# LOW LEVEL AUREOMYCIN CONTAMINATION IN A PELLETED PONY RATION

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# TABLE OF CONTENTS

	Page
LIST OF TABLES	ii
LIST OF FIGURES	iii
LITERATURE REVIEW	1
Introduction: Antibiotics and the Equine	1
Antibiotics: General Considerations	3
Aspects of Antibiotic Effects in Nonruminants	
and Ruminants	5
Anaerobic Microbes and Antibiotics	13
Microbiology of the Equine Gut	17
Digestibility and Utilization of Feedstuffs	
By the Nonruminant Herbivorus Equine	27
OBJECTIVE OF STUDY	34
MATERIALS AND METHODS	35
RESULTS AND DISCUSSION	39
CONCLUSION	52
LITERATURE CITED	53
APPENDIX	61

# LIST OF TABLES

	vē.	Page
TABLE 1.	LATIN SQUARE DESIGN USED IN PONY DIGESTION TRIALS	36
TABLE 2.	COMPOSITION OF RATIONS FED TO PONIES	36
TABLE 3.	N.R.C. VALUES OF NUTRIENT COMPOSITION OF FEEDSTUFFS VS. ANALYSIS OF FEEDSTUFFS	40
TABLE 4.	N.R.C. NUTRIENT REQUIREMENTS FROM CALCULATED AND ACTUAL ANALYSIS	41
TABLE 5.	MEAN APPARENT DIGESTIBILITY COEFFICIENTS OF MEASURED NUTRIENTS AND BALANCE VALUES FOR NITROGEN, CALCIUM, AND PHOSPHORUS	42
TABLE 6.	MEAN APPARENT DIGESTIBILITIES OF PROXIMATE AND VAN SOEST COMPONENTS OF THE COMPLETE FEED	48
3 %	TABLES IN APPENDIX	
TABLE 1.	ABSOLUTE GROWTH RATE	61
TABLE 2.	RELATIVE GROWTH RATE	61
TABLE 3.	ANALYSIS OF FEEDSTUFFS	61
TABLE 4.	URINARY AND FECAL OUTPUT THE ANALYSIS	62
TABLE 5.	NUTRIENT DIGESTIBILITY	64

## LIST OF FIGURES

		Page
Figure 1.	Mean Nitrogen Balance (g/day) vs. Aureomycin Level (ppm/day)	44
Figure 2.	Mean Calcium Balance (g/day) vs. Aureomycin Level (ppm/day)	45
Figure 3.	Mean Phosphorus Balance (g/day) vs. Aureomycin Level (ppm/day)	46

#### LITERATURE REVIEW

Introduction: Antibiotics and the Equine

The beneficial use of antibiotics in animal feeds has been recognized since the early 1950's. Their use in poultry, swine and ruminant rations has lead to increased growth and/or efficiency of feed utilization with resultant decreases in the costs of production. However, their effects upon the nutritional status of the equine has received little attention and often it is believed that antibiotics have little to no effect, or are even detrimental to the horse and pony. These views are based upon early research work and maladies encountered in horses ingesting feedstuffs intended for other livestock, or the accidental contamination of a horse feed.

The early work of Taylor et al. (1954) compared the growth of thoroughbred foals given 50 and 100 mg of aureomycin hydrochloride twice daily from birth to 13 weeks of age, and from 13 weeks to 39 weeks of age, respectively. In those foals given the antibiotic as an electuary, their mean relative growth rates (see Appendix) were 30.1% and 26.8% greater than the control foals at weaning in 21 weeks and 39 weeks, respectively. The absolute (see Appendix) growth rate of the supplemented foals for the two periods, respectively, were improved by 14.1% and 10.5% over the control thoroughbred foals. Also, the antibiotic fed foals tended to have a smaller standard deviation in their absolute growth rates during the total time they were given aureomycin than the control foals. This last observation reversed itself when the antibiotic was withdrawn from 39 weeks to

the end of the experimental period at 56 weeks. Further, the mean relative growth rate and the absolute growth rate of the previously supplemented foals were only 2.6% and 7.0%, respectively, better than the nonsupplemented foals during the final period.

While Taylor, et al. (1954) established antibiotic supplementation increased the growth rate in the young horse and that it was dependent upon the level of supplementation, they also concluded that there was an actual decrease of between 8 and 10% in the relationship of the percentage growth increase and the age of foals once the aureomycin was withdrawn. They saw no difference in the density of the metacarpal bones or the degree of union of the epiphyses with the shaft between the two groups, though each of the six aureomycin supplemented foals ate about 0.1 lb. less of an oats-based diet than each of the control foals during the period from weaning to 56 weeks.

Clifford et al. (1956) fed penicillin or vitamin  $B_{12}$  to mature horses to test the concept that either antibiotic or cyanocobalamin could improve the overall condition of a debilitated mature horse. All 12 horses they chose displayed some degree of poor physical condition and had staring coats, prominent ribs, and drop over the hind quarters. After nine weeks of supplementing an oat-hay ration with 60,000 units of penicillin per day given in an ounce carrier of distillers' spent yeast, no improvement was observed in the condition of any of the horses. Instead, the condition of debilitated mature horses was improved by supplementation with 15  $\mu g$  of vitamin  $B_{12}$  per day in an ounce of the carrier. They postulated that the source of

aureomycin in which Taylor et al. (1951) used may have been contaminated with  $B_{12}$ , although Taylor et al. make no mention of such an occurrence. The overall condition in both the penicillin and  $B_{12}$  supplemented groups was visually appraised and no quantitative data were taken in either trial.

Further work with antibiotic supplementation in feedstuffs for the equine is lacking and much of the recent work by Cook (1973), Andersson et al. (1971), Bennett et al. (1969), Manahan (1970), Miller et al. (1961), and Teske et al. (1973) has dealt with the adverse effects that therapuetic levels of antibiotics may have in the equine by causing colitis, diarrhoea and/or death. These are valid concerns as it is well known that monensin and lasalocid, both polyether antibiotics, can result in the death of horses at minute levels. Lincomycin can likewise be lethal to the equine since it can essentially eliminate all anaerobic bacteria (Finegold, 1970), and as Krieger (1982) has stated, antibiotic poisoning of horses has become more frequent due to the increased use of antibiotics in livestock feeds. But just as important as the detrimental aspects of antibiotic use, are the beneficial effects that antibiotics have not only for other livestock, but perhaps for the equine as well.

#### Antibiotics: General Considerations

It is generally accepted that any beneficial effects that growth promoting concentrations of antibiotics may have are due to the modification of the microflora or their products of metabolism and the host's gastrointestinal tract (Visek, 1978). In comparing germ-free,

conventional, and antibiotic-fed animals, the antibiotic-fed animals are usually between the other two with respect to morphological differences (Combe et al., 1976, and Visek, 1978) and excretion products (Visek, 1978 quoting DeSomer et al. 1965). The major morphological differences between gnotobiotic and conventional animals appear in those portions of the intestinal tract which are closely associated with or harbor the intestinal flora (Gordon and Pesti, 1971). Schaedler (1971) likewise has concluded these effects, but has further stated that the microflora could also affect the growth and development of the host and prevent the establishment of foreign microorganisms. Thus, the intimate relationship between the host and its microflora is altered by the use of antibiotics.

Visek (1978) has outlined four proposed mechanisms whereby the host-flora relationship could be modified by antibiotics and result in a stimulatory effect. These hypotheses include the belief that microorganisms are responsible for mild but unrecognized infections that are constantly being suppressed by the host's defenses, and that there is a reduction in the microbial production of growth depressing toxins. Thirdly, it is believed that antibiotics reduce microbial destruction of essential nutrients either by enhancing bacteria that synthesize the essential nutrients, or by reducing competition between the various microorganisms (Maynard et al., 1979). Fourthly, antibiotics have a stimulatory effect because there is increased effeciency of absorption and utilization of nutrients due to the thinning of the alimentary canal. Because of the nutrient-sparing, inhibition of toxin-producing or decrease in disease-level effects, antibiotics have promoted the growth

of animals and their efficiency of feed utilization.

In addition to these accepted concepts of antibiotic feeding, it has also become established that the greatest stimulatory effects are seen in animals which are young, unthrifty, maintained in poor sanitary and environmental conditions, and are borderline in their nutritional requirements. Those agents which are absorbed more readily and have a broad spectrum of activity, have been shown to result in greater responses than those antibiotics that are narrow in their spectrum and ill absorbed.

Finally, concern has increased that the use of antibiotics in animal feeds will lead to the increased resistance of microorganisms. During the past 30 years of their use, Fulghum et al. (1968), Finegold (1970), and Hungate (1966) have documented the appearance of antibiotic-resistant forms of ruminal bacteria, but, depending upon animal specie, antibiotic(s), and environmental conditions, the average rate of growth enhancement has remained between 4 and 8% (Visek, 1978). Antibiotics have also been shown to decrease animal losses due to reduced liver abcesses in feedlot cattle, enhance an animal's ability to adapt to stresses, and reduce mycoplasmic pneumonia in poultry (Maynard, 1979 and Visek, 1978). The economic importance of sub-therapeutic levels of antibiotics is not based exclusively upon growth promotion and feed efficiency.

# Aspects of Antibiotic Effects in Nonruminants and Ruminants

Interpreting the data on the effects that sub-therapeutic levels of antibiotics have in both ruminants and nonruminants is difficult.

This is due to the use of both broad and narrow spectrum antibiotics, the various forms, combinations, and levels in which they have been used, the types of diets, and the variations seen in different animal species, as well as within a given specie. However, the influence that antibiotics have via the microflora upon the host can conveniently be seen as those which are histologically expressed in the gastrointestinal tract (GIT), and the effects upon the digestion and utilization of proteins, carbohydrates, fats, vitamins, and minerals.

Combe et al. (1976) have reviewed and summarized these aspects for monogastrics. Histologically, they have found that the cell renewal rate is slower and the gastrointestinal mucosa thinner in the germ-free and antibiotic-fed animal. Thinning of the gastrointestinal tract is not the same throughout its entire length. No differences have been found between the conventional and germ-free guinea pig stomach, but in the small intestine, the structure and cellular arrangement of the intestinal mucosa is more regular. The epithelial layer in the rat has been found to be more uniform, the lamina propria containing fewer lymphocytes and histocytes, and that there are fewer leuocytes. Peyer's patches have been observed to be smaller, having few reactive centers, low mitotic activity, and few plasmocytes in the germ-free animal. The amount of musculature present in the ileal portion of the small intestine has been found to be equal in both the conventional and gnotobiotic animal, although the latter has been shown to have less connective tissue.

The cell renewal rate in the antibiotic-fed animal has been shown to be 30 to 40% slower due to the decreased mitotic activity of the

crypts. Visek (1978) has calculated that the savings in nutrients due to the slower renewal rate alone could account for about 4.5% of the overall increase in the daily weight gain of the rat, which falls within the range of added response seen in antibiotic-fed animals. Further, Savage and Blumershine (1974) have shown that mouse crypts of Lieberkuhn are filled with anaerobic bacteria which are attached by filaments penetrating the epithelial surface. While cell attrition undoubtedly plays a role in the renewal rate of cells, Gordon and Pesti (1971) believe it is also enhanced by other unknown factors.

Besides these two histological aspects, the villi of the small intestine (Combe et al., 1976) have been shown to be of greater height and thinner in the antibiotic-fed animal. The villi are longer in the proximal portions of the intestine and decrease in length as one moves distally. In piglets that have been contaminated with bacteria, the villi decrease in relative length, have a higher mitotic index, and have deeper crypts (Kenworthy and Allen, 1966). It has also been noted that the brush borders in germ-free piglets are wider, well defined, and regularly arranged, but that those of contaminated piglets are reduced and sometimes indistinguishable. The increase in the height of the villi partially explains the slower cell renewal rate due to the increased distance that cells must travel.

Contrarily, Gustafson et al. (1968) have observed that treatment levels of antibiotics can markedly shorten villi and enlarge villous crypts in the duodenum and jejunum of horses. Although there were no gross lesions, there was a pattern loss to the villous epithelium and the normal ratio of villous length to crypt of 7 to 1 was reduced to about 1 to 1. The lamina propria and submucosa had more leukocytes

and lymphocytes in both the small intestine and colon.

Another common feature of the antibiotic-fed animal is the increase in the size of the cecum. So far, no valid explanation has been presented for this effect, though some experiments (Combe et al., 1976) have shown that some bacterial genera can modify cecal histology. Their mode has been theorized to be neurally mediated due to the response of the cardiovascular system after cece tomy (Gordon and Pesti, 1979).

The net result of these histological changes seen in the germ-free and antibiotic-fed animal is a thinner absorptive surface of the GIT, the epithelium of which contains fewer and less well developed lymphoid and reticuloendothelial elements. The wet weight of the small intestine is less, but that of the cecum is greater. Changes in other portions of the large intestine include the reduction of mucous cells, and irregular villi becoming more regular and smoother in the contaminated animal.

The increased absorption of minerals generally seen in germ-free and antibiotic-fed animals can be partially explained by the thinner intestinal epithelium. The rest of the explanation involves the microorganisms and the greater absorptive capacity of the brush borders (Reddy, 1971) due to increased adenosine triphosphatases (ATPases). alkaline phosphatases, and calcium-binding proteins in the gnotobiotic animal. The latter can increase the absorption of calcium (Ca) and magnesium (Mg) beyond that of the conventional animal. The former can affect mineral metabolism either by directly competing with the host or by releasing toxic substances and enzymes that are capable of inactivating or destroying the activity of the ATPases and alkaline

phosphatases. Fitt et al. (1972) demonstrated the ability of rumen microorganisms to perferentially bind Mg in their cell walls, thus rendering it unavailable to the host, and to release substances which inactivated enzymes involved in the active transport of Ca and Mg (Reddy, 1971). Combe et al.(1976) have stated that the germ-free condition results in greater retention of calcium, magnesium, and phosphorus regardless of age. This helps to increase skeletal weight and reduce the incidence of osteoperosis in older animals. It can also lead to possible calcification of the soft tissues and urethra.

Indirectly, the bacteria can modify mineral metabolism via the bile salts (Combe et al., 1976). Bacteria are capable of deconjugating bile salts by hydrolases (Norman and Widstrom, 1964), but because conjugated bile salts are capable of solubilizing and maintaining in solution Ca and Mg, and maintaining intestinal membrane permeability, conventional animals have shown a decrease in mineral absorption. There are differences in the extent to which different antibiotics can counteract this phenomenom. Penicillin can increase the absorption of Ca and Mg, but tetracycline can sometimes have the opposite effect by inhibiting collagen synthesis and forming insoluble calcium complexes (Combe et al., 1976).

Calcium absorption, in turn, is affected by vitamin D so that the conjugated bile salts also favor the increased absorption of vitamin D through micelle formation (Combe et al., 1976). Indeed, increased vitamin D absorption is seen in the germ-free state, not only for this reason, but also because bacteria are capable of metabolizing steroid compounds (Hungate, 1966).

While bacteria can be detrimental to mineral metabolism, Hungate (1966) has shown that they can be helpful in some instances. Some of the anaerobic ruminal bacteria have phytases that can hydrolyze phytic acids and salts. Therefore, the availability of phosphorus could be increased due to the digestion by these bacteria of phytic phosphorus, an otherwise poorly digested source of phosphorus.

The affect of metabolism on copper, zinc, manganese, sodium, potassium, chlorine, and iron between conventional and germ-free animals is variable: no effect to unfavorable with certain antibiotics. Requirements for copper, zinc, and manganese are generally increased in the contaminated animal (Combe et al., 1976). There is no effect or a slight increase in sodium, potassium, and chlorine requirements in the conventional animal. However, iron concentrations have been shown to be lower in the kidneys, liver, and spleen of germ-free rats, rabbits, and mice.

Other than the affect which microorganisms have on the absorption of minerals, is their effect upon the utilization of dietary lipids. Working via bile acid metabolism, the hydrolases and dehydrogenases of bacteria decrease the absorption of dietary fat both by histological modification of the intestine and by producing toxic unconjugated derivatives of bile acids. As Combe et al. (1976) have outlined, the differences seen in lipid absorption between floranated and defloranated animals can be due to the interaction of three factors: gastrointestinal transit is slower in the germ-free animal, conjugated salts are absorbed only in the distal portions of the small intestine of germ-free animals as compared to free acids being absorbed at all levels of

the intestine in conventional animals, but by different mechanisms; and that the liver is freed from the task of reconjugating free bile acids, rehydroxylation, and reduction of ketones in the germ free animal.

Lipid metabolism is also mediated by bacterial hydrogenation and endogenous excretion of lipids, the latter is usually higher in conventional animals due to the rapid cell turnover rate, but the absorption of unsaturated fatty acids is not higher in conventional animals. Saturated fatty acids are utilized better in the germ-free animal.

The extent to which the microflora participate in carbohydrate metabolism is dependent upon the source(s) present and site within the GI tract. Within the low pH of the stomach, Lactobacilli are abundant and their principle end-product of carbohydrate metabolism is lactic acid. In the small intestine and caudally, the end-products of the numerous anaerobic bacteria are the volatile fatty acids (VFA). The VFA are the principle products found in the ruminant animal (Hungate, 1966) and the cecum of nonruminant herbivores (Alexander and Davies, 1963, and Griffiths and Davies, 1963). The role that bacteria have in carbohydrate metabolism is, therefore, to either precede or complete digestive enzyme degradation. Any effect that antibiotics would have upon carbohydrate metabolism would depend upon affected bacteria and their mode of utilization. Generally, the more complex the carbohydrate, the less the response seen by a given antibiotic.

Lastly, the differences seen in protein digestion between gnotobiotic and conventional animals have shown several biochemical differences (Combe et al., 1976). Most of the nitrogen in the ceca of germ-free rodents has been found to be in the form of uric acid, urea, and hexosamines. Depending upon the level of protein in the diet, the concentration of free amino acids is 10 to 30 times higher in the germ-free cecum. Independent of the protein level and source, the germ-free animal will usually contain 2 to 4 times more serine and threonine, but 2 times less alanine. Also, Garson and Hill (1960) have observed adepression in amine formation in the ileum of pigs fed chlortetracycline. Soluble nitrogen compounds often accumulate in the distended cecum of germ-free animals.

Bacteria in conventional animals degrade nitrogenous compounds to ammonia with the largest fraction being found in the insoluble nitrogen component. The concentration of ammonia is lower in the portal vein of germ-free rats and guinea-pigs, and is not affected by the nitrogen content of the diet. However, the ammonia level in the conventional animal fluctuates with the nitrogen content of the diet and, especially in ruminants, the form of available carbohydrate. Harbers et al., (1963) have also shown that antibiotics depress urea hydrolysis in the GIT of rats. Likewise, McKinley et al. (1970) determined that urea hydrolysis in the rabbit could be depressed with neomycin to about one-sixth of the usual microbial synthetic rate.

Combe et al. (1976), and Visek et al. (1978) have stated that ammonia is toxic to intestinal cells and can have adverse biological effects. Dang and Visek in 1964 (as cited by Visek, 1978) found that ammonia increases the wet weight, alters nucleic acid synthesis, and increases protein in the intestinal mucosa. Therefore, any antibiotic action which could decrease ammonia synthesis by bacteria would help

decrease intestinal cell turnover rate and increase the efficiency of feed utilization.

One final aspect of the whole germ-free animal is the decrease seen in metabolic rate (Visek, 1978). Sherry et al. (1981) have found similar results in pigs given a combination of aureomycin, sulfamethazine, and penicillin. Rérat in 1978 has shown that the metabolic rate of the bacterial population within the gut could be depressed by antibiotics. Levenson et al. (1963, 1966) demonstrated in rats that the extent to which the metabolic rate of the host is depressed is in fact dependent upon the requirements of the indigenous bacteria. The net result of the decrease seen in metabolic rate would be a savings in heat increment and nutrients for cell renewal. This, in turn, could also enhance the feed efficiency of antibiotic-fed animals.

#### Anaerobic Microbes and Antibiotics

Over ninety percent of the bacteria inhabiting the gastrointestinal tract upon which antibiotics have their effects are anaerobic microorganisms. Hungate was the first to successfully culture these obligate anaerobes during the 1950's, but research on consequences of antibiotic use upon the microflora is limited. It has been limited toward the effects upon the metabolism of the total microbial population and the nutritional significance that this has for the animal. Further, research had been limited mainly to ruminant species and little is known about contributions possibly made by the equine's autochtous microflora and fauna in their host.

Of the bacteria that have been isolated from the equine's gut,

mostly from the large intestine, by Kern et al. (1973, 1974), Alexander et al. (1952), Alexander and Davies (1963), Davies (1968, 1979), and Reithour et al. (1970) many are also found in the rumen and have similar metabolic pathways. Fulghum (1968) and el Akkad and Hobson (1969) have tested some of the dominant anaerobic bacteria found in the rumen against 15 different antibiotics in vitro. Those microbes tested included the following: Bacteroides amylophilus, Bacteroides melaninogenicus, Bacteroides ruminicola ss. brevis, Bacteroides ruminicola ss. ruminicola, Bacteroides succinogenes, Butyrivibrio fibrisolvens, Eubacterium ruminantium, Lachmospira multiparus, Peptostreptococcus elsdenii, Eubacterium limosum, Ruminococcus albus, Ruminococcus flavefaciens, Selenomonas ruminantium, Spirillum, Streptococcus bovis, Succinimonas amylolytica, and Succinivibrio dextrino-Most all of the cultures were sensitive to bacitracin, solvens. chloramphenicol, chlortetracycline, erythromycin, novobiocin, oleandomycin, oxytetracycline, penicillin, tetracycline, tylosin, and vancomycin. Some of the bacteria were not suppressed by kanamycin, neomycin, polymycin, and streptomycin, and S. ruminantium was stimulated by tylosin and vancomycin.

Growth of Spirillum, L. multiparus, R. albus, S. ruminantium, and S. dextrinosolvene was stimulated by small concentrations of novobiocin, oleandomycin, polymycin, tylosin, and vancomycin as seen by the appearance of single and double Arndt-Schulz rings. Klatte and Thomas (1967) also observed enhanced growth of S. bovis in medium containing streptomycin.

The metabolism of these and other bacteria have been shown to

influence the nutritional requirements of the host animal. Their relative numbers fluctuate from one animal to another, regions of the country, and from country to country. Because of the degree of variation in the indigenous bacteria and the use of different antibiotics, no conclusions have been generally drawn upon the effects of an individual microflora genera. Rather, the population has been viewed as a whole and how antibiotics influence the nutrition of an animal by alterations in fermentation patterns, as well as those expressed morphologically and histologically.

Hungate et al. (1955) supplemented steers with 5 mg of chlortetracycline (aureomycin) per pound of feed. The level did not prevent fermentation but did alter the composition of the rumen microflora as did supplementation with streptomycin. Klopfenstein et al. (1964) fed graded levels of aureomycin to lambs and found that gas production was increased above control animals in proportion to the level of antibiotic. They also showed that both the viable and total counts of bacteria were not significantly altered by antibiotic addition. Apparent nitrogen digestibility increased significantly, and both the nitrogen balance and urinary nitrogen tended to increase with supplementation. Klopfenstein and his coworkers also showed conclusively that protozoal concentrations were a third greater in antibiotic-fed lambs than in the conventional lambs. The nutritional significance of the increased Entodinium population is not fully known, although protozoan protein has been found to be of high nutritional quality.

Purser et al. (1965) have confirmed the conclusions of the previous

work and have shown that rumen fermentation can be affected to different extents depending upon the antibiotic used. Tylosin was found to modify the bacterial population to a greater extent than aureomycin. Both antibiotics increased the proportion of propionic acid in the rumen, but total VFA concentration was not found to be significantly different due to either antibiotic.

In contrast, Beede and Farlin (1977) have generally found that antibiotic supplementation decreases VFA production. In an in vitro evaluation of 16 antibiotics fed at levels of 10, 40, and 200 ppm, only one antibiotic, capreomycin disulfate, increased apparent total VFA production and decreased the acetate to propionate (A:P) ratio. Oxamycin at 10 and 40 ppm was found to always result in higher total VFA production, but not at the 200 ppm. Monessin and novobiocin at 10 ppm decreased the A:P ratio, and decreased total VFA production at 40 and 200 ppm. Thiram decreased VFA and the A:P ratio at all treatment levels. In a follow up study, Beede and Farlin (1977b) observed decreased molar concentrations of propionate, and increased acetate and butyrate in sheep fed capreomycin disulfate.

Capreomycin disulfate has also been shown to reduce the production of lactic acid by as much as 65% (Beede and Farlin, 1977a). Bartley et al. (1979) have observed that lasalocid can also depress lactic acid production, and that both monensin and lasalocid are effective in increasing propionate and decreasing acetate. Neither affect total VFA concentration. Ruminal gas, specially methane, is decreased by both polyether antibiotics, although total amino acid concentration of ruminal bacterial protein appears not to be greatly affected. Lasalocid

increases the methionine and tyrosine concentration by 39% and 79%, respectively (Bartley et al., 1979). Monsein increases the concentration of leucine.

Muir et al. (1980), and Muir and Barreto (1979) have screened the growth of *S. bovis*, the initial lactate producer in grain engorged cattle, with penicillins, thiopeptin and thiopeptin-like antibiotics in vivo. Neither penicillin G nor ampicillin, due to the fact that many rumen bacteria have penicillinases, were found effective in inhibiting *S. bovis* for more than 16 hours in vivo. Thiopeptin was found to be effective against *S. bovis* and prevented acute lactic acidosis by reducing rumen lactate 80 to 90%.

Thus it seems that antibiotics affect rumen fermentation patterns by inhibiting microbial protein synthesis, depressing methane gas formation, increasing the molar proportion of propionate while depressing that of acetate and yet not altering total volatile fatty acid concentration, decreasing the production of lactate, and increasing total protozoa counts. The extent to which any of these are altered depends upon the antibiotic and its dosage level. The interrelationship of these factors has tended to reduce feed intake but improve feed efficiency. A recent exception to the last generality is lasalocid in which feed intake is not affected and feed efficiency is improved.

## Microbiology of the Equine Gut

As previously stated, many of the anaerobic bacteria isolated from the equine gut are commonly found in ruminants. It is often inferred from this that the cecum is a fermentation vat similar in function to that of a rumen (Alexander, 1952). However, though both permit a close association between microorganism and substrate, the anatomical arrangement of the cecum is such that feedstuffs are subjected to digestive enzymes prior to any microbial attack in the equine. The anaerobic bacteria, in turn, inhabiting the intestines are affected by the nutritive substrates upon which they must survive. There are also differences in the passage rate of digesta between these species as well as in reference to feed preparation. Together these variables have often made it difficult to draw any type of conclusion concerning the influence of the microbial ecology of the intestinal tract of the equine.

Vander Noot and Gilbreath (1970) found that geldings and steers were able to digest the protein and nitrogen free extract (NFE) components of forages equally as well, but that the steers were more efficient for the remaining proximate components. Hintz et al. (1971), believing that the ratio of hay to grain in the diet could possibly have some associative affects, found that there was a linear relation between percent of nutrient fraction in the diet and the digestibility of that nutrient. Further, Hintz et al. (1970) also demonstrated that the major site of neutral detergent fiber (NDF) digestion was in the cecum and colon and that protein and available carbohydrate (ACHO) was digested precedally. The digestion coefficients for ACHO indicated that at least 72% of the digestible ACHO disappeared before digesta intered the lower gut.

While these observations appear true, it is also true that Hintz, et al. (1971) found that the total concentration of volatile fatty acids in cecal fluid of ponies fed a high forage diet was greater than that of

ponies fed a high grain diet. The high grain diet also produced a lower percentage of acetate and higher percentages of propionate, isovaleric, and valeric. However, in one trial a higher percentage of butyrate (P < .05) was found in the cecal fluid, but not in an identical second trial. Glinsky et al. (1976) estimated that the VFA production within the cecum accounts for roughly 30% of the digestible energy intake. Thus, it is possible to alter the microbial population.

Applegate and Hershberger (1969) lend support to the fact that the microbial ecology of an individual pony or horse can vary considerably between individuals. Their nylon bag experiments demonstrated that fermentation rates varied between ponies and between the type of forage diet. They contradict Hintz's work by stating that alfalfa supplied nutrients to the cecum which increased the rate of fermentation. Digestibilities of timothy hay, orchard grass, and wheat straw between hay adapted ponies and steers, and hay-grain adapted ponies show that the digestibility was greater when exposed to ruminal microbes than for the hay adapted pony or the grain-hay adapted cecal microbes (Koller et al., 1978). They found no difference between any of the three groups in the digestibility of alfalfa, though it was fermented faster.

Thus it is evident that the diet does have an affect upon the microbial population and that differences in the individuals have yielded conflicting results. To complicate the matter even further, Kennedy et al. (1966) found that there were differences between age groups in the acetic, propionic, butyric and total volatile fatty acids of the cecum for weanlings, yearlings, 4-year olds, and aged animals.

The means, respectively, for acetic, propionic, butyric, and total VFA were (moles/ml): 72, 25, 13, and 109 for weanlings; 78, 17, 8, and 102 for yearlings; 89, 19, 9, and 117 for 4-year olds; and 85, 26, 11, and 123 for aged animals.

To understand why there are differences in digestibilities of feed between the nonruminant herbivorous horse and the bovine, and between individuals, it is necessary to examine the bacteria populating the intestines of the equine. Earlier work by Alexander et al. (1952) revealed that pentoses were not readily attacked in the right dorsal and ventral colons, but that cellobiose, fructose and lactose were more readily attacked in the dorsal colon than even in the ventral colon. Among the streptococci isolated were *Streptococcus equinus* and *Streptococcus bovis*. The chief fermentation product of the colon streptocci is lactic acid. Little lactic acid is found in the colon due to the presence of *Veillonella gazogenes* which ferments only lactate to volatile fatty acids. It is present throughout the tract in counts from 1 to 6 x 10<sup>8</sup> per ml (Alexander and Davies, 1963).

Crawford et al. (1968) used inoculum from the cecum and dorsal colon of horses being fed an oats-hay ration while others had access to green pasture. Their findings showed that starch digestion in the cecum was greater than that for the dorsal colon, 52.1% versus 25.3%, respectively, in horses fed the oats-hay ration. When the horses had access to green pasture, the figures were 21.0% and 31.9% for starch digestibility in the cecum and colon, respectively. Values for cellulose digestion between the cecum and dorsal colon, and between oats-hay

fed and pasture fed horses were, respectively, 2.0% and 5.1%, and 3.6% and 9.7%.

Kern et al. (1973,1974), in comparative studies, found that the microbial population increased in total and viable bacteria per ml of ingesta, and μg DNA increased when oats were fed to ponies, but not to steers. In general, there were no significant morphological or physiological microbial changes in the ponies due to diet, although there tended to be more Gram-negative rods when oats were fed or more Gram-positive or variable cocci when the diet consisted of mainly hay.

In ponies and steers maintained on timothy hay, the isolation of Gram-positive cocci was greater in the stomach and ileum for ponies but not for steers, compared to that in the cecum and terminal colon (Kern et al., 1974). The numbers of celluloytic bacteria (  $\times$  10 $^6$  ) in the pony were greater in the cecum, 43, than in either the terminal colon or stomach, 7 and 0.0003, respectively. There are few coliform bacteria found in the pony, and there are more viable bacteria in the fundic area of the stomach than in the pyloric. The proteolytic activity of the ileum is 30-fold that of the cecum or colon in ponies and Kern et al. (1973) have estimated that 19.7% of the cecal bacteria are proteolytic.

Of the bacteria isolated by Kern et al. (1973, 1974), Strepto-coccus bovis and Streptococcus equinus were the predominant Grampositive cocci, while Bacteroides and Propionibacterium increased in number when all hay or hay plus oats were fed, respectively. Alexander and Davies (1963) have found typical counts of lactate producing streptococci were in the order of 2 x 10<sup>8</sup> per ml and Streptococcus lactis

has been isolated from the equine stomach. Other anaerobic bacteria that have been isolated from the large intestine of the horse by Davies (1968, 1979) and Reitnaur et al. (1970) include: cocci, Grampositive organisms such as Ruminococcus flavefaciens, Ruminococcus albus, Peptostreptococcus elsdenii, Peptostreptococcus intermedius, and Peptostreptococcus parvulus, and the bacillary, Gram-negative microbes of Fusobacterium necrophorum, Bacteroides clostridiiformis, Bacteroides ruminicola ss. brevis, Bacteroides ruminicola ss. ruminicola, Bacteroides succinogenes, Bacteroides amylophilus, Butryvibrio fibrisolvens, Succinivibro dextrosolvens, Succinimonas amylolytica, and Selenomonas ruminantium. Many of these anaerobic bacteria are found in the rumen. However, Davies (1968) has suggested that they may differ in their metabolic activities within the equine somehow due to the fact that they grow better in medium containing some equine liquor than if grown in rumen fluid medium. The author has also confirmed this observation in experimental work done in this lab.

McCreery et al. (1971) published results obtained from one pony on a 14% protein pellet plus hay ration which required direct microscopic clump counts (DMCC) ranging from 1 x  $10^6$  to 2.4 x  $10^{11}$  organisms per m1 of cecal fluid. DMCC were highest 5 to 6 hours after feeding. Colony counts made by Hungate's roll tube technique were highest on medium containing 40% (v/v) horse cecal fluid, or "H" medium. "H" medium supported growth of  $3.5 \times 10^9$  to  $4.3 \times 10^9$  organisms per m1 of cecal fluid which was 11.3 to 14.0% of the DMCC, respectively. Medium containing 40% (y/y) rumen fluid supported  $3.2 \times 10^9$  organisms per m1 or 10.5% of DMCC. Also, McCreery estimated that approximately 45% of

the cecal bacteria were obligately anaerobic and  $1.25 \times 10^7$  or 45% of the DMCC were proteolytic. Reitnour and Mitchell (1979) found lower proteolytic counts in cecal fluid ranging from  $2 \times 10^5$  to  $8 \times 10^5$  organisms per ml, perhaps due to different medium, ponies and diets.

The relative numbers of the above anaerobic bacteria within the intestinal tract of the horse do vary with respect to diet, medium, and individual, and therefore, no numbers are reported here. Reitnour and Mitchell (1979) have stated that once the animal is adapted to a ration, the microbial population remains relatively stable as to number and type. In their anaerobic proteolytic study, in which cecal samples were taken at 2 hour intervals, there was less than a twofold difference between the lowest (2 hours after feeding) and the highest (6 hours after feeding) counts, which ranged from  $5.0 \times 10^5$  to  $9.5 \times 10^5$  organisms per ml, respectively.

Reitnour et al. (1969, 1972), and Wooton and Argenzio (1975) have both concluded that the major portion (40%) of the apparent protein digestion is caudal to the cecum. Also, urea feeding is accompanied by an increase in the volume of the large intestinal contents, especially the ventral and dorsal colon. Wysocki and Baker (1971, 1975) demonstrated using <sup>14</sup>C-glucose that only 7.15% of the <sup>14</sup>C appeared in the amino acid fraction of the cecal bacteria. Houpt and Houpt(1971) demonstrated that oral treatment with phthalylsulphathazole and neomycin sulphate increased daily urea-nitrogen excretion. The rise of 1.7 g urea-nitrogen seen with antibiotic treatment could have been due to lysis of bacterial cells and thereby cause a transitory rise or by the decreased utilization of urea for protein synthesis as a result of suppressing the bacteria.

Further study of this aspect has not been done.

Besides these observations mentioned, Alexander and Davies (1963) have isolated lactobacilli from the equine intestinal tract. Lactobacilli were isolated from the stomaches in 14 of 16 horses, and in the cecum and colon of 6 horses. Bacterial counts ranged from 2 x  $10^6$  to 3 x  $10^8$  down to below 1 x  $10^3$  per ml in the respective regions. Morphologically, the lactobacilli resembled Lactobacillus bifidus.

The lactic acid producing bacteria have been shown to be correlated to lactic acidosis in the equine which can result in mild to severe cases of laminitis or even death. Garner et al. (1977, 1978) have shown that lactic acidosis is the result of soluble carbohydrate or grain overload in which the lactic acid producing bacteria proliferate and produce L-lactate. This, in turn, decreases the pH, which decreases other bacterial populations and damages the mucosal lining of the intestinal tract.

The decline in pH decreases another bacterium found in the gut, namely Escherichia coli. Upon lysis of E. coli, endotoxin is released. If enough endotoxin is released, the mean arterial blood pressure is elevated along with other systemic body functions (Burrows, 1971). These can result in systemic arterial hypotension, central venous hypertension, neutropenia, hyperglycemia, hemoconcentration, and eventually peripheral vascular perfusion failure followed by death.

Besides the flora mentioned so far, equines also have protozoa that are likewise influenced by the diet and pH of the intestinal environment. In the late 1920's and early 30's, Hsiung compiled a monograph on the protozoa found in the large intestine of the horse in which four

classes of protozoa are represented: Rhizopoda, Mastigophora, Ciliata, and Suctoria. The order Coccidia was not represented, and the ciliates were the predominate class in both numbers and variety of forms. Kern et al. (1973. 1974) have shown that the cecum of ponies contains approximately 5,668 protozoa per ml. They found no protozoa in other regions of the digestive tract, and when oats were fed, Blephacerys uncinata increased, but when the diet consisted of timothy hay plus or minus oats, Cycloposthium bipalmatum increased in numbers proportionately. Generally, the protozoa thrive best near a neutral pH.

The work of Hsiung (1930) and Adam (1951) has, however, shown that protozoa inhabit other regions of the large intestine and not just the cecum. Adam suggested that the fauna populating the large intestine could be divided into two parts:

- Those species which are characteristic of the cecum, right ventral colon, and left ventral colon, and occur only occasionally in the posterior region of the large intestine;
- Those species which are in the left dorsal colon and are characteristic of the caudal portion of the large intestine.

There is also a change in the population between the left dorsal colon and the right dorsal colon.

Adam (1951) found that Blepharcorys uncinata is typical of the cecum, but that few are found in the left or right ventral colon. Cycloposthium was dominant in the left and right ventral colons with C. bipalmatum the dominant protozoa. In the left dorsal colon, Bundleia postciliata, Blepharocors curvigula and B. angusta(?) were the typical dominant species. The species differed at the pelvic flexure.

The total concentration of ciliates in the left dorsal colon is greater than in the right ventral colon, though the total fauna bulk does not differ. The bulk does not differ because the species Cycloposthium in the right ventral colon is greater in size. *C. bipal-matum* and *C. edentatum* have mean lengths of 101 and 190 $\mu$ , and widths of 42 and 80 $\mu$ , respectively. *Bundleia postciliata*, *Blepharocorys curvigula* and *B. angusta* have mean lengths of 42, 70, and 87 $\mu$ , and mean widths of 25, 22 and 32 $\mu$ , respectively.

In comparison, the average number of ciliates found in ruminants is between 500,000 and 1,000,000 per ml, but in the horse there is generally never more than 500,000. However, ciliates may increase in the horse if fed a hay-grain diet instead of just a hay diet. Unlike the bacteria which have been isolated often from both ruminants and the equine fed like diets, Adams and Hsiung have found that the fauna of the equine are unique and specific. Upon infusion of equine ciliates into rumens, the protozoa did not infect and generally died out completely.

Besides the bacteria and the protozoa found in the equine's intestine, Alexander et al. (1970) have found bacteriophage-like particles throughout the intestinal tract. There is no noticable difference in concentration of phages between the various regions. Like the protozoa and the bacteria, it is not known to what extent the bacteriophages may have upon the nutritional status of the equine.

### Digestibility and Utilization of Feedstuffs By the Nonruminant Herbivorus Equine

Squibb (1958) made the comment that little advancement had been made in the knowledge of horse nutrition. Though research in this area has increased during the past 15 years spurred by renewed interest in the light horse, the same statement can still be said today. Much of the early work was done with draft horses, but the shift to the light horse has brought with it changes in ownership from farmer to urbanite, from pasture to pens, and from work to recreational purposes. The years have also seen changes in the feedstuffs and manner in which we feed our other livestock. However, this has not been the case with the horse since most owners yet rely on the staple oat, corn and hay type ration.

Several researchers have reviewed the digestibility and utilization of various grass hays, the major crude fiber component of a horse diet. In 1967 and 1968, Fonnesbeck et al., and Darington and Hershberger, respectively, determined the digestibility of the proximate components of canary grass, brome grass, fescue, bermuda grass, alfalfa, red clover, timothy, and orchard grass. Fonnesbeck et al. (1967) found that horses perfer to ingest legumes (P < .05) more than grasses, and that forages contain significantly more digestible crude protein and nitrogen free extract (NFE). This resulted in higher digestible crude protein and total digestible nutrient (TDN) values for the legumes.

Darington and Hershberger (1968) reported that the apparent digestibility for alfalfa, timothy and orchard grass of dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE), NFE, TDN, and digestible energy (DE) decreased as forages matured. And although

ruminants generally have a higher digestibility of the crude fiber fraction of any feed than horses (Vander Noot and Gilbreath, 1970; Olsson and Ruudvere, 1955), Darlington and Hershberger (1968) reported that the digestibility of the crude fiber fraction of alfalfa was 25% greater than that reported for ruminants. In conclusion, they stated that the ability of the horse to digest DM, CP, CF, NFE, and energy was inversely related to the percentage of crude fiber in the diet.

Alexander (1963) and Hintz (1970) have stated that the horse digests the crude fiber of hay only 2/3 as well as cattle, and Koller et al. (1978) have shown that ruminal bacteria are more efficient than cecal bacteria in degrading forages. Vander Noot and Gilbreath (1970) have obtained similar results in comparison trials between steers and geldings fed four different forages. Again, significant differences (P < .10) were obtained for all forage proximate components with the exception of protein and nitrogen free extract.

Fonnesbeck (1968) has calculated the true digestibility of the fibrous fractions of forages by regression analysis. He has estimated the average digestibility and true digestibilities of cellulose, hemicellulose, and lignin are, respectively, 45.2 and 43.4%, 46.9 and 49.5%, and 1.9 and -0.3%. The respective apparent digestibility and true digestibility of total crude fiber was 43.0 and 54.3%. Fonnesbeck (1968) concluded that the apparent and true digestibilities were the same for fibrous carbohydrates and lignin since there is no endogenous fecal excretion.

Fonnesbeck (1968) has also estimated the apparent and true digestibility of the soluble fractions of forages. The cellular content, which represents the total soluble nutrients, had an average apparent and estimated true digestibility of 61.1 and 101.8%, respectively; endogenous excretion accounted for 12.7%. The values calculated for the soluble carbohydrate, protein, ether extract, and ash fractions of forages for apparent and true digestibility were 70.5 and 105.7%, 51.2 and 81.7%, 28.8 and 75.1%, and 61.5 and 90.5%, respectively. However, Fonnesbeck (1968) stated the digestibility, whether true or apparent, of the soluble fractions of forages could not be compared between forages of different content.

Hintz et al. (1972) have shown that as the proportion of crude fiber in the diet increases, the importance of microbial fermentation in the cecum and colon increases. Volatile fatty acid synthesis in the cecum accounts for approximately 30% of the digestible energy intake (Glinsky et al., 1976), and hintz et al. (1971) have shown that the proportions of acetate, propionate and butyrate change when grain is added to the diet. As previously stated, total VFA and the percentage of acetate decreases while the percentages of propionate, isovalerate and valeric increase on high grain diets. Although changes do occur with respect to forage:grain ratios, Hintz et al. (1971) have shown that the relationship between percent of nutrient fraction in the diet supplied by either forage or grain is linear and that there are no associative effects.

The VFA formed via fermentation of grains or forages are absorbed from the cecum and colon (Argenzio and Stevens, 1975). Unlike rumen epithelium, Giddings and Stevens (1968) have demonstrated that horse cecal epithelium metabolizes less of the absorbed VFA into ketone bodies. Rumen epithelium absorbs about 3 times more butyrate

than acetate, but due to epithelial metabolism it is transported to the blood side only one half that of acetate. Equine cecal epithelium transports both acetate and butyrate at equal rates. Hintz, et al. (1972) have found that the propionate disappearance rate from venous blood is similar in both the pony and cow.

Alexander (1952) found that witholding food decreased the concentration of fatty acids and increased them with feeding, and that the proportions of the acids varied from dorsal and ventral colon. More butyrate and longer acids formed in the dorsal colon, and more propionate was found in the dorsal colon. It has been suggested that these differences are a reflection of the variation in metabolism of the resident microbial population.

The lipid fraction of forages and grains can be digested by the equine in the small intestine due to the constant secretion of bile. Hintz and Schryver (1978) state that the primary site of absorption is within that portion of the gut and that the composition of body fat can be influenced by the dietary fat source. Earlier work by Olsson Ruudvere (1955) erroneously reported low and negative apparent digestibility coefficients for the lipid fraction of forages due to the inability of ether extract to quantitatively extract plant lipids (Street and Chang, 1964; as cited by Fonnesbeck et al., 1967).

Proteins in feedstuffs are digested both by enzymes of the gut and the microflora. Hintz et al. (1970) have determined that about 70% of the digestible protein fraction of feeds is absorbed precedally. Alexander (1954) found that the ileum was the major site of protein digestion and absorption, and any which reached the large intestine

was degraded to ammonia by the microflora. Alexander and Davies (1963) have found urea in all parts of the equine intestinal tract and particularly in the large intestine in spite of the presence of the microflora. Wysocki and Baker (1975) have found no appreciable absorption of amino acids across the epithelium of the lower gut. Slade et al. (1971), using <sup>15</sup>N-labeleld bacterial protein, have shown that lysed bacterial amino acids can be absorbed from the cecum and colon. The microbial synthesis of protein can upgrade poor quality protein sources and make them available to the equine.

Like the ruminant, horses can utilize non-protein nitrogen sources due to the presence of the microflora (Houpt and Houpt, 1971; Reitnour and Salsbury, 1972; and Nelson and Tyznik, 1971) and the urea cycle proposed by Slade et al. (1970) was later proven correct by Prior et al. (1974). Wooton and Argenzio (1975) have shown that the adaptation to an urea diet is accompanied by the increase in volume of the ventral and dorsal colon. An explanation of this aspect has not been researched.

The calcium and phosphorus requirements and absorption sites have been reviewed by Schryver et al. (1970; 1971a,b; 1972a,b; and 1974). The availability of both minerals varies according to the feedstuff, but Schryver and his coworkers have determined that calcium is absorbed mainly from the upper portion of the small intestine. Some calcium may also be absorbed from the lower part of small intestine but essentially none is absorbed from the large intestine. Phosphorus is absorbed from the dorsal and small colon of the large intestine. Requirements of calcium and phosphorus were determined

to be 2.5 g/100 kg and 2.1 g/100 kg of body weight per day, respectively, by Schryver and his colleagues.

These requirements were based upon the assumption that calcium and phosphorus are absorbed 50 and 45%, respectively. An additional 0.15 to 0.23 g of calcium and 0.1 to 0.17 g of phosphorus is required for each kg of weight gain in young horses.

These requirements for calcium and phosphouus are also based upon the assumptions that the main endogenous loss of calcium is in the feces, and that of phosphorus in the urine. The N.R.C. (1978) recommended levels of 16.6 - 22.8 g of calcium and 11.1 - 14.3 g of phosphorus for a 500 kg mature horse are based upon the assumption that each, respectively, is absorbed 55 - 75% and 35 and 55%. Stillons et al. (1968) have shown that old horses may require more calcium and phosphorus than young equines.

Forages are generally higher in calcium and fiber, and lower in phosphorus, lipid, and crude protein content whereas grains are higher in these components. Feeding only hay at the recommended level of 2% of the total body weight (N.R.C., 1978) has sometimes resulted in deficiencies of energy and protein (McNally, 1979). And it has been suggested to feed cereal grains to balance the requirements. Of those grains most commonly fed, oats and corn have been the choice feed grains for many years. Recently, research with sorghum grain has found it to be of similar value to the horse.

Because of its small kernel size, it has been recommended by Henry since 1911 that sorghum be processed by grinding before it is fed to the horse. Morrison (1948, 1956; as cited by Householder, 1978)

suggested that if it could not be ground that it should be soaked.

Hintz (1977, as cited by Householder, 1978) has also recommended that sorghum grain be processed by grinding, rolling, crimping, or steam flaking.

An evaluation of growth performance of both weanlings and yearlings, which require a high protein diet, have been found to be comparatively the same on diets containing sorghum, oats, and corn. Word in 1968 (as cited by Householder, 1978) found that either 30% corn or 30% sorghum resulted in no significant differences for digestible crude protein or energy in weanlings. Likewise, Aber and Potter (1975) found that the replacement of corn in an oat-corn diet formulated to contain 14.5% crude protein with 0, 22.5, 45.0 and 67.0% rolled sorghum, resulted in no statistically significant differences of apparent digestible dry matter, energy or crude protein. The apparent digestibilities, respectively, for the respective percentages of sorghum were: 74, 77, 80; 80, 81, 82; 78, 79, 77; and 78, 80, and 75.

Householder (1978) evaluated prececal, postileal and total tract digestion and growth performance of horses fed concentrate rations of oats and sorghum. The grains were processed either by crimping or micronizing. While micronizing oats did not improve overall performance of the horses, micronized sorghum did. Digestibilities of dry matter, crude protein and gross energy were significantly (P < .05) improved over crimped sorghum. Householder and the others have shown sorghum grain to be an alternative to feeding oats or corn.

### OBJECTIVE OF STUDY

The objective of the present study was to determine what effect, if any, contamination levels of a broad spectrum antibiotic had on the nutritional status of the mature equine. Sorghum grain was chosen as the grain source since there is little available literature on its feeding value and none as a pelleted form for the equine. The antibiotic used was aureomycin, not only because of its effects on the Gram-negative and positive bacteria, but since it is a common antibiotic used in other livestock feeds.

### MATERIALS AND METHODS

Four gelded ponies ranging in weight from 133 to 213 kg were fed aureomycin at levels of 2, 4, and 8 ppm at .2% of their body weight in pelleted sorghum using a Latin square design (Table 1). The four geldings were wormed and allowed to adjust to their individual pens for a period of 3 weeks prior to experimental trials. During this period the ponies were placed on a 14% crude protein corn-oat feed mix at .2% of their weight and given prairie hay free choice.

At the end of this period, the ponies were weighed and placed on their respective rations according to the 4 x 4 Latin square experimental design used. The diets consisted of pelleted sorghum, alfalfa pellets, and praire hay. The ration composition is given in Table 2. The rations were calculated to meet suggested N.R.C. (1978) maintenance requirements for mature ponies and were fed at the recommended 2% level of body weight.

Each period consisted of 15 days: a 10 day adjustment period followed by a 5 day collection period. Half of the total ration allowance was fed in the morning and the other half fed 12 hours later. The ponies were allowed one hour of free exercise outside of their individual concrete pens prior to the evening meal during the 10 day adjustment period. During the collection period the ponies remained tied within their pens unless walked to water. The ponies were watered three times a day: after each meal and once during the middle of the day. At the end of each collection period, the collection apparatus was removed, the ponies untied, and the ration switched.

Table 1. LATIN SQUARE DESIGN USED IN PONY DIGESTION TRIALS

		Per	iod		
Pony	1	2	3	4	
1	С	Α	В	D	
2	В	D	Α	С	
3	Α	С	D	В	
4	D	В	С	A	

# Treatments:

A -- Control diet C -- 4 ppm aureomycin B -- 2 ppm aureomycin D -- 8 ppm aureomycin

Table 2. COMPOSITION OF RATIONS FED TO PONIES

<u>Ingredients</u>	Dry Matter (%)
Sorghum <sup>a</sup>	10.79
Dehydrated Alfalfa (17% protein)	7.11
Prairie Hay	78.95
Molasses	2.02
Monosodium Phosphate	. 07
Salt	.005

<sup>&</sup>lt;sup>a</sup>Aureomycin levels included in this ingredient

The sorghum grain was prepared for pelleting by grinding through a 1.16 mm (1/16 in.) screen. The molasses, salt, and monosodium phosphate were added to the sorghum and thoroughly mixed. The total batch was then divided into four equal lots and the aureomycin levels (Table 2) added to each lot. The lots were then pelleted under 60-70°C temperature range and about 1500 psi through a 4.8 mm (3/16 in.) die. The first pellets of each batch through the pelleter were discarded to prevent cross contamination with subsequent pelleting operations. The sorghum pellets were allowed to cool at ambient temperature.

Pelleted alfalfa was obtained from a local vendor with a guaranteed 19% crude protein analysis. The paririe hay was obtained locally.

Feed, urine, and feces were collected and weighed at 12 hour intervals during the collection period. Feed samples of the sorghum, alfalfa and hay were collected each period, combined, and then stored until analyzed. Any feed rejected was removed from the feed bunk, weighed, and the total for each day combined and stored for future analysis.

A 5% representative sample of the feces and urine were also collected; at the end of each 24 hour period a composite 10% sample was stored for further analysis. The fecal samples were frozen and the urine samples placed in the refrigerator at 4°C. After collection of the urine, dilute sulfuric acid (50 ml) was placed in the bottom of the collection buckets to acidify the urine and prevent ammonia loss.

Urine samples for each pony by period were thoroughly mixed and proportionately combined into a single sample and analyzed for calcium, phosphorous, and nitrogen (A.O.A.C., 1975). Feed and fecal samples were both dried at 45°C in a draft oven, weighed, and then ground in a Christy Norris mill. The fecal samples were proportionately mixed on a

dry matter basis, and feed and fecals were analyzed for both proximate (A.O.A.C., 1975) and Van Soest (Goering and Van Soest, 1970; Robertson and Van Soest, 1977) components, gross energy, calcium, and phosphorus by A.O.A.C. methods (1975).

Coefficients of digestibility were calculated for dry matter, crude protein, crude fiber, nitrogen free extract, ether extract, energy, cell wall constituents, cell soluble constituents, acid detergent fiber, acid detergent nitrogen, hemicellulose, and total digestible nutrients (Maynard et al., 1979). Nitrogen, calcium, and phosphorus balances were calculated. Significant means were analyzed using the SAS 79 procedure (SAS Institute, Inc.) by Duncan's new multiple range test (Snedecor and Cochran, 1967).

### RESULTS AND DISCUSSION

Analysis of the diets fed are shown in Table 3. Individual feed analysis and metabolic data are found in Tables 3, 4, and 5 in the appendix. Although the ration was calculated to meet the N.R.C. (1978) suggested levels of digestible energy, crude proteins, calcium, and phosphorus, actual analysis of the feedstuffs (Table 3) revealed lower values for percent crude protein, calcium, and phosphorus, and higher crude fiber than those suggested by N.R.C. The crude protein level as calculated met 98% of the requirement due to the addition of salt, monosodium phosphate and molasses, and this should have been adequate to meet the needs of the ponies. However, the prairie hay and sorghum grain both contained less crude protein and the diet was actually meeting only about 77% of the required crude protein level. The sorghum, alfalfa, and prairie hay also contained less calcium and phosphorus and this resulted in slightly less (.05%) phosphorus in the diet than required (Table 4). The calcium requirement, however, was met.

These data confirm the observation by McNally (1979) that hay can be deficient in crude protein and possibly could not meet maintenance requirements when eaten at 2% of the equine's total body weight. And although the crude protein level was still deficient upon the addition of the pelleted sorghum grain and alfalfa, none of the four ponies were in negative nitrogen balance at any time. Neither were they found to ever be in negative balance for calcium and phosphorus.

The addition of 0, 2, 4 and 8 ppm of aureomycin to the diet resulted in greater retention of two of these components (Table 5). Nitrogen balance increased from 149.96 g/day to 152.41 g/day for the 0 to 8 ppm

TABLE 3. N.R.C. VALUES OF NUTRIENT COMPOSITION OF FEEDSTUFFS VS. ANALYSIS OF FEEDSTUFFS

	Sor	ghum	Dehydrat	ed Alfalfa	Prai	rie Hay
	N.R.C	Analysis	N.R.C.	Analysis	N.R.C.	Analysis
Dry Matter %	90.	90.54	92.	92.29	90.	91.09
Crude Protein %	12.6	10.96	19.7	19.57	6.7	4.71
Crude Fiber %	3.0	3.64	27.0	26.32	33.0	36.20
Cell Wall %		11.48	45.0	46.79		73.37
ADF %		5.88	35.	32.01	***	43.86
Calcium %	0.03	0.02	1.50	1.49	0.41	0.32
Phosphorus %	0.33	0.30	0.26	0.24	0.15	0.11

TABLE 4. N.R.C. NUTRIENT REQUIREMENTS FROM CALCULATED AND ACTUAL ANALYSIS

Item	DE(Mcal/gm)	CP(%)	Ca (%)	P(%)
Pony Maintenance Requirements	2.2	8.5	0.30	0.20
Calculated	2.2	8.3	0.45	0.20
over (+) or deficient (-)	<u>+</u> 0	2	+.15	±0
Actual	2.7 <sup>a</sup>	6.5	0.28	0.15
over (+) or deficient (-)	+.5	-2.0		05

<sup>&</sup>lt;sup>a</sup>based upon average dry matter digestibility of 63.15% of control diet

MEAN APPARENT DIGESTIBILITY COEFFICIENTS OF MEASURED NUTRIENTS AND BALANCE VALUES FOR NITROGEN, CALCIUM, AND PHOSPHORUS. TABLE 5.

		Aur	Aureomycin Levels (ppm fed/day)	els (ppm fe	ed/day)		
	0	2	4	8	Mean	S.D.	Ь
Nitrogen g/day	149.96 <sup>a</sup>	146.76 <sup>b</sup>	150.11 <sup>a,b</sup>	152.41ª,b	150.56	±1.37	101.
Calcium g/day	3.28 <sup>a</sup>	2.94ª	4.51 <sup>b</sup>	4.93 <sup>b</sup>	3.91	±.53	.005
Phosphorus g/day	1.43	1.20	1.74	1.95	1.58	7.56	.324
Digestible Dry Matter %	63.15	61.23	22.09	61.83	61.74	±3,36	.773
Crude Protein %	42.85a,b	36.56 <sup>b</sup>	46.15 <sup>a</sup>	46.74 <sup>a</sup>	43.09	±5.23	.109
Crude Fiber %	62.69	67.95	65.44	66.48	66.26	±3.53	.849
Ether Extract %	45.84 <sup>a</sup>	37.46 <sup>a</sup> ,b	29.20 <sup>b</sup>	36.25°,b	37.19	±7.62	.104
Nitrogen Free Extract %	68.64	65.35	65.58	66.48	66.51	±3.65	. 599
Ash %	40.41	36.32	36.36	35.62	37.18	₹6.58	.733
Energy %	59.90	61.29	59.60	60.95	60.43	±5.18	.958
Total Digestible Nutrients	58.74	56.98	56.55	57.68	57.49	₹1.66	
Acid Detergent Fiber %	55.68	48.19	48.06		50.28	±5.70	.280
Acid Detergent Nitrogen	58.65ª	12.23 <sup>b</sup>	40.74a,b	46.13 <sup>a,b</sup>	39.44	±21.02	060.
Hemicellulose %	69.12	79.98	73.39	74.11	73.65	7€.86	.413
Cell Walls %	61.37	60.20	58.46	29.67	59.95	±4.11	.795
Cell Solubles %	65.34	64.10	64.58	57.68	64.85	±2.61	.875
				The second secon			

 $^{\text{d}}\text{Means}$  indicated by the same superscript letter are not significantly different for the rations at  $\alpha$  = .05 by Duncan's Multiple Range test. No superscript letter indicates no significant difference.

level of antibiotic, respectively. The mean nitrogen balance was 150.56, and though it was not significant at the .05 level, it was significant at P < .10. Figure 1 shows the trend toward greater nitrogen retention as the antibiotic level increased and that the ration was more than adequate for even the control diet.

Figure 1 also shows a slight depression of N absorption at the 2 ppm aureomycin level. Likewise, a depression is seen in Figures 2 and 3 for the absorption of calcium and phosphorus. The observed depression is likely to be due to individual pony differences since the absorption of nitrogen was significantly different (P < .05) for each pony. Individual differences were also significant for calcium absorption. Pony 1 was significantly different from pony 2 and both were different from 3 and 4 at the .05 level of significance for calcium absorption. There was no difference between any of the ponies for phosphorus absorption at P < .05, yet there was a depression in phosphorus absorption at the 2 ppm aureomycin level.

A depression is also seen at the 2 ppm level of antibiotic in CP, NFE, EE, Ash, ADF, ADN, cell wall, and cell soluble mean digestibility coefficients (Table 5). This is in agreement with Combe et al. (1967). Whether this antibiotic level affects the microbial population, the gastrointestinal tract, or both to cause this effect is unknown. The mean digestibility coefficients for NFE, Ash, ADF, cell wall, and cell soluble were not significantly different from the means at 0, 4, and 8 ppm. Mean digestibility coefficients for CP, EE, and ADN were significantly different at P < .05.

The significant digestibility coefficient for the ADN fraction is due to low and negative results (Appendix, Table 5) obtained for the

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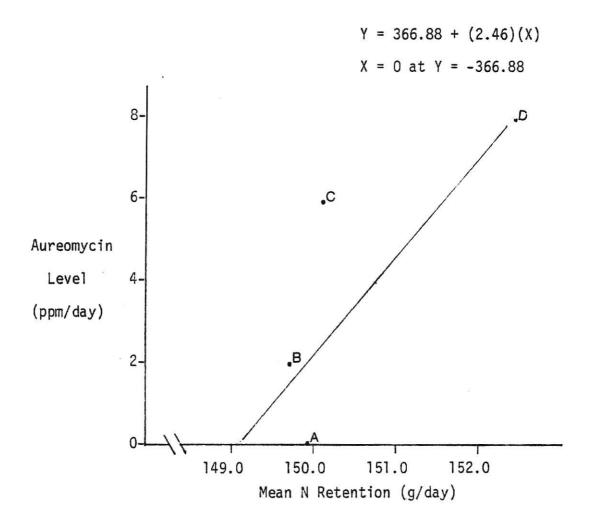


Figure 1. Mean Nitrogen Balance (g/day) vs.

Aureomycin Level (ppm/day)

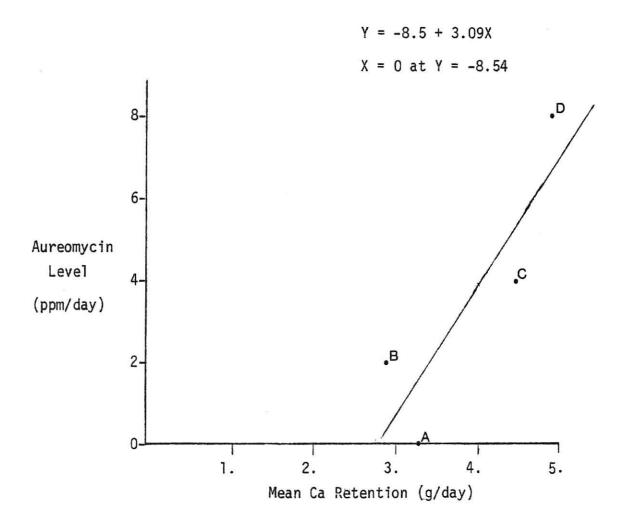


Figure 2. Mean Calcium Balance (g/day) vs.

Aureomycin Level (ppm/day)

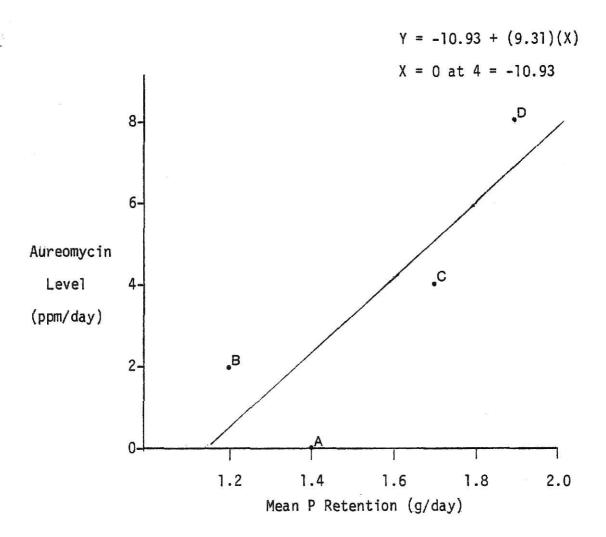


Figure 3. Mean Phosphorus Balance (g/day) vs.

Aureomycin Level (ppm/day)

ponies on the 2 ppm level ration. There may have been unknown variations in the sample collection, analyses, or feed to explain the low results.

Though most digestibility coefficients were depressed, CF and hemicellulose were enhanced at the 2 ppm aureomycin level. Neither was significant at the .05 level nor determined different from the means for CF and hemicellulose at 0, 4, and 8 ppm level of aureomycin. The enhanced digestion of both fractions would be the result of increased cellulolytic activity by the microflora of the gut as the equine has no cellulase to digest the Fibrous fractions of feedstuffs. Whether there is an actual increase in population number or decreased competition among the cellulolytic bacteria remains to be determined.

Besides the increased nitrogen balance, calcium retention and phosphorus retention increased from 3.28 to 4.93, and from 1.43 to 1.58 g/day, respectively (Table 6). Phosphorus balance was not statistically different in this study, but the trend toward increased balance was evident at 4 and 8 ppm of aureomycin. This result may have been due to the fact that dietary phosphorus in the diet was too low (Table 4) to result in any statistically significant increase in absorption. However, the calcium in the diet was more than adequate so higher levels of antibiotic could have a greater effect on the absorption of calcium within the equine gut. Calcium balance was significant at the .005 level, and both the phosphorus and calcium levels were adequate to maintain positive balance. Again this is in agreement with the findings of Combe et al. (1976).

These findings are similar to those discussed previously for other monogastric animals in which antibiotic levels increased calcium

MEAN APPARENT DIGESTIBILITIES OF PROXIMATE AND VAN SOEST COMPONENTS OF THE COMPLETE FEED. TABLE 6.

	B d l l	Aureom	ycin Level	Aureomycin Levels (ppm fed/day)	lay)		
	0	2	4	œ	Mean	S.D.	Ь
Crude Protein g/day	80.68ª,b	69.43 <sup>b</sup>	87.26 <sup>a</sup>	88.28ª	81.41	±9.24	680.
Crude Fiber g/day	605.64	628.28	609.05	599.10	610.58	±22.45	.373
Ether Extract g/day	29.77 <sup>a</sup>	24.29ª,b	17.72 <sup>b</sup>	23,37 <sup>a</sup> ,b	23.79	±5.14	.081
Nitrogen Free Extract	1029.00	986.686	1001.72	1007.96	1007.16	±43.08	.652
Ash g/day g/day	115.57	102.57	100.52	103.11	105.44	±15.73	. 557
Energy Mcal/day	7.83	8.06	7.89	8.03	7.95	±.52	.907
ADF g/day	624.78	542.95	553.80	556.72	569.56	±60.22	.300
ADN g/day	3.35ª	q09°	2.44a,b	b 2.56 <sup>a,b</sup>	2.44	±1.35	901.
Hemicellulose g/day	498.33	570.14	536.83	542.90	537.05	±47.33	.293
Cell Walls g/day	1141.99	1132.12	1109.72	1124.29	1127.13	±54.16	.857
Cell Solubles g/day	706.68	649.84	705.55	711.84	704.73	±23.83	.785
							11

 $^{\text{a}}\text{Means}$  indicated by the same superscript letter are not significantly different for the rations at  $\alpha$  = .05 by Duncan's Multiple Range test. No superscript letter indicates no significant difference.

and phosphorus absorption. They would suggest that the entire gastrointestinal tract is influenced by the antibiotic in the pony since
calcium and phosphorus are essentially absorbed from the small intestine,
and the dorsal and ventral colon, respectively (Shryver et al., 1970,
1971a, 1972b). There are no comparative studies with which to compare
these findings as neither Taylor et al. (1954) nor Clifford et al.
(1956) quantitatively measured calcium or phosphorus.

The increased nitrogen retention is reflected in the increased apparent crude protein digestibility coefficient and g/day digestibility. Both are significant at P < .10 (Tables 5,6). Again, whether this is the result of greater digestion and absorption in the small intestine, reduced ammonia systhesis, or better microbial degradation and utilization within the large intestine is unknown at this time. It may be the result of the combination of these effects.

The digestibilitities of ether extract were significant at P < .10 and decreased inversely with increased aureomycin levels. The decreased digestibility of lipids in antibiotic-fed animals has already been noted and it appears to follow a like trend in this experiment with ponies. However, this may not be an accurate assessment as the ether extraction of lipids from forages is difficult and can lead to erroneous results.

There were no significant differences due to antibiotic levels for the crude fiber, nitrogen free extract, ash, energy, total digestible nutrients, ADF, hemicellulose, cell walls, or cell solubles. Total digestible dry matter was not significantly different for the various rations.

From the above discussion it would appear that the proximate scheme of analysis is better than Van Soest's for partitioning the coefficients of digestibility for the equine. None of the Van Soest fractions were significant except for ADN, which may not have been representative as previously discussed, and at least differences were seen in the crude protein and ether extract components of proximate analysis. The cell wall fraction, representing the hemicellulose, cellulose and lignin fraction, showed no significance (P < .80), nor did the ADF fraction or hemicellulose with levels of significance of .28 and .41, respectively. However, the crude fiber fraction of the proximate scheme was less significant (P < .85 for apparent digestibility coefficient and P < .37 for apparent g/day digestibility) than the ADF fraction of the Van Soest analysis. The apparent digestibility coefficient and daily g/day digested for ADF was significant at P < .28 and P < .30, respectively. Thus, the ADF fraction perhaps reflects a greater digestibility of cellulose rather than hemicellulose (P < .29) or lignin at the higher antibiotic levels.

Householder (1978) reported apparent total tract digestibilities of dry matter, crude protein, and energy of 63, 64, and 63%, and 68, 70, and 68%, respectively, for crimped and micronized sorghum grain rations, respectively, in yearling horses. Results obtained in this experiment reveal digestibilities of 63, 43, and 60 respectively, for the control ration (Table 5). These more closely approximate the results that Householder obtained in mature horses for crimped and micronized sorghum grain in which digestible dry matter was 63 and 65%, digestible crude protein 62 and 65% and digestible energy 61

and 63%, respectively. The difference in crude protein digestibility may be due to the low level in this experiment and the fact that Householder fed his animals 50:50 concentrate to roughage. The percent of digestible NFE was also lower in this experiment compared to Householder's (1978); 69% compared to 76 and 77%, respectively, for crimped and micronized sorghum grain, respectively.

These results indicate that pelleting of sorghum may not improve its digestibility over that of crimping sorghum grain for the horse.

### CONCLUSION

Low contamination levels of 2,4, and 8 ppm aureomycin can improve the calcium, phosphorus and nitrogen balance, and the digestibility of crude protein. The digestibility of ether extract tended to decrease with increased levels but was not found to be significant at these low levels of aureomycin. The pelleting of sorghum grain possibly does not have any more of a beneficial effect than crimping or micronizing the grain for use in horse rations. The analysis of Van Soest components revealed no significant differences in the apparent digestibility of ADF, ADN, hemicellulose, cell walls, or cell solubles.

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APPENDIX

TABLE 1. ABSOLUTE GROWTH RATE

$$= \frac{w_2 - w_1}{t_2 - t_1}$$

$$= \frac{w_2 - w_1}{t_2 - t_1}$$

$$= \frac{w_2 - w_1}{t_2 - t_1}$$

$$= \frac{w_1}{t_2} = \text{Weight at beginning of period}$$

$$= \text{Weight at beginning of period}$$

$$= \text{Time at beginning of period}$$

TABLE 2. RELATIVE GROWTH RATE

$$= \frac{w_2 - w_1}{w_1}$$
  $w_1$  = Weight at birth  $w_2$  = Weight at end of period

TABLE 3. ANALYSIS OF FEEDSTUFFS

	Sorghum	Dehydrated Alfalfa	Prairie Hay
Dry Matter %	90.542	92.292	91.091
Nitrogen mg %	17.529	33.310	7.529
Crude Protein %	10.956	19.569	4.705
Crude Fiber%	3.640	26.321	36.198
Ether Extract %	3.262	3.003	1.910
NFE %	74.525	39.769	47.973
Ash %	7.67	11.339	8.220
Calcium %	0.021	1.486	0.323
Phosphorus %	0.301	0.235	0.105
Cell Wall %	11.478	46.789	73.370
Cell Solubles %	88.522	52.211	26.630
ADF %	5.882	32.012	43.858
Hemicellulose %	5.596	14.770	28.703
ADN %	0.234	0.243	0.183
Kal/gm	4.145	4.361	4.480

					1	1
* Ana	4321	4321	4321	4321	Pony	
*Analysis o	0000	0000		AAAA	Ration	
of Rejected	2.41 2.61 3.20 3.87	2.41 2.61 3.20 3.87	2.41 2.61 3.20 3.87	2.41 2.61 3.20 3.87	100% DM Basis Daily Feed	
ected Feed	4,461. 7,095. 9,955. 14,545.	6,810. 12,241. 11,364. 10,500.	5,398. 7,806. 17,058. 10,085.	6,997. 7,187. 12,241. 11,038.	Intake (kg) Average Urinary Output (m1)	
ا ا	5.991 5.322 5.882 2.617	5.269 4.800 5.465 5.269	4.835 6.397 4.961 2.978	5.697 6.187 5.311 3.461	Nitrogen mg/g	-
L	805.120 612.946 656.724 548,916	737.110 733.297 356.579 763.633	1262.274 1411.141 935.603 1008.042	1162.370 1444.775 746.171 1008.042	Calcium (ppm)	
	40.988 41.297 29.013 41.336	27.291 40.352 35.073 32.585	46.734 55.746 51.662 46.223	42.004 40.215 50.662 34.071	Phosphorus (ppm)	
	3411.34 2898.46 2895.26 4698.52	3089.96 3078.20 3462.46 4695.42	3295.96 3039.18 3411.78 4778.16	3141.08 3412.36 2796.90 4988.62	Average Daily Fecal Output (gr)	
	32.194 33.003 33.292 31.340	37.003 32.063 32.603 28.165	32.032 32.025 32.701 32.702	31.929 31.156 28.861 28.278	%Dry Matter	
7 529	12.204 10.896 12.618 17.984	10.936 11.913 12.789 17.269	14.909 13.684 15.497 20.178	12.994 13.428 13.749 19.196	ng/gn	
4 705	7.627 6.810 7.887 11.240	6.835 7.466 7.993 10.793	9.318 8.553 9.686 12.612	8.121 8.393 8.593 11.997	100% DM	
36 108	27.146 39.248 27.049 25.860	29.021 27.735 27.484 25.509	26.569 25.776 26.472 23.892	30.115 29.167 29.283 26.442	Basis %CF	
1 910	3.575 3.415 3.707 3.766	3.302 3.918 4.176 4.714	3.295 3.661 3.613 3.434	3.195 3.343 3.431 2.831	%EE.	
47.973	46.356 43.848 42.043 45.826	44.166 47.011 46.290 41.285	45.832 46.837 44.960 44.451	42.575 43.657 42.039 45.164	%NFE	
. 323	4539.826 3767.731 3702.969 4293.000	4195.196 4390.679 4936.534 3601.849	4976.887 4691.260 5407.992 5400.419	5952.329 3857.363 5983.682 4625.913	Ca(ppm)	

TABLE 4. URINARY AND FECAL OUTPUT -- THE ANALYSIS continued

ř	100%	DM Basis										
1 1	Pony	Ration	P(ppm)	Kcal/ gm′	%Ash	%ADF	%ADN	%Hemi.	%Cell Wall	%Cell Soluble	Rejected Feed(gm)*	
16		А	2853.263	4.253	15.953	47.230	0.178	19.644	66.874	- 1	9.929	
	2	A	3437.657	4.533	15.440	46.999	0.188	20.269	67.267		49.462	
	ω	Α	5332.947	4.460	16.654	44.660	0.181	20.980	65.640		1175.347	
	4	Α	3448.137	4.645	13.565	43.045	0.297	21.933	64.979	35.021	572.689	
	_	В	2526.683	4.378	14.986		0.436	15.641	66.479	33.521	98.834	
	2	<del></del>	2923.683	4.449	15.182	52.043	0.327	14.504	66 553	33.447	160.593	
	ω	В	3668.084	4.196	15.270		0.467	14.272	66.290	33.710	484.695	
	4	В	3772.929	4.406	15.621		0.559	12.146	62.766	37.234	668.152	is.
		0	2350.874	4.455	16.676	49.390	0.226	21.381	70.771	29.299	0	
	~	C	2254.257	3.726	13.895	55.355	0.396	9.837	65.192	34.803	74.239	
	ω	C	2271.645	4.572	14.056	52.143	0.360	12.903	65.046	34.954	288.759	
	4	C	4022.492	4.643	17.700	43.938	0.207	21.661	65.599	34.401	810.983	
	_	D	2251.293	4.474	15.296		0.348	14.448	67.166	32.834	14.757	
	2	D	2605.645	4.536	16.678	48.466	0.131	20.044	68.510	31.490	7.378	
	· w	-	3269.273	4.521	19.314		0.176	17.826	65.933	34.007	780.194	
	4	0	2611.154	4.573	13.307		0.403	15.288	65.326	34.674	406.539	
	Ana	Analysis of	of Feed - 105	181	8 220	/3 8E8	0 103	28 702	73 370	oe 600		
	3				1				70.070	20.000		e.

\*Only the hay was ever rejected

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TABLE 5. NUTRIENT DIGESTIBILITY

		Pony 1	y 1			Pon	Pony 2	
	0	2	4	œ	0	2	4	8
% Fecal DM	31.929	32.032	37.003	32.194	31.156	32.025	32.063	33.003
% DM dig.	58.424	55.910	52.641	54.455		62.313	62.038	63.395
N g/day	119.896	119.806	121.235	122.145	128.772	129.067	128.987	134.628
CP g/day	67.526	52.525	71.717	67.798	70.885	75.333	84.137	97.321
Ca g/day	1.072	1.993	2.874	2.961	3.185	2.522	3.217	4.916
P g/day	1.126	1.310	1.324	1.537	0.661	1.417	2.045	1.831
EE g/day	20.041	16.958	14.369	12.804	20.719	20.204	17.547	23.754
CF g/day	450.211	465.70	421.544	454.166	502.230	553.498	536.797	535.590
NFE g/day	800.672	735.266	723.638	718.104	861.726	859.344	859.516	910.469
Kcal/gm	6134.370	5808.223	5585,686	5750.118	5650.750	7090.126	6980.201	7221.114
Ash net flux	67.710	53.407	37.201	59.640	81.822	96.381	108.430	148.065
ADF g/day	443.119	399.135	352.961	337.407	498.841	473.235	441.014	529.594
ADN g/day	2.898	.278	2.103	.860	3.060	1.835	1.835	4.318
Hemi. g/day	398.738	433.781	351.858	436.802	427.496	495.442	544.480	453.668
Cell Wall g/day	857.495	848.410	720.473	789.839	934.220	985.311	1002.327	1000.203
Cell Soluble g/day 547.632	y 547.632	538.936	546.194	519.008	602.838	619.385	606.034	651.859
Wt. change 1b/period +8.000	iod +8.000	+8.000	+3.000	+2.000	+16.000	+2.000	±0.000	+2.000

TABLE 5. NUTRIENT DIGESTIBILITY continued

		Po	Pony 3			pony 5	y 5	
	Ō	2	4	œ	0	2	4	8
% Fecal DM	28.861	32.701	32.603	33.292	28.278	29.202	28.165	31.340
% DM dig.	72 754	64.020	64.094	68.312	62.456	62.672	64.352	61.154
N g/day	160.760	159.347	159.338	161.583	190,414	190.827	190.873	191,290
CP g/day	114.727	86.263	102.770	113.081	69.597	63.590	90.435	74.906
Ca g/day	4.074	3.316	4.920	6.109	4.791	3.912	7.022	5.713
P g/day	2.486	1.071	2.657	1.989	1.431	0.993	0.932	2.440
EE g/day	36.852	26.877	20.793	30.327	41.469	33.125	18.155	26.585
CF g/day	676.382	667.441	666.684	608.649	793.797	826.483	812,467	797.996
NFE g/day	1175.226	1079.230	1077.080	1147.244	1278.358	1286.092	1346.647	1256.037
Kcal/gm	9481.766	8974.580	8725.469	9100.586	10068.237	10380.796	10260.542	10028.450
Ash net flux	148.065	123.490	138.398	102.833	164.677	136.632	118.047	162.821
ADF g/day	751.885	592.617	601.682	683.206	814.274	706.824	819.574	676.686
ADN g/day	4.318	.821	2.039	4.227	3.119	-0.526	4.484	1.425
Hemi. g/day	553.019	602.792	627.610	573.238	614.084	748.536	623.356	707.910
Cell Wall g/day	1323.736	1215.359	1249.401	1275.490	1452.522	1479.384	1466.695	1413.616
Cell Soluble g/day 788.850	ay 788.850	764.193	756.134	796.764	887.408	856.831	913.820	879.714
Wt. change 1b/period+10.000	iod+10.000	+10.000	+6.000	+2.000	-10.000	-3.000	-13.000	6.000

# LOW LEVEL AUREOMYCIN CONTAMINATION IN A PELLETED PONY RATION

by

# LONIE BURCH

B.S., Kansas State University, 1979

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the requirements for the degree

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Four gelded ponies were fed aureomycin at levels of 2, 4, and 8 ppm at .2% of their body weight in pelleted sorghum using a Latin square experimental design. Each period consisted of 15 days: a 10 day adjustment period followed by a 5 day collection period. Fecal and urine samples were collected, and digestibility coefficients were calculated for dry matter, crude protein, crude fiber, nitrogen free extract, ether extract, energy, cell wall constituents, cell soluble constituents, acid detergent fiber, acid detergent nitrogen, hemicellulose, and total digestible nutrients. Nitrogen, calcium, and phosphorus balance values were also calculated.

From this study it was found that the prairie hay and sorghum grain used in this study contained less crude protein, calcium and phosphorus, and higher fiber than those levels suggested by N.R.C. (1978). This resulted in lower crude protein and phosphorus levels in the ration than those recommended by N.R.C. Further, the addition of aureomycin at contamination levels resulted in significantly greater balances for nitrogen and calcium as well as an increase in phosphorus balance. Of those proximate and Van Soest components, only crude protein, ether extract, and ADN were found to approach significance at these levels of aureomycin.