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Title: DIETARY FACTORS INFLUENCING ENTERIC DISORDERS OF RABBITS

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An extensive literature review into the causes and controls of diarrhea was followed by three weanling rabbit performance trials to examine the influence of dietary modifications on diarrhea in the rabbit.

In the first set of experiments, the ion exchangers clinoptilolite (0, 1.5 and 3.0%) and sodium bentonite (0 and 5.0%) were added to a low fiber (20% alfalfa) and high fiber (54% alfalfa) diet to examine performance and mortality of weanling rabbits. Gains, intakes, feed efficiencies and mortalities did not differ between clinoptilolite or bentonite levels. For both alfalfa levels, clinoptilolite added at 3.0% reduced mortality by 50% over the controls. Bentonite addition slightly depressed gains and intake with an increase in mortality. Rabbits preferred the non-bentonite diets 2:1 for the low fiber and 5:1 for the high fiber diets in a two choice feed preference test.

In the second set of experiments, the effects of dietary

alfalfa level and supplementation with copper sulfate (Cu) or oxytetracycline (TM-10) on the growth rate, feed conversion, mortality and tissue accumulation of Cu were examined. The inclusion of Cu with or without TM-10 had no effect on gains, feed efficiencies or mortality. Copper sulfate fed at 0, 50, 100, 250, or 500 ppm supplemental Cu had no effect on gains, feed efficiencies or mortality. A level of 1000 ppm supplemental Cu impaired growth performance.

In the third experiment, the switching between a high (54% alfalfa) and low (20% alfalfa) fiber diet 2 weeks post weaning was examined for effects on gains, feed efficiencies and mortality of growing rabbits. Overall performance was not affected by the switching of the diets. Mortalities were lower for animals fed the high fiber diet for the first 2 weeks post weaning, while feed efficiencies were better for rabbits eating the low fiber diet at some time during the growing period.

In conclusion, clinoptilolite fed at 3.0% proved the most beneficial for improving growth performance and reducing mortality. The inclusion of Cu and TM-10 had no effect on growth performance or mortality. Diets containing high fiber (54% alfalfa) improved gains and reduced mortality while diets low in fiber (20% alfalfa) had the best feed conversions.

DIETARY FACTORS INFLUENCING ENTERIC DISORDERS OF RABBITS

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CHAPTER 1

INTRODUCTION

Enteritis, characterized by profuse diarrhea, is a major cause of mortality in weanling rabbits. Losses to the extent of 61.2 % from enteritis complex have been reported for rabbits under eight weeks old (Greenham, 1962). A general figure for commercial losses to diarrhea is about 20% of all rabbits between weaning and marketing. This is a significant loss of income in the rabbit industry where high labor and feed costs result in a low profit margin. Not only is there a loss of net income from the deaths, but feed and labor expeditures to raise the rabbit must also be considered in the overall economic impact.

Although there is a general agreement on the significance of losses due to diarrhea, the causes have not been well defined. Numerous attempts at isolating etiological factors have proven less than fruitful with the influences of dietary factors accepted as playing a major role. Methods of control would therefore require changes in the diet. When considering alterations of diets, not only is the reduction in mortality important, the performance of the rabbits on these altered diets must be weighed. A diet that reduces mortality, while significantly reducing gains and feed efficiencies, would be of little benefit economically.

After an extensive review of the literature regarding causes and controls of diarrhea in rabbits, several studies using various feed additives in the diets of weanling rabbits were conducted to examine their effects on not only mortality, but on growth and feed efficiency.

CHAPTER 2

DIARRHEA IN THE RABBIT:

A REVIEW

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INTRODUCTION

There are several factors limiting the current profitability of the rabbit industry. Among these are high feed costs, intensive labor requirements and high mortality due to diseases such as enteritis that often causes large losses to rabbit growers. Synonyms for enteritis including mucoid enteropathy, enteritis complex, mucoid enteritis, mucoid diarrhea, scours and hypoamylasemia (Flatt, et al., 1974). It is the focus of this paper to review the current understanding of diarrhea in the rabbit.

The earliest cases of mortality in the rabbit associated with diarrhea in the United States were recorded in Los Angeles in 1929 (Hurt, 1949). The reports of mortality attributed to diarrhea vary from a low of 7.0% (Casady, Damon and Suitor, 1961) to 100% (Gordon, 1943) in domestic herds. Most likely, diarrhea has occurred in both wild and domestic rabbits prior to the early report. However, it was not until the implementation of intensive production systems that the incidence was of noticeable proportion. Increased disease incidence generally follows the implementation of intensive production systems, incidence rises with an increase in the numbers of animals at risk, increase in stress and increased concentration of disease organisms.

Until recently, rabbits dying and exhibiting signs of diarrhea were diagnosed as having enteritis. The name has been widely used by both lay persons and professionals. Enteritis is to rabbits as flu is to humans. When someone speaks of having the flu, symptoms of diarrhea, nausea, intestinal discomfort, general uneasiness and weakness are communicated. The symptoms are lumped into a general category, flu, which gives no indication of the cause of the symptoms. It is apparent that enteritis is a complex of diseases, rather than a single pathogenic entity. An improved understanding of the etiology of diarrhea in the rabbit will bring about changes in terminology employed, although enteritis will probably remain as the most commonly used term.

Enteritis is defined as an inflammation of the intestines. Inflammation may or may not be associated with diarrheal diseases of rabbits. Diarrhea may also be associated with constipation or an impaction of the cecum that does not involve the colon.

CATEGORIES OF INTESTINAL DISEASE

At the Second World Rabbit Congress in Barcelona, Spain, Loliger (1980) identified four categories of diseases of the intestine. In the past, research into the etiology of diarrhea has generally been limited to the specific type identified by the investigator. In light of this report, the possibilities of several unique diarrheal diseases existing as well as the possibility of a combination of these within a given herd are being investigated. This could explain the wide variation in symptoms described in the literature.

The four categories described by Loliger are:

1. The tympanites (bloat)
2. Intestinal catarrh (mucoid enteritis)
3. Acute dysentaries (enterotoxemia, infectious enteritis, etc.)
4. Constipation of the large intestine

These categories for the most part pertain to the non-specific enteritis or enteritis of unknown etiology as described by Whitney (1976).

Tympanites - Bloat is described as a specific disease showing little pathology of the intestinal tract. This condition appears to occur primarily from the consumption of lush green feeds, although animals on pelleted diets have exhibited signs of bloating. It is unknown whether this is related to the cause of the diarrhea or is a post mortem effect. In the case of most livestock species, post mortem bloating is observed. Bloat may indicate disruption of the microflora of the rabbit gastrointestinal tract leading to abnormal fermentation. In practical terms, bloat is rarely observed.

Intestinal catarrh - Intestinal catarrh (mucoid enteritis) involves either the small or large intestine. General symptoms include anorexia (loss of appetite), weight loss, rough hair coat, listlessness, squinted eyes, excessive water intake, irregular defecation and mucous in the stool. Similar symptoms are described by others (Templeton, 1953; Greenham, 1962; Pout, 1971; Meshorer, 1976; Sinkovics, 1976; Willis and Jensen, 1979). The prominent characteristic of this category is the large quantities of mucous excreted. However, excretion of mucous is not diagnostic as will be discussed for constipation of the large intestine. Most diarrhea related diseases reported in the literature tend to be in line with the symptoms described for mucoid enteritis.

Acute dysentery - Acute dysentery is characterized by profuse diarrhea accompanied by symptoms described for mucoid enteritis. The onset of symptoms is so rapid that rabbits appearing to be healthy sometimes succumb overnight and are found dead in the morning exhibiting profuse, sometimes foul smelling diarrhea. The rabbits appear to be dehydrated, and have a significant decrease in Na^+ and Cl^- ions in the blood. Rabbits may die as a result of an electrolyte imbalance or of toxins produced by bacteria. A number of pathogenic bacteria have been isolated from individual cases, some of which produce potent toxins. This is apparently true of Clostridium perfringens, a toxin producing bacteria of the genus associated with botulism production, which has been isolated by several researchers from the hindgut of the rabbit (Patton, et al., 1978; Baskerville, et al., 1980; Eaton and Fernie, 1980), and

Escherichia coli (Prescott, 1978). More recent research associates the toxin, thought to be produced by C. perfringens, to C. spiroforme (Borriello and Carman, 1983).

Constipation - constipation of the large intestine is typified by a mucous discharge as well as the other symptoms described for mucoid enteritis. Diagnosis is confirmed by post mortem examination revealing a semi-dry clay-like impaction in the cecum. This impaction may involve the distal end only, the entire cecum or the entire cecum and haustra region of the colon. Diarrhea may accompany the impaction if the haustra region is not involved. Although the cecum is impacted, passage from the small intestine to the colon need not be hindered. Mucous may be produced in response to the impaction (Sinkovics, 1976) to lubricate the colon and pass the obstruction. This mucous may cause malabsorption of water and lead to the watery diarrhea.

Whether the categories described are separate conditions, variations of or different stages of the same disease has yet to be elucidated. The variation of the description of symptoms not only between reports but by the same investigator within a report suggests there is more than one type of diarrhea. The varying response to widely differing therapeutic regimens further suggests several different etiological factors may be involved. Therefore, many investigators refer to all the diarrheal diseases of rabbits as enteritis complex.

PHYSIOLOGY

Diarrhea is the result of disturbed function of the gastrointestinal tract. The disturbance deals with the abnormal absorption and secretion of solutes and fluids that circulate between the blood and intestinal lumen. In general, there are four conditions that can lead to diarrhea (Argenzio, 1978). These are abnormalities in permeability, motility, ion transport and microbial digestion.

Abnormal permeability - Movement of solutes and fluid or the permeability of the mucosa is governed by the integrity of the cell membranes and tight junctions. Disrupting the integrity of the mucosa through intensive damage causes dissipation of the intercellular osmotic and hydrostatic pressure gradient, making net absorption nearly impossible. Such is the case of parasitic invasion.

In contrast, damage to the epithelium may result in a loss of surface area with decreased permeability due to the absence of mucosal entry mechanisms. In both cases, net absorption is reduced or abolished with no net secretion. Electrolyte imbalances may result, leading to heart failure. Excess water being excreted in the feces produces diarrhea.

Abnormal motility - A delay in transit time (time of passage through the gut) is required for microbial digestion and maximal absorption of water and electrolytes. In the rabbit, motility plays an important role in retaining substrate and water for microbial digestion in the cecum. The haustral section of the colon selectively retains small particles and water, transporting them by antiperistalsis to the cecum (Jilge, 1982). Peristalsis is the rhythmic muscular contraction of the intestine which propels digesta along the digestive tract. Peristaltic contractions flow from mouth to anus with antiperistalsis being the reverse flow. Removal or blockage of the antiperistaltic activity of the haustra would result in stopping of the selective retention of water. Excessive water would reach the colon resulting in diarrhea.

Increased motility may result in diarrhea through a decrease in the absorption of water. Rate of passage may be too quick for proper water uptake by the intestinal mucosa. Some laxatives act by increasing the transit time allowing for softer, wetter feces to be formed. In contrast, a slowing of the rate of passage would result in increased contact of microorganisms with the intestinal mucosa and decrease the removal rate of fermentation by-products which exit by way of the anus. By allowing longer mucosal contact, the chances of invasion with resultant damage and inflammation are increased. Delayed passage also allows longer contact with fermentation by-products which may be toxic. Toxins may be absorbed at a rate greater than can be detoxified or the by-products, such as volatile fatty acids, may damage the mucosa resulting in abnormal permeability.

Abnormal ion transport - The colon is capable of net electrolyte secretion. With an increased secretion of ions there is a decrease in the absorption of water. This occurs even though permeability remains unchanged. Water is retained in the lumen of the intestine not due to a lack of permeability, but due to an increase in electrolyte concentration. Water secretion is coupled with electrolyte secretion; an increase in ion secretion results in an increase in water secretion. Ion transport is mediated by cyclic adenosine monophosphate (c-AMP), which when present in greater concentrations increases net secretion. Several factors have either been implicated (enterotoxins) or are known (prostaglandins) to increase ion secretion. Prostaglandins are released from inflamed intestinal mucosa produced by invasive pathogens (Giannella et al., 1977).

Abnormal microbial digestion - A change in the microflora can evoke diarrhea in three ways. First, a change in microflora and fermentation products is conducive to the establishment of pathogens (Grady and Keusch, 1971). The competition for substrate which holds pathogenic bacteria in check is removed, allowing for their proliferation. Second, the end-products of normal fermentation are required for proper absorption. Leng-Peschlow and Marty (1979) demonstrated net secretion in rabbit cecal pouches supplied with synthetic cecal contents in contrast to net absorption when normal contents are present. Finally, over-production of end-products changes the osmotic balance. Hyperosmolity results in net water secretion. However, large osmotic pressure differences are necessary to overwhelm the normal mucosal permeability. An accumulation of acids also occurs affecting the osmotic balance as well as causing diarrhea through intestinal mucosal damage and inflammation.

CAUSES OF DIARRHEA

Numerous factors have been thought to influence the onset and outcome of digestive disorders which cause diarrhea. These factors include stress, weather, diet and feeding practices, bacteria, protozoa, viruses, genetic variation, or any combination of these.

Stress - Stress has received little consideration as a cause of diarrhea. Through intensive production practices, rabbits are exposed to increasing amounts of stress. Artificial environments, high densities and feed changes each can impose varying degrees and types of physiological responses that are considered stressor effects. Stress has been demonstrated experimentally to render animals more susceptible to disease, decrease growth rate or cause actual weight loss and influence reproductive performance. Stressing an already infected animal reduces resistance to certain diseases. In shipping fever pneumonia in yearling feedlot cattle, an interaction of stress, viruses and bacteria is implicated as the cause (Jensen et al., 1976). A majority of cases occur during the first 45 days on feed when the cattle are exposed to the greatest amount of stress (shipping, new environment, new feed, etc.) This stress lowers disease resistance making them susceptible to infective agents. A similar occurrence is seen in newly weaned rabbits. Increased mortality is seen in rabbits within the first two weeks post weaning. This initial onset of diarrhea and mortality has been attributed to post weaning stress. Weaning stress not only includes environment changes, but often changes in diet. Diet changes and stress will be discussed later.

Stress can change physiological homeostasis in the gut, often resulting in diarrhea. This diarrhea may be due to a mild infection as a result of a lowered resistance; however, the role of stress appears as the primary causative factor. Further research to ascertain the full role of stress on influencing diarrhea is warranted.

Weather - Seasonal variation in mortality has been noted by Whitney (1970) and Rollins and Casady (1967a). The peak losses occurred in March and August with minimal losses in October and November. It appears that mortality increases in the winter and late summer. This effect could be a combination of weather stress and feed intake.

In most areas late summer is a time of great daily temperature fluctuation. Temperatures can rise during the day and drop dramatically at night. This temperature fluctuation not only stresses rabbits, but has a tendency to disturb their normal feed intake and fecal excretion. It has been observed that during hot weather rabbits will consume larger quantities of feed during the cooler night than during the day. Leplace (1978) suggests such a disturbance of normal feed intake can be deleterious to the intestinal tract and eventually lead to diarrhea.

During the colder winter months, feed consumption increases because more feed is necessary to maintain body temperature. It appears that rabbits will adjust their feed intake to meet their energy requirements (NRC Rabbits, 1977). Brooks (1978) suggests that an increased feed intake may predispose rabbits to enteric disorders. Overconsumption results in an excessive food mass in the stomach that is poorly penetrated by gastric acids. This reduces the digestive efficiency of the stomach leading to decreased digestion and absorption in the small intestine. Fermentable substrate is then allowed to reach the cecum and colon forming a suitable environment for colonization, multiplication and toxin production by noninvasive enterotoxic bacteria.

Poor penetration of the acids also decreases the destruction of microorganisms. Pathogenic bacteria present in feeds may pass into the small intestine and colon where they will find a suitable environment to proliferate. An increase of pathogenic organisms coupled with a lowered resistance appears to play an important part in producing diarrhea.

Diet - Of all factors, diet plays a major role in influencing diarrhea, whether direct or indirect. Direct causes would be related to fiber level, possible allergic reactions, toxins and related components of feed. Indirect causes would be related to dietary factors which will initiate conditions resulting in diarrhea such as a carbohydrate overload of the hindgut.

Fiber, or lack of fiber, appears to influence diarrhea in most livestock species. Lebas (1975) suggests indigestible fiber at appropriate levels may prevent diarrhea. Work conducted by Cheeke and Patton (1978) confirms that indigestible fiber exhibits a protective effect. Fiber may be required to stimulate the lining of the stomach and intestinal tract without which the mucosa loses vitality and becomes susceptible to bacterial invasion. Bulk, supplied as fiber, maintains tonus to the musculature of the intestines. Muscle tone is important in the movement and dilution of digesta and by-products through the tract.

The size of the fiber particle may be crucial to whether it has a protective effect against diarrhea. With a rise in the reported incidence of cecal impactions comes the speculation that fine grinding of fiber may be at fault. The cecum actively collects fine particles and fluids separated from coarse particles in the proximal colon (Jilge, 1982). Overloading of the cecum with fine indigestible particles may lead to the formation of a clay-like impaction. The accumulation of fine particles may also lead to an altered microflora leading to diarrhea as previously discussed. The change in microflora may also result in an altered fermentation that upsets the normal balance within the cecum. Although reports vary as to the populations of bacteria present within the cecum of both healthy rabbits and those exhibiting diarrhea (Carmicheal, 1945; Gouet and Fonty, 1973; Sinkovics, 1976; Prescott, 1978; Emaldi, et al., 1979; Sinkovics et al., 1979), the importance of "normal" cecal contents in maintaining appropriate absorptive function has been demonstrated (Leng-Peschlow and Marty, 1979).

Abnormal fermentation may lead to a change in transport across the cecal mucosa leading to an impaction. This may in turn lead to an increased output of mucous as described by Sinkovics (1976) and result in a mucin coating of the colon decreasing the absorption of water. Besides water retention by the cecum, a large proportion of water is absorbed by the distal spiral and descending colon. Although the cecum may be impacted, flow from the small intestine to the colon need not be hindered. Coupled with the reduced absorption, a watery, green diarrhea may accompany excretion of mucous. The possibility that impaction is a secondary effect must not be overlooked.

Impaction may be the result of decreased motility with no change in permeability or absorption. Under normal conditions, net absorption of water, Na, Cl and volatile fatty acids occurs. If water is not constantly added to the cecum, enough nutrients will eventually be absorbed to form a semi-dry impaction. Once this impaction forms it would be difficult if not impossible to remove and could lead to diarrhea as previously described. There are many factors and naturally occurring compounds that stimulate or inhibit gastrointestinal motility and could be effective agents in cecal impactions.

Of interest, but little concern, are naturally occurring plant derivatives that can either directly cause diarrhea or be converted to a cathartic by bacterial metabolism. A cathartic is a compound that induces evacuation of the bowel. Glycosides, compounds that yield one or more sugars upon hydrolysis, are one class of such derivatives. Glycosides are found in several plants such as Rhamnus purshianus, which is native to the Northwest. The dried bark, referred to as cascara sagrada, is used in supplying glycosides for laxatives. Aloe vera is another plant commonly harvested with extracts being used for laxative production. There appears to be little likelihood that there are problems with feedstuffs causing diarrhea. However, there exists the possibility that bacteria could produce compounds with a similar mode of action. Further research on this subject is needed.

Hypersensitivity to antigens of the food or colonic flora has been postulated to be a cause of mucoid enteritis. Histologically, there is a considerable resemblance between mucoid enteritis and ulcerative colitis in humans (Meshorer, 1976). In ulcerative colitis, antigens resembling lipopolysaccharides of E. coli have been isolated. The presence of eosinophils, lymphocytes and plasma cells in the intestinal wall of rabbits having died of mucoid enteritis is suggestive of an allergic or similar type immunological process. The evidence is suggestive of hypersensitivity to antigens. Although there is a tendency for an increased number of E. coli in rabbits having died of mucoid enteritis, their role has yet to be confirmed.

Rapid diet changes, overfeeding and high carbohydrate diets (diets high in cereal grains) appear to play a major role in initiating diarrhea. All basically have a similar proposed mode of action, causing a change in the microflora of the gut leading to diarrhea. In all instances, it has been suggested that the gastrointestinal tract is loaded with readily fermentable nutrients, overwhelming the small intestinal digestive and absorptive capacity. This readily fermentable substrate reaches the hindgut where a proliferation of microorganisms occurs.

Cheeke and Patton (1980) hypothesized that the proliferation of Clostridium perfringens Type E was due to a carbohydrate overload of the hindgut on high grain diets. Patton et al., (1978) have demonstrated that a high proportion of enteritis cases are enterotoxemia caused by a toxin that is neutralized by antisera for iota toxin produced by this bacterium. Incomplete digestion of starch in the small intestine would provide a substrate for pathogen growth in the hindgut. In cattle, it has been demonstrated that the pH of the small intestine is suboptimal for amylase activity, with the result that some intact starch passes through the small intestine (Russell et al., 1981). The possibility of a similar situation was explored in rabbits (Harris et al., 1982). These results suggest that the addition of buffers to the diet increases rather than decreases mortality due to enteritis. This is consistent with Brooks's previous description of decreased acidity of the stomach predisposing rabbits to diarrhea by allowing pathogens to pass through the stomach without being destroyed by the normally highly acidic environment.

Recently, Borriello and Carman (1983) have isolated Clostridium spiroforme, which has been associated with enterotoxemia in rabbits. During the isolation, it was found that the iota-like toxin leading to the death of the animal could only be produced if the bacteria were grown in the presence of glucose. This is further support for the carbohydrate overload theory.

McQuinton (1964) suggested that young rabbits forced to eat carbohydrate developed hypoamylasemia and gastro-enteritis. Hypoamylasemia, low blood sugar, associated with gastro-enteritis suggested that the low levels of amylase present in young rabbits would result in starch passing through to the hindgut undigested allowing for bacterial proliferation. Although amylase was found in low concentrations in young rabbits, the alimentary tract of the unweaned rabbit was able to hydrolyze soluble starch (Alus and Edwards, 1976). However, the rate that this starch is absorbed is unknown and there is a possibility that insoluble starch could serve as a substrate for bacterial proliferation.

The gut of the neonate rabbit is sterile due to the presence of several antimicrobial factors in the stomach (Smith, 1966). At this time, the pH of the stomach is relatively high (5.0) limiting the antimicrobial activity of gastric acids. The level of acidity increases with age, dropping the pH near to that of an adult rabbit by 45 days of age (table 1). An especially critical time occurs when the acidity of the stomach is still low and the presence of milk in the stomach has decreased. The dam's milk supplies substrates that are converted to the antimicrobial factors by enzymes of the young rabbit's stomach. The ability of pathogenic bacteria to pass through to the hindgut is great, while a corresponding decrease in efficiency of digestion and absorption provides fermentable substrate to the hindgut.

Bacteria - A variety of bacteria have been associated with diarrhea in the rabbit. These include species of Proteus, Clostridium, Bacillus, Bacteriodes, Salmonella and Escherichia.

In rabbits with diarrhea, there is a tendency for an increase in total numbers of bacteria cultured from various parts of the digestive tract. The increase is predominantly in the small intestine (Brooks, 1978). This increase could be a result of the primary proliferation causing the diarrhea or the result of stasis of the small intestine. In diarrheal outbreaks, variable results are obtained in isolating a causative bacteria. It may be that the bacteria found in greatest numbers may be those best suited to the environment produced when diarrhea has developed.

Proteus: Early work by Larson and Bell (1913) showed that Proteus spp. in association with other bacteria were capable of causing gastro-enteritis in young rabbits. Little work involving Proteus has followed this initial report, but work has instead focused on the associated bacteria; E. coli and C. perfringens (welchii). Proteus spp. appear to be secondary either initiating infection by other pathogenic organisms or proliferating in response to changes in the environment as a result of the diarrhea.

Escherichia coli: E. coli is not usually an inhabitant of the rabbit intestine (Prescott, 1978), but has been isolated in large numbers from the cecum of rabbits with diarrhea and healthy rabbits in herds with a high incidence of diarrhea (Ostler, 1961; Greenham, 1962; Savage et al., 1973; Sinkovics, 1976; Prescott, 1978). Whitney (1970) describes work of D.H. Neil suggesting E. coli are confined to the large bowel of the healthy rabbit. In cases of enteritis, these organisms were disseminated throughout the small intestine with an associated increase in numbers in the cecum.

Several hemolytic strains (Ostler, 1961) and enterotoxin-producing E. coli (Glantz, 1970) have been isolated from all levels of the intestine of rabbits with diarrhea. Some workers have produced diarrhea by intraintestinal inoculation of certain serotypes of E. coli and those isolated from the ceca of rabbits exhibiting acute diarrhea (Prescott, 1978). A variety of biochemically-distinct groups can be found in healthy and scouring rabbits, none of which appear to have any primary clinical significance (Whitney, 1970). Sinkovics (1976) suggests the multiplication and colonization of E. coli may be a consequence rather than a cause of diarrhea, primarily when impaction occurs. Evidence suggests E. coli proliferation is secondary to diarrhea; however, the interaction of bacterial proliferation and initiation of diarrhea has yet to be ascertained.

<u>Clostridia</u>: Blount (1945) described cases of acute enterotoxemia associated with clostridial organisms. Morcos (1932) reported cases of tympanites from which clostridia were isolated. <u>Clostridium perfringens</u> was found in healthy young rabbits by Smith and Crabb (1961). Whitney (1976) describes Neil's isolation of <u>C. perfringens</u> and several other clostridial organisms from healthy rabbits. Neil observed these organisms to multiply and invade the small intestine in cases of enteritis. Vaccination against <u>C. perfringens</u> Types A, B, C, and D failed to influence mortality from enteritis and it was concluded clostridial organisms had no primary role in the etiology of these diseases.

Flatt et al. (1974) postulated that clostridial isolates may parallel <u>E. coli</u> in having secondary effect rather than being the primary cause of enteritis complex. Kunstyr, Matthiesen and Matthiesen (1975) suggest that <u>C. perfringens</u> Type A is the cause of enterotoxemia through the examination of several rabbits exhibiting clinical signs. Patton et al. (1978) demonstrated the presence of <u>C. perfringens</u> Type E iota toxin in cecal contents of rabbits which died of enteritis. Furthermore, toxicity tests showed the cecal contents were lethal to young rabbits. Antiserum for <u>C. perfringens</u> Type E iota toxin was protective against toxicity confirming the presence of iota toxin. Baskerville et al. (1980) and Eaton and Fernie (1980) reported similar results suggesting the presence of the iota toxin. Borriello and Carman (1983) associated C. spiroforme, an anaerobic, gram-positive bacillus with an iota-like toxin.

Bacillus: Bacillus piliformis is the causative organism for Tyzzer's disease, a bacterial infection that produces a severe acute diarrheal disease with high mortality in rabbits (Allen et al., 1965; Prescott, 1977). In rodents, outbreaks of Tyzzer's Disease follow stressful factors such as overcrowding, transportation, dietary decomposition and poor sanitation (Ganaway et al., 1971). Wild rodents serve as a potential source of infection in rabbitries. Rabbits with Tyzzer's Disease exhibit anorexia, depression, dehydration and diarrhea, often succumbing within 24-48 hours after its onset. With symptoms similar to other forms of diarrheal diseases, Tyzzer's Disease is diagnosed by demonstrating the presence of the intracellular organism in affected tissues.

Bacteriodes: The primary organisms isolated from the large bowel of healthy rabbits are bacteriodes (Smith and Crabb, 1961). Similar numbers were located in the cecum of rabbits exhibiting cases of diarrhea. These organisms are the normal flora and have no apparent significance in relation to diarrhea.

Salmonella: This organism is uncommon in the rabbit although several isolations of Salmonella typhimuirium are reviewed by Whitney (1976). Salmonellosis or infection with

Salmonella spp. can occur as a systemic disease involving a variety of organs including the gastrointestinal tract. It is associated with sudden death or, in less acute cases, a acute hemorrhagic enteritis with yellowish diarrhea. Infection by Salmonella spp. can be associated with abortions and infertility and can be spread through contamination of feed or water, or by carrier animals such as wild rodents and birds.

Protozoa - Many Eimeria occur in the intestinal tract of rabbits, but the most important species are E. irresidua, E. magna, E. media, and E. perforans (Olster, 1961). Of these, E. magna and E. irresidua are regarded as being more pathogenic. Swarbrick (1961) observed large numbers of coccidia in clinically healthy rabbits.

Intestinal coccidiosis and enteritis complex exhibit many similar symptoms. Clinical cases of coccidiosis show anorexia, acute depression, debility, a harsh staring cast and anemia (Chapman, 1929; Blount, 1945). Diarrhea is usually evident on the last day of life starting with a watery discharge and becoming mucoid. There is question as to the clinical diagnosis of coccidiosis based on the presence of organisms in the rabbits gut and the absence of post mortem lesions (Harriss, 1961).

The principal differentiation between the two conditions lies in the presence of large numbers of coccidia in the stool; however, large numbers may also be present in clinically diagnosed cases of enteritis complex. Often infections are mild and no clinical signs are noted. Cases may range in severity from causing decreased weight gains to producing diarrheal death related to dehydration and secondary bacterial infection. Whitney (1970) demonstrated a close relationship between coccidiosis and the enteritis complex. Where death diagnosed as coccidiosis increased, there was a rise in death attributed to enteritis complex.

Other protozoa have been isolated from the alimentary tract of the rabbit, but the general consensus of opinion is that they do not cause any illness in rabbits (Whitney, 1976). Effective treatments are available and as a result, the incidence of coccidia related diarrhea in rabbits has declined over the last 20 years.

Viruses - Recent isolation of adenovirus from a rabbit exhibiting enteritis has brought about speculation that viruses may play a more prominent role in enteritis complex (Borden and Prohaska, 1980). Earlier, Brown et al. (1969) concluded that there was no evidence suggesting that viruses played a role in enteritis. However, with the advances in isolation and identification of viruses along with the finding that viruses may be secondary, predisposing factors, their role is being re-evaluated.

Several classes of viruses have been isolated and implicated in the etiology of diarrhea in many livestock and lab animal species. Rotavirus (Bryden et al., 1977) and

Parvovirus (Matsunaga et al., 1977) have also been isolated from rabbit stools although the extent of their role in the etiology of diarrhea is unknown. General clinical features of a viral infection include sudden vomiting and profuse diarrhea. The diarrhea may also contain fresh blood and mucous. Damage to the epithelial cells is extensive giving rise to diarrhea through disruption of mucosal integrity, allowing for colonization of invasive bacteria. In general, viruses are the initiating factor with with secondary infection by bacteria being responsible for death.

Fungi, yeasts and molds - *Saccharomycopsis guttulatus* is considered pathogenic for rabbits although it is thought to be a contributory rather than a primary cause of the enteritis complex (Whitney, 1976). There are currently no reports implicating fungi or molds as primary or contributory to enteritis complex, although enteritis associated with presence of molds in feed is said to occur. Loliger (1980) and Dahle et al. (1980) report heavy mold contamination of feed did not induce enteritis, but had more of an influence on the prevention of diarrhea.

GENETIC ROLE

Some breeds are said to be less susceptible to enteric diseases than others. Whitney et al. (1976) found that mortality incidence in crossbred rabbits was less than that in Californian or New Zealand White purebreds. Lukefahr et al. (1981) in comparing New Zealand White and Flemish Giant purebreds and three-breed terminal-cross rabbits found no difference in deaths attributed to enteritis. A considerable difference among breeds and crossbreds was observed for mortality by Heckmann and Mehner (1970). New Zealand White purebreds had the highest mortality in their experiment. Rollins and Casady (1967a) obtained a heritability estimate of .12 for deaths resulting from enteritis and pneumonia from 15-56 days of age, as derived from a New Zealand White population. Lukefahr et al. (1982) suggested coat color may influence early survival and growth performance through pleiotropy. Pleiotropism is a phenomenon where genes exhibiting a major effect on one trait may cause detectable variation in another unrelated trait. Further work examining the role of genetics in enteritis is needed.

TREATMENT AND PREVENTION

Treatment of enteric disorders requires early detection. In most cases detection is usually after the disease has reached its full course. Most reports indicate sudden and rapid death with evidence of diarrhea as the principal sign of enteritis complex. Anorexia, a non-specific symptom, is considered a fairly reliable indicator of enteric disease.

Once enteritis is detected, the first objective should be the determination of the "trigger factor" inducing the diarrhea. Ostler (1961) as well as others suggest dietary factors are important even where the etiology is unknown. Hurt (1949) recommends complete removal of food at the onset of diarrhea. After 2 days food can then be slowly re-introduced. Castor and mineral oils as well as soapy enemas have also been prescribed with varying success. Treatment with chloramphenicol (Hill and MacDonald, 1956) and dimetridazole (Whitney, 1974) were both used with considerable success. Pout (1971) suggests that mucosal damage by the time of enteritis detection may be so extensive that chemotherapy would be of no value with the only action likely to resolve the condition to be nutritional in origin. Pout (1971) points out that anorexia accompanies this stage and forced feeding is impractical. Oral rehydration (the rapid replacement of sodium, chloride, base, potassium and blood volume) is of limited value especially in acute dysentaries where death is rapid and unexpected. Prevention seems the only likely course in reducing mortality due to enteritis.

Preventative measures include a number of prophylactic antibiotics and drugs as well as changes in feed constituents and management practices. In the United States, Chlortetracycline and oxytetracycline are the two most commonly used antibiotics being added either in the feed or water. Levels of 10 to 20 g per ton have met with inconsistent results with respect to control of disease (Lawrence and McGinnis, 1952; Huang et al., 1954; Templeton, 1953; Kruijt, 1976). Casady et al. (1964) reported significant depression in the incidence of diarrhea and mortality attributed to enteritis with the inclusion of 50 mg oxytetracycline and 100 mg chlortetracycline per ton. There was no apparent effect on enteric rabbits once they exhibited signs of diarrhea. Whitney (1976) reported the use of chloramphenicol, furazolidone and nitrofurazone with variable results. Meshorer (1976) reports the use of a combination of anti-*E. coli* substances: colimycin, tetracycline and furazolidone in rotation to eradicate mucoid enteritis.

Sulfa containing compounds used primarily to control coccidiosis have met with variable success in controlling enteritis complex. Sulfadimidine, sulfaquinidine (toxic), sulfaquinoxaline, sulfadimethoxine and sulfamonomethoxine are reported effective in controlling coccidiosis with a concurrent reduction in enteritis complex (Whitney, 1976).

Two comments about the inclusion of drugs in rabbit rations to control enteritis are appropriate. Sometimes all animals will be placed on a treatment with no adequate controls to compare the effects of the actual treatment on the incidence of enteritis. This fact may mask a change if feed or management as the true source of prevention. Secondly, there are only two of the above drugs approved in the United States for use in rabbits, oxytetracycline and sulfaquinoxaline. These should also be administered under the supervision of a veterinarian.

Acid addition to feed has been used to control enteritis. Templeton (1957) found beneficial results in the control of enteritis complex when ascorbic acid was added to the diet. The addition of tannic acid (Lund, 1952) and lactic acid at 2% (Kruijt, 1976) failed to elicit a similar response.

Morisse (1979) attempted to prevent Ampicillin induced colibaccilosis type enteritis by administering acetic acid, lactic acid and lactulose. Resistance of rabbits was markedly enhanced by the addition of acetic acid and lactulose with a mortality rate of 19% and 8.7% respectively compared to 55% mortality found in the controls. In an attempt to buffer the small intestine to increase the pH to that for optimum amylase activity, an increase in mortality was noted (Harris et al., 1980). This along with the results of Templeton (1957) and Morrisse et al. (1980) suggest an acidic stomach environment has some protective effect following the reasoning of Brooks (1978), as previously discussed.

Limiting feed intake has been suggested by several authors to prevent enteritis (Nikkels, 1976; Brooks, 1978; Sinkovics et al., 1980a,b). In contrast, Templeton (1953) and Kruijt (1976) failed to find any benefit from restrictive feeding. A change of diet, particularly when the fiber level increased provides a reduction in the mortality due to enteritis. Lebas (1975) suggests fiber has a scabrous effect stimulating the intestinal mucosa and may possibly aid in removal of pathogenic bacteria attempting to colonize the epithelium. Fiber is also important in preserving the normal intestinal flora and digestion function hindering a bacterial change (Loliger, 1980), which is reported to initiate diarrhea.

Addition of fiber to the diet is commonly suggested as a treatment for high outbreaks of diarrhea. Sinkovics (1976) suggests feeding grass hay in conjunction with a pellet to increase fiber. Willis and Jensen (1979) found the addition of fiber in the form of supplemental whole oats and oat hay was beneficial. A further change to whole grains was implemented with a decrease in the isolation of *E. coli*, but mucoid enteritis still caused death in the young at the same rate.

Copper sulfate is currently being examined for its protective effect. Patton et al. (1982) reported a decrease in mortality primarily related to enterotoxemia when supplemental copper was fed.

Willis and Jensen (1979) vaccinated young rabbits with a bovine rotavirus vaccine. The vaccine proved most beneficial in rabbits vaccinated before 2 weeks of age, decreasing mortality from 65% to 14%. Preliminary results from China (Tung et al., 1981) indicate that inoculation of rabbits with a vaccine prepared against *Clostridium* *perfringens* was protective when lethal doses of toxin were administered. However, duration of the immunity and shelf-life of vaccine were not tested and a great deal more work is necessary in this area.

CONCLUSIONS

This review is an update of that previously published by Whitney (1976). The complications, contradictions and inadequacies of our knowledge of enteric diseases in the rabbit remain. Work since the original review has expanded knowledge on enteritis. Aside from the conclusion that much work is still needed to unravel the complexities of enteritis, several of the conclusions of Whitney are reaffirmed as new ones are drawn. It is more evident that enteritis complex is multifactorial in origin. Initiating factors relating to diet appear to be of primary concern. The role of bacteria appears to be a secondary infection, but the interactions and interrelations of diet and microorganisms in inducing diarrhea need further work. Viruses, which earlier were discredited, are receiving more attention as an initiating factor.

With an increasing understanding of the multiple factors involved in initiating diarrhea and their interactions will come more appropriately designed experiments to uncover the etiology of diarrhea. A possible limiting factor of earlier studies is the disregard of environmental and managerial influences when examining treatments and possible etiologies of diarrhea. This and a lack of adequate controls may have masked important information concerning the etiology of diarrhea in the rabbit.

Another important limiting factor which could hinder the development of treatments is that of economics. A closer examination of cost vs. benefits may slow advances. The question of whether it is more economical to market more rabbits after feeding them longer or sending less animals in a shorter amount of time must be answered. Some treatments, no matter how effective, may be economically unfeasible.

As the physiology of the rabbit digestive tract is better understood, primarily in the areas of water and electrolyte transport and the structure-function relationships, a better understanding of the etiology of enteritis complex will come about. Until these problems of etiology have been overcome, little solid progress can be made on the control of these diseases (Mack, 1962).

CHAPTER 3

THE EFFECT OF DIETARY CLINOPTILOLITE AND SODIUM BENTONITE ON GROWTH AND MORTALITY OF WEANLING RABBITS

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The Effect of Dietary Clinoptilolite and Sodium Bentonite on Growth and Mortality of Weanling Rabbits

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SUMMARY

Two experiments utilizing a low fiber (20% alfalfa) and high fiber (54% alfalfa) diet with added clinoptilolite (zeolite) or sodium bentonite were conducted to examine performance and mortality of weanling rabbits. Experiment 1 consisted of a 21 day performance trial in which 96 weanling rabbits were randomly allotted to six treatment groups consisting of the two diets with zeolite added at 0, 1.5, or 3%. Experiment 2 consisted of two 28 day performance trials in which 72 (trial 1) and 80 (trial 2) weanling rabbits were randomly allotted to four treatment groups consisting of the two diets with or without added bentonite (5%). In experiment 1, average daily gains (ADG) were greater ($p<.05$) by an average of 6.7 g per day for rabbits consuming the high fiber diet compared to the low fiber diet. Zeolite addition did not have a significant effect on ADG (avg = 29.5 g per day), feed efficiency (avg = 4.46 g feed/g gain) or mortality (avg = 21.9%) within or between the diets. There was a significant interaction for mortality between zeolite addition and fiber that can be attributed to an unexplainable high mortality for rabbits consuming the 54% alfalfa, 1.5% zeolite diet. For both fiber levels, mortality was lower by 50% for the 3% zeolite level over the

control.

In experiment 2, ADG (avg = 37.7 g per day) and average daily feed consumption (avg = 111.3 g/day) were not different ($p>.05$) among treatments, with ADG and average daily feed consumption (ADFC) lowered by an average of 1.88 and 3.8 g per day, respectively, for the diets containing bentonite compared to the control. There were no differences ($p>.05$) in mortality (avg = 23.9%) or feed efficiency (avg = 2.96 g feed/g gain) between bentonite levels although mortality averaged 5.9% higher in groups where bentonite had been added. ADFC was higher ($p<.01$) by 23.9 g/day for the high fiber over the low fiber diet, while the feed efficiency was better ($p<.01$) for the low fiber diet. Decreased palatability may account for the decreased intake and gains with rabbits preferring the non-bentonite diet 2:1 for the low fiber diet and 5:1 for the high fiber diet. Zeolite addition at the 3% level proved most beneficial in decreasing mortality while bentonite addition slightly depressed gains and intake with an increase in mortality.

INTRODUCTION

Feeding high grain diets to weanling rabbits has been shown to induce enteritis, while high fiber, low grain diets have a protective effect (Pote et al., 1980). Patton et al. (1978) demonstrated that a high proportion of enteritis cases are enterotoxemia, speculated to be caused by iota toxin produced by Clostridium perfringens Type E. Kunstyr et al. (1975) suggest C. Perfringens Type A is involved while in other cases, E. Coli has been implicated (Prescott, 1978). Cheeke and Patton (1980) hypothesized that the proliferation of the organism resulting in excessive toxin production is due to carbohydrate overload of the hindgut on high grain diets. Incomplete digestion of starch in the small intestine would provide a substrate the pathogen growth in the hindgut. One pathogen, C. spiroforme has been shown to elaborate toxin only when grown in the presence of glucose (Borriello and Carman, 1983). Thus, modification of the diet to reduce the amount or availability of carbohydrates reaching the hindgut may be beneficial in reducing enteritis in rabbits.

In cattle, it has been demonstrated that the pH of the small intestine is suboptimal for amylase activity, with the result that some intact starch gets through the small intestine (Russell et al., 1981). The use of dietary buffers to raise the intestinal pH increased starch digestion (Wheeler, 1980) This offers a tenable explanation for the carbohydrate overload in the rabbit and further suggests that dietary supplementation with buffers could increase intestinal pH, increase amylase activity, increase intestinal starch digestion and reduce starch reaching the hindgut.

Zeolites (crystalline, aluminosilicates having ion exchange properties) might be employed to reduce the incidence of enteritis. These inexpensive and naturally occurring minerals have been shown to be effective in reducing diarrhea in other weanling animals (Mumpton and Fishman, 1977). England and George (1975) suggest that zeolites may act to change the density of hydrogen ions within the stomach and intestines, thus acting as a buffer to increase carbohydrate digestion, reducing enteritis. Zeolite may also act by stimulating the intestinal mucosa, increasing antibody production (Mumpton and Fishman, 1977) or to lower free ammonia concentrations in the digestive tract limiting bacterial proliferation as has been the suggested role of antibiotics (Visek et al., 1959; Zuidema et al., 1962).

Sodium bentonite (a colloidal hydrated aluminum silicate), which is a common feed additive used to increase pellet firmness may also act to reduce enteritis in the rabbit. Bentonite has also been found to lower the free ammonia in the digestive tract (Martin et al., 1969). Kurnick and Reid (1960) reported a slight delay of digesta passage time along with an increase in intakes of chicks on diets containing bentonite resulting in a highly significant improvement in growth rate. This slight delay may result in a more efficient utilization of carbohydrates, limiting the amount reaching the hindgut.

Although possible benefits can be seen, concern of the practice of adding bentonite to rabbit rations has been raised with an increase observation of cecal impactions in commercial rabbit production. Impactions are characterized by a dry, clay-like material filling either the distal portion or entire cecum. Because the cecum acts as a reservoir of fine particles and fluids, which are actively separated from coarse particles in the proximal colon (Jilge, 1982), it may trap particles of bentonite initiating an impaction.

Two experiments were designed to determine the effects of feeding zeolite and bentonite in either a high or low fiber diet upon mortality of weanling rabbits. A further objective was to determine the effect of both on growth and feed efficiency. It might be that either or both may reduce the incidence of enteritis, but could be of little value to the rabbit industry if weight gains and feed efficiency are reduced.

MATERIALS AND METHODS

Experiment 1. Ninety-six New Zealand White (NZW) and NZW X Blanc de Hotot weanling rabbits (avg initial weight = 808 g) were randomly allotted to six groups. Treatments consisted of two basal rations: 20 and 54% alfalfa (table 2) and three levels of zeolite (clinoptilolite; Castle Creek, Idaho): 0,1.5 and 3 %. Prior to weaning, the rabbits were fed the 54% alfalfa diet. The chemical analysis of the various feeds is shown in table 3. Diets were analyzed for crude protein content using a micro-Kjeldahl method (AOAC, 1975). Fiber analysis was accomplished using the micro-method of Waldern (1971) and acid-insoluble ash was determined by the methods of Van Keulen and Young (1977). The zeolite was ground through a 1 mm screen and incorporated into the basal rations without replacing any of the ingredients leading to a small nutrient dilution at the 1.5 and 3 % levels.

Rabbits were taken at weaning (four weeks of age), randomized and adapted to the experimental diets for 5 days. During randomization, care was taken to distribute littermates among the treatments. Rabbits were housed four per cage in 76 X 76 X 46 cm all wire quonset-style cages with four pen replicates per treatment. Water was offered ad libitum through an automated watering system while feed was offered in preweighed lots to insure a constant supply in front of the rabbits at all times. The experiment lasted 21 days, during which beginning and ending weights, feed consumption and mortality were recorded. Results were analyzed by least-squares analysis of variance according to procedures of Harvey (1975) using pen means as observations. Linear contrast comparisons were used to examine differences between fiber and zeolite levels and fiber X zeolite interactions.

Experiment 2 Two feed performance trials comparing diets with and without added bentonite lasting 28 days were conducted. In trial 1, 72 NZW and NZW X Blanc de Hotot weanling rabbits (avg initial weight = 782 g) were allotted to one of four groups. Treatments consisted of the same basal rations used in experiment 1 (table 2) with or without added bentonite (5 %). Diets were analyzed as in experiment 1 with the results listed in table 4. Bentonite (American Colloid Co., Skokie, Illinois) was incorporated into the basal rations on top of the other ingredients without replacing any one ingredient resulting in a nutrient density dilution in the diets with added bentonite. Rabbits were housed 6 per cage in 76 X 76 X 46 cm all wire quonset-style cages with three pen replicates per treatment.

In the second trial, 80 NZW and NZW X Blanc de Hotot weanling rabbits (avg initial weight = 819 g) were randomly allotted to four groups. Diets and and bentonite additions were the same as in trial 1. Rabbits were housed 5 per cage in 76 X 76 X 46 cm all wire quonset-style cages with four pen replicates per treatment. Rabbits in both trials were taken at weaning (four weeks of age), randomized and adapted to the experimental diets for four days. During randomization, care was taken to distribute littermates among treatments. Water was offered ad libitum through an automated water system while feed was offered in preweighed lots to insure a constant supply in front of the rabbits through the feeding period.

Both feeding trials lasted 28 days, during which beginning and ending weights, feed consumption and mortality were recorded. Postmortem examinations were performed on all animals dying during each trial. Cause of death was grossly determined and attributed to respiratory disease, non-specific enteritis, cecal impaction or other. Initial analysis indicated no difference between trials ($p<.05$), therefore, results of both trials were combined and analyzed by least-squares analysis of variance according to procedures of Harvey (1975). Pen means were used as observations. Linear contrast comparisons were used to examine differences between fiber and bentonite levels and fiber X bentonite interactions.

Preference Trials A two choice preference test was conducted with the feeds used in trials 1 and 2 of experiment 2. NZW and NZW X Blanc de Hotot rabbits (5-7 weeks of age) were offered either the 20% or 54% alfalfa diet with or without added bentonite. Water was offered ad libitum through an automated watering system while the feeds were offered in preweighed amounts to insure an ample supply of each diet was in front of the rabbits during the entire trial. Feed was place in one of two identical feeders (7 inch metal J feeders) with feeder position being switched daily to eliminate feeder bias.

Rabbits were housed 3 per pen in 76 X 76 X 46 cm all wire quonset-style cages with five pens per treatment. Three replicates were conducted for a total of fifteen observations per comparison. Feed consumption was measured for fourteen days. Consumption of each diet is expressed as a percent of total consumption. Means for consumption were compared by Students' t-test according to procedures of Rowe and Brenne (1982).

RESULTS AND DISCUSSION

Experiment 1 Least-squares means, standard errors of the means and selected comparisons for the performance traits for experiment 1 are presented in tables 5 and 6. Initial analysis showed breed (NZW vs NZW X Blanc de Hotot) to be a non-significant effect for all dependent variables and was dropped for the final analysis of variance. All traits are reported on a pen basis. Average daily gain (ADG) was calculated only for animals surviving the 28 day trial by subtracting the initial weight from the final weight and dividing this value by 28 days. Average daily consumption was calculated by dividing the total feed consumed by a pen by the total number of animal days for that pen. Animal days is the sum of the days alive for each animal within a pen. Feed efficiency represents pen efficiencies, calculated as the ratio of average daily feed consumption to ADG representing the grams of feed per gram of gain.

ADG (avg = 29.5 g/day) did not differ between zeolite treatments ($p>.05$). ADG averaged 6.7 g/day more for rabbits consuming the 54% alfalfa diet compared to those fed the 20%

alfalfa diet. Zeolite did not have a consistent effect on ADG, being numerically lowest (22.6 g/day) at the 1.5% level in the 20% alfalfa diet while being numerically similar between the levels when added to the 54% alfalfa diet (avg = 33.9 g/day). These results contrast with those of Kondo and Wagai (1968) where young pigs fed 5% clinoptilolite exhibited from 25 to 29% greater gains than that of animals receiving normal diets.

There were no differences (p>.05) in feed efficiencies (avg = 4.46 g feed/ g gain) between the fiber levels or zeolite treatments. Feed efficiencies were numerically highest, 6.18 and 5.19, for the animals consuming the 54% alfalfa, 1.5% zeolite and 20% alfalfa, 0% zeolite respectively. The numerically low value was 3.2 g feed/g gain for animals consuming the 20% alfalfa, 3.0% zeolite diet. The poor feed efficiencies, noted by the large values, occur in the groups with the numerically highest mortality. Animals succumbing to enteritis may linger for several days, while not consuming feed, affecting the determination of daily consumptions. This coupled with feed wastage could be the cause of the excessively high values noted. As with gains, feed efficiencies were not improved by the inclusion of zeolite in the diet in contrast to reports of Onagi (1966) and Kondo and Wagai (1968) with poultry and swine respectively. Mumpton and Fishman (1977) suggest a level of 5% to be beneficial; it may be that the levels used in this study were below optimum.

Mortalities were a result of enteritis as characterized by profuse diarrhea. Percent mortality (avg = 21.9%) represents the percentage of the 16 animals within each treatment group that died during the course of the data collection. Percent mortality was numerically highest (62.5%) for the animals consuming the 54% alfalfa, 1.5% zeolite diet and lowest (6.5%) for those consuming the 54% alfalfa, 3.0% zeolite. There was a significant zeolite (1.5% vs. 3.0%) by fiber (54% vs. 20% alfalfa) interaction. This can be partly explained by the high mortality in the animals consuming the 54% alfalfa, 1.5% zeolite diet. Except for this group, mortality was numerically lower on diets with added zeolite than without.

The reduction in mortality attributed to enteritis is consistent with results of England and George (1975) with pigs. However, work of Brooks (1978) suggesting that an insufficient acid stomach prevents pathogenic bacteria naturally present in foods from being destroyed contradicts this theory. This suggests another mode of action for this effect. Possibly, zeolites may act by stimulating antibody production of the mucosa of the digestive tract (Mumpton and Fishman, 1977) or simply by maintaining the mucosal integrity. The ammonia selectivity of zeolites suggest that they may act as a nitrogen reservoir, resulting in a slower release of ammonium in the cecum, modulating the proliferation of pathogenic organisms. A final mechanism of action may be hypothesized that zeolites, by nature of their absorptive properties, may trap and remove toxins from the rabbit gastrointestinal tract. Brooks (1978) reports a four-fold increase in blood urea nitrogen (BUN) in rabbits exhibiting diarrhea. Zeolites might play a role in reducing the available ammonium ions, decreasing any toxic effect associated with an the increase in BUN.

Experiment 2 Least-squares means, standard errors of the means and selected comparisons for performance traits are presented in table 7 and 8. As in experiment 1, all traits are reported on a pen basis. ADG, average daily consumption and feed efficiency were calculated as in experiment 1. Percent mortality is the percentage of the 38 animals within each treatment group that died during the course of data collection expressed on a pen basis.

Variation between trials and breeds as well as the interactions between trial X treatments and fiber X bentonite levels were non-significant for all dependent variables, thus, the data were combined for the final analysis. ADG did not differ ($p>.05$) between fiber or bentonite levels (avg = 37.7 g/day) although gains averaged 1.88 g/day lower for the diets containing bentonite. Hollister and Kienholz (1979) and Sellers et al. (1979) reported a similar depression in gains with 4.8% and 5.0% bentonite fed to ducks and broilers, respectively. In contrast, Collings et al. (1980) reported an improved rate of gain when levels of 2-3% were fed to swine.

Feed consumption (avg = 113.3 g per day) between bentonite levels did not differ ($p>.05$). Intakes were numerically lower by an average of 3.8 g/day for both diets containing bentonite. Intake between fiber levels differed ($p<.01$) with rabbits consuming an average of 23.9 g more feed on the 54% alfalfa diet. This difference between diets would be predicted from the lower energy level of the 54% alfalfa diet. The depressed intakes for diets containing bentonite contrast results of others (Collings et al., 1980; Sellers et al., 1979; Hollister and Kienholz, 1979; Kurnick and Reid, 1960). In general, results indicate a decrease in intake with increasing bentonite addition, this decrease never droping below the intake of the basal ration.

Feed efficiency, which is dependent on ADG and feed intake, did not differ ($p>.05$) between bentonite levels; the average feed efficiency was 2.96 g feed/g gain. Rabbits consuming diets without added bentonite had a slight advantage in feed conversion (4.1%) when compared to those with bentonite. This is in agreement with the work of Collings et al. (1980), reporting a non-significant increase in the amount of feed required per gain in swine on diets containing 2 or 3% bentonite. Hollister and Kienholz (1979) reported a similar response in feed efficiency in growing ducks with levels of 2.4, 4.8 and 9.1% added bentonite and

Sellers et al. (1979) in broiler chicks fed 5% bentonite. There was a difference ($p<.001$) in feed efficiencies for the 20% vs 54% alfalfa levels. Rabbits consuming the 54% alfalfa diet required an average 0.7 g more feed per g of gain as compared to the 20% alfalfa diet. This is expected due to the energy density differences between the two diets.

The slight decrease in ADG and intake may be the result of a lowered preference for the diets containing bentonite. Based on energy densities, a slight increase in intakes by rabbits consuming diets diluted with bentonite was expected. In this experiment, the opposite was true.

To determine if a decreased palatability was responsible, a two-choice preference test using feed remaining from trials 1 and 2 was conducted. Rabbits (5-7 weeks of age) were fed either the 20 or 54% alfalfa diet with or without added bentonite. Diets of the same fiber level, differing in bentonite addition, were compared and the percent of the total consumption attributed to each diet calculated. Results of the palatability trials are summarized in figure 1. Mean consumption differed ($p<.01$) between diets with or without added bentonite. The diets without bentonite were preferred 2 to 1 when compared in the 20% alfalfa diet and 5 to 1 in the 54% alfalfa diet. A depressed intake with increasing levels of added bentonite is reported by others (Collings et al., 1980; Sellers et al., 1979; Hollister and Kienholz, 1979; Kurnick and Reid, 1960).

Mortality (avg = 23.9%) did not differ ($p<.05$) between fiber or bentonite levels. Mortality was numerically higher by an average of 5.9% for diets containing bentonite with the highest mortality (29.9%) for the 54% alfalfa diet with added bentonite. The numerically lowest mortality was on the 54% alfalfa diet without added bentonite (18.6%). The low mortality on the 54% alfalfa diet is similar to the results of the first experiment. Although there are no reports of mortality associated with feeding bentonite to livestock, the unique separation and delay of fine particles in the rabbit hindgut led to the concern for the addition of bentonite to rabbit diets. Visual observation of cecal contents of impacted rabbits reveals a semi-dry, clay-like material. This material is higher in dry matter (29.7 vs 22.6%) and, in this experiment, ash content (21.81 vs 12.0%) than cecal contents of the normal animal (unpublished data). Furthermore, there are reports of a slowing of feed passage rate through the digestive tract when clays are added to poultry rations (Quisenberry and Bradley, 1964; Almquist et al., 1967; Kurnick and Reid, 1960; Ousterhout, 1967). Impactions are attributed to a slowing of digesta passage or dysfunction of the separation of coarse from fine particulate matter in the formation of cecotrophes by the hindgut (Grobner, 1982).

To examine for impactions, a gross post mortem examination was performed on each animal dying during the course of the experiment. Partitioning of mortalities (figure 2) by dietary treatment and gross diagnosis indicates a shift in the incidence of enterotoxemia and cecal impactions on the 20% alfalfa diet. Although overall mortality is constant, there is a decrease in the incidence of non-specific enteritis (generally attributed to enterotoxemia) with a corresponding increase in cecal impactions. This shift was not evident on the 54% alfalfa diet, although there were cecal impactions in rabbits consuming the bentonite diet, while none were noted on the 54% alfalfa diet without bentonite

The partitioning of deaths suggests that since bentonite is not absorbed by the animal, it may have one of two actions. First, a reduction in acute toxicity may occur through absorption and excretion of toxins or toxic factors such as ammonia (Zuidema et al., 1962) or toxin production may be limited through its manipulation or inhibition of bacterial fermentation (Galyean and Chabot, 1981; Martin et al., 1969; Zuidema et al., 1962). With the exact causes of enteritis as yet conclusively undetermined, speculation into the etiology abounds. Although enterotoxemia and cecal impactions (constipation) have received separate classification and are thought to be of different etiological origin (Loliger, 1980), the above observations may support the hypothesis that a cecal impaction may be the result of a chronic, rather than acute case of enterotoxemia. Potent toxins have been isolated from rabbits diagnosed as enterotoxemic (Patton et al., 1978). Stasis of the gastrointestinal tract has been observed in animals with symptoms of enterotoxemia, which were sacrificed and immediately submitted for post-mortem exam. This same toxin has not been isolated in rabbits with cecal impactions, which means the toxin is either not present or present in a concentration below the level of detection (Patton et al., 1978). If this were the case, the toxin may act by slowing the peristaltic movement of the gastro-intestinal tract or inhibiting the selective retention of water resulting in an impaction.

CHAPTER 4

EFFECT OF DIETARY COPPER AND OXYTETRACYCLINE ON GROWTH AND MORTALITY OF WEANLING RABBITS

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Effect of Dietary Copper and Oxytetracycline on Growth and Mortality of Weanling Rabbits

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SUMMARY

The effects of dietary alfalfa level and supplementation with copper sulfate (Cu) or oxytetracycline (TM-10) on the growth rate, feed conversion, mortality and tissue accumulation of Cu in weanling rabbits were studied in two experiments. In both experiments, weanling rabbits (28 $\pm$ 2 d of age) were assigned to each of 12 dietary treatments and fed the diets ad libitum for 28 d. In experiment 1, dietary treatments included two alfalfa levels (54% and 20%), two Cu sources (hydrated and anhydrous copper sulfate at 250 ppm) and TM-10 (10 g per 906 kg), with 10 animals per treatment. Average daily gain (ADG), feed efficiency and mortality did not differ among the Cu sources and TM-10 ($p>.05$). The ADG and feed intakes were greater ($p<.01$) for the animals receiving the 54% alfalfa diet than for those on the 20% alfalfa level. Feed conversion was less efficient ($p<.01$) for the animals fed the 54% alfalfa diets while mortality was lower ($p<.05$) for rabbits consuming the 54% alfalfa diet than for the 20% level.

In experiment 2, the same alfalfa levels were used with Cu (as anhydrous copper sulfate) added at 0, 50, 100, 250, 500, and 1000 ppm, with 20 animals per treatment. Liver, kidney, muscle, fecal and cecal contents were analyzed for

Cu content. The addition of 1000 ppm Cu resulted in decreased ($p<.01$) ADG, feed intake and feed efficiencies over other Cu levels. There were no differences ($p>.05$) in these parameters among the other Cu levels. Liver Cu concentrations were increased ($p<.01$) while kidney and muscle concentrations did not change with increasing levels of Cu. There was a trend to thinning of the ceca of rabbits consuming increasing levels of Cu. The results indicate better rabbit performance on a high alfalfa diet than with one low in alfalfa and no response to Cu and/or TM-10. A level of 1000 ppm Cu is impairs growth performance in rabbits.

INTRODUCTION

Enteritis is a major cause of rabbit mortality, and appears to have a strong relationship with dietary factors. In swine, the inclusion of copper (Cu) or subtherapeutic levels of antibiotics in the diet have been shown to reduce mortality and morbidity from enteritis, while improving growth rate and efficiency of feed utilization (Braude, 1975; Hays and Muir, 1979; Stahly et al., 1980). Feeding antibiotics to rabbits has shown little beneficial effect in improving growth and feed efficiency (Lawrence and McGinnis, 1952; Huang et al., 1954; King, 1962; Casady et al., 1964), but appears to have some effect in reducing rabbit mortality, thereby increasing total production per doe

(Casady et al., 1964). Increased growth rate has been reported in rabbits fed 200 ppm Cu as Copper sulfate ($CuSO_4$), although there were no significant increases in feed efficiency (King, 1975; Omole, 1977). Patton et al. (1982) report an improvement in gains in rabbits fed 400 ppm Cu as anhydrous $CuSO_4$ and a significant reduction in mortality associated with enterotoxemia. In swine, the response from feeding Cu is similar to that of antibiotics. The improved rate and efficiency of gain and reduced mortality and morbidity of pigs fed antibiotics are attributed to a reduction in numbers of pathological microorganisms (Stahly et al., 1980). There is evidence that the response from supplemental Cu and antibiotics in weanling pigs is additive (Beames and Lloyd, 1965; Barber et al., 1978; Mahan, 1980; Stahly et al., 1980). In the rabbit, the mode of action of copper and the possible interactions between Cu and antibiotics have not been established.

The purposes of these experiments were to (1) examine the possible interaction between supplemental Cu and the broad spectrum antibiotic, oxytetracycline, (2) determine the effect of the source of Cu ($CuSO_4$ either anhydrous or hydrated), (3) determine the optimal level of supplemental Cu and (4) determine if fiber level has an interactive effect with these treatments on the growth, efficiency of gain and survival of 28-day-old weanling rabbits.

MATERIALS AND METHODS

Exp. 1. One hundred twenty weanling New Zealand White (NZW) and NZW X Blanc de Hotot crossbred rabbits rabbits were weaned at 28 $\pm$ 2 days of age (weighing an average 680 g) and allotted to twelve dietary treatments, with littermates distributed among the treatments. Rabbits were housed five per pen in 76 X 76 X 46 cm all wire quonset-style cages with two pen replicates per treatment. Feed and water were offered ad libitum.

Treatments consisted of two basal diets: a low fiber (20% alfalfa) and a high fiber (54% alfalfa) diet (table 9). A 900 kg batch of each basal ration was prepared and split into 150 kg lots to which the appropriate level of Cu and antibiotic was added before pelleting as outlined in table 10. All rabbits were fed the 54% alfalfa ration with no added Cu or antibiotics before weaning. At weaning, rabbits were offered the appropriate basal ration during a four day adaptation period, after which they were given the test diets for 28 d. Body weights and feed consumption were measured at 7 day intervals. Dead animals were necropsied to determine cause of death. The date of death was recorded to facilitate the calculation of animal days for the pen. Total feed consumption for a pen was divided by the total animal days to estimate average daily feed consumption. The weight gain and estimated feed consumption of rabbits that died during the trial were not included in the determination of pen gains and feed efficiencies.

Diet mixtures analyzed for crude protein content by the Kjeldahl method (AOAC, 1975), acid detergent fiber by the micro methods of Waldern (1971). For Cu content, samples were dry ashed at 600 ^{o}C, diluted in .1 N HCl and analyzed against corresponding standards on a flame atomic absorption spectrophotometer (Perkin-Elmer model 303).

The data, using pen as the experimental unit, were subjected to factorial analysis by analysis of variance techniques according to the procedures of Snedecor and Cochran (1967) with diet, antibiotic and Cu source as main effects. In the initial analysis, breed of rabbit was included as an effect, but being non-significant (P>.05), it was dropped from the final analysis. A second analysis, consisting of orthogonal treatment comparisons, was conducted to compute the average differences between and compare the combinations of copper, antibiotic and fiber treatments and their interactions.

Exp. 2. In each of two trials one hundred twenty NZW and NZW X Blanc de Hotot crossbred rabbits were weaned at 28 $\pm$ 2 d of age (weighing an average 810 g) and allotted to twelve dietary treatments, with littermates distributed among the treatments. Rabbits were housed as in experiment 1 with two pen replicates per treatment per trial for a total of four pen replicates per treatment. Feed and water was offered ad libitum.

Treatments consisted of the same 2 basal diets as in experiment 1 (table 9) with Cu added as anhydrous $CuSO_4$ to supply the following supplemented levels: 0, 50, 100, 250, 500, 1000 ppm Cu. A 900 kg batch of each basal ration was prepared as before and split into 150 kg lots to which the appropriate levels of Cu were added before pelleting. At weaning, the rabbits were offered the appropriate basal ration during a four day adaptation period, after which they were given the test diets for 28 d. Performance and mortalities were obtained as in experiment 1.

During trial 1, feces from each pen were collected over a 24 hr period by suspending hardware cloth (60 mesh) beneath each cage. At 28 days, four animals from each treatment group were killed by cervical dislocation. Livers, kidneys and right fore limbs were removed, avoiding mineral contamination. Ceca were removed from 4 rabbits on each level of Cu on the 54% alfalfa diet by ligating the ileum approximately 2 cm from the Sacculus rotundus and the proximal colon, 2 cm from the Ampulla cecalis. Cecal contents were removed, weighed, lyopholized and reweighed. The ceca were flushed with saline to remove digesta, blotted dry and weighed. A two cm square of cecum was then cut from the ampulla cecalis, avoiding the folds, and weighed.

Weighed samples of feed, feces and cecal contents were oven dried at 100° C, reweighed and dry ashed at 600° C for 8 hrs. Weighed samples of liver, kidney and muscle from the fore limb were oven dried at 100° C, reweighed and wet ashed in 4:1 conc. HNO_3:$HClO_4$ according to the procedures of Swick et. al. (1981). Each sample was diluted with .1 N HCl and analyzed against corresponding standards by flame atomic absorption spectrophotometry for Cu content. Standards were found to be within acceptable limits when checked against National Bureau of Standards bovine liver and oyster tissue. Mineral content was determined as a dry matter concentration in parts per million (ppm). Diets were analyzed for crude protein, dry matter and acid detergent fiber content as in experiment 1.

The performance data, using pen as the experimental unit, and tissue and fecal Cu levels were subjected to factorial analysis by analysis of variance techniques according to the procedures of Snedecor and Cochran (1967) with alfalfa and Cu levels and their interaction as main effects. As in experiment 1, breed of rabbit was included in the initial analysis, but being non-significant, it was dropped from the final analysis. Orthogonal treatment comparisons were used to compute average differences between alfalfa levels and compare differences between combinations of Cu levels. Cecal data were analyzed by analysis of variance with Cu as the main effect. Orthogonal treatment comparisons were used to determine differences between combinations of Cu levels.

RESULTS AND DISCUSSION

Exp. 1. Performance traits for experiment 1 are summarized in table 11. Gains, intakes, feed efficiencies and mortalities did not differ (P>.05) between Cu and TM-10 fed rabbits. Two and three-way interactions between alfalfa, Cu and TM-10 inclusion were also non-significant for all traits. The ADG averaged 35.0 g per day being numerically highest for rabbits consuming the 54% alfalfa diet with added TM-10 and lowest for rabbits consuming the 20 % alfalfa diet with added TM-10. Numerically, the gains were similar between Cu treatments and the inclusion or absence of TM-10 when compared across both alfalfa levels (table 13). The lack of growth response to TM-10 confirms earlier studies of Lawerence and McGinnis (1952), Huang et. al. (1954) and Casady et. al. (1964) while contrasting with the improved growth reported by King (1962,1967), all using oxytetracycline at similar levels in rabbits between the ages of three and nine weeks. This also contrasts with the 10.8% improvement in daily gains of swine in the starter and grower-finisher phase as reviewed by Cromwell (1983). No improvement in feed efficiency (feed efficiencies were not mentioned in earlier rabbit experiments) is also not in agreement with the 6.3 and 3.9% improvement noted in starter and grower-finisher swine respectively (Cromwell, 1983).

The level of Cu used was that found to improve performance in swine as reviewed by Braude (1975). This level is different than the 200 ppm (Omole and Adegbola, 1976; Omole, 1977; King, 1975) and 400 ppm (Patton et al., 1982) levels reported to improve gains and feed conversions in rabbits. Of interest is the difference ($p<.05$) in feed conversion between rabbits consuming Cu in the two sources. Rabbits fed hydrated $CuSO_4$ ate a greater quantity of feed while gaining slightly less than those fed anhydrous while the feed intake and gains for the anhydrous fed rabbits were similar to those not receiving added Cu.

Average daily intakes were similar between Cu and TM-10 fed animals and the controls averaging 113.7 g of feed consumed per day. Rabbits fed the 54% alfalfa diet with hydrated CuSO4 and TM-10 added had the numerically highest consumption (134 g per day) while the lowest consumption was for those consuming the 20% alfalfa diet with anhydrous $CuSO_4$ and TM-10 (84.8 g per day). Consumption was numerically similar for rabbits when comparing Cu source or the inclusion or absence of TM-10 (table 13).

Cu and TM-10 inclusion produced no significant effect on feed efficiencies (avg = 3.23 g feed/g gain). Rabbits consuming the 20% alfalfa diet with added Cu (anhydrous) and TM-10 required the numerically lowest amount of feed per gain (2.73 g feed/g gain) while those consuming the 54% alfalfa diet with added Cu (hydrated) and TM-10 required the most (3.50 g feed/g gain). In comparing the feed efficiencies between Cu sources, rabbits consuming Cu as anhydrous CuSO4 required .40 g less (P<.05) feed per g of gain than those consuming Cu from the hydrated source (table 12). The lack of improvement in feed conversion, although not measured in earlier rabbit experiments, is also not in agreement with the 6.3 and 3.9% improvement noted in starter and grower-finisher swine respectively (Cromwell, 1983). Lawrence and McGinnis (1952) report no effect on mortality when oxytetracycline was added at a similar level while Casady et al., (1964) found a significant reduction with levels greater than those used here. Stahly et al. (1980) reported an additive effect for both improved growth and feed conversion in swine when oxytetracycline was fed in combination with Cu. No additive effect was noted here due to a lack of response to the addition of either singularly or in combination.

Mortality (attributed to enteric disorders) averaged 12.5 percent over all treatments. Mortality was not different (p>.05) between the Cu, TM-10 and control groups. The numerically highest mortalities were recorded in rabbits consuming the 20% alfalfa diets with all but two of the 54% alfalfa groups having no mortality (table 11).

Exp. 2. Performance results for experiment 2 are summarized in table 14. Inclusion of Cu at the various levels had an effect (p<.01) on gains, intake and feed efficiencies and no effect (p>.05) on mortality. Two way interactions between alfalfa and Cu levels were non-significant for all traits. The ADG averaged 32.0 g being numerically highest for rabbits consuming 100 ppm. The ADG for rabbits consuming either basal ration with 1000 ppm added Cu was 12.4 g less ($p<.01$) than rabbits consuming any of the other levels of Cu (table 15).

Average daily intakes (avg = 100.8 g feed per day) did not differ ($p>.05$) between rabbits receiving supplemental Cu and those on the basal ration without added Cu (table 15). Rabbits fed 1000 ppm supplemental Cu had a lower ($p<.01$) consumption by an average of 17.8 g feed per day compared to rabbits receiving the other levels of Cu.

The addition of Cu did not improve ($p>.05$) feed efficiencies (avg = 3.43 g feed/g gain) compared to rabbits not receiving supplemental Cu. Feed efficiencies were poorest ($p<.01$) for 1000 ppm Cu addition requiring rabbits an average of 2.1 g more feed per g of gain than for the other levels of Cu. In comparing feed efficiencies for rabbits fed 1000 ppm Cu with those of rabbits fed the other levels, a significant interaction with alfalfa was noted (table 15). Rabbits fed the 20% alfalfa diet with 1000 ppm Cu required twice as much feed per unit gain as those fed the 54% alfalfa diet (table 14).

The lack of response to Cu supplementation at 250 ppm while reports at other levels indicate improved performance suggested that rabbits respond to differing levels of Cu in their diet. Results from experiment 2 would confirm this hypothesis. The best performance was seen for rabbits consuming 100 ppm supplemental Cu having the numerically highest gains and a feed conversion. Values for gains and feed efficiencies indicate levels above and below 100 ppm Cu are not as beneficial. This is in line with the results of King (1975), Omole and Adegbola (1976) and Omole (1977,1979) in which levels of 100 or 200 ppm were found to be effective while contradicting the findings of Patton et al. (1982) suggesting higher levels are effective. One possible explanation for the difference in levels between the present study and that of Patton et al. (1982) could be the difference in the rabbit's response associated with different ambient temperatures. The greatest response in the previous study was found during a period of a high average ambient temperature while the present study was conducted in a period of low average ambient temperature.

Percent mortality (avg = 18.75 percent) did not differ ($p>.05$) between Cu supplementation and no added Cu. In comparing low to high Cu supplementation, mortality averaged 10 percent less ($p<.05$) for rabbits receiving 50 ppm compared to 100 ppm and for 500 ppm compared to 250 ppm. In comparing the mortality between rabbits consuming 250 and 500 ppm added Cu, a Cu by alfalfa interaction is indicated ($p<.05$).

Mortality varied over all the Cu levels fed suggesting no effect of Cu on reducing mortality due to enteritis.

This contradicts the results of Patton et. al. (1982) in which deaths due to enterotoxemia were markedly reduced by the inclusion of 400 ppm Cu as anhydrous $CuSO_4$. In the present study, fifteen rabbits (representing rabbits from each of the treatments) having died and exhibiting diarrhea, were tested for the presence of a toxin in the cecal contents according to the procedures of Patton et al. (1982). Of the fifteen rabbits, fourteen were confirmed to have toxic cecal contents (eleven of which were on Cu supplementation), although only one was confirmed iota toxin. This, along with no differences in mortality suggests Cu may not protect against enterotoxemia as previously thought.

Quarterman (1967) indicated that rabbits will tolerate excessive Cu levels (500 ppm) in the diet as measured by normal health and growth. The present study agrees that levels up to 500 ppm are tolerated. At 1000 ppm added Cu, impaired performance demonstrated by a decreased feed intake, reduced gains and poor feed efficiencies was noted. Possibly the impaired performance was the result of a decrease in palatability of the diets high in Cu limiting the intake of essential nutrients. This could explain the alfalfa by Cu interaction for feed efficiency comparing the 1000 ppm level to the other levels fed. The intake was the poorest on the 20% alfalfa diet as were the ADG and feed efficiencies. This is supported by a lack of obvious lesions that could be associated with the high level of Cu in histological examinations of rabbits sacrificed for tissue collection on the diets containing 1000 ppm Cu.

Tissue and fecal Cu levels are summarized in tables 17 and 18. Copper addition had a significant effect on liver Cu content while not affecting muscle or kidney levels ($p>.05$). There was a 90 fold increase ($p<.01$) in liver Cu levels in the 1000 ppm supplemented rabbits over the controls while kidney levels doubled, although they were not significantly different from the controls (table 18). Although non-significant, the liver Cu levels were numerically lower and the kidney Cu levels were numerically higher for the 20% alfalfa fed rabbits when compared to those fed the 54% alfalfa based diets. Fecal Cu levels increased 40 fold when Cu was added at 1000 ppm (table 17). Rabbits consuming the 20% alfalfa diet on the average excreted twice the dry matter content of Cu as those on the 54% alfalfa diet.

The 50-fold increase of liver Cu content at 500 ppm supplementation is in line with the 40 and 20-fold increases noted by Quarterman (1967) and Patton et al. (1982) with supplemented Cu levels of 500 and 400 ppm respectively. The difference between liver Cu levels for the two alfalfa level treatments is probably because rabbits consumed less feed on the 20% alfalfa diets and thus had a lower Cu intake. This is especially evident on the 1000 ppm diets. There was a non-significant increase of kidney Cu level on the higher levels of Cu supplementation. This could be the result of an increase in urinary Cu excretion due to elevated plasma Cu levels. Such is the case in humans with cirrhosis accompanied by biliary obstruction and other abnormalities resulting in elevated plasma Cu concentrations (Underwood, 1977).

The addition of Cu increased ($p<.01$) the cecal Cu levels by 60-fold for rabbits fed 1000 ppm Cu compared to the controls (table 19). The cecum tended to decrease in size with an increase in the level of Cu added as measured by total cecal weight, weight of 2 cm squares of ceca and the cecal weight as expressed as a percent of the total body weight (table 19). This trend is similar to reports of King (1975), Omole and Adegbola (1976) and Omole (1977,1979), in which they suggest a decreased cecal weight is related to a thinning of the wall or the digestive tract.

In both experiments, rabbits fed the 54% alfalfa diet had higher ADG, ate more feed, required more feed per unit gain and had a lower mortality than those on the 20% alfafla diet. ADG were 5.8 and 5.3 g greater ($p<.05$) per day for rabbits fed the 54% alfalfa diet for experiments 1 and 2 respectively. Consumption was greater ($p<.01$) by an average of 32.7 and 28.9 g per day for rabbits consuming the 54% alfalfa diet in experiments 1 and 2 respectively. Feed efficiencies were poorer ($p<.01$) requiring .42 g more feed per g gain for rabbits consuming the 54% alfalfa diet in experiment 1 and did not differ ($p>.05$) in experiment 2. In both experiments, mortality averaged 10.4 percent less ($p<.05$) for rabbits fed the 54% alfalfa diet. Gains were greatest for the rabbits fed the 54% alfalfa diets as has been previously demonstrated (Grobner et al., 1982,1983; Cheeke and Patton, 1978). The 20% alfalfa diet also contains a lower percent crude protein which could also be responsible for the decreased gains. Feed intake was also greater on the 54% alfalfa diet owing to its increased fiber content and decreased energy density. Mortality was lower for rabbits consuming the 54% alfalfa diets as has been previously reported (Grobner et al., 1982, 1983). This could be the result of an increased fiber to starch ratio that has been reported to reduce the incidence of enteritis in rabbits (Cheeke and Patton, 1978).

CHAPTER 5

DIET SWITCHING AS A PREDISPOSING FACTOR FOR DIARRHEA IN WEANLING RABBITS

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Diet Switching as a Predisposing Factor for Diarrhea in Weanling Rabbits

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SUMMARY

Seventy-two New Zealand White weanling rabbits (28 ± 2 d of age) were evaluated for performance and mortality on either a high fiber or low fiber diet when maintained for 28 days on their respective diet or switched to the other diet at 14 days. Average daily gains did not differ (P>.05) between regimens although rabbits starting on the 20% alfalfa diet and those switched to the 20% alfalfa diet averaged 1.4 and 2.0 g per day greater gain than those consuming the 54% alfalfa diets. Average daily consumption was lower (P<.05) for rabbits starting on the 20% alfalfa by 15.1 g of feed per day. Feed per gain was improved (P<.01) by .35 g feed per g gain for rabbits started on the 20% diet and by .52 g feed per g gain for those ended on the 20% diet. Mortality did not differ (P>.05) between the dietary regimens although it was numerically highest for rabbits starting on the 20% alfalfa diet. These results suggest a maximum performance feeding system requiring a highly palatable, high fiber post-weaning diet with a switch to a high carbohydrate fattening ration. However, the labor investments and economics of feeding two rations must be carefully considered before implementing such a system.

INTRODUCTION

Of all the predisposing factors postulated for enteric disorders in weanling rabbits, diet appears to take a leading role. A variety of dietary factors has been implicated. Lebas (1975) suggested indigestible fiber at the appropriate level is required to prevent enteritis. Cheeke and Patton (1978) found a favorable response to increasing levels of fiber supplied by alfalfa meal. It was suggested that it might not be the type of fiber, but the ratio of indigestible carbohydrate to highly digestible carbohydrate producing the beneficial response. A high energy, low fiber diet, according to the carbohydrate overload of the hindgut theory (Cheeke and Patton, 1980), predisposes the hindgut to bacterial proliferation resulting in toxin production and a diarrhetic death.

Sinkovics et al. (1980a,b) feels that faulty feeding, with respect to form of diet (granulated vs hay supplementation) and type of fiber supplementation (alfalfa meal vs meadow hay) of weanling rabbit plays a decisive role in parasitosis leading to dysentery. Diets supplemented with meadow hay were superior to diets high in or supplemented with alfalfa meal. Not only was the type of roughage supplying the fiber of importance, but the time of offering hay was critical in preventing enteritis. It was found that fiber should be supplied between the ages of three and seven weeks to be of benefit. Whitney (1970) speculates that the peak mortality from enteritis complex at weaning may be precipitated by a change in diet with a lowered absorption of protein and carbohydrate. Ostler (1961) gives similar accounts where a change in diet precipitated death from enteritis complex.

It was the objective of the following study to determine the effects of switching from high to low and low to high fiber diets on mortality due to enteritis and performance of weanling rabbits.

MATERIALS AND METHODS

Seventy-two New Zealand White weanling (28 ± 2 d of age) rabbits (avg initial wt. = 760 g) were allotted to one of four treatment groups, with littermates equally distributed across treatments. The rabbits were fed either of the following diets: 1) 54% alfalfa, 2) 20% alfalfa (diet composition in table 20). Preweaning, the rabbits were fed the 54% alfalfa diet. The treatment groups consisted of the following regimens:

1) 54% alfalfa - 4 weeks
2) 20% alfalfa - 4 weeks
3) 54% alfalfa - 2 weeks switched to 20% alfalfa
4) 20% alfalfa - 2 weeks switched to 54% alfalfa

Both changes were made abruptly at two weeks with the feed remaining from the previous diet being removed, weighed and the new feed directly offered. Rabbits were fed the diets for two weeks after the switch.

Rabbits in this experiment were weaned at four weeks of age and allowed three days adaptation to the respective diets. Diet switches occurred when rabbits were approximately 6 weeks of age. Rabbits were housed 6 per cage with three cage replicates per regimen and offered food and water ad libitum. The study lasted 28 days during which gains, feed consumption and mortality were recorded. Results were analyzed by least squares analysis of variance according to procedures of Harvey (1975). Mean comparisons were made using selected contrast comparisons.

RESULTS AND DISCUSSION

Least squares means, standard errors of the means and selected comparisons are shown in table 21. Average daily gain (ADG) did not differ ($P>.05$) between dietary regimens. Rabbits starting on the 20% alfalfa diet averaged 1.4 g per day gain more than those starting on the 54% alfalfa diet. The interaction comparisons indicate that animals that were on the 20% alfalfa diet for the last two weeks of the trial averaged 2.0 g more gain per day over those on the 54% alfalfa diet.

Average daily feed consumption did not differ ($P>.05$) between switching and not switching or for regimens ending with the same diet. However, rabbits starting on the 20% alfalfa diet consumed an average 15.1 g less ($P<.05$) feed per day than those starting on the 54% alfalfa diet. Intakes were thus increased by offering the 54% diet immediately post weaning. This could be related to the lower palatability of the 20% alfalfa diet (unpublished data). The supplying of a palatable diet at weaning appears to increase total consumption, even when a less palatable diet is substituted at a later date.

Feed efficiency was improved ($P<.01$) when the switch was made to the 20% alfalfa diet or when the rabbits were started on the 20% alfalfa diet. Animals started on the 54% alfalfa diet, then switched to the 20% diet averaged 0.35 g less feed per g of gain while rabbits consuming the 20% alfalfa diet for the first two weeks averaged 0.52 g less feed per g of gain. This indicates an overall improvement in efficiency for the 20 % diet since its feeding at any time resulted in a lower feed to gain ratio. This would be expected owing to the higher energy density of the 20% alfalfa diet.

Mortality did not differ ($P>.05$) between any of the dietary regimens due to the high variability between pen replicates. Mortality was numerically highest for the dietary regimens starting on the 20% alfalfa diet (avg = 16.6% higher). Comparing mortality between groups switched and not switched or those ending on the same diet showed no difference ($P>.05$). The frequency of mortality by week of the study indicates 50% of the mortalities occurred during the first two weeks with over 90% of all the mortalities occurred before the end of the third week the animals were on the diets (table 22). Of the mortality occurring within one week of the diet switch, 3 occurred in the groups switched, 1 in the group on the 20% alfalfa diet and none on the 54% alfalfa diet. These results, although not significant, suggest that the indigestible fiber to digestible carbohydrate ratio has a more prominent role in enteric interactions in newly weaned animals. These results fall in line with those reported by Sinkovics et al. (1980a, 1980b), who suggested that the three to seven week age is the period of greatest risk and requires added fiber.

Comparing the means for mortality of animals starting on the 20% alfalfa diet to the mean of the animals started on the 54% alfalfa diet and then switched to the 20% diet suggests that higher fiber or low readily digestible carbohydrate is required post weaning. However, it cannot be determined if the high susceptibility to high carbohydrate diets is the result of an interaction between weaning stress and diet or possibly due to the weanling rabbit's inability to digest starch at the time the diet was first offered. Results of Sinkovics et al. (1980a, 1980b) would suggest the latter, that there may be a lower ability of the weanling rabbits digestive tract to digest and/or absorb starch. Undigested starch reaching the hindgut would allow bacterial proliferation leading to diarrhea. However, a palatable feed offered at weaning (or familiar feed since all weanlings dams were maintained on the 54% diet) may reduce the post weaning stress.

The gut of the pre-weanling rabbit is sterile due to the presence of several antimicrobial factors in the stomach produced from the dam's milk (Smith, 1966). At the time of weaning, the milk intake is reduced (Lebas, 1971) and the pH is still relatively high, limiting the stomach's ability to destroy pathogenic bacteria and to completely digest feed. As a result, the rabbits are susceptible to infiltration and proliferation of pathogenic bacteria. The acidity of the stomach increases with age, dropping to near that of an adult by 45 days of age (Brooks, 1978). Thus, time immediate post weaning period appears critical in relation to diet and its role in producing enteritis.

CHAPTER 6

CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH

From the results of the literature review and experiments reported in the thesis, the following conclusions and recommendations for further research can be made:

1) Enteritis complex is multifactorial in origin, with initiating factors related to diet of primary concern. Bacteria appear to play a secondary role while the interactions with other factors as contributors to the complex require further work.

2) The addition of clinoptilolite at 3% of the diet proved beneficial in reducing enteritis. Levels above 3% should be examined for their effect on mortality and growth. Determination of the mode of action may increase the knowledge of the mechanisms of diarrhea in the rabbit,

3) The inclusion of copper at various levels, with or without oxytetracycline, has no effect in reducing mortality caused by diarrhea. Levels of antibiotics above those allowed in rabbit feeds and the use of different antibiotics could be explored for their effects on the control of diarrhea. Until an etiological agent can be identified, research in this area may prove futile.

4) Based on the favorable results for the 54% alfalfa diets when compared to the 20% alfalfa diets, diets high in

fiber and low in starch should be used for weanling rabbits. Once the rabbits have overcome the stresses of weaning, a diet higher in starch can be used for fattening. Further work examining the use of a weaning and fattening ration for decreased mortality and maximal growth should include the labor and other economic factors.

TABLE 1. THE pH VALUES OF THE STOMACH CONTENTS OF HEALTHY RABBITS AT VARIOUS AGES

Age in days	Stomach Contents	pH range
1-7	Milk curd	5.0
7-14	Milk curd	5.0-6.5
14-21	Milk curd 1st solid feed 1st night feces	4.0-6.5
21-28	Less milk curd	4.0-6.5
28-35	Increasing solid feed	3.0-5.0
35-42	Night feces semisolid	2.0-5.0
42-49	Liquid to suspensions of solid feeds	1.0-3.0

Adapted from Brooks (1978)

TABLE 2. COMPOSITION OF BASAL RATIONS FOR ZEOLITE AND BENTONITE ADDITIONS

Ingredient[a]	20% Alfalfa	54% Alfalfa
Alfalfa meal (suncured)	20.0	54.0
Soybean meal (44% CP)	21.0	21.0
Ground corn	54.0	-
Wheat mill run	-	20.0
Dicalcium phosphate	.25	.25
Limestone	.25	-
Trace mineral salt[b]	.50	.50
Soybean oil	1.00	1.25
Molasses	3.00	3.00

[a]Ingredients expressed on a percent of the total diet on an as-is basis.

[b]Mortons Farm and Ranch iOFIXT T-M SALT. Provides NaCL and the following elemental levels in mg/kg of complete diet: Zn, 17.5; Mn, 14; Fe, 8.75; Cu, 1.75; Co, .35.

TABLE 3. CHEMICAL ANALYSIS OF DIETS FOR ZEOLITE ADDITION[a]

Diet	% DM	% CP	% ADF	% CWC	% AIA
20% Alfalfa					
0% Zeolite	89.4	12.9	11.6	25.3	1.08
1.5% Zeolite	89.8	13.3	12.4	24.1	3.58
3.0% Zeolite	90.1	16.9	12.1	24.8	5.13
54% Alfalfa					
0% Zeolite	89.7	24.6	24.5	36.1	1.30
1.5% Zeolite	90.6	22.2	25.9	37.4	3.07
3.0% Zeolite	89.8	21.4	25.9	39.9	5.99

[a]Values (except dry matter) are expressed on a dry matter basis.

TABLE 4. CHEMICAL ANALYSIS OF DIETS FOR BENTONITE ADDITION[a]

Diet	% DM	% CP	% ADF	% AIA
20% Alfalfa				
0% Bentonite	91.0	16.1	12.8	.70
5% Bentonite	91.0	16.1	18.0	4.33
54% Alfalfa				
0% Bentonite	91.5	19.4	22.9	1.49
5% Bentonite	92.2	20.1	24.0	4.01

[a]Values (except for dry matter) are expressed on a dry matter basis.

TABLE 5. PERFORMANCE OF WEANLING RABBITS FED VARIOUS LEVELS OF ZEOLITE

Diet Treatment	No. of rabbits	Avg daily gain, g	Feed:Gain ratio	Percent mortality
20% Alfalfa				
0% Zeolite	16	24.5	5.19	25.0
1.5% Zeolite	16	22.6	4.57	12.5
3.0% Zeolite	16	27.8	3.20	12.5
54% Alfalfa				
0% Zeolite	16	33.7	3.77	12.5
1.5% Zeolite	16	33.9	6.18	62.5
3.0% Zeolite	16	34.2	3.84	6.3
Standard error		2.1	.45	10.0

TABLE 6. SELECTED COMPARISONS FOR THE PERFORMANCE OF RABBITS FED VARIOUS LEVELS OF ZEOLITES[a]

Comparison	Avg daily gain, g	Feed:Gain ratio	Percent Mortality
20% vs 54% alfalfa	8.9 ± 2.1**	.38 ± 1.13	10.4 ± 8.6
0% vs 1.5 + 3.0%	.5 ± 2.1	.26 ± 1.20	4.7 ± 9.1
0% + 1.5% vs 3.0%	2.4 ± 2.1	1.40 ± 1.20	-18,8 ± 9.1
Fiber X zeolite	3.6 ± 1.1**	-.06 ± .63	6.3 ± 4.8
Fiber X hi-lo zeolite	2.5 ± 2.1	1.58 ± 1.16	-25.0 ± 8.8*

[a]Values represent mean differences between treatment comparisons with the standard error of these means.

*P<.05.

**P<.01.

TABLE 7. RESPONSE OF WEANLING RABBITS FED DIETS WITH OR WITHOUT SODIUM BENTONITE

Item	Alfalfa	20%		54%		
	Bentonite	0%	5%	0%	5%	SE[a]
No. of rabbits		38	38	38	38	
Avg daily gain, g		38.7	37.5	38.7	36.1	1.4
Daily feed intake, g		99.6	99.1	126.8	119.7	5.7
Feed:Gain ratio		2.57	2.64	3.30	3.31	.12
Percent mortality		23.2	23.8	18.6	29.9	6.3

[a]Standard error of the mean.

TABLE 8. SELECTED COMPARISONS FOR THE RESPONSE OF WEANLING RABBITS FED DIETS WITH OR WITHOUT SODIUM BENTONITE[a]

Item / Comparison	0% vs 5%	20% vs 54%	Interaction	SE[b]
Avg daily gain, (g)	-1.9	.7	.7	1.4
Daily feed intake, (g)	-3.8	23.9**	3.3	4.9
Feed:Gain ratio	.041	-.700**	.027	.10
Percent mortality	5.9	-.7	-5.4	6.3

[a]Comparisons made were; no bentonite vs 5% bentonite (0% vs 5%), 20% alfalfa vs 54% alfalfa diets (20% vs 54%), interaction - bentonite X alfalfa level.

[b]Standard error of the difference.

**P<.001

TABLE 9. COMPOSITION OF BASAL RATIONS FOR COPPER AND OXYTETRACYCLINE SUPPLEMENTATIION

Ingredient[a]	20% Alfalfa	54% Alfalfa
Ground corn	54.0	--
Alfalfa meal (suncured)	20.0	54.0
Soybean meal	21.0	21.0
Wheat mill run	-	20.0
Dicalcium Phosphate	.25	.25
Limestone	.25	--
Trace mineral salt[b]	.50	.50
Soybean oil	1.00	1.25
Molasses	3.00	3.00

[a]Ingredients expressed on a percent of diet on as fed basis.

[b]Mortons Farm and Ranch iOFIXT T-M SALT. Provides NaCL and the following elemental levels in mg/kg of complete diet: Zn, 17.5; Mn, 14; Fe, 8.75; Cu, 1.75; Co, .35.

TABLE 10. DIETARY TREATMENTS FOR THE FEEDING OF OXYTETRACYCLINE AND CU AS COPPER SULFATE EITHER ANHYDROUS OR HYDRATED.

	Diet	
Antibiotic	20% Alfalfa	54% Alfalfa
None	No Cu	No Cu
	250 ppm Cu Hydrated	250 ppm Cu Hydrated
	250 ppm Cu Anhydrous	250 ppm Cu Anhydrous
Oxytetracycline[a] 10 g per 906 kg	No Cu	No Cu
	250 ppm Cu Hydrated	250 ppm Cu Hydrated
	250 ppm Cu Anhydrous	250 PPm Cu Anhydrous

[a]Oxytetracycline supplied as Terramycin, soluble powder (Pfizer Inc., Agricultural division, New York, N.Y.).

TABLE 11. THE PERFORMANCE OF WEANLING RABBITS FOR THE DIETARY INCLUSION OF COPPER AS $CuSO_4$ EITHER ANHYDROUS OR HYDRATED WITH OR WITHOUT OXYTETRACYCLINE.

Diet	No. of rabbits	Avg daily gain, g	Daily intake, g	Feed:Gain ratio	Percent mortality
54% Alfalfa					
Regular	10	36.6	127.0	3.48	20.0
$CuSO_4$ (Anhy)	10	37.9	127.8	3.37	0.0
$CuSO_4$ (H_2O)	10	38.0	131.7	3.46	0.0
TM-10	10	38.6	128.8	3.38	0.0
$CuSO_4$ (Anhy)	10	38.2	131.7	3.45	0.0
$CuSO_4$ (H_2O)	10	38.4	134.0	3.50	10.0
20% Alfalfa					
Regular	10	35.4	99.2	2.82	30.0
$CuSO_4$ (Anhy)	10	33.4	92.2	2.76	10.0
$CuSO_4$ (H_2O)	10	32.2	109.9	3.41	10.0
TM-10	10	30.6	89.7	2.89	20.0
$CuSO_4$ (Anhy)	10	31.0	84.8	2.73	30.0
$CuSO_4$ (H_2O)	10	30.8	108.5	3.54	20.0
Standard Error[a]		3.6	12.8	.26	10.4

[a]Standard error of the mean.

TABLE 12. SELECTED COMPARISONS FOR PERFORMANCE OF WEANLING RABBITS FOR THE INCLUSION OF COPPER AS $CuSO_4$ EITHER ANHYDROUS OR HYDRATED WITH OR WITHOUT OXYTETRACYCLINE.

Comparisons	Avg daily gain, g	Daily intake, g	Feed:Gain ratio	Percent Mortality
54% vs 20% alfalfa	5.8 $\pm$ 2.1[a]*	32.7 $\pm$ 7.4**	.42 $\pm$ .15**	-15.0 $\pm$ 6.0*
Regular vs + TM-10	1.0 $\pm$ 2.1	1.8 $\pm$ 7.4	-.03 $\pm$ .15	-1.7 $\pm$ 6.0
Anhy vs H_2O $CuSO_4$	.3 $\pm$ 2.5	-11.9 $\pm$ 9.1	-.40 $\pm$ .18*	.0 $\pm$ 7.4
Cu vs Cu + TM-10	.8 $\pm$ 2.5	.6 $\pm$ 9.1	-.06 $\pm$ .18	-10.0 $\pm$ 7.4
Interactions				
TM-10 * alfalfa	-1.8 $\pm$ 2.1	-4.3 $\pm$ 7.4	.03 $\pm$ .15	5.0 $\pm$ 6.0
Cu source * alfalfa	-.4 $\pm$ 2.5	8.8 $\pm$ 9.1	.33 $\pm$ .18	-5.0 $\pm$ 6.0
Cu vs Cu+TM-10 * alf	1.1 $\pm$ 2.5	-3.7 $\pm$ 9.1	-.01 $\pm$ .18	5.0 $\pm$ 6.0

[a]Mean difference with standard error of the mean.

*P<.05

**P<.01

TABLE 13. PERFORMANCE OF WEANLING RABBITS FOR THE INDIVIDUAL DIETARY TREATMENTS OF ALFALFA, COPPER AND OXYTETRACYCLINE

Diet	No. of rabbits	Avg daily gain, g	Daily intake, g	Feed:Gain ratio	Percent mortality
54% Alfalfa	60	37.9 ± .4[a]	130 ± .9	3.4 ± .02	5.0 ± .8
20% Alfalfa	60	32.1 ± .3	97 ± 1.5	3.0 ± .04	20.0 ± 1.4
No Copper	40	35.2 ± .8	111 ± 3.2	3.1 ± .05	17.5 ± 2.1
Cu (Anhydrous)	40	35.1 ± .5	109 ± 3.0	3.1 ± .05	10.0 ± 1.9
Cu (Hydrated)	40	34.8 ± .6	121 ± 2.2	3.5 ± .04	10.0 ± 1.9
No Antibiotic	60	35.5 ± .4	114 ± 1.7	3.2 ± .03	11.7 ± 1.1
TM-10	60	34.6 ± .4	112 ± 2.0	3.3 ± .04	15.4 ± 1.5

[a]Mean and standard error of the mean.

TABLE 14. THE PERFORMANCE OF WEANLING RABBITS FOR THE DIETARY INCLUSION OF COPPER AS ANHYDROUS $CuSO_4$ AT VARIOUS LEVELS.

Diet	No. of rabbits	Avg daily gain, g	Daily intake, g	Feed:Gain ratio	Percent mortality
54% Alfalfa					
0 ppm	20	31.7	103.7	3.28	10.0
50 ppm	20	37.4	124.1	3.31	10.0
100 ppm	20	38.3	119.7	3.14	15.0
250 ppm	20	34.1	114.0	3.61	30.0
500 ppm	20	37.5	123.7	3.33	10.0
1000 ppm	20	29.2	106.6	3.71	20.0
20% Alfalfa					
0 ppm	20	31.8	89.1	2.81	30.0
50 ppm	20	32.9	94.2	2.91	10.0
100 ppm	20	34.5	94.4	2.81	25.0
250 ppm	20	33.6	83.1	2.49	20.0
500 ppm	20	28.3	89.2	3.16	20.0
1000 ppm	20	15.1	68.3	6.63	25.0
Standard Error[a]		1.2	2.8	.21	2.1

[a]Standard error of the mean.

TABLE 15. SELECTED COMPARISONS FOR PERFORMANCE OF WEANLING RABBITS FOR THE INCLUSION OF COPPER AS ANHYDROUS $CuSO_4$

Comparisons	Avg daily gain, g	Daily intake, g	Feed:Gain ratio	Percent Mortality
54% vs 20% alfalfa	5.3 ± .2[a]*	28.9 ± 3.4**	-.07 ± .21	-5.8 ± 2.2*
Some Cu vs no Cu	.7 ± 5.3	10.6 ± 9.0	-.93 ± .55	-3.0 ± 5.8
Added Cu vs 1000	12.4 ± 2.7**	17.5 ± 4.6**	-2.07 ± .28**	-5.0 ± 4.8
50 ppm vs 100 ppm	-1.3 ± 3.4	2.1 ± 5.8	.14 ± .36	-10.0 ± 3.8*
250 ppm vs 500 ppm	1.0 ± 3.4	-7.9 ± 5.8	-.20 ± .36	10.0 ± 3.8*
Hi Cu vs Lo Cu	-2.4 ± 2.4	-5.6 ± 4.1	.11 ± .25	5.0 ± 2.7
Interactions				
Some Cu vs no Cu	6.5 ± 5.3	17.1 ± 9.0	-.66 ± .55	17.0 ± 5.8
Added Cu vs 1000	-9.6 ± 5.4	8.1 ± 9.2	3.42 ± .56**	2.5 ± 5.9
50 ppm vs 100 ppm	.3 ± 3.4	2.3 ± 5.8	.03 ± .36	5.0 ± 3.8
250 ppm vs 500 ppm	-4.4 ± 3.4	-1.8 ± 5.8	.47 ± .36	10.0 ± 3.8*
Hi Cu vs Lo Cu	.4 ± 2.4	2.6 ± 4.1	.14 ± .55	2.5 ± 2.7

[a]Mean difference with standard error of the mean.

*P<.05

**P<.01

TABLE 16. PERFORMANCE OF WEANLING RABBITS FOR THE INDIVIDUAL DIETARY TREATMENTS OF ALFALFA AND COPPER LEVELS

Diet	No. of rabbits	Avg daily gain, g	Daily intake, g	Feed:Gain ratio	Percent mortality
54% Alfalfa	120	34.7 ± 1.2[a]	115 ± 2.6	3.4 ± .13	10.0 ± 2.7
20% Alfalfa	120	29.4 ± 2.0	86 ± 2.6	3.5 ± .40	21.7 ± 2.9
Copper levels					
0 ppm	40	31.7 ± 1.2	96 ± 4.3	3.0 ± .13	20.0 ± 5.3
50 ppm	40	35.2 ± 1.7	109 ± 6.3	3.1 ± .12	10.0 ± 3.7
100 ppm	40	36.4 ± 2.1	107 ± 6.6	3.0 ± .17	20.0 ± 5.3
250 ppm	40	33.9 ± 2.6	99 ± 1.9	3.1 ± .34	25.0 ± 6.4
500 ppm	40	32.9 ± 2.3	106 ± 7.4	3.3 ± .13	15.0 ± 5.0
1000 ppm	40	22.1 ± 4.8	87 ± 8.2	5.2 ± .10	22.5 ± 4.5

[a]Mean and standard error of the mean.

TABLE 17. MUSCLE, KIDNEY, LIVER AND FECAL CU CONCENTRATIONS FOR RABBITS CONSUMING VARIOUS LEVELS OF CU

Cu Level Fed (ppm)	Muscle		Kidney		Liver		Feces	
	54[a]	20	54	20	54	20	54	20
0	2.6[b]	4.5	9.6	17.0	12.3	23.7	57.6	73.2
50	3.0	2.3	15.7	15.3	26.1	25.5	87.5	166.8
100	3.0	7.5	11.4	17.8	116.4	36.9	171.2	186.6
250	3.6	2.8	10.9	14.4	195.4	84.8	352.2	568.5
500	3.4	5.5	14.6	24.6	890.2	981.4	661.4	1325.9
1000	2.0	6.3	37.1	17.2	1743.9	1390.4	1741.3	3518.1
Mean	3.9		17.2		465.8			
Standard error	2.3		10.1		275.6			

[a]Alfalfa level in the diet.

[b]Cu levels expressed in ppm on a dry matter basis.

TABLE 18. MEAN TISSUE CU CONCENTRATIONS FOR RABBITS FED VARIOUS LEVELS OF CU.

Cu Level Fed (ppm)	Muscle	Kidney	Liver
0	3.6[a]	13.3	18.0
50	2.7	15.5	25.8
100	5.3	14.6	76.7
250	3.2	12.6	140.1
500	4.4	19.6	910.8
1000	4.2	27.2	1567.2
Standard error	1.6	7.2	194.9

[a]Cu concentrations expressed as ppm on a dry matter basis.

TABLE 19. CECAL WEIGHTS AND CU CONCENTRATIONS FOR RABBITS FED VARIOUS LEVELS OF CU.

Cu Level Fed (ppm)	Cecal Cu Conc.	Total Cecal Weight	Two cm Section Weight	Percent Body Weight
0	31.8[a]	33.2	.190[b]	1.71[c]
50	77.9	26.3	.218	1.67
100	107.9	26.1	.158	1.62
250	239.4	24.1	.120	1.57
500	1176.5	25.2	.124	1.43
1000	1977.5	18.9	.107	1.44
Mean	601.8	25.6	.153	1.57
Standard error	373.3	14.5	.088	.40

[a]Cu concentrations expressed as ppm on a dry matter basis.

[b]This is the weight of a 2 cm square of the cecum taken from the Ampulla cecalis.

[c]The percent body weight is the amount of the body weight represented by the cecum.

TABLE 20. COMPOSITION AND CHEMICAL ANALYSIS OF DIETS FOR FEED SWITCHING EXPERIMENT

Ingredient[a]	20% Alfalfa	54% Alfalfa
Corn	54.0	--
Alfalfa meal (suncured)	20.0	54.0
Soybean meal	21.0	21.0
Wheat mill run	--	20.0
Dicalcium Phosphate	.25	.25
Limestone	.25	--
Trace mineral salt[b]	.50	.50
Soybean oil	1.00	1.25
Molasses	3.00	3.00
Chemical Analysis[c]		
Dry matter	91.04	91.47
Crude protein	18.63	22.25
Acid detergent fiber	9.05	17.25
Cell wall constituents	34.05	36.23
Ash	6.58	10.31

[a]Ingredients expressed on a percent of diet on as fed basis.

[b]Mortons Farm and Ranch iOFIXT T-M SALT. Provides NaCL and the following elemental levels in mg/kg of complete diet: Zn, 17.5; Mn, 14; Fe, 8.75; Cu, 1.75; Co, .35.

[c]Nutrient composition (except dry matter) is expressed on a dry matter basis.

TABLE 21. PERFORMANCE OF WEANLING RABBITS GROWN ON A SINGLE DIET OR WEANED TO ONE DIET AND SWITCHED TO A SECOND DIET.

DIET	Avg daily gain, g	Daily feed intake, g	Feed: Gain ratio	Percent Mortality
Over-all mean	38.3	113.0	2.96	19.4
20% Alfalfa	39.9	102.4	2.54	27.8
54% Alfalfa	36.5	124.1	3.41	16.7
20% switch to 54%	38.0	108.5	2.86	27.8
54% switch to 20%	38.6	116.9	3.02	5.6
Standard error	9.8	2.5	.03	4.8
Selected comparisons				
Interaction (end)[a]	2.0	6.7	.35**	5.6
Interaction (start)[b]	1.4	15.1*	-.52**	16.6
Switch vs no switch	.1	-.5	- .04	-5.6
SE[c]	2.0	5.0	.06	9.8

[a]This contrast is comparing means for the dietary regimens ending on the same diet (i.e. the average of 20% Alfalfa + 54% Alfalfa sitched to 20% Alfalfa vs the average of the other two means).

[b]This contrast is comparing means for the dietary regimens starting on the same diet (i.e. the average of the 20% Alfalfa + 20% Alfalfa sitched to 54% alfalfa vs the average of the other two means).

[c]Standard error of the mean difference.

*P<.05

**P<.01

TABLE 22. THE FREQUENCY OF MORTALITY RELATIVE TO DIETARY REGIMEN AND WEEK.

DIET	Week[a] 1	2	3	4	Total
20% Alfalfa	1	2	1	1	5
54% Alfalfa	1	2	0	0	3
20% switch to 54%	2	1	2	0	5
54% switch to 20%	0	0	1	0	1
Weekly total	4	5	4	1	14
Relative frequency	28.6	35.7	28.6	7.1	
Cumulative frequency	28.6	54.3	92.9	100	

[a] Diet switches were made at the start of week three.

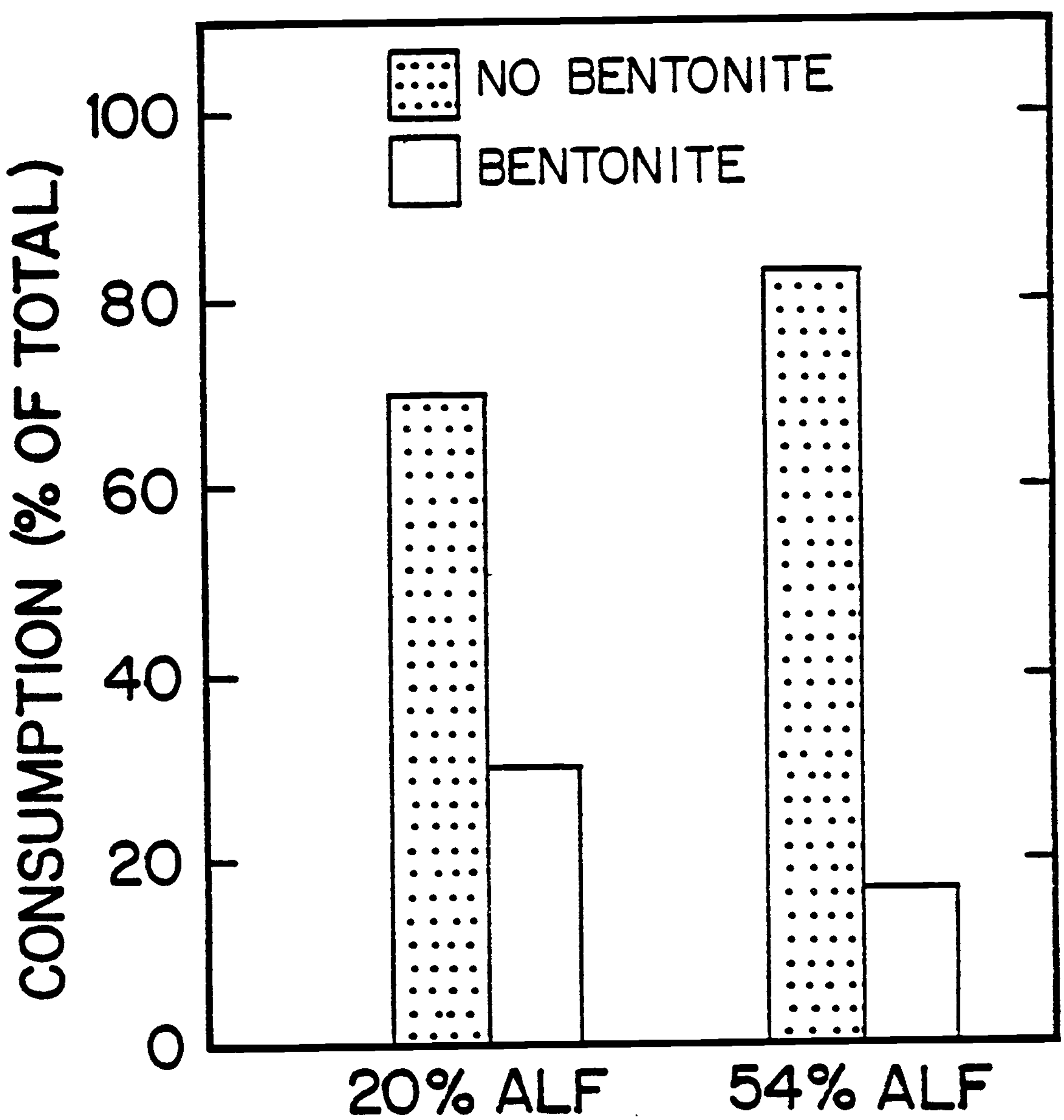

FIGURE 1. DIET PREFERENCE FOR BENTONITE ADDITION.

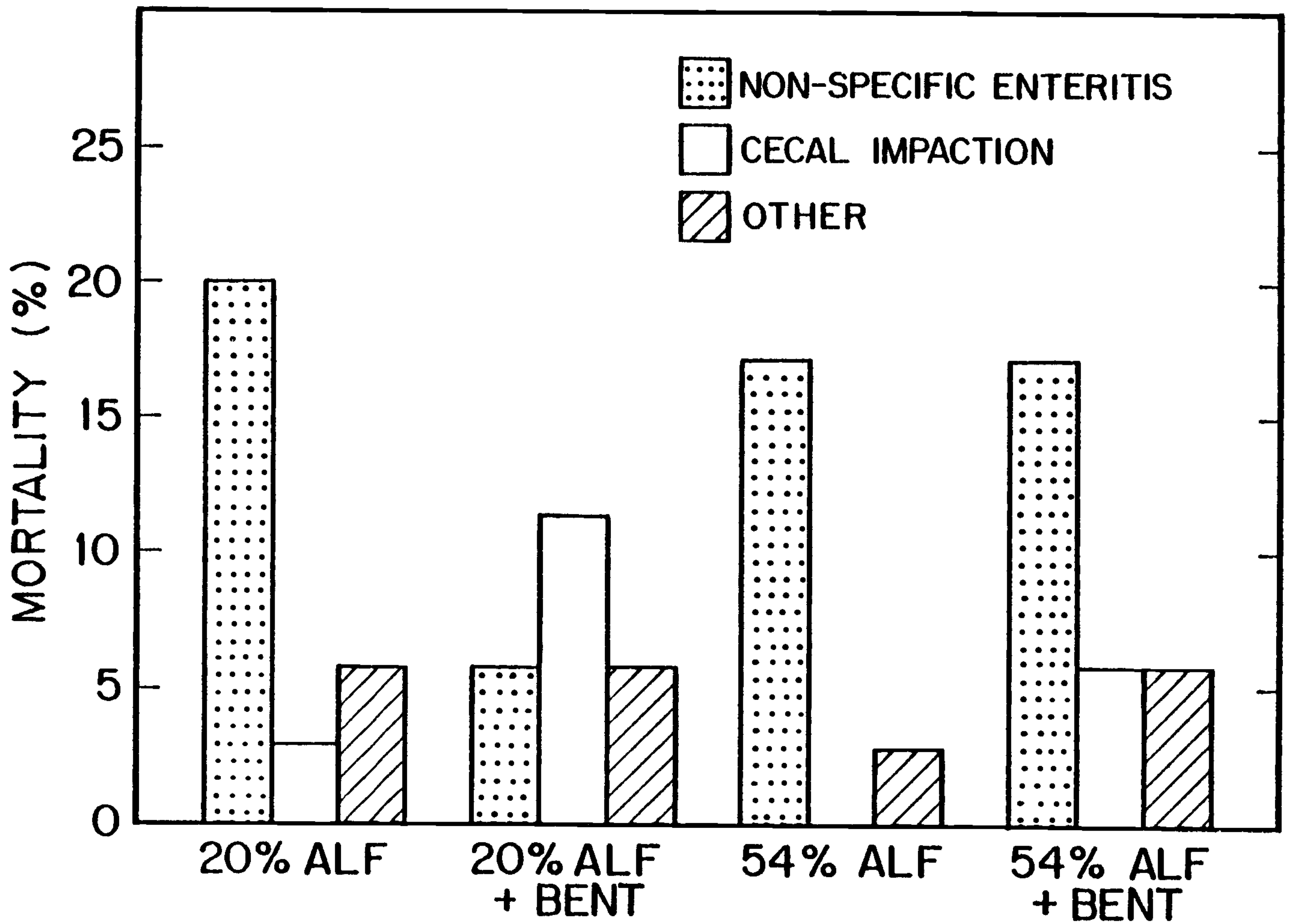

FIGURE 2. PARTITIONING OF MORTALITY BY DIET AND DIAGNOSIS.

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