bacteria elicit different effects, the conclusions cited here are not necessarily relevant to the use of other combinations of microorganisms.

Diet effects in this study heavily support the use of high fat, high fiber feeds in weaning situations. The fecal butyric, valeric, isobutyric and isovaleric acid concentrations indicate less disruption of microbial populations due to change in forage for the foals fed the fat and fiber feed. Presumably this is due to a more diverse microbial population promoted by adaptation to the range of fibers in the FF diet. If more were known regarding specific microbial strains and which strains most benefit the horse, a carefully prepared probiotic supplement might be able to promote optimal microbial populations. Providing the horse with a feed supplement that inherently supports a more diverse microbial population, enabling the horse to cope with inevitable changes in forage, may be a more logical choice.

Implications

LAB supplemented in the diet appears to survive transit through the gastrointestinal tract and colonize in the hindgut. However, the use of supplemental lactic acid bacteria was not found to be beneficial or detrimental for the purpose of reducing the effects of stress seen during weaning. A high fat, high fiber diet, however, may improve humoral immune status and encourage a more diverse intestinal microbial population in the hindgut, enabling foals to better cope with the nutritional changes that contribute to physiological stress during weaning.

Literature Cited

Abe, F., N. Ishibashi, and S. Shimamura. 1995. Effect of administration of bifidobacteria and lactic acid bacteria to newborn calves and piglets. *J. Dairy Sci.* 78:2838-2846.

Apter, R. C., and D. D. Householder. 1996. Weaning and weaning management of foals: a review and some recommendations. *J. Equine Vet. Sci.* 16:428-435.

Argenzio, R. A., and C. E. Stevens. 1984. The large bowel, a supplementary rumen? Pages 13-23 in *Proc. Nutr. Soc.*

Art, T., D. Votion, K. McEntee, H. Amory, A. Linden, R. Close, and P. Lekeux. 1994. Cardio-respiratory, haematological and biochemical parameter adjustments to exercise: effect of a probiotic in horses during training. *Vet. Res.* 25:361-370.

Barker, S. B. and W. D. Summerson. 1941. The colorimetric determination of lactic acid in biological material. *J. Biol. Chem.* 138:535-540.

Buchko, S. J., R. A. Holley, W. O. Olson, V. P. J. Gannon, and D. M. Veira. 2000. The effect of different grain diets on fecal shedding of *Escherichia coli* 0157:H7 by steers. *J. Food Protection* 63:1567-1474.

Bergman, E. N. 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiological Reviews.* 70:567-590.

Brennan, L. A., G. M. Morris, G. R. Wasson, B. M. Hannigan and Y.A. Barnett. 2000. The effect of vitamin C or vitamin E supplementation on basal and H₂O₂-induced damage in human lymphocytes. *Br. J. Nutr.* 84:195-202.

Clarke, L. L., M. C. Roberts, and R. A. Argenzio. 1990. Feeding and digestive problems in horses. *Vet. Clin. North Amer. Equine Pract.* 6:433-450.

Covalesky, M. E., C. R. Russoniello and K. Malinowski. 1992. Effects of show-jumping performance stress on plasma cortisol and lactate concentrations and heart rate and behavior in horses. *J. Equine Vet. Sci.* 12:4 244-251.

Erwin, E. S., G. J. Marco and E. M. Emory. 1961. Volatile fatty acid analysis of blood and rumen fluid by gas chromatography. *J. Dairy Sci.* 44:1768.

Fulkerson, W. J., and P.A. Jamieson. 1982. Pattern of cortisol release in sheep following administration of synthetic ACTH or imposition of various stressor agents. *Aust. J. Biol. Sci.* 35:215-222.

Fuller, R. 1989. Probiotics in man and animals. *J. Appl. Bacteriol.* 66:365-378.

Fuller, R. *Probiotics: The Scientific Basis.* 1992. Chapman and Hall. New York.

Fuller, R. *Probiotics 2: Applications and Practical Aspects.* 1997. Chapman and Hall. New York.

Fuller, R. 1999. Probiotics for farm animals. Pages 15-21 in *Probiotics: A Critical Review* G.W. Tannock, ed. Horizon Scientific Press, Norfolk, England.

Glinsky, M. J. 1976. Measurement of volatile fatty acid production rates in the cecum of the pony. *J. Anim. Sci.* 42:1465-1470.

Glade, M. J., and L. M. Biesik. 1986. Enhanced nitrogen retention in yearling horses supplemented with yeast culture. *J. Anim. Sci.* 62:1635-1640.

Glade, M. J., and M. Campbell-Taylor. 1990. Effects of dietary yeast culture supplementation during the conditioning period on equine exercise physiology. *J. Equine Vet. Sci.* 10:434-443.

Glade, M. J. 1991. Effects of dietary yeast culture supplementation of lactating mares on the digestibility and retention of the nutrients delivered to nursing foals via milk. *J. Equine Vet Sci.* 11:323-329.

Hall, R. R., S. G. Jackson, J. P. Baker, and S. R. Lowry. 1990. Influence of yeast culture supplementation on ration digestion by horses. *J. Equine Vet. Sci.* 10:130-134.

Henneke, D. R., G. D. Potter, J. L. Kreider, and B. F. Yeates. 1983. Relationship between condition score, physical measurement, and body fat percentage in mares. *Equine Vet. J.* 15:371-372.

Huis in 't Veld, J. H. J. and Havenaar, R. 1993. Selection criteria for microorganisms for probiotic use. Pages 11-19 in *Probiotics and Pathogenicity*. COVP-DLO Het Spelderholt, Beekbergen, The Netherlands.

Hoffman, R. M., D. S. Kronfeld, J. L. Holland, and K. M. Greiwe-Crandell. 1995. Preweaning diet and stall weaning method influences on stress response in foals. *J. Anim. Sci.* 73:2922-2930.

Hoffman, R. M., D. S. Kronfeld, J. H. Herbein, W. S. Swecker, W. L. Cooper, and P. A. Harris. 1998. Dietary carbohydrates and fat influence milk composition and fatty acid profile of mare's milk. *J. Nutr.* 128:2708S-2711S. (Abstr.)

Hoffman, R. M., K. L. Morgan, M. P. Lynch, S. A. Zinn, C. Faustman, and P. A. Harris. 1999. Dietary vitamin E supplemented in the periparturient period influences immunoglobulins in the equine colostrums and passive transfer in foals. Pages 96-97 in *Proc. 16th Equine Nutr. and Physiol. Soc.*, Raleigh, NC.

Holland, J. L., D. S. Kronfeld, R. M. Hoffman, K. M. Greiwe-Crandell, T. L. Boyd, W. L. Cooper, and P. A. Harris. 1996a. Weaning stress is affected by nutrition and weaning methods. *Pferdeheilkunde.* 12:257-260.

Holland, J. L., D. S. Kronfeld, and T. N. Meacham. 1996b. Behavior of horses is affected by soy lecithin and corn oil in the diet. *J. Anim. Sci.* 74:1252-1255.

Hoyos, G., L. Garcia, and F. Medina. 1987. Effects of feeding viable microbial feed additives on performance of lactating cows in a large dairy herd. *J. Dairy Sci.* 70:217-220.

Jay, J. M. 1982. Antimicrobial properties of diacetyl. *Appl. Environ. Microbiol.* 44:525-532.

Julliand, V. 1992. Microbiology of the equine hindgut. *Pferdeheilkunde.* 1:42-47.

Kern, D. L., L. L. Slyter, E. C. Leffel, J. M. Weaver, and R. R. Oltjen. 1974. Ponies vs. steers: microbial and chemical characteristics of intestinal injeesta. *J. Anim. Sci.* 38:559-563.

Kim, L., P. S. Morley, J. L. Traub-Daragatz, M. D. Salman, and C. Gentry-Weeks. 2001. Factors associated with salmonella shedding among equine colic patients at a veterinary teaching hospital. *J. Am. Vet. Med. Assoc.* 218:740-748.

Kleeman, E. G. 1982. Adherence of lactobacillus species to human fetal intestinal cells. *J. Dairy Sci.* 65:2063-2069.

Kronfeld, D. S., T. N. Meachem, and S. Donoghue. 1990. Dietary aspects of developmental orthopedic disease in young horses. *Vet. Clin. N. Am. Equine Pract.* 6:451-465.

Kronfeld, D. S. 1998a. A practical method for ration evaluation and diet formulation: An introduction to sensitivity analysis. Pages 77-88 in *Proc. Equine Nutr. Conf. for Feed Manufacturers*, Versailles, KY.

Kronfeld, D. S. 1998b. Clinical assessment of nutritional status of the horse. Pages 185-217 in *Metabolic and Endocrine Problems of the Horse*. W. B. Saunders, New York.

- Kulshrestha, D. C. and E. H. Marth. 1974. Inhibition of bacteria by some volatile and non-volatile compounds associated with milk. *J Milk Food Technol.* 37:539-544.
- Lema, M., L. Williams, and D. R. Rao. 2001. Reduction of fecal shedding of enterohemorrhagic *Escheria coli* 0157:H7 in lambs by feeding microbial feed supplement. *Small Ruminant Research.* 39:31-39.
- McCall, C. A., G.D. Potter, J. L. Kreider and W. L. Jenkins. 1987. Physiological responses in foals weaned by abrupt or gradual methods. *J. Equine Vet. Sci.* 7:368-374.
- Malinoski, K., N. A. Hallquist, L. Helyar, A. R. Sherman and C. G. Scanes. 1990. Effect of different separation protocols between mares and foals on plasma cortisol and cell-mediated immune response. *Equine Vet. Sci.* 10:5 363-368.
- Mathew, A. G., M. A. Franklin, W. G. Upchurch, and S. E. Chattin. 1996. Influence of weaning age on ileal microflora and fermentation acids in young pigs. *Nutr. Res.* 16:817-27.
- McDowell, L. R. 2000. *Vitamins in Animal and Human Nutrition.* Iowa State University Press. Ames, Iowa.
- Montes, A. J., and D. G. Pugh. 1993. The use of probiotics in food-animal practice. *Vet. Med.* 45:282-288.
- Naidu, A. S., W. R. Bidlack, and R. A. Clemens. 1999. Probiotic spectra of lactic acid bacteria (LAB). *Critical Reviews in Food Science and Nutrition.* 38:13-126.
- Oliveira, J. S., J. T. Huber, J. M. Simas, C. B. Theurer, and R. S. Swingle. 1995. Effect of sorghum grain processing on site and extent of digestion of starch in lactating dairy cows. *J. Dairy Sci.* 78:1318-1327.

Omole, O. O., G. Nappert, J. M. Naylor, and G. A. Zello. 2001. Both l- and d- lactate contribute to metabolic acidosis in diarrheic calves. *J. Nutr.* 131:2128-2131.

Parraga, M. E., S. J. Spier, M. Thurmond, and D. Hirsh. 1997. A clinical trial of probiotic administration for prevention of salmonella shedding in the post-operative period in horses with colic. *J. Vet. Inter. Med.* 11:36-41.

Pennington, R. J. and T. M. Sutherland. 1956. Ketone-body production from various substrates by sheep-rumen epithelium. *Biochem. J.* 63:353.

Peridgnon, G. 1986. Enhancement of immune response in mice fed with *Streptococcus thermophilus* and *Lactobacillus acidophilus*. *J. Dairy Sci.* 70:919-926.

Peridgnon, G. 1990. The oral administration of lactic acid bacteria increases the mucosal intestinal immunity in response to enteropathogens. *J. Food Protect.* 53:404-410.

SAS. 1999. SAS/STAT® User's Guide. (Version 8). SAS Inst. Inc., Carey, NC.

Spring, P. 2000 The role of probiotics in equine nutrition. Pages 41-49 in Proc. Dodson & Horrell Ltd 3rd Int. Conf. on Feeding Horses, Northamptonshire, England.

Stephens, D. B. 1980. Stress and its measurement in domestic animals: a review of behavioral and physiological studies under field and laboratory situations. *Advances in Veterinary Science and Comparative Medicine.* 24:179-204.

Stevens, C. E., R. A. Argenzio, and E. T. Clemens. 1980. Microbial digestion in the rumen versus large intestine. Pages 95-102 in *Digestive Physiology and Metabolism in the Ruminant.* Y. Ruckebush and P. Thivend ed. MTP Press, Lancaster, England.

Tannock, Gerald W. *Probiotics: A Critical Review.* 1999. Horizon Scientific Press. Norfolk England.

Topliff, D. R. and T. Monin. 1990. Growth of weanling horses fed yeast, soybean meal or milk based protein supplements. Oklahoma Agricultural Experiment Station Research Report. Pages 182-187.

Weese, J. S. 2001. A review of probiotics: are they really “functional foods”? Pages 24-28 in Proc. Amer. Assoc. Equine Pract. San Diego, California.

Weese, J. S. 2002. Microbiologic evaluation of commercial probiotics. J. Amer. Vet. Med. Assoc. 220:794-797.

Wohlt, J. E., M. E. Allyn, P. K. Zajac, and L. S. Katz. 1994. Cortisol increases in plasma of Holstein heifer calves from handling and method of electrical dehorning. J. Dairy Sci. 77:3725-3729.

Yoon, I. K. and M. D. Stern. 1995. Influence of direct-fed microbials on ruminal microbial fermentation and performance of ruminants: a review. Asian-Austr. J. Anim. Sci. 8:533-555.

Figure 1.

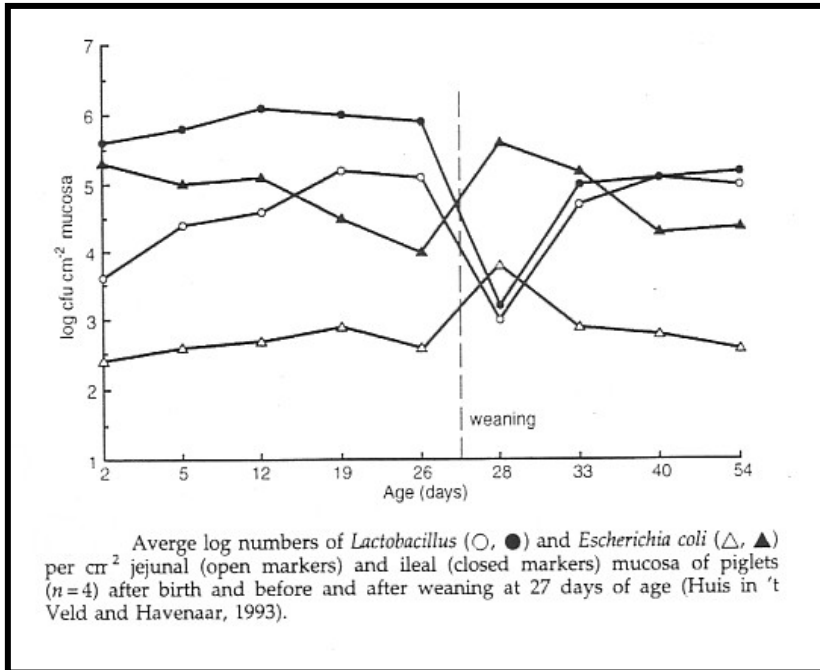
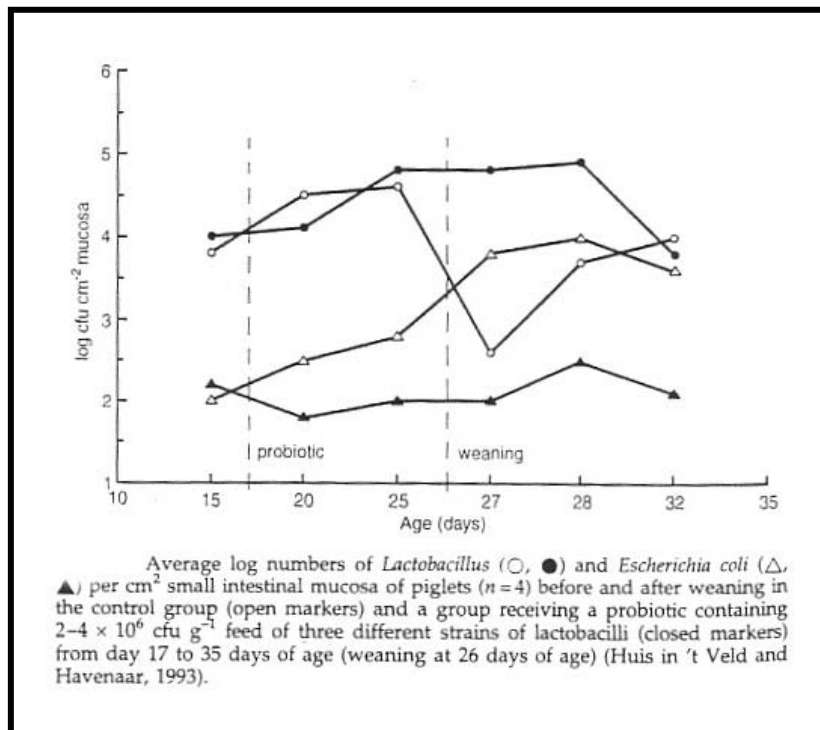
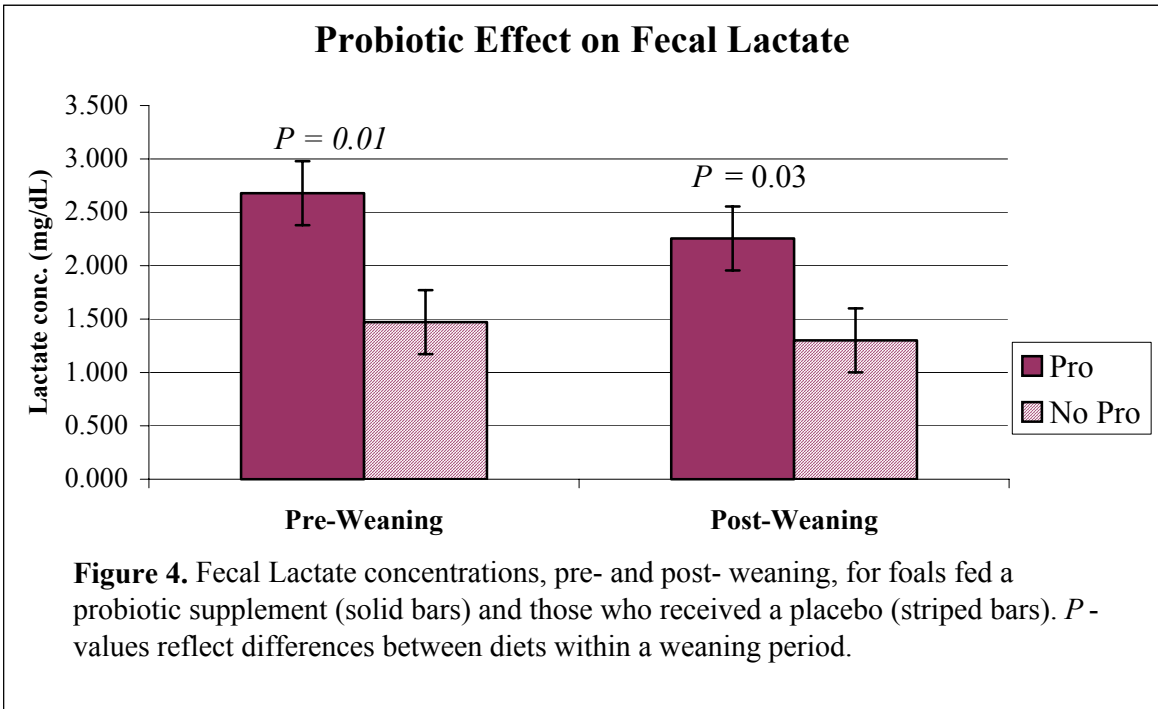
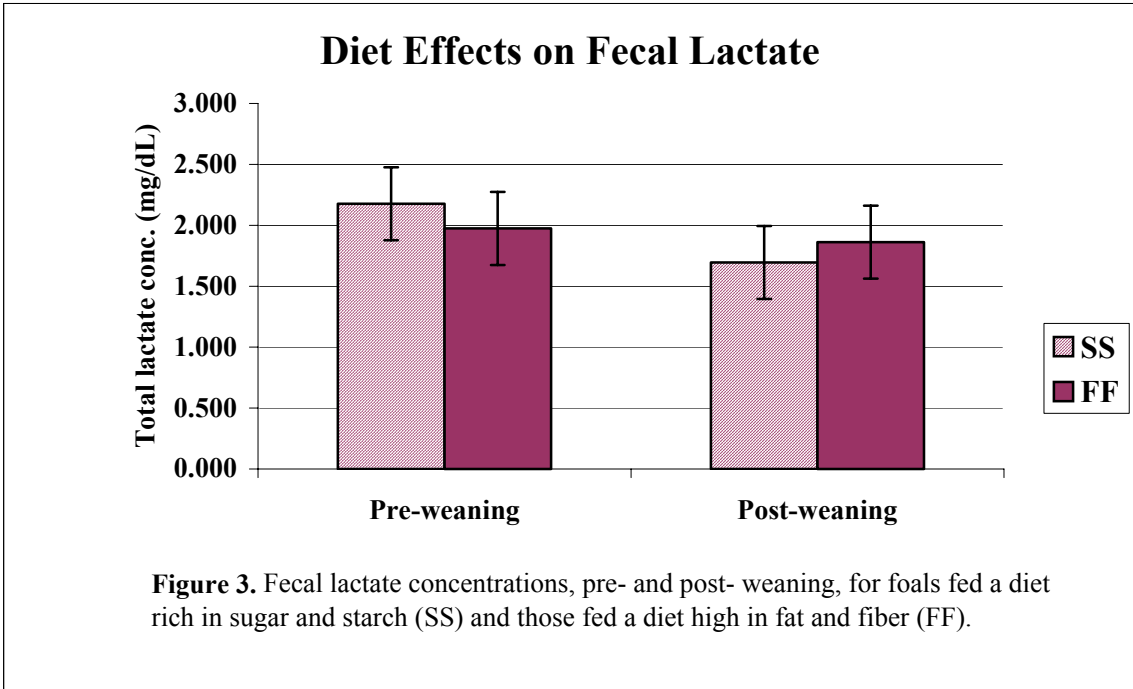


Figure 2.





Probiotic Effects on Acetic Acid

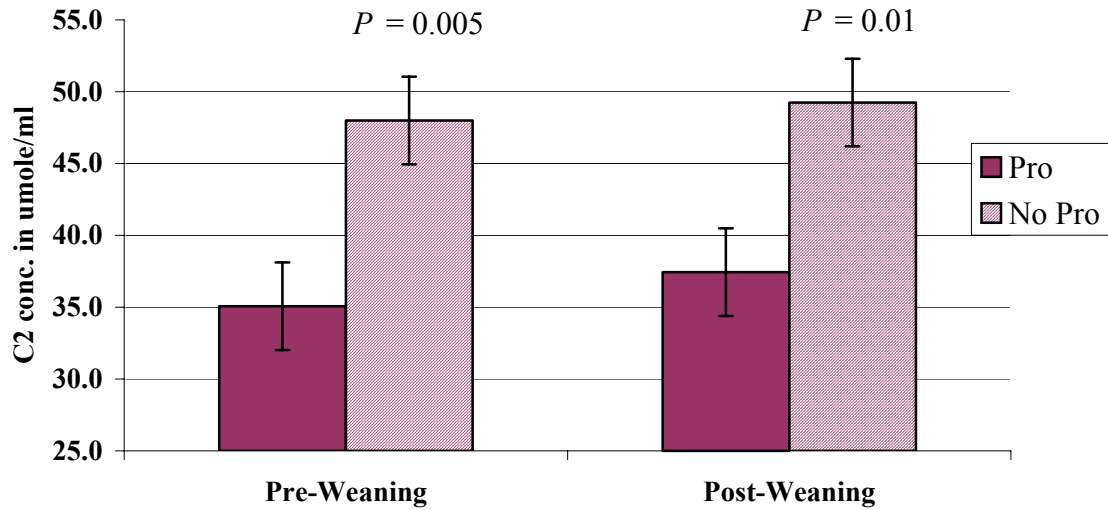


Figure 5. Fecal acetic acid concentrations pre- and post- weaning, for foals fed a probiotic supplement (solid bars) and those who received a placebo (striped bars). *P*-values reflect differences between diets within a weaning period.

Diet Effects on Acetic Acid

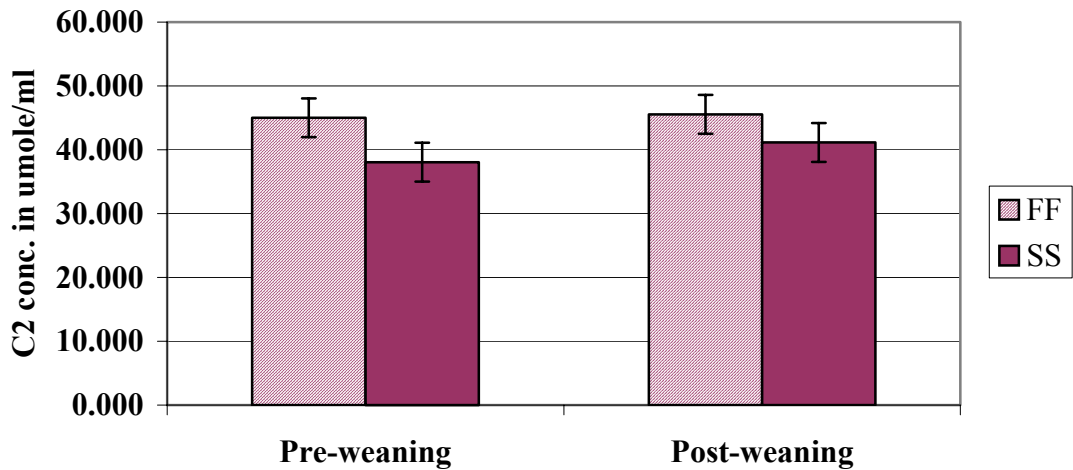


Figure 6. Fecal acetic acid concentrations pre- and post- weaning, for foals fed a diet high in fat and fiber (FF) and those fed a diet rich in sugar and starch (SS). *P*-values reflect differences between diets within a weaning period.

Diet Effects on Propionic Acid

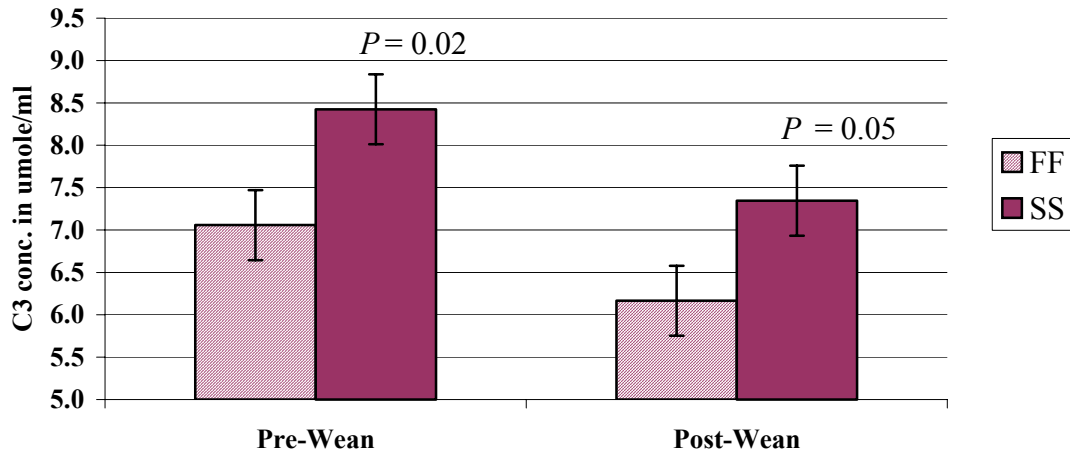
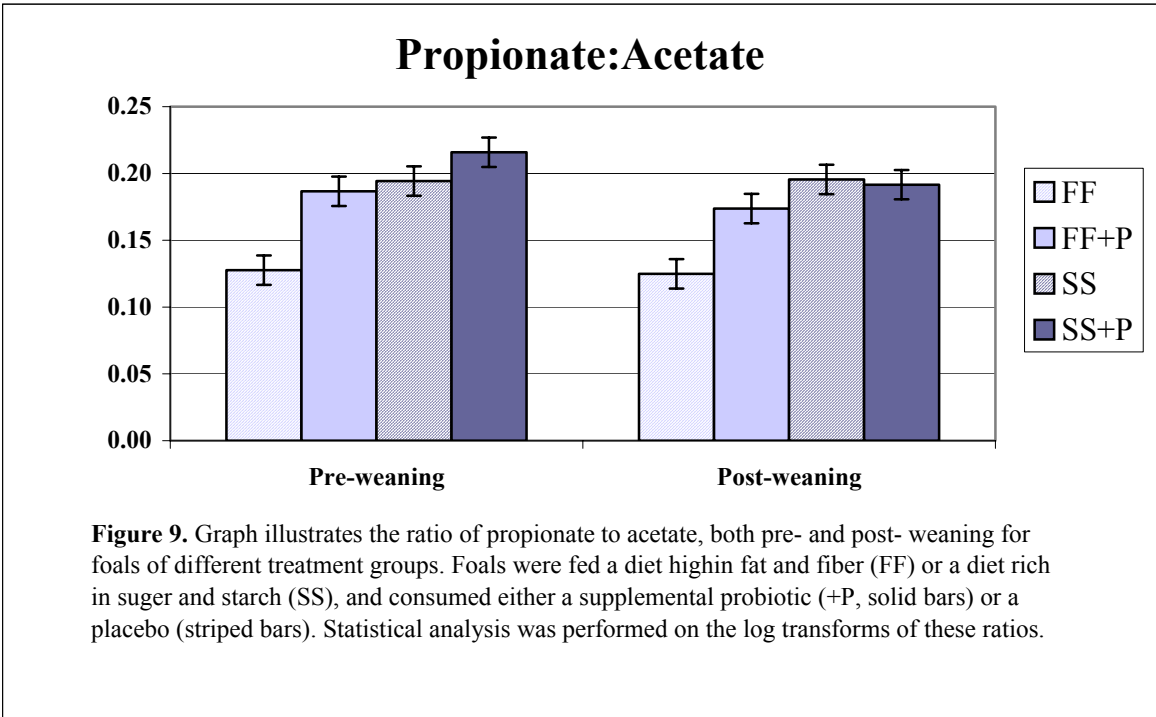
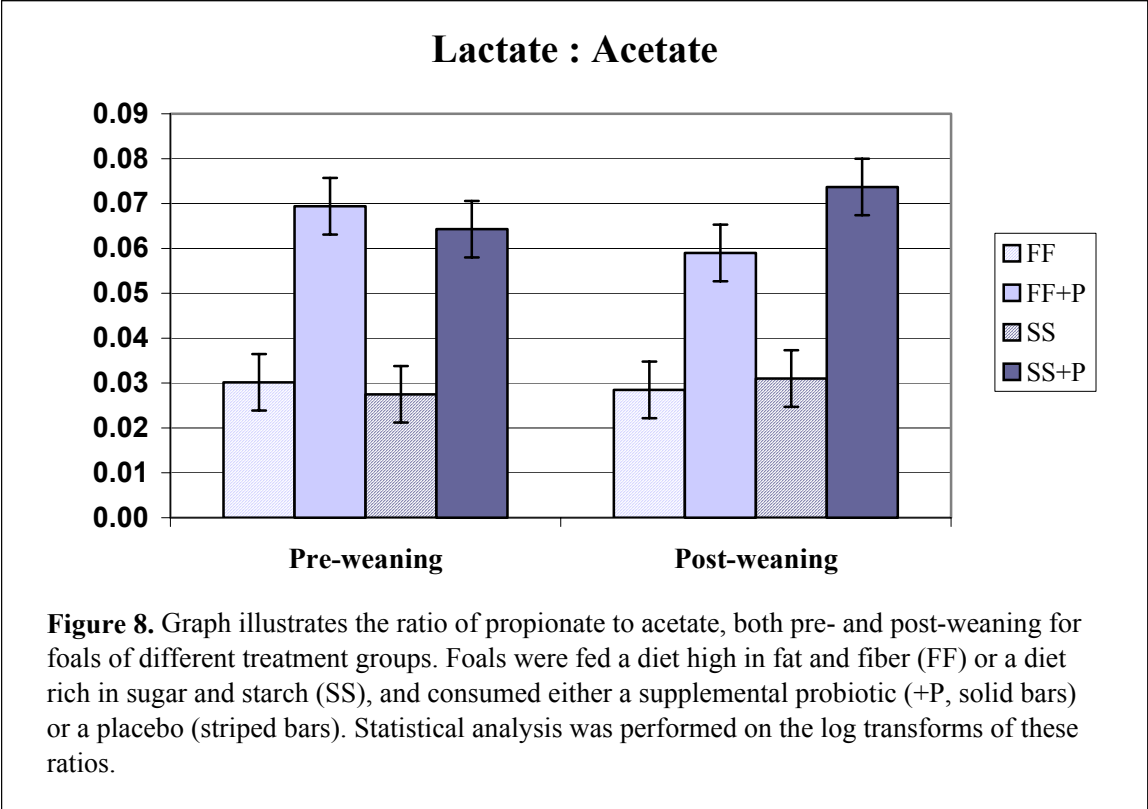


Figure 7. Fecal concentrations of propionic acid, pre- and post- weaning, for foals fed a high fat, high fiber diet (FF) and those fed a sugar and starch rich diet (SS). *P*-values reflect differences between diets within a weaning period.



Diet Effects on Butyric Acid

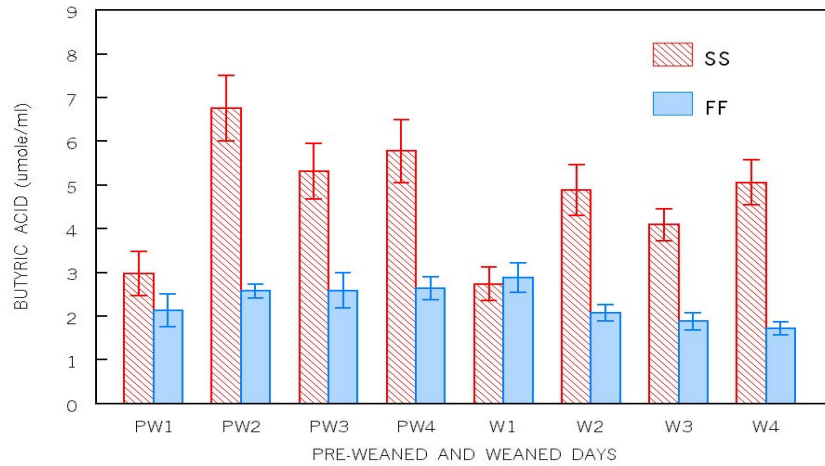


Figure 10. Fecal concentrations of butyric acid for foals fed a diet rich in sugar and starch (SS) and those fed a diet high in fat and fiber (FF). Comparisons for each collection day, both pre-weaning (PW) and post-weaning (W) are shown.

Diet Effects on Valeric Acid

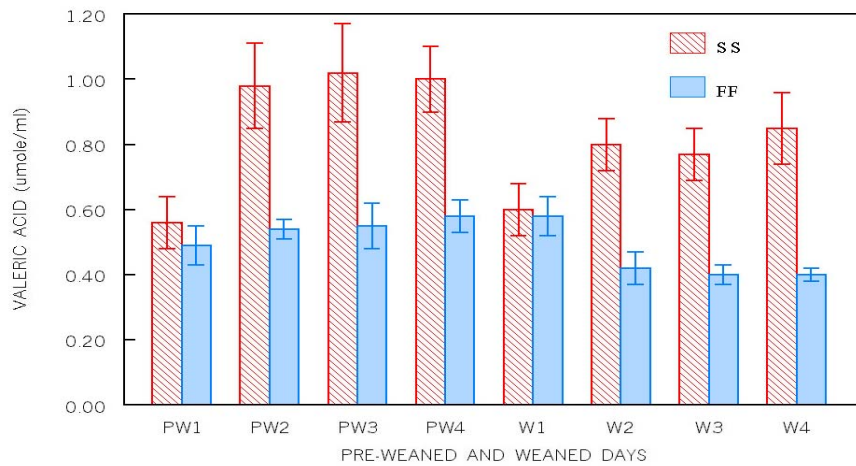


Figure 11. Fecal concentrations of valeric acid for foals fed a diet rich in sugar and starch (SS) and those fed a diet high in fat and fiber (FF). Comparisons for each collection day, both pre-weaning (PW) and post-weaning (W) are shown.

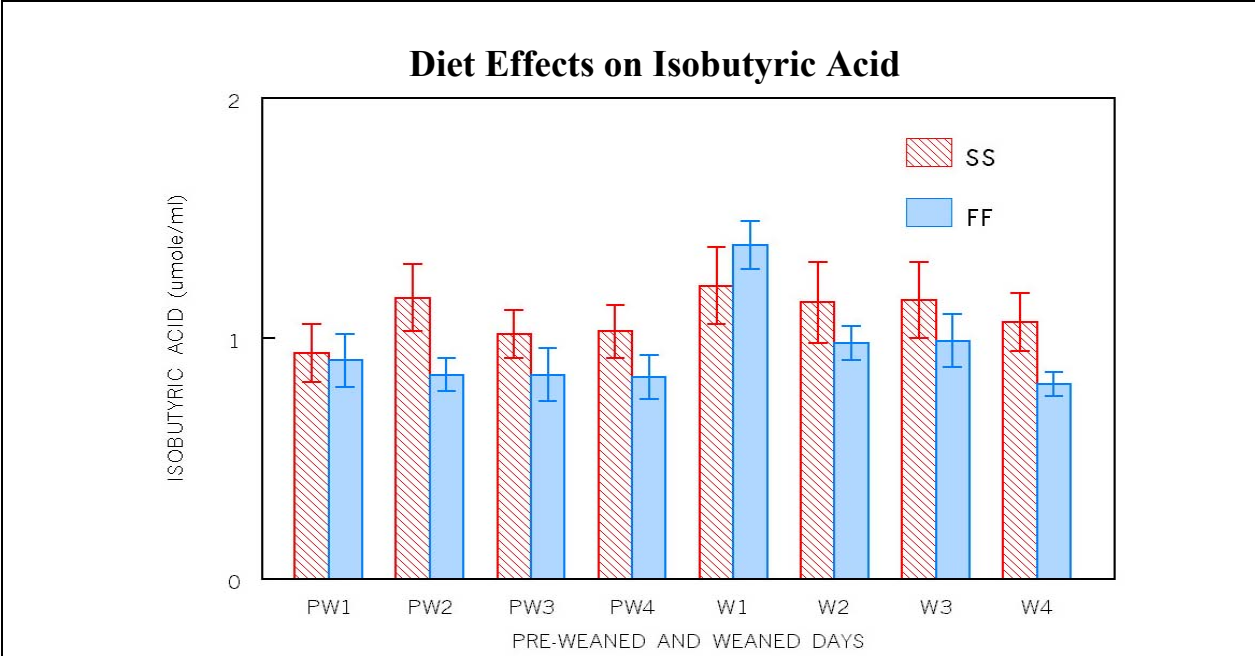


Figure 12. Fecal concentrations of isobutyric acid for foals fed a diet rich in sugar and starch (SS) and those fed a diet high in fat and fiber (FF). Comparisons for each collection day, both pre-weaning (PW) and post-weaning (W) are shown.

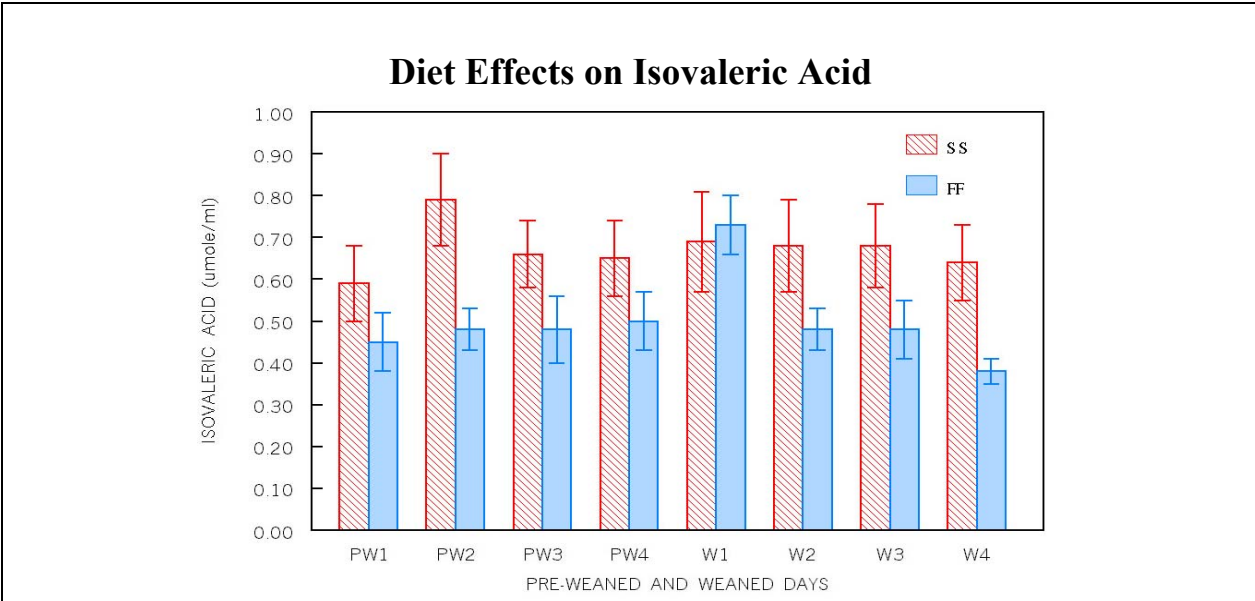
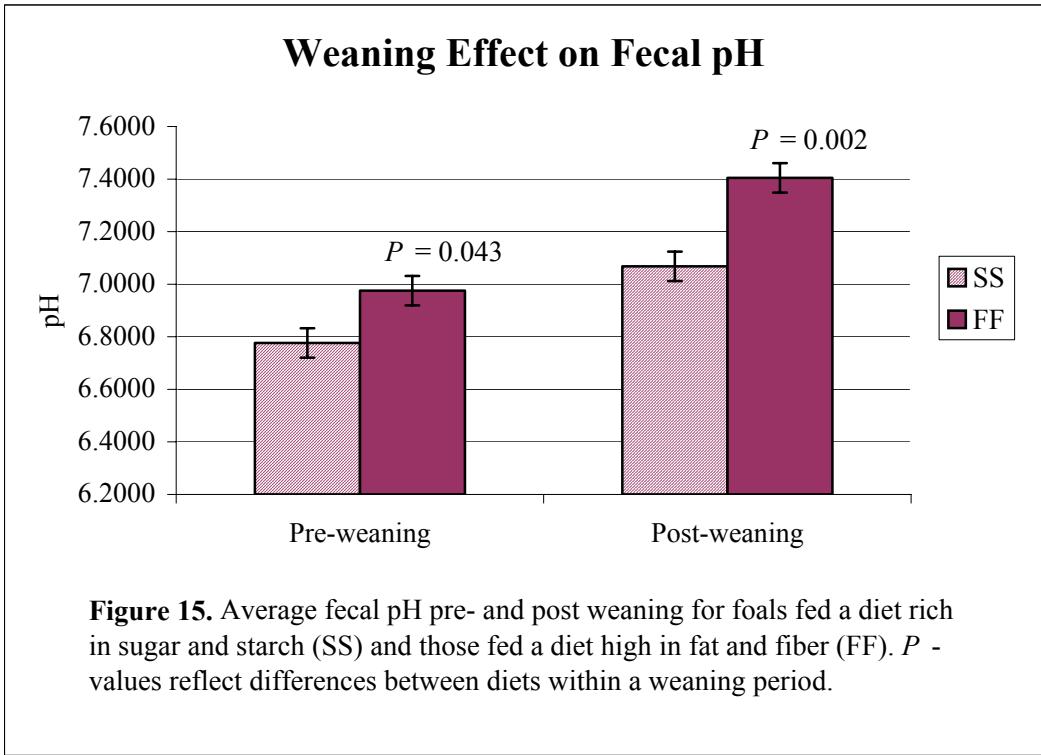
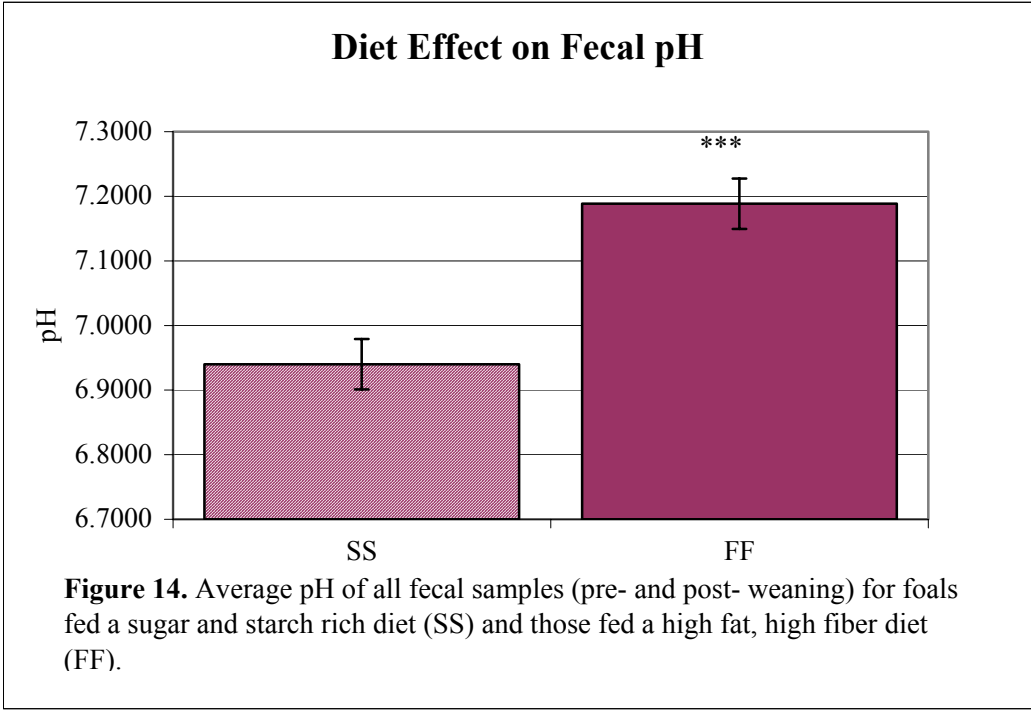
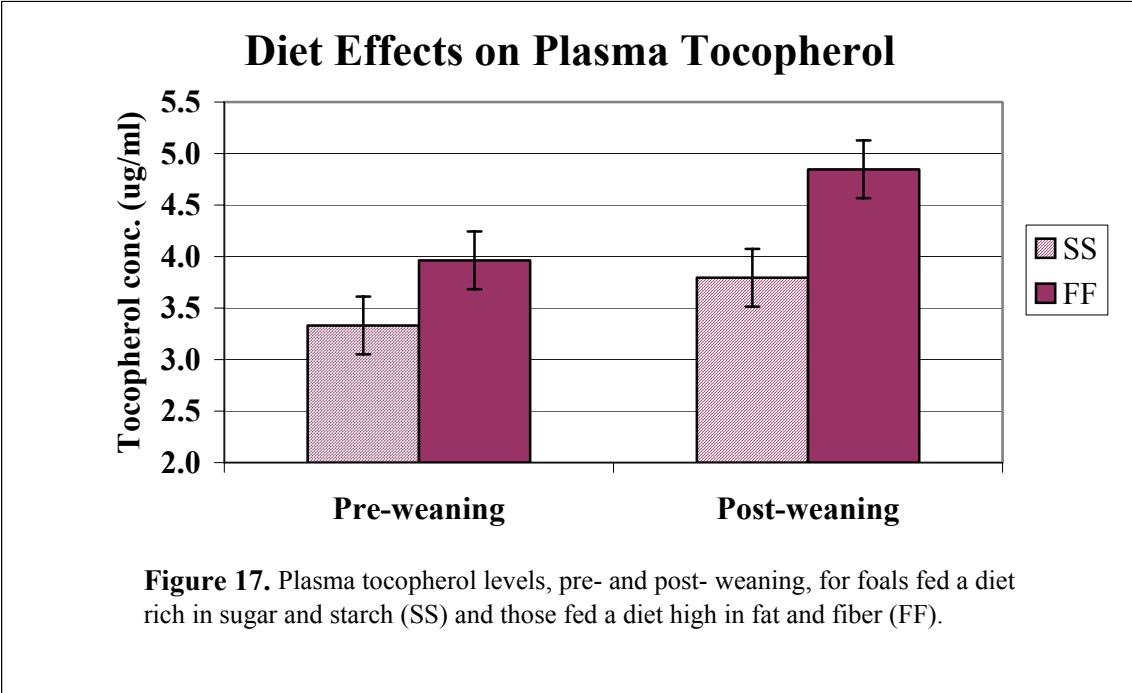
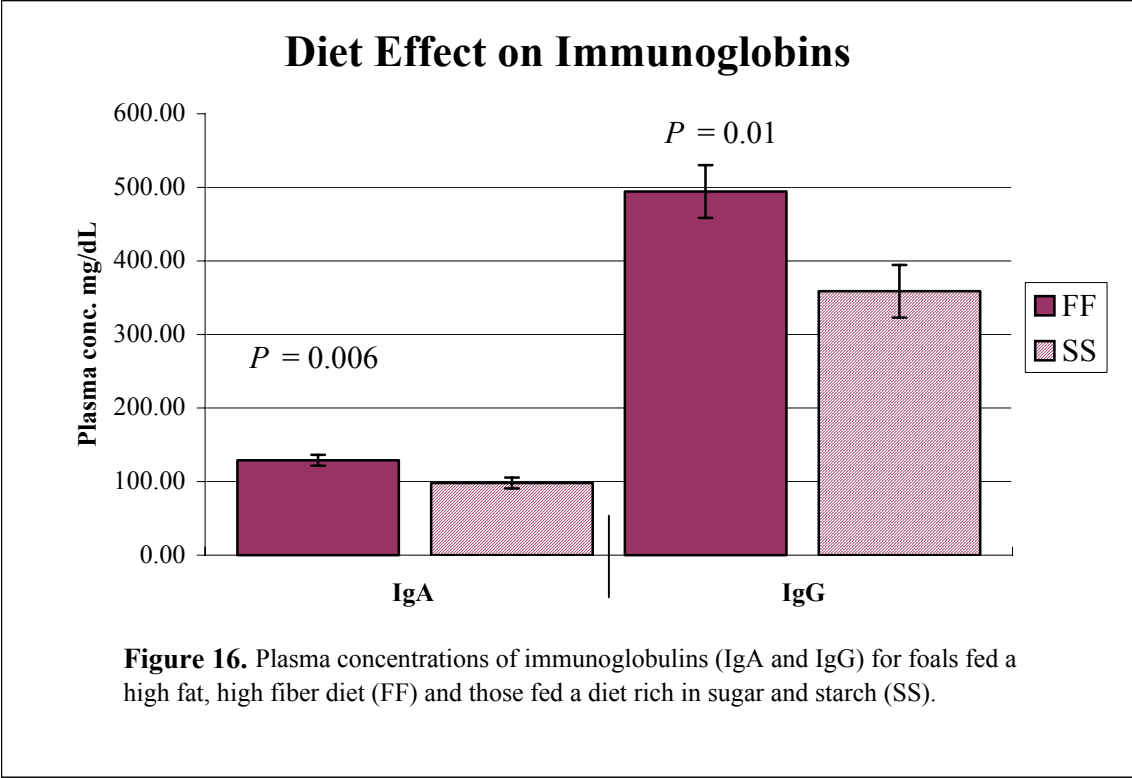


Figure 13. Fecal concentrations of isovaleric acid for foals fed a diet rich in sugar and starch (SS) and those fed a diet high in fat and fiber (FF). Comparisons for each collection day, both pre-weaning (PW) and post-weaning (W) are shown.





Weaning Effects on Behavior

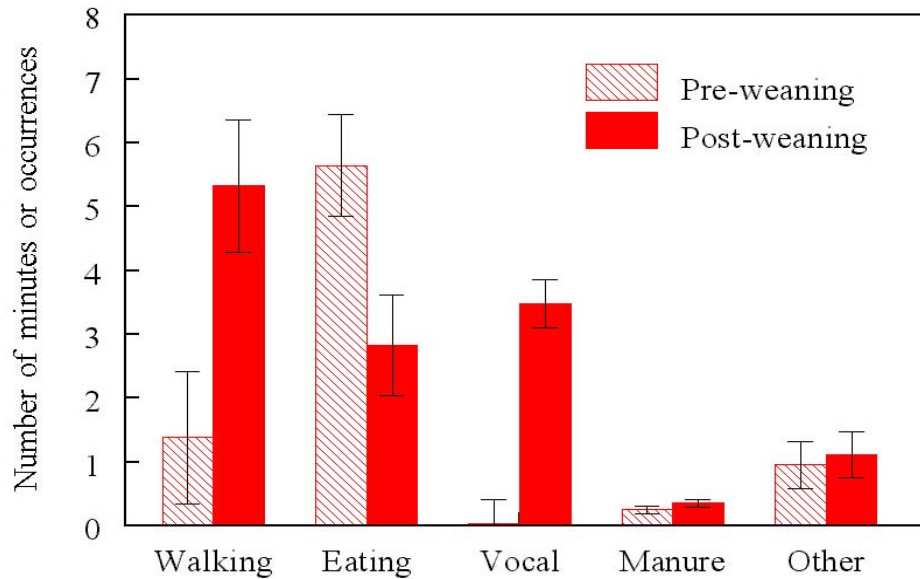


Figure 18. Graph depicts the average number of minutes spent walking or eating, the number of vocalizations, and the number of defecations in a 30 min time period. Other denotes negative behaviors such as kicking, pawing, weaving and chewing on stall.

Weaning Effect on Plasma Cortisol

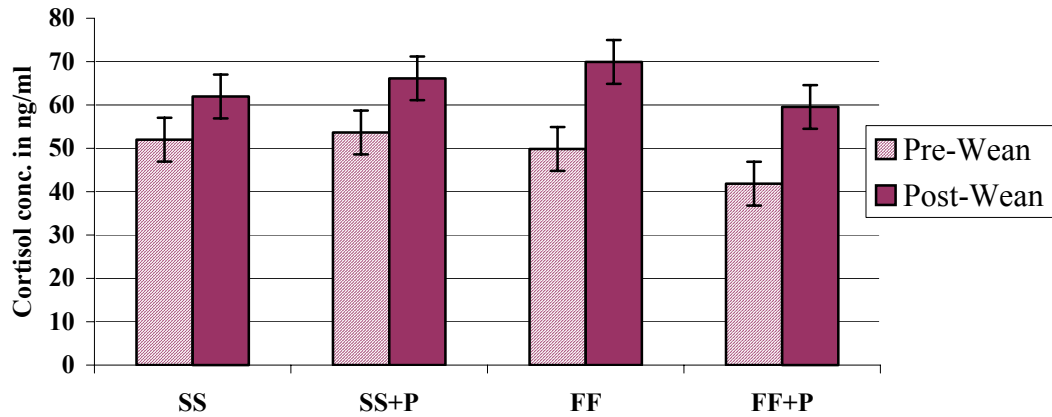


Figure 19. Plasma cortisol concentrations pre- (striped bars) and post- (solid bars) weaning for foals fed a sugar and starch rich diet, with (SS+P) or without (SS) probiotic, as well as those fed a diet high in fat and fiber, with (FF+P) and without (FF) probiotic supplement.

ACTH Response Test

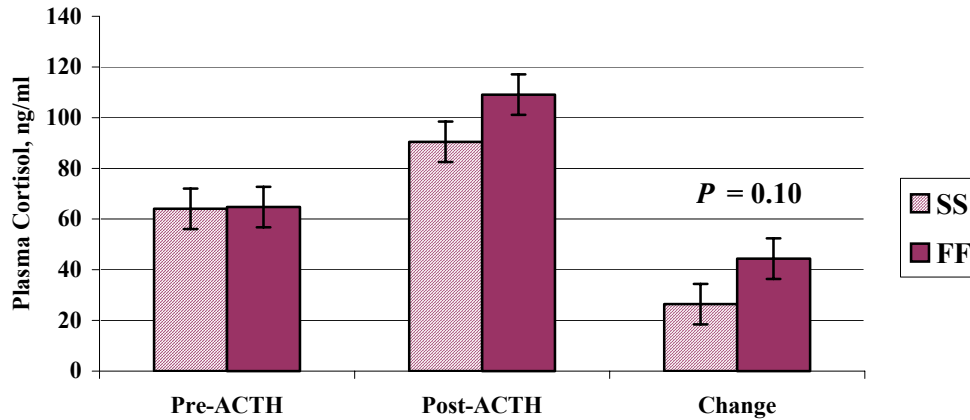


Figure 20. Plasma cortisol concentrations before (Pre-ACTH) and after (Post-ACTH) an ACTH challenge, as well as the response, or difference between these two values (Change) for foals who were fed a diet rich in sugar and starch (SS) and those fed a diet high in fat and fiber (FF).

Vita

Carrie Ann Swanson was born July 3, 1974 in Fairfax, VA. She graduated from James W. Robinson Jr. Secondary School in Fairfax, VA in 1992. She then attended Virginia Polytechnic Institute and State University, receiving her Bachelor of Science in Animal Science in 1996. Carrie was employed for three years as a veterinary technician at the Marion du Pont Scott Equine Medical Center in Leesburg, VA. In August 2000, Carrie returned to Virginia Tech to pursue a Master of Science in Equine Nutrition. Most of her research was done at the Middleburg Agricultural Research and Extension Center in Middleburg, VA. Carrie has accepted a position with Cornell University Cooperative Extension and will be moving to Orange County, New York upon completion of her M.S.