

AN ABSTRACT OF THE THESIS OF

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Title: UTILIZATION OF HIGH ROUGHAGE DIETS BY RABBITS

**Redacted for privacy**

Abstract approved: \_\_\_\_\_

Dr. Peter R. Cheeke

A series of experiments were conducted to determine the utilization of fibrous feeds by the rabbit. A review of literature was performed to ascertain the results of similar studies. The first two experiments involved various levels of alfalfa substituted for corn or barley. Results indicated that a 50 percent alfalfa diet with an acid detergent fiber (ADF) level of 19 percent could be fed without adversely affecting growth rate or dressing percentages. The group fed a 74 percent alfalfa diet, where alfalfa completely replaced corn, had lower growth rates but was the only group with no mortality.

Experiment 3 was performed to determine the effects of fresh greens supplementation on pellet consumption. Pellet intake was significantly less in the group given free choice of greens versus those fed pellets only. Average daily gains were the same in both groups. Even when pellet consumption was limited, weight gains were

not adversely affected until less than 50 grams (g) of pellets were allotted. The lowest weight gains were in the group fed greens and no pellets. Dressing percentages were calculated in the ad libitum greens, the 75 g pellets and ad libitum greens and the pellets only groups. There were no significant differences among the three groups.

Lastly an attempt was made to improve the fiber digestibility in alfalfa, millrun and corn cobs with sodium hydroxide treatment. The only apparent increase in fiber digestibility occurred in the millrun group. The energy digestibility did not increase in the treated millrun group, indicating another factor may have been involved in the elevated fiber digestibility in this group.

UTILIZATION OF HIGH ROUGHAGE DIETS  
BY RABBITS

by

Linda Marie Wayland Pote

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# UTILIZATION OF HIGH ROUGHAGE DIETS BY RABBITS

## I. INTRODUCTION

The domestic rabbit (*Oryctolagus cuniculus*) has been used as a pilot animal in nutritional studies, for biological and medical research, and for fur and meat production. In spite of its widespread use, there has been little interest in the nutritional requirements of the rabbit until recently.

The rabbit is a nonruminant herbivore, so in the wild, fibrous plants are a large part of its diet. This would suggest a need for fiber, but little is known about required levels, beneficial effects, and digestibility of fiber in the domestic rabbit.

Due to the competition for grains with the monogastric animal, there is a need to investigate cheaper alternative feed sources such as by-products for the rabbit. Increasing the digestibility of fibrous feed-stuffs via chemical treatment is another area of rabbit feeding which should be considered.

One of the objectives of this study was to determine the effects of replacing corn or barley with alfalfa on growth performance, feed conversion and carcass yield of the rabbit. Another objective was to observe the effects on growth performance and dressing percentage when

rabbits were fed Purina Rabbit Chow supplemented with fresh greens. Lastly, an attempt was made to determine the effects of sodium hydroxide treatment of alfalfa, millrun, and corn cobs on fiber digestibility by rabbits.

## II. REVIEW OF LITERATURE

### Rabbit physiology

The domestic rabbit is physiologically classified as a nonruminant herbivore. This means that the rabbit has a monogastric digestive system, but also has a fermentative area located in the hindgut or the cecum, colon, and large intestine (Hintz, 1978).

Digestion of feedstuffs begins in the mouth of the rabbit. The rabbit has two sets of teeth which enable it to masticate fibrous plant material. Like other mammals, enzymes begin to break down the food through hydrolytic reactions (Swenson, 1977). The digesta then passes through the esophagus to the stomach.

The stomach acts as a reservoir, and is the first site for the digestion of protein and fat. In the stomach the digesta comes in contact with hydrochloric acid and pepsin, produced by the parietal and chief cells, respectively (Guyton, 1976). The stomach pH in the rabbit is more acidic than in other nonruminant herbivores. The pH in both the anterior and posterior portion of the stomach is 1.9, whereas the pH of the anterior and posterior portions of the horse's stomach is 5.4 and 3.3 (Smith, 1965). This would indicate that the parietal cells in the rabbit stomach function differently than those in other nonruminant herbivores.

The digesta then passes through the pyloric sphincter, which limits the particle size entering the small intestine. The small intestine is divided into three parts; the duodenum, ileum, and jejunum (Swenson, 1977). The pH at the beginning of the small intestine is 6.0 and increases to 7.5 to 8.0 (Smith, 1965). This would indicate that the duodenum has a remarkable buffering capacity (Kametaker, 1967). The primary function of the small intestine is absorption of nutrients from the small intestine and into the blood stream (Swenson, 1977).

The digesta, via peristaltic movement, then moves to the large intestine, or the hindgut, rapidly (Swenson, 1977). The pH of the cecum, or the blind sac in the large intestine is 6.6 and in the colon is 7.2 (Smith, 1965). It is in the hindgut where absorption of water and electrolytes takes place. It is also the region where microbes proliferate, especially in the cecum (Swenson, 1977).

The environment of the cecum in the rabbit is ideal for microflora. There is a steady refluxing of water (Pickard and Stevens, 1972), bringing the microbes in contact with the food supply. The food supply itself is constant, consisting of food residues which passed intact through the stomach and small intestine, cellular debris, and endogenous secretions (Hungate, 1966).

The microbial population in the rabbit is unique when compared to that of ruminants and even other non-ruminant herbivores. The rabbit unlike the horse does not normally have Escherichia coli, or Lactobacilli in the gastrointestinal tract. Streptococci are present, but only in the cecum. Instead the flora of the rabbit consists almost exclusively of Bacteroides (Smith, 1965).

The genus Bacteroides belongs to the family Bacteroidaceae. They are classified as gram negative and are slender rods with rounded ends. They are non-spore forming, strict anaerobes, and fermentative (McFadden, 1976). In rumen microbiology Bacteroides are considered capable of utilizing cellulose (Bacteroides succinogenes), hemicellulose and starch (Bacteroides ruminicola), and amino acids (Bacteroides amylophilus). Bacteroides ruminicola are also ammonia producers (Hungate, 1966). Evidence that these species may also be present in the rabbit is seen in the presence of volatile fatty acids, which are by-products of bacteria in the fermentation of sugars (Barcroft et al., 1944; Elsdon et al., 1946; Cools and Jeuniaux, 1961; Hoover and Heitman, 1972).

Bacteria capable of utilizing cellulose may also be present in the cecum (Hall, 1952). When carboxy methyl cellulose was mixed with rabbit cecal contents in vitro, free reducing sugars were liberated (Cools and Jeuniaux, 1961).

The flora in the hindgut can vary depending on such things as the diet of the animal, antibiotics and other bactericidal and bacteriostatics administered, the health of the animal, and interaction between the microbes themselves.

When rabbits were fed raw cabbage diets or a control diet for 24 days, no change in the flora was observed. However, when rabbits were fed dried milk, the flora remained unchanged until day 12 when lactobacilli appeared (Smith, 1965). This would indicate that some diets can alter the flora.

Antibiotics can inhibit or kill bacteria. They usually exert their effect by interfering with some stage of protein or nucleic acid synthesis, depending on the antibiotic used (Nester et al., 1973). Often antibiotics are used in rabbit production as growth promotants (NRC, 1977). Nitrofurans have also been used. They are bacteriostatic and bactericidal against a wide range of gram positive and gram negative bacteria. It is believed they interfere with enzymes within the bacteria involved in the metabolic process (Titus and Fritz, 1971). Coccidiostats, such as sulfonamides, are frequently used, but they alter the protozoan population in the cecum (Titus and Fritz, 1971).

Animals are also capable of producing their own immunological substances (Nester et al., 1973). Thus the

health of the animal would also be a determining factor in the type of microbial population in its hindgut.

It has been observed repeatedly that only a few bacteria occupy a specific microbial niche (Nester et al., 1973). When different microbes compete for the same nutrients, the microbe with the faster growth rate is the one which predominates (Nester et al., 1973). This faster growth rate is usually due to ideal conditions such as pH and temperature (Hungate, 1966). The predominance of *Bacteroides* in the rabbit could possibly be explained in this manner.

The age of the rabbit will also influence the type of flora found in the hindgut. It was found that in the first week of life *Lactobacilli*, *Streptococci*, and *Clostridium perfringens* were present. *Bacteroides* appeared between the first and second week and remained constant until the study ended at week 17. *Escherichia coli* appeared at week two and fluctuated from week 14 until the end of the study. At week 14 *E. coli* and *Bacteroides* were the only microbes present (Smith and Crabb, 1961). The presence of *E. coli* at week 14 disagrees with the later findings by Smith (1965).

Exposure of an animal to bacteria could also alter the micro-population in that animal. *E. coli*, *Cl. perfringens*, *Str. faecalis*, *L. acidophilus*, and *C. bovine* were introduced into the alimentary tract of the rabbit.

by various methods (Smith, 1960). When they were given in drinking water, E. coli was the only culture found in the feces. This occurred two days out of the six day trial. Introducing the cultures orally in an aqueous suspension to fasting rabbits had similar results, but only 50 percent of the rabbits had E. coli. Injection of the cultures directly into the small intestine resulted in all cultures flourishing until day three, when they disappeared. Injection of the cultures into the stomach resulted in destruction of the cultures, even when the stomach was made more alkaline. Smith (1965) concluded that the stomach was the chief antibacterial mechanism in the rabbit.

#### Volatile fatty acids

One type of nutrient utilized by the bacteria is carbohydrate. Carbohydrates can be classified as monosaccharides, disaccharides, and polysaccharides. Monosaccharides such as glucose can be readily used by the microbe for energy. They have the empirical formula  $(CH_2O)_n$ , where n equals three or some larger number, and are referred to as simple sugars (Lehninger, 1975). Disaccharides are composed of two monosaccharides joined together, such as sucrose, lactose, or maltose. Polysaccharides are composed of more than two monosaccharide units, such as starch or cellulose (Lehninger, 1975).

Both disaccharides and polysaccharides are too complex to enter a bacterial cell for degradation. They first have to be catabolized to monosaccharides. This is done by exocellular enzymes produced by certain species of bacteria (McFadden, 1976).

The monosaccharide is then ingested by the bacteria and metabolized via fermentation, yielding energy for the bacteria and various end products (McFadden, 1976). Some bacteria are capable of fermenting amino acids, fatty acids, purines and pyrimidines (Lehninger, 1975).

Fermentation is an anaerobic oxidation-reduction metabolic process where the organic substrate is the final hydrogen acceptor instead of oxygen (Stainer et al., 1963). The fermentation of organic substrates such as carbohydrates results in both oxidized and reduced products (Stainer et al., 1963).

There are several factors which determine the final end products in carbohydrate fermentations. Some of these are: (1) The type of organism involved, (2) the substrate being fermented, and (3) environmental factors, such as pH and temperature (Stainer et al., 1963). The end products which result are hydrogen, carbon dioxide, a few volatile fatty acids (VFA's), a few alcohols, and one ketone (Leng, 1970). The chief VFA's found in the rabbit hindgut are butyric, propionic, and acetic acid (Elsden et al., 1946; Henning and Hird, 1972; Hoover and Heitman, 1972).

Acetic acid is a two-carbon fatty acid,  $\text{CH}_3\text{COOH}$  (Kice and Marvell, 1974). Several types of bacteria produce acetic acid as a by-product, using various fermentative pathways. One type of bacteria which is capable of producing propionic acid from glucose may also produce acetic acid as a by-product (Zinsser, 1968). Another type of bacteria which excretes butanediol also produces acetic acid (Zinsser, 1968).

There are several pathways in which acetic acid is produced. One pathway involves pyruvate becoming acetyl CoA when  $\text{CO}_2$  and  $\text{H}_2$  are released. Acetyl CoA in a series of steps becomes acetate (Leng, 1970).

Propionic acid is a three-carbon fatty acid,  $\text{CH}_3\text{CH}_2\text{COOH}$ . Propionic acid fermentators are closely related to Lactobacilli (McFadden, 1976). Pyruvate with the addition of two hydrogens can be converted to lactate, which in several steps is converted to propionic acid (Leng, 1970). Another pathway in which propionic acid is produced is via the citric acid cycle. Using this pathway, certain bacteria are able to reverse it yielding propionic acid from succinate (Leng, 1970).

Butyric acid is a four-carbon fatty acid,  $\text{CH}_3\text{CH}_2\text{CH}_2\text{COOH}$  (Kice and Marvell, 1974). Bacteria which produce this also produce other products such as butanol and acetone, depending on the pathway. The major reaction in the production of butyric acid involves

pyruvate releasing  $\text{CO}_2$  and  $\text{H}_2$  to become acetyl CoA which, through a series of hydrogenation and dehydration reactions becomes butyrate (Leng, 1970). This pathway also produces acetic acid as a by-product. There is also some interconversion of acetic acid to butyric acid (Maynard et al., 1979).

After the VFA's are produced by the bacteria they are excreted into the cecum and large intestine. Henning and Hird (1972) reported that acetic acid was the most abundant acid, followed by n-butyric, then propionic in the cecum and proximal colon. The acids are then absorbed through the intestinal mucosa of the animal. It has been observed that blood samples from the cecal veins contained higher VFA concentrations than did samples from the mesenteric and carotid arteries (Cools and Jeuniaux, 1961). Henning and Hird (1972) also found that volatile fatty acid content decreased from the cecum to the rectum of the rabbit, again indicating absorption of acids through the mucosa.

The mechanism involved in the transport of fatty acids is not clearly understood. One theory is that fatty acid absorption is partly dependent on sodium absorption (Leng, 1978). It is also believed that water and VFA absorption in the colon occur via separate mechanisms (Henning and Hird, 1972).

Once in the blood of the animal fatty acids may be utilized in two major physiological roles. They may act as building blocks for phospholipids and glycolipids, or fuel molecules for the animal (Stryer, 1975). VFA's in the rabbit may supply as much as 30 percent of the maintenance energy required (McBee, 1970; Parker, 1976).

As mentioned previously, the diet of the animal can determine the type and quantity of VFA's produced in the hindgut. Orskov et al. (1971) showed that a fluctuation in starch content in a sheep caused a fluctuation in VFA production in the cecum. Hoover and Heitmann (1972) observed that butyric acid production was lower with an increase of fiber in the rabbit diet. This same study also indicated that acetic acid was in the greatest concentration, followed by butyric and then propionic acid. These results disagree with earlier studies by Barcroft et al. (1944) and Elsdon et al. (1946) who found propionic to be higher than butyric acid, but agreed with the more recent findings of Henning and Hird (1972) and McMillan et al. (1975). Feeding high grain diets may also result in a greater proportion of propionate (Maynard et al. 1979).

The feed intake in the rabbit may also determine the amount and type of VFA's produced. Parker (1976) fed one group of rabbits 100 grams (g) per day and another group ad libitum. The ad libitum group produced more butyric

acid, but there was a higher turnover rate of VFA's in the rabbits fed 100 g per day.

There appears to be no diurnal variation of VFA production in the hindgut of the rabbit (Henning and Hird, 1972; McMillan et al., 1975).

### Coprophagy

There is another way the rabbit benefits from the microbial population in the hindgut. The bacteria not only produce VFA's but also produce some of the B-complex vitamins (Kulwich et al., 1953). The rabbit benefits from this production via coprophagy.

Coprophagy is the act of eating feces, either the animal's own or that of other animals (Arrington and Kelley, 1976). Coprophagy in the rabbit was reported as early as 1882 (Morot, 1882). This has been observed not only in caged rabbits, but in wild rabbits as well (Myers, 1955). Alus et al. (1977) observed that the rabbit does not practice coprophagy until approximately 20 days after birth. It is also at this time that the rabbit's diet consists almost entirely of solid food (Alus, 1977).

The way the rabbit practices coprophagy is rather unique. Instead of producing one type of feces, they produce both hard and soft feces (Arrington and Kelley, 1976). Rabbits ingest the soft feces which are eaten

directly from the anus (Arrington and Kelley, 1976). It was observed that when the anus was surgically removed, large quantities of soft feces were not ingested (Bezille et al., 1973). This may indicate that receptors in the anus stimulate coprophagy.

It was once believed that soft feces were produced only at night and hard feces during the day (Eden, 1940). More recent research indicates that the production of hard feces is at a maximum from 8 p.m. to 8 a.m. (Lebas and Laplace, 1974). Feeding time may also influence fecal production. Proto (1975) observed that hard feces were excreted two to eight hours after feeding, while soft feces were excreted eight to 14 hours after feeding. There was also a lapse of 30 to 60 minutes prior to coprophagy, where no fecal production occurred (Proto, 1975).

There have been several proposals to explain the formation of hard and soft feces. One proposal suggested that soft feces originated in the cecum, while hard feces consisted of material which by-passed the cecum (Thacker and Brandt, 1954; Eden, 1940). Another proposal was that hard and soft feces both originated in the cecum (Bjornhag, 1972; Henning and Hird, 1972), and that the physical difference occurred in the proximal colon (Henning and Hird, 1972).

There are several distinct differences between hard and soft feces. Soft feces contain a tough mucous outer

membrane, which encloses Lactobacilli and a phosphate buffer (Griffiths and Davies, 1963). It is believed that this buffer causes a high pH inside the pellet, even when the pellet is in the stomach, which allows fermentation to occur within the fecal pellets (Griffiths and Davies, 1963).

Another important difference between hard and soft feces is their nutritional content. Soft feces contain more nitrogen than hard feces (Thacker and Brandt, 1954), three to four times more niacin and riboflavin, six times more pantothenic acid, two to three times more vitamin B<sub>12</sub> (Kulwich et al., 1953), and more VFA's (Henning and Hird, 1972). Soft feces contain less fat soluble components, crude fiber, nitrogen free extract, and more total ash contents.

The rabbit, upon ingestion of soft feces, is able to utilize these nutrients. When diets deficient in such nutrients as vitamin B<sub>12</sub>, pantothenic acid, riboflavin, niacin, and thiamin were fed to rabbits, deficiencies did not result. It was found that these nutrients were present at a higher concentration in the feces than in the diet, suggesting abundant intestinal synthesis (Olcese et al., 1948; Olcese et al., 1949; Kulwich et al., 1953; Reid et al., 1963).

The bacteria also provide nitrogen and some amino acids to the host. When the bacteria are ingested via coprophagy, they are then broken down into amino acids

(Maynard et al., 1979). These amino acids are either utilized by the animal, if needed, or undergo transamination and deamination reactions (Stryer, 1975). The amino acids are converted to  $\text{NH}_3$  and  $\alpha$ -ketoglutarate. The  $\text{NH}_3$  enters the urea cycle and the carbon atoms are transformed into acetyl CoA, acetoacetyl CoA, pyruvate, or one of the intermediates of the citric acid cycle (Stryer, 1973).

It was shown in one study that conventional rabbits had a higher percentage of total essential amino acids in their feces than did germ free animals (Yoshida et al., 1963). Although the bacteria may supply some amino acids for the rabbit, there are still requirements for certain amino acids (Adamson and Fisher, 1973; Cheeke, 1971).

When coprophagy in the rabbit is prevented, digestibility of certain nutrients decreases. Yoshida et al. (1968) showed this in a study using a roughage diet versus a purified diet. On the roughage diet rabbits allowed to practice coprophagy had higher apparent digestibilities of dry matter and ash and higher retention of nitrogen, but cellulose, ether extract, and carbohydrate digestibilities were not improved. When the purified diet was fed, all digestibilities were increased in the rabbits allowed to practice coprophagy.

## Nutrition of the rabbit

In general an animal consumes food to meet its energy requirements (Scott et al., 1976). With a diet extremely high in energy, the animal will eat less and if other nutrient levels are not increased to compensate for this decrease in intake, deficiencies can result (Scott et al., 1976). If the diet is too low in energy the animal may not have the gut capacity to eat enough to meet its energy requirements, thus starvation can occur (Scott et al., 1976).

Comparatively little work has been done on the energy requirements of the rabbit during various stages of production such as growth, lactation, and gestation. Lebas (1975) found that for every gram of weight gain in the growing rabbit, 9.5 Kcal of digestible energy are required. He concluded the best results for growth would occur when the growing rabbit was fed 2500 Kcal per kilogram (kg) of diet.

The growing rabbit seems to be able to tolerate high quantities of fat. Thacker (1956) found there was greater weight gain when the growing rabbit was fed ten percent fat in the diet versus five percent fat. Arrington (1974) fed levels of fat ranging from 2.4 to 14.4 percent fat to growing rabbits. He observed that there was significantly more growth in the 14.4 percent fat group than in the 2.4 percent group. It was also

observed that intake was reduced with an increase in fat and a better feed conversion occurred (Arrington, 1974).

Cheeke (1974) found there was actually a preference for the higher fat diets by the growing rabbit. He observed that rabbits preferred five percent fat in the diet to no fat in the diet. However, when 20 percent fat was included in a diet, the rabbits still preferred the diet containing five percent fat. It would appear that the range of fat the rabbit prefers lies somewhere between five and twenty percent.

Minerals: The calcium level required for the growing rabbit is .4 percent per kg of diet (NRC, 1977). Calcium metabolism in the rabbit is unique, in that it is able to absorb large quantities of calcium and excrete the excess in the urine (Cheeke, 1973). This excess of calcium appears not to be detrimental to the animal. More research needs to be done to determine how the rabbit is able to metabolize such large quantities without hypercalcemia occurring.

The suggested requirements for phosphorus and magnesium in the growing rabbit are .22 percent per kg of diet and 300 to 400 mg per kg of diet, respectively (NRC, 1977). The growing rabbit's maximum requirements for potassium, sodium and chlorine are .6 percent, .2 percent, and .3 percent per kg of diet, respectively (NRC, 1977). The growing rabbit also requires 3 g of copper,

.2 milligrams (mg) of iodine and 8.5 mg of manganese per kg of diet (NRC, 1977). The requirement for zinc and iron in the growing rabbit's diet is not known, though a dietary need has been established (NRC, 1977). See Table 1.

Vitamins: Vitamins are divided into two groups, fat soluble or water soluble vitamins. The fat soluble vitamins, vitamins A, D, E, and K, are apparently absorbed by the same mechanism as are fats (Scott et al., 1976).

The growing rabbit requires 580 IU's of vitamin A (NRC, 1977). Vitamin D requirements for the growing rabbit are not known (NRC, 1977). It has been estimated that 40 mg of vitamin E are required by the growing rabbit in every kg of feed (NRC, 1977). It is assumed that there are no requirements for vitamin K, due to its synthesis in the rabbit hindgut by the microbes (NRC, 1977).

As mentioned previously, water soluble vitamins are supplied partially or completely to the rabbit via coprophagy (Kulwich et al., 1953). Although there is microbial synthesis of niacin and thiamine in the hindgut of the rabbit (Kulwich et al., 1953), there is still a dietary requirement for both (Wooley and Sebrell, 1945; Reid et al., 1963). It is recommended that the growing rabbit be fed 180 mg of niacin per one kg of diet (NRC,

TABLE 1. NUTRIENT REQUIREMENTS OF RABBITS FED AD LIBITUM  
(PERCENTAGE OR AMOUNT PER KG OF DIET)

Nutrients <sup>a</sup>	Growth	Maintenance	Gestation	Lactation
Energy and protein				
Digestible energy (kcal)	2500	2100	2500	2500
TDN (%)	65	55 <sub>b</sub>	58	70
Crude fiber (%)	10 <sub>b</sub> 12 <sup>b</sup>	14 <sub>b</sub>	10 <sub>b</sub> 12 <sup>b</sup>	10 <sub>b</sub> 12 <sup>b</sup>
Fat (%)	2 <sub>b</sub>	2 <sub>b</sub>	2 <sub>b</sub>	2 <sub>b</sub>
Crude protein (%)	16	12	15	17
Inorganic nutrients				
Calcium (%)	0.4	-- <sup>c</sup>	0.45 <sup>b</sup>	0.75 <sup>b</sup>
Phosphorus (%)	0.22	-- <sup>c</sup>	0.37 <sup>b</sup>	0.5
Magnesium (mg)	300-400	300-400	300-400	300-400
Potassium (%)	0.6 <sub>b,d</sub>	0.6 <sub>b,d</sub>	0.6 <sub>b,d</sub>	0.6 <sub>b,d</sub>
Sodium (%)	0.2 <sub>b,d</sub>	0.2 <sub>b,d</sub>	0.2 <sub>b,d</sub>	0.2 <sub>b,d</sub>
Chlorine (%)	0.3 <sub>b,d</sub>	0.3 <sub>b,d</sub>	0.3 <sub>b,d</sub>	0.3 <sub>b,d</sub>
Copper (mg)	3	3	3	3
Iodine (mg)	0.2 <sub>b</sub>	0.2 <sub>b</sub>	0.2 <sub>b</sub>	0.2 <sub>b</sub>
Iron	-- <sub>c</sub>	-- <sub>c</sub>	-- <sub>c</sub>	-- <sub>c</sub>
Manganese (mg)	8.5 <sup>e</sup>	2.5 <sup>e</sup>	2.5 <sup>e</sup>	2.5 <sup>e</sup>
Zinc	-- <sub>c</sub>	-- <sub>c</sub>	-- <sub>c</sub>	-- <sub>c</sub>
Vitamins				
Vitamin A (IU)	580	-- <sup>c</sup>	>1160	-- <sup>c</sup>
Vitamin A as carotene (mg)	0.83 <sup>b,e</sup>	-- <sup>f</sup>	0.83 <sup>b,e</sup>	-- <sup>f</sup>
Vitamin D	-- <sub>g</sub>	-- <sub>g</sub>	-- <sub>g</sub>	-- <sub>g</sub>
Vitamin E (mg)	40 <sub>h</sub>	-- <sub>c</sub>	40 <sub>h</sub>	40 <sub>h</sub>
Vitamin K (mg)	-- <sub>i</sub>	-- <sub>j</sub>	0.2 <sub>b</sub>	-- <sub>i</sub>

TABLE 1. (CONTINUED)

Nutrients <sup>a</sup>	Growth	Maintenance	Gestation	Lactation
Vitamins (cont'd)				
Niacin (mg)	180	---	---	---
Pyridoxine (mg)	39	---	---	---
Choline (g)	1.2 <sup>b</sup>	---	---	---
Amino acids (%)				
Lysine	0.65	---	---	---
Methionine + cystine	0.6	---	---	---
Arginine	0.6	---	---	---
Histidine	0.3 <sup>b</sup>	---	---	---
Leucine	1.1 <sup>b</sup>	---	---	---
Isoleucine	0.6 <sup>b</sup>	---	---	---
Phenylalanine + tyrosine	1.1 <sup>b</sup>	---	---	---
Threonine	0.6 <sup>b</sup>	---	---	---
Tryptophan	0.2 <sup>b</sup>	---	---	---
Valine	0.7 <sup>c</sup>	---	---	---
Glycine	---	---	---	---

<sup>a</sup>Nutrients not listed indicate dietary need unknown or not demonstrated.

<sup>b</sup>May not be minimum but known to be adequate.

<sup>c</sup>Quantitative requirement not determined but dietary need demonstrated.

<sup>d</sup>May be met with 0.5 percent NaCl.

<sup>e</sup>Converted from amount per rabbit per day using an air-dry feed intake of 60 g per day for a 1-kg rabbit.

<sup>f</sup>Quantitative requirement not determined.

<sup>g</sup>Probably required, amount unknown.

<sup>h</sup>Estimated.

<sup>i</sup>Intestinal synthesis probably adequate.

<sup>j</sup>Dietary need unknown.

1977). The growing rabbit also requires 39 mg of pyridoxine and 1.2 mg of choline per kg of diet (NRC, 1977).

Protein: Protein is necessary for structural components such as muscle, connective tissue, collagen, hair, and skin (Scott et al., 1976). The blood proteins are necessary for balance of osmotic pressure and are also involved in blood clotting, red blood cell structure, and cellular membrane structure (Scott et al., 1976).

There has been some debate concerning the protein requirements of the growing rabbit. In the past it was assumed the rabbit obtained a significant amount of protein via coprophagy. Spreadbury (1978) observed that bacterial protein from the cecum and colon supplied the rabbit with only two grams of crude protein per day. Spreadbury (1978) recommended 15.6 percent crude protein in the diet and found that protein in excess of this did not improve growth. Yet Merkusin and Rost (1966) found that greater carcass yield resulted with 16.77 percent crude protein. The National Research Council (1977) recommends a level of 16 percent crude protein for the growing rabbit.

The quality of the protein is also an important factor to consider. Evidently the rabbit can not be supported on incomplete proteins, such as zein and gelatin (Cheeke, 1971). This would indicate that the

rabbit has specific amino acid requirements. The growing rabbit requires per kg of diet: .65 percent lysine, .6 percent methionine and cystine, .6 percent arginine, .3 percent histidine, 1.1 percent leucine, .6 percent isoleucine, 1.1 percent phenylalanine and tyrosine, .6 percent threonine, .2 percent tryptophane, and .7 percent valine (NRC, 1977). There is a requirement for glycine, but the amount is unknown (NRC, 1977). The previously mentioned amino acids are probably required for maintenance, gestation, and lactation, but the amounts required are unknown (NRC, 1977).

The rabbit, like other non-ruminant herbivores, has the ability to extract protein from such forages as alfalfa. In one digestibility study it was shown that when a rabbit and pig were fed alfalfa meal, the protein in the meal was 74 percent digestible for the rabbit (Slade and Hintz, 1969), but less than 50 percent for the pig (NRC, 1978). When comparing the digestibility of alfalfa protein in the horse, pony, and rabbit the digestibility coefficients were 74.0, 76.2, and 73.7, respectively (Slade and Hintz, 1969). Thus the rabbit is able to take leafy forages, which are poorly used by most monogastrics, and digest the protein fraction efficiently.

#### Fiber utilization

The term "fiber" has been defined in various ways.

Southgate et al. (1978) defined fiber as the sum of lignin and the polysaccharides that are not digested by the endogenous secretions. The terms acid detergent fiber (ADF) or crude fiber (CF) are often used in reference to fiber levels in the diet. In the Weende system crude fiber consists of plant structural carbohydrates such as cellulose and hemicellulose, plus lignin (Maynard et al., 1979). This is determined by boiling the sample in dilute acid, then in dilute base (Maynard et al., 1979) ADF consists of cellulose, lignin, and silica (Figure 1) and is measured by boiling the sample in acid detergent (Goering and Van Soest, 1975). It is the residue insoluble in acid detergent.

Lignin is one of the cellular components in the plant. It is in a class of noncarbohydrate compounds and gives structural support to plant cell walls. Basically it consists of carbon-to-carbon bonds and ether linkages, and it is found in the woody portions of the plant (Maynard et al., 1979). As the plant matures, the quantity of lignin increases, reducing the digestibility of the plant (Maynard et al., 1979).

Cellulose is a complex linear polysaccharide, consisting of glucose molecules linked together at the  $\beta$  1,4 position (Lehninger, 1975). When broken down via bacteria and protozoa through enzymatic action, cellobiose units result. Cellobiose is a two glucose unit, again

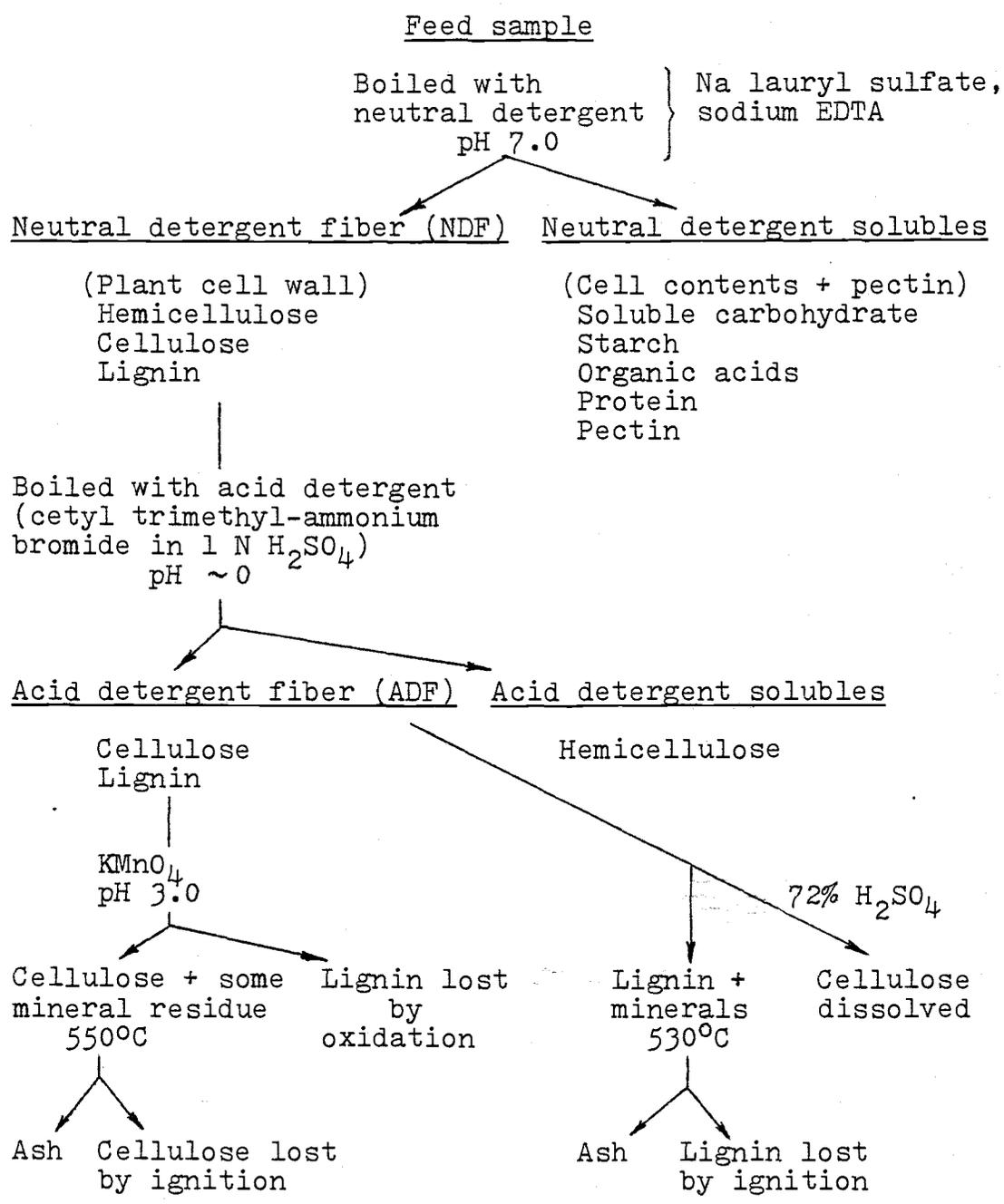


Figure 1. The Van Soest method of partitioning fiber in feeds. (Goering and Van Soest, 1975).

joined at the  $\beta$  1,4 (Lehninger, 1975). Bacterial cell-obiose breaks these units into glucose which is then metabolized by the microorganisms. VFA's are produced as a result (Hungate, 1966).

Hemicellulose refers to the parts of the plant that are insoluble in boiling water, soluble in dilute alkali, and degraded by acid. The two most prevalent hemicelluloses are xylan and polyglucuronic acid (Scott et al., 1976). These can be partially broken down by the hydrochloric acid and pepsin in the stomach (Scott et al., 1976).

As mentioned earlier, microbial enzymes are necessary to break cellulose down into digestible components. The rabbit needs these microbial organisms if it is to utilize cellulose at all. Bacteroides have been isolated in the hindgut of the rabbit (Smith, 1965), and some bacteroides are capable of cellulolytic hydrolysis (Hungate, 1966). Hall (1952) isolated a cellulolytic cocci from the rabbit's hindgut, which may also indicate fiber digesting bacteria exist in the cecum.

Although some microbial species in the rabbit may be capable of breaking down cellulose, the rabbit is not as efficient as ruminants or even other herbivores in digesting and utilizing fiber. Crude fiber digestibility of alfalfa was compared in horses, ponies, rabbits, and guinea pigs. The horse, guinea pig, and pony

digestibility coefficients for crude fiber were between 34 and 39 percent, while the rabbit digestibility coefficient was 16.2 percent (Slade and Hintz, 1969). Fannesbeck et al. (1974) compared the apparent digestibility of cellulose and hemicellulose in sheep, swine, rats, rabbits, and chickens. Fiber digestibility in the rabbit was lower than in the other species.

Poor fiber digestibility in the rabbit is also obvious when using the rabbit as a pilot animal for forage digestibility by ruminants. Watson and Bodden (1935) found that the rabbit was not a suitable animal to be used as a pilot animal for sheep. In another study the rabbit was shown to have a greater sensitivity to forages at later stages of harvest than did the sheep (Ingalls et al., 1963). They also noted that the weight gain in the rabbit was directly related to the percent of dry matter digestibility of the forage, rather than dry matter intake, as it is in sheep.

There is some debate as to the minimum level of fiber required in the rabbit diet and the maximum levels of fiber that can be used to maintain optimum growth. Several investigators have found that fiber in the diet should be considered as a requirement. Davidson and Spreadbury (1975) observed that dietary fiber levels of less than six percent fiber resulted in diarrhea in the rabbits. Heckman and Mehner (1970) found that eight to

nine percent crude fiber gave the best results for weight gain and feed efficiency in the growing rabbit. Lebas (1975a) noted diarrhea even when the crude fiber level was lower than 12 percent. Heckman and Mehner (1970) also observed that the mortality rate was highest in the five percent crude fiber groups. This agreed with Cheeke and Patton (1978) who found a higher mortality rate in the group fed diets containing 2.7 or 5.2 percent crude fiber. Franck and Coulmin (1979) fed rabbits diets containing nine or 12 percent crude fiber. Again the higher mortality rate was in the diet containing less fiber.

There is also some concern about balancing the maximum amount of fiber that can be used in the growing rabbit's diet and also maintaining optimum growth. Besidina and Perel'dik (1971) found that with every one percent increase in crude fiber in the diet there was also a 14.5 percent decrease in the digestibility of organic matter. They found that when 20 percent crude fiber was used, the digestibility of organic matter was above 70 percent, but with the addition of one percent crude fiber this fell to ten to 15 units. In another study Cheeke and Patton (1978) fed growing rabbits diets containing 0, 10, 20, 30, and 40 percent sun-cured alfalfa. They reported that the best growth, 38.6 to 44.7 grams per day (g/day), occurred with 20 percent alfalfa, containing 7.8 percent crude fiber. Even with a level of 40 percent alfalfa or

12.8 percent crude fiber the growth rate was 32.8 to 35.9 g/day.

When growing rabbits were fed two levels of straw in a diet, resulting in crude fiber levels of 9 and 12 percent the growth rates at both levels were around 34.4 g/day (Franck and Coulmin, 1979). In another study Joyce *et al.* (1971) fed rabbits pasture grass ad libitum, grass pasture ad libitum plus 2.5 percent barley, or grass pasture ad libitum plus 5 percent barley. The highest mortality rate in this study was in the grass pasture ad libitum group, due to starvation. The weight gain was the highest in the five percent barley group. The weight gain in that group was only 13.6 g/day which may be accounted for by the low energy level of the diet, giving an intake of only 279 Kcal/day.

There are several ways in which fiber digestibility may be improved. One way is to reduce the particle size of the fiber by grinding the plant. Laplace and Lebas (1977) found smaller particles to be retained longer in the hindgut, resulting in better dry matter digestibility.

Chemical treatment of fiber may be another way of improving fiber digestibility. One such treatment involves the use of sodium hydroxide. It has been found that treatments such as sodium hydroxide dissolve hemicellulose and increase the extent of cellulose and hemicellulose digestion and the rate at which they are

digested (Klopfenstein, 1978). Since lignin contents are usually not reduced via chemical treatment, the increase in digestion of hemicellulose and cellulose is probably due to the breaking of bonds between lignin and hemicellulose or cellulose, without the actual removal of the lignin (Klopfenstein, 1978). Once the solubilization occurs the cellulose and hemicellulose are then made more available for bacterial digestion (Klopfenstein, 1978).

Swick et al. (1978) treated rabbit feces with two percent sodium hydroxide. Diets containing 75 percent treated or untreated feces were then fed to rabbits. It was found that ADF digestibilities for the alkali treated versus the untreated feces were 39.4 and 8.1, respectively.

Another study showed that sodium hydroxide solubilized corn cobs and in vitro digestion of that solubilized material resulted in further digestion of the corn cobs (Waller, 1976). The in vitro digestion of barley straw treated with sodium hydroxide increased the digestibility by 22 units, and cell wall constituents were 35 percent more digestible. The pH of the feed sample increased from 6.92 to 10.35 with alkali treatment (Hartley and Jones, 1978).

#### Transit rate

The time taken by digesta to move through the gastrointestinal tract, or the rate of passage, is of

nutritional importance. The rate of passage of digesta influences the digestion and absorption of a feed. Also once the rate of passage of an animal is established the fasting period prior to a digestibility study may be determined.

The rate of fluid passage through the animal may be measured with the use of water soluble markers, such as polyethylene glycol and EDTA-Cr (Pickard and Stevens, 1972). The rate of passage of solid matter can be measured with insoluble markers such as lignin or plastic tubing. To measure the flow of both solid and liquid digesta simultaneously, such things as  $Ce^{141}$  are used (Laplace et al., 1974). All of these markers are effective only if they are not secreted, digested, or absorbed by the gut (Swenson, 1977).

With the use of markers several things have been observed about the herbivore large intestine. Elliott and Barclay-Smith (1904) observed that the large intestine was divided into three sections; (1) the proximal segment, where digesta was similar to that of the cecum and anti-peristaltic motor activity occurred (2) the intermediate section, where formed feces first appear and peristaltic movement exists (3) the distal segment which is similar to the intermediate, except it is emptied in one single movement. This anti-peristaltic movement is believed to aid in retention of microbes and water in the hindgut

(Christensen, 1971).

There have been several studies involving the digesta flow through the rabbit. Pickard and Stevens (1972) used both liquid and particulate markers and found when both of the markers were placed in the stomach, the liquid marker left the stomach and accumulated in the cecum within a few hours, while 60 percent of the particulate marker remained in the stomach at the end of 24 hours. In the same study the markers were placed in the cecum and the particulate marker left more rapidly than did the liquid marker. It was also noted that there was a continuous flow of digesta between the apex and the base of the cecum, and a periodic reflux between the cecum and proximal colon (Pickard and Stevens, 1972). Bjornhag (1972) also observed that water soluble substances and fine particles were brought back to the cecum by anti-peristaltic movement and retrograde flow, and the coarse particles went directly to the colon.

With the use of  $Ce^{141}$  Laplace et al. (1974) observed the marker in the cecum in 30 to 60 minutes, in the proximal colon after 90 minutes, and in the distal colon after 180 minutes. It was also observed that the stomach and the cecum had a constant level of Ce, 77 percent, even though the level of radioactivity in other regions varied (Laplace et al., 1975).

In a study of flow, not rate of flow of digesta, it

was found that 95 to 97 percent of the digesta moved from the small intestine to the cecum and three percent went directly to the proximal colon (Kametaker, 1967). During the same study, of the three percent of the digesta in the proximal colon, 16 to 18 percent moved back towards the cecum instead of going to the distal colon (Kametaker, 1967).

There are many factors which might influence the rate of passage of digesta. One needs to consider the type of diet, particle size of the feed, and coprophagy. Yoshida and Kandatsu (1968) found the indicator,  $\text{Cr}_2\text{O}_4$ , was excreted slower in the rabbits practicing coprophagy by about four to eight hours. Thus coprophagy allows ingesta to remain in the rabbit longer.

The physical form of a feed may not be a factor in rate of passage of digesta. Lebas and Laplace (1977) found there to be no difference in the retention time of a pelleted versus an unpelleted feed. The particle size does influence the rate of flow of digesta. Fine ground pellets were found to be retained six hours longer than coarser pellets (Laplace and Lebas, 1977).

The type of feed fed may also affect the flow rate of digesta, especially when considering fiber in the diet. Mangold and Behm (1956) fed rabbits diets containing fiber levels of 10, 20 and 30 percent. Using straw stained with fuschin as an indicator, they found the passage rate of

digesta to be the same. Another study comparing diets with 29.4 versus 14.7 percent ADF also showed no difference in the transit times of the diets (Hoover and Heitman, 1972). When comparing a cellulose free diet or a diet containing cellulose, the transit time was found to be fastest in the cellulose diet (Lebas and Laplace, 1977b).

The type of fiber used also affects the transit time. When diets containing barley straw, oak sawdust, or purified wood cellulose were compared, it was observed that the wood cellulose diet was retained the longest, while the straw containing diet was excreted first (Lebas and Laplace, 1977a). The investigators explained this difference may have been due to difference in particle size, starch content, energy content, or possibly all three.

### Feed consumption

Factors affecting feed intake are important in rabbit nutrition. Some of these are diet composition, age of the animal, and feeding practices. Fiber levels, energy content, palatability of the diet, and feed form all affect feed intake. It has been shown that as the fiber level of the diet increased and energy decreased, the consumption of the diet likewise increased until gut capacity was exceeded (Spreadbury and Davidson, 1978). In the same study rabbits fed a diet with an ADF of 3.9

percent consumed 80 g/day, while rabbits fed a diet with and ADF of 2.7 percent consumed 115 g/day (Spreadbury and Davidson, 1978). When the diets were made isocaloric the difference in feed consumption was not significant.

Feed consumption can also be affected by the form of the diet. Rabbits fed the same diet in meal or pelleted form preferred the pelleted form (King, 1974).

Palatability problems may arise when feeding rabbits. It was observed that saponin content in alfalfa meal affected the feed consumption (Cheeke et al., 1977). Rabbits preferred the high saponin alfalfa when it made up 20 percent of the diet, but when the diet contained over 35 percent alfalfa the low saponin alfalfa was preferred (Cheeke et al., 1977). Adult rabbits have been shown to prefer sucrose in the diet (Cheeke, 1974).

The time of day the rabbit is fed may also affect the feed consumption. Lebas and Laplace (1975) fed rabbits 70 or 100 g/day either in the morning or the evening. When fed in the morning the ration was not consumed completely, whereas when fed in the evening it was finished.

The age of the rabbit also determines the feed consumption. With New Zealand rabbits intake of food and water increased until 15 weeks of age, then leveled off and began to decrease by week 18 (Prud'hon et al., 1975).

### Growth rate

In rabbit production the growth rate, or the average daily gain of the rabbit, is of vital importance to the producer. The growth rate of the rabbit can be affected by such things as the diet, age of the animal, breed, environment, and many other factors.

The primary factor in weight gain is the diet. When a diet consisting of 100 percent pasture, with a crude fiber level of 31.1 percent, was fed the average daily gain was 3.8 g. With the supplementation of five percent barley the gain rose to 13.6 g per day (Joyce et al., 1979). In another study where the diet consisted of 40 percent oat husk with a crude fiber level of 23 percent, the average daily gain was 39.3 g (Spreadbury and Davidson, 1978). In the same study when 40 percent cellulose was included, with a crude fiber of 33 percent, the average daily gain was 46.5 g. With a fiber level of 11.1 percent, the average daily gain was found to be 35.3 g (Parigi-bini and Chiericato, 1974). In the same study limiting the feed consumption to 70 percent resulted in an average daily gain of 30.8 g. Another study in which rabbits were fed diets with a fiber level of 12 percent, the average daily gain was 34.4 g (Franck and Coulmin, 1979).

Cheeke and Patton (1978) found when growing rabbits were fed a diet containing no alfalfa with a crude fiber

level of 2.7 percent, the average daily gain was between 23.5 and 28.1 g. When they fed a 40 percent alfalfa diet with a crude fiber content of 12.8 percent, the average daily gain was 32.8 to 35.9 g. The highest growth rates (38.6 to 41.5 g per day) were in the group fed 7.8 percent fiber.

It was also observed that environmental temperature may affect growth rate in the rabbit. Masoero and Auxilia (1977) found as the temperature rose above the 18 to 24 C range, the average daily weight gain and average daily intake both decreased.

In an effort to improve growth rates, additives such as antibiotics have been used. Studies using virginiamycin, terramycin and aureomycin showed no growth improvement (Huang et al., 1954; King, 1974).

### Meat analysis

Meat yield and carcass quality are important concerns of rabbit production. The carcass yield and quality is dependent on the type of diet fed to the rabbit and the intake. When rabbits were fed pasture ad libitum or pasture plus five percent barley, the greater body weight was in the barley group. The dressing percentage, including the heart and the liver, was 37 percent in the ad libitum group and 51.9 percent in the group fed pasture ad libitum plus five percent barley (Kirton et al., 1971).

They also observed that the highest water content in the carcass was in the group fed pasture ad libitum.

When comparing carcass quality in a standard ration versus a standard ration plus vegetable waste ad libitum, the greater carcass yield was in the latter group (Titarev and Vojnickij, 1963). In a high fiber diet, the body fat and dry matter of the carcass was lower and the nitrogen content was higher than for the lower fiber diet (Spreadbury and Davidson, 1978). Franck (1979) found there to be no difference in dressing percentages when comparing nine percent crude fiber diet and a 12 percent crude fiber diet. Both had dressing percentages between 61.2 and 62.4 percent. Chen et al. (1978) found rabbits fed Purina Rabbit Chow and slaughtered at 8 to 12 weeks of age had dressing percentages (excluding organs) from 46.4 to 49.9 percent.

The age of the ~~animal~~ animal can also affect the carcass. As the animal increased in age the dry matter content rose, as did the meat quality of the carcass (Zupka et al., 1978). The organ weight and fat content also increased with age but the crude protein remained the same (Rao et al., 1978). Thus it has been recommended that the most economical age to slaughter was at eight weeks (Chen et al., 1978). Rao et al. (1978) also observed that the weaning age of the rabbits did not affect growth, carcass yield, or meat to bone ratios.

### III. MATERIALS AND METHODS

#### Experiment 1

The objectives of this study were to determine the effect of substituting alfalfa meal for grain in diets for growing rabbits on growth performance, feed conversions, carcass quality, and incidence of enteritis.

Eighty-five four to five week old New Zealand White rabbits were purchased locally. They were housed singly in stainless steel cages with an interior floor space of either 61 x 64 x 42 or 46 x 61 x 38 centimeters (cm). The floors of the cages consisted of wire mesh under which was a removable tray, filled with sawdust. The cages were in racks of either six or nine cages.

The cages were equipped with either automatic waterers or one liter water bottles. Both provided water ad libitum. If the rabbits failed to adjust to either of these, ceramic crocks were used instead. A stainless steel hopper-feeder was inserted in each cage door. The cage trays and water bottles were changed twice each week and the rack was sterilized once a week.

The room in which the rabbits were housed was kept in a temperature range of 17 to 18 C. The lights were controlled by an automatic timer, which turned the lights on at 7:00 a.m. and off at 7:00 p.m. The ventilation rate in the room was 10 to 20 air changes per hour.

Upon arrival at the facility the rabbits were placed in quarantine for five days. During this period the rabbits were fed Purina Rabbit Chow ad libitum. Fifty milligrams of Tetracycline Hydrochloride per kilogram of body weight was administered in the drinking water for three days.

At the end of the quarantine period the 80 healthiest rabbits were divided into eight groups with ten rabbits in each group. Rabbits in the same group were housed in close proximity to each other. Each group was fed a different diet, ad libitum. A corn-soy basal diet (Table 2) was used. Various levels of ground sun-cured alfalfa were used to replace equal amounts of corn in the basal diet. The diets contained levels of 0, 10, 20, 30, 40, 50, 60, or 74 percent alfalfa. The corn was completely replaced with alfalfa in the 74 percent alfalfa diet.

The diets were mixed in a large mixer and pelleted. The diameter of each pellet was .95 cm and the length 1.3 cm. The 0 and 10 percent alfalfa diets were refrigerated to prevent rancidity. A binder was not used in the diets, thus the 0, 10, and 20 percent alfalfa diets tended to crumble.

Prior to the study the rabbits went through a three-day adaptation period. On the first day the rabbits were fed one-fourth experimental diet and three-fourths

TABLE 2. DIET COMPOSITION (PERCENTAGE). EXPERIMENT 1

Ingredient	Percent alfalfa							
	0	10	20	30	40	50	60	74
Alfalfa meal (1-00-111) <sup>a</sup>	0	10	20	30	40	50	60	74
Ground corn (4-02-992)	69	62	54	44	34	24	14	--
Soybean meal (5-04-604)	26	23	21	21	21	21	21	21
Trace mineral salt <sup>b</sup>	.5	.5	.5	.5	.5	.5	.5	.5
Dicalcium phosphate	.5	.5	.5	.25	.25	.25	.25	.25
Limestone	.5	.5	.5	--	--	--	--	--
Molasses (4-04-696)	3	3	3	3	3	3	3	3
Corn oil	.5	.5	1.0	1.25	1.25	1.25	1.25	1.25
% of corn replaced in diet by alfalfa	0	10.1	21.7	36.2	50.7	65.2	79.7	100.0

<sup>a</sup>International reference number.

<sup>b</sup>Provides NaCl and the following mg/kg elemental levels: Zn, 17.5; Mn, 14; Fe, 8.75; Cu, 1.75; I, 0.35; and Co, 0.35.

Purina Rabbit Chow; day two, one-half experimental diet and one-half Purina Rabbit Chow; day three, three-fourths experimental diet and one-fourth Purina Rabbit Chow. On the fourth day the rabbits were fed only the experimental diets and the study began. Body weights and feed intakes were measured weekly.

Any feed spillage was weighed and recorded. When the feed was crumbly, the powdery substance was removed from the feeder and appropriate adjustments were made in the feed consumption data. Average daily gains, feed intakes and feed conversion were calculated using data only from animals that survived the full length of the study. Data were analyzed by analysis of variance and means compared by Duncan's Multiple Range Test (Barr et al., 1976).

Rabbits dying during the experiment were necropsied within 24 hours, when possible. During the external examination, the skin was checked for discoloration, hemorrhages, wounds, ulcers, tumors, and parasites. The fur condition was also observed. Internally, the thoracic cavity was examined and cultures and samples were collected. The heart, lungs, pericardial sac, spleen, liver, kidneys, stomach, cecum, diaphragm, genital organs, and bladder were also examined. Lungs, heart, kidneys, liver, and spleen were removed and tissues specimens of each were fixed in a 10 percent neutral

buffered formalin for histopathological examination later.

On the last day of the study final rabbit weights and feed intakes were recorded. The rabbits were not fasted 24 hours before the study ended. Six rabbits from each of the following groups were sacrificed for further analysis: 0, 40, and 74 percent alfalfa.

The 18 rabbits were dressed out, with the removal of all organs. The kidneys were later weighed and included in dressing percent values. The carcasses were placed in individual plastic bags and were refrigerated at 1 C for 24 hours to facilitate cutting of meat. Samples of the cecal contents were collected for measurement of pH and volatile fatty acid (VFA) content. Stomach pH's were also measured. The pH of each sample was measured immediately. The samples to be used for VFA measurements were refrigerated at 1 C for two to three hours, or until slaughter was finished.

Two hours after slaughtering a five milliliter (ml) sample was drawn from each of the cecal contents collected for VFA measurements. Each of these samples was mixed with one ml metaphosphoric acid. This mixture was allowed to stand for 30 minutes. Each mixture was then poured into individual centrifuge tubes, and centrifuged at 12,500 rpm for 20 minutes. The supernatant in each

tube was poured into individual containers and frozen at -15 to -20 C for five months prior to VFA determination.

Twenty-four hours after slaughter, the carcasses were weighed. Each carcass was sawn in half, from neck to tail through the spinal column. The left half was cut into three pieces: the loin, rack, and leg. The rack consisted of the arm and rib area combined. The pieces were frozen until lab analysis could be done.

Laboratory analysis: The leg of each animal was allowed to thaw slightly and the meat was removed from the bone with a knife. The meat was chopped into pieces 1 cm x 1 cm. The pieces were placed in a mesh, copper basket and lowered into liquid nitrogen for approximately ten seconds, or until solidly frozen. The frozen pieces were then put into a Waring blender and ground until a powdery consistency was achieved.

The ground meat samples were freeze dried for 48 hours at 50 millimeters (mm) Hg. Until analysis for crude protein and fat could be done, the dried samples were put in sealed plastic containers and kept in a large desiccator.

The meat was measured for protein content, using the AOAC (1970), macro kjeldahl method for crude protein determination. The fat content was measured via ether extraction as recommended by AOAC (1970).

Samples of each diet were ground through a .4 mm screen. The samples were analyzed for crude protein content using a modified version of the micro-kjeldahl as recommended by AOAC (1970). Each sample was also analyzed for fiber content using a micro-method for acid detergent fiber determination (Waldern, 1971).

The samples for VFA determination were thawed at room temperature for one to two hours. The instrument used for VFA analysis was a Varian Aerograph Series 1200 gas chromatograph. It was equipped with a hydrogen flame detector. The column in the gas chromatograph was stainless steel and was 5 feet x 1/8 inch, and was packed with Chromosorb 101. The integrator was a Spectral-Physics Autolab Minigrator. The recorder was a Sargent SRG. The nitrogen flow rate was 25 ml per minute and the hydrogen flow rate was 25 ml per minute. The temperatures of the injector, detector, and column were 88 C, 115 C, and 82 C, respectively. The paper speed in the recorder was 1.27 cm per minute. Each sample was injected with 10 ml Hamilton micro syringe, which was then thoroughly rinsed. VFA's were calculated using the following equation:

$$\frac{\text{Area of unknown}}{\text{Area of standard}} \times \mu \text{ mole/ml standard} = \mu \text{ mole/ml unknown.}$$

The area of the unknown and standard was the area of the VFA peak calculated by the chromatograph integrator. The standard was the average of two trial runs, where a known amount of each VFA was injected into the chromatograph.

## Experiment 2

The objective of this study was to determine effects of substituting alfalfa meal for corn or barley in diets for growing rabbits on growth performance, feed conversion, and enteritis.

Ninety four to five week old New Zealand White rabbits were purchased locally. Unlike the first study, however, the rabbits were arranged so that rabbits of the same group were not housed close together. The quarantine period, antibiotic treatment and necropsy procedures were the same as for experiment 1.

The rabbits also went through an adaptation period. Eighty of the healthiest rabbits were chosen and divided into eight groups with ten rabbits in each group. The diet composition was identical to that of experiment 1, except barley was directly substituted for corn in some of the diets, thus sun-cured alfalfa directly replaced either corn or barley. Group 1 was fed a corn based diet with 20 percent alfalfa; group 2, a barley based diet with 20 percent alfalfa; group 3, a corn based diet with 30 percent alfalfa; group 4, a barley based diet with 30 percent alfalfa; group 5, a corn based diet with 50 percent alfalfa; group 6, a barley based diet with 50 percent alfalfa; group 7, no grain and 74 percent alfalfa; group 8, no grain, 74 percent alfalfa and Rabbit Flavor F-203 (Agrimerica, Inc., Northbrook, IL)

at 1.1 g of flavor per kg of diet (Table 3). The adaptation procedure was identical to the one followed in experiment 1.

On the first day of the study all rabbits were weighed. They were weighed weekly thereafter. Feed was given ad libitum and feed consumption was recorded at least once a week. Feed spillage was recorded and adjustments in feed consumption data were made accordingly. When large quantities of spilled feed were urinated on, it was collected and oven dried at 100 C for 24 hours, then weighed.

Feed intake, feed consumption, and average daily gain data were analyzed as in experiment 1.

At the end of the study final weights and feed consumption were recorded. The rabbits were then sent to market.

Lab analysis: Feed samples, which had been collected from each batch of diet mixed, were analyzed for fiber and protein content. The micro methods for acid detergent fiber and crude protein determination were used as described in experiment 1.

### Experiment 3

The objective of this experiment was to determine the response of growing rabbits to greens substituted for

TABLE 3. DIET COMPOSITION (PERCENTAGE). EXPERIMENT 2

Ingredient	Percent alfalfa with corn or barley							
	20	20	30	30	50	50	74	74
Alfalfa (1-00-111) <sup>a</sup>	20	20	30	30	50	50	74	74
Molasses (4-04-696)	3	3	3	3	3	3	3	3
Trace mineral salt <sup>b</sup>	.5	.5	.5	.5	.5	.5	.5	.5
Dicalcium phosphate	.25	.25	.25	.25	.25	.25	.25	.25
Limestone	.25	.25	---	---	---	---	---	---
Corn oil	1.0	1.0	1.25	1.25	1.25	1.25	1.25	1.25
Ground corn (4-02-992)	54	---	44	---	24	---	---	---
Ground barley (4-07-939)	---	54	---	44	---	24	---	---
Soybean meal (5-04-604)	21	21	21	21	21	21	21	21
Feed flavor	---	---	---	---	---	---	---	.11

<sup>a</sup>International reference number.

<sup>b</sup>Provides NaCl and the following mg/kg elemental levels: Zn, 17.5; Mn, 14; Fe, 8.75; Cu, 1.75; I, 0.35; and Co, 0.35.

<sup>c</sup>Flavor F-203 (Agrimerica, Inc., Northbrook, IL).

a pelleted diet.

Seventy four to five week old New Zealand White rabbits were purchased locally. The housing and management were identical to those used in experiment 1, except rabbits in the same group were not housed close together. Rabbits dying during the study were examined with the procedures outlined in experiment 1.

The rabbits were quarantined for five days, during which time they were fed Purina Rabbit Chow ad libitum. They were also administered five g of Nitrofurazone per gallon of drinking water (of 4.59 percent active ingredients). There was no adaptation period prior to the beginning of the study.

On the first day of the study 60 of the healthiest rabbits were chosen. They were divided into six groups with ten rabbits in each group. Group 1 was fed Purina Rabbit Chow ad libitum; group 2, ad libitum Purina Rabbit and greens; group 3, 75 g of Purina Rabbit Chow and greens ad libitum; group 4, 50 g of Purina Rabbit Chow and greens ad libitum; group 5, 25 g of Purina Rabbit Chow and greens ad libitum; and group 6, greens only.

The greens consisted of such things as grape and bean vines, all parts of the corn plant, dandelions, red clover, lettuce, cabbage, cauliflower, green peppers, radishes, carrots, celery, cucumbers, parsley, bean

sprouts, comfrey, amaranthus and turnips. The largest portion of the greens consisted of lettuce, cabbage, and red clover.

The greens were fed fresh daily in the late morning and in the evening. The Purina Rabbit Chow was given during the morning feeding. All debris accumulated in the cages was removed daily.

The rabbits were weighed once a week. The rabbits fed Purina Rabbit Chow ad libitum had their feed consumption recorded weekly, while the rabbits with limited Purina Rabbit Chow had theirs recorded daily. Calculations and analysis of data were done following procedures given in experiment 1.

At the end of the study final weights and feed consumption data were recorded. Groups 1, 3 and 6 were kept on the study for another week. They were fasted for 24 hours and six rabbits from each of these groups were sacrificed. The carcasses were refrigerated for 24 hours. The carcasses were then cut in half, vertically, down the spine. The left half was then divided into the leg, loin, and rack.

Lab analysis: The leg was prepared for lab analysis following the procedures outlined in experiment 1. After the meat was freeze dried, it was analyzed for fat and crude protein, again following procedures used in experiment 1.

#### Experiment 4

The objective of this study was to determine whether alkali treatment of fibrous feeds increased the fiber utilization in the rabbit.

Six full grown New Zealand White rabbits were confined to metabolism cages manufactured by Hoeltge Company. They were housed in separate aluminum cages which were part of a six cage unit. Each cage had an interior floor space of 46 x 64 x 36 cm. The floors consisted of wire, under which was a large funnel shaped apparatus, designed to catch all feces. At the bottom of the funnel was a rectangular metal cone, which allowed urine, but not feces, to pass around its edges. The urine was then collected in one liter jars located underneath the cages. Housing and management were identical to that in experiment 1.

Rabbits were fed various levels of fibrous feeds, treated or untreated with sodium hydroxide. Composition of the basal diet is given in Table 4. The fibrous feeds were substituted for corn in the basal diet. The diets fed were as follows: (a) untreated sun-cured alfalfa, 20 percent; (b) sodium hydroxide treated sun-cured alfalfa, 20 percent; (c) untreated sun-cured alfalfa, 40 percent; (d) treated sun-cured alfalfa, 40 percent; (e) untreated sun-cured alfalfa, 60 percent; (f) untreated sun-cured

TABLE 4. DIET COMPOSITION (PERCENTAGE)  
EXPERIMENT 4

Ingredient	Percent in diet
Ground corn <sup>a</sup> (4-02-992) <sup>b</sup>	84.5
Soybean meal (5-04-604)	10.0
Dicalcium phosphate	1.0
Vegetable oil	4.0
Salt	.5

<sup>a</sup>Alfalfa, millrun, and corn cobs were put in diet in place of corn.

<sup>b</sup>International reference number.

alfalfa, 60 percent; (g) untreated millrun, 40 percent; (h) treated millrun, 40 percent; (i) untreated ground corn cobs, 40 percent; (j) treated ground corn cobs, 40 percent. The feeding period was for five days. Prior to and after each trial, the animals were fasted for one day.

During the first week, three animals were on diet (a) and three were on diet (b). During the second week the reverse was true. During week 3, three animals were fed diet (c) and three were fed diet (d). During week 4, the reverse was true. This pattern was continued throughout the study. The feeds and water were given ad libitum. The feed intake was recorded daily. The feces were collected daily and stored in the refrigerator for five to seven days. They were then frozen at -10 to -20 C until lab analysis was done.

The fibrous feeds were treated with two percent sodium hydroxide in a ratio of three kg of meal to four liters of two percent sodium hydroxide solution. The feed was mixed until all of the meal was moist and covered with aluminum foil and left at room temperature for 24 hours. The treated feed was dried in aluminum pans at 55 C and stirred every five to six hours to prevent mold growth. The treated feeds were mixed with other ingredients in the diets following procedures outlined in experiment 1.

Lab analysis: The feces and feed samples were dried at 100 C for 24 hours and moisture contents were calculated. The dried feces and feeds were ground through a .44 mm screen and placed in sealed containers. Prior to lab analysis the samples were redried to ensure no moisture accumulation during storage. All feeds were analyzed for ADF and crude protein. All feces were analyzed for ADF content. The 40 percent millrun feces and feed were also analyzed for crude protein, cell wall constituents (CWC), and energy. The ADF determination was performed using the micro method (Waldern, 1971). The micro-kjeldahl method was used for protein determination (AOAC, 1970). The CWC in the feces were determined by procedures outlined by Van Soest and Marcus (1964). The feed CWC's were analyzed using amylase digestion to remove starch (McQueen, 1979). The gross energy of the diet and feces was measured using a Parr Adiabatic Calorimeter, Model 1241.

## III. RESULTS AND DISCUSSION

Experiment 1

Average daily gains of the 0 percent group were significantly lower than those of the 10 percent and 50 percent alfalfa groups ( $P < .05$ ). The 10 percent alfalfa group had the highest daily gain of 44 g and the 0 percent alfalfa group the lowest, 31.4 g (Table 5). The remaining groups with 20, 30, 40, 60, or 74 percent alfalfa were not significantly different from each other or from the 0, 10, and 50 percent groups. ADF in the 20, 30, 40, 60, and 74 percent alfalfa diets ranged from 6.6 to 24.5 percent (Table 6), yet the average daily gains were not significantly different from each other. Cheeke and Patton (1978) also observed no significant differences in weight gains when rabbits were fed alfalfa diets containing 2.7 or 12.8 percent crude fiber.

This indicated that an increase in dietary fiber did not lower the growth rates. One rabbit in the 0 percent group had an average daily gain of 8.8 g. This partially accounted for the lower average daily gain in that group.

The average daily feed intake tended to increase as the alfalfa levels or fiber levels in the diet increased (Table 5). The lowest intake was in the 0 percent alfalfa group. The 10, 20, 30, and 40 percent alfalfa

TABLE 5. PERFORMANCE OF RABBITS. EXPERIMENT 1

Treatment	Daily gain (g)	Daily feed intake (g)	Feed Conversion	Mortality	Daily DE intake (Kcal) <sup>d</sup>
0% alfalfa	31.4 <sup>b</sup> ±3.9	84.2 <sup>d</sup> ±4.8	3.2 <sup>abc</sup> ±.6	2	309.7
10% alfalfa	44.0 <sup>a</sup> ±1.8	107.5 <sup>c</sup> ±5.3	2.4 <sup>c</sup> ±.05	2	374.3
20% alfalfa	36.6 <sup>ab</sup> ±3.8	105.3 <sup>c</sup> ±6.4	3.0 <sup>bc</sup> ±.2	1	350.3
30% alfalfa	40.1 <sup>ab</sup> ±.3	110.4 <sup>c</sup> ±6.5	2.8 <sup>bc</sup> ±.2	7	348.9
40% alfalfa	36.4 <sup>ab</sup> ±2.3	115.8 <sup>c</sup> ±6.2	3.2 <sup>abc</sup> ±.1	1	344.4
50% alfalfa	41.1 <sup>a</sup> ±1.2	130.9 <sup>b</sup> ±3.1	3.2 <sup>abc</sup> ±.1	1	364.9
60% alfalfa	37.3 <sup>ab</sup> ±2.2	134.3 <sup>ab</sup> ±5.5	3.6 <sup>ab</sup> ±.2	1	349.4
74% alfalfa	38.2 <sup>ab</sup> ±1.7	147.6 <sup>a</sup> ±4.1	3.9 <sup>a</sup> ±.2	0	345.7

a, b, c Means for each performance trait with different superscripts significantly different (P<.05).

<sup>d</sup> Calculated using digestible energy values in Table 6.

TABLE 6. DIET ANALYSIS. EXPERIMENT 1

Percent alfalfa	Crude protein (%)	Acid detergent fiber (%)	Digestible <sup>a</sup> energy (Kcal/kg)
0	17.4	3.8	3678
10	16.4	6.6	3482
20	17.2	9.4	3327
30	17.6	13.7	3160
40	17.8	15.7	2974
50	18.1	19.1	2788
60	20.7	20.1	2602
74	21.5	24.5	2342

<sup>a</sup>Calculated assuming 1800, 3660, 2500, 4000, 7600 Kcal/kg for alfalfa, corn, molasses, soybean meal, and corn oil, respectively.

diets had a significant increase in intake when compared to the 0 percent diet but were not significantly different from each other. The average daily intakes were highest in the 50, 60, and 74 percent groups.

This pattern of feed intake would be expected, since the dietary energy level was reduced as the percent dietary alfalfa increased (Table 6). Lebas (1975b) recommended growing rabbits be fed 2500 kcal per kg of diet. The amount of diet consumed also needs to be considered. Although several of the high alfalfa diets in this study fell short of 2500 kcal/kg of diet, the average daily intake of digestible energy remained the same as the lower fiber, higher energy diets due to increased intake (Table 6). This data demonstrated that rabbits adjust their feed intake to meet their energy requirements.

Feed conversion, or grams of feed required per gram of gain, in groups with 0, 10, 20, 30, 40, or 50 percent alfalfa was not significantly different for any group. (Table 5). The 60 percent alfalfa group had a higher average feed conversion than the 10 percent group, but was not significantly different from any other group. The 74 percent alfalfa group had a higher feed conversion than the 10, 20, and 30 percent alfalfa groups. Although more feed was required in the higher alfalfa diets to achieve similar weight gains than in the lower alfalfa groups, less corn was also used in the higher fiber diets.

According to these results a more expensive feedstuff, such as corn could be replaced with alfalfa without significantly reducing weight gains.

For the 0, 40, and 74 percent alfalfa groups, the dressing percentages (dressed weight  $\div$  live weight) were not significantly different (Table 7). The dressing percentages included the kidney weights. There was some concern that the live weight gains recorded in the higher fiber groups may have included a significantly higher gut weight, due to an increased gut capacity than was present in the lower fiber groups. The dressing percentages, however, indicated this did not occur since there were no significant differences in dressing percentages.

The crude protein content of the meat was similar in all three groups with the highest value for the groups fed 74 percent alfalfa. The fat content of the meat, calculated via ether extract, was significantly lower ( $P < .05$ ) in the 74 percent group, but there was no significant difference between the 0 and 40 percent alfalfa groups (Table 7). This higher nitrogen and lower fat content of the carcasses in the highest fiber diet confirmed Spreadbury and Davidson's (1978) findings. The higher protein, lower fat content of the rabbits fed high fiber may have important market appeal.

TABLE 7. MEAT ANALYSIS. EXPERIMENT 1

Treatment	Dressing (%)	Crude protein (%)	Ether Extract (%)
0% alfalfa	48.4 <sup>a</sup> ±1.3	73.3 <sup>a</sup> ±2.0	17.9 <sup>a</sup> ±1.7
40% alfalfa	48.1 <sup>a</sup> ±.8	72.0 <sup>a</sup> ±1.6	17.9 <sup>a</sup> ±.8
74% alfalfa	49.2 <sup>a</sup> ±.4	76.4 <sup>a</sup> ±2.0	11.0 <sup>b</sup> ±.8

a, b Means for each trait with different superscripts significantly different ( $P < .05$ ).

Acetic acid was significantly lower ( $P < .05$ ) in the group fed 74 percent alfalfa (Table 8). There were two rabbits in this group with very low acetate values (5 and 10 micromoles/ml) which could have accounted for the lower mean acetate values in that group. A significant difference was not observed between the propionic and butyric levels in any group. These findings disagreed with those of Hoover and Heitman (1972) who found butyric levels to be higher in the higher fiber diet and those of Maynard (1970) who reported a greater proportion of propionic acid in higher grain diets.

When comparing the volume of all three acids, acetic was found to have the highest volume which confirmed earlier observations (Barcroft et al., 1944; Elsdon et al., 1946; Henning and Hird, 1972; and McMillan et al., 1975). The ratio of propionic to butyric was approximately one to one, which disagreed with the observations of those workers previously mentioned.

There was a large variation in the butyric acid levels in all rabbits (Table 8). The group fed 0 percent alfalfa also showed a large variation in propionic acid production. These variations in acid production could be due to several factors: (1) the animals had eaten different quantities and at different times (2) the flora in the cecum was varied (3) the rate of conversion of acetic acid to butyric may have varied.

TABLE 8. VOLATILE FATTY ACID AND pH MEASUREMENTS. EXPERIMENT 1

Treatment	Acetic μmoles/ml	Propionic μmoles/ml	Butyric μmoles/ml	Cecum pH	Stomach pH
0% alfalfa	36.3 <sup>a</sup> ±2.8	5.7 <sup>c</sup> ±1.0	13.6 <sup>c</sup> ±2.8	6.32	2.42
40% alfalfa	39.7 <sup>a</sup> ±3.8	5.1 <sup>c</sup> ± .6	13.0 <sup>c</sup> ±1.1	6.04	2.35
74% alfalfa	24.0 <sup>b</sup> ±6.6	4.3 <sup>c</sup> ± .9	6.3 <sup>c</sup> ±1.6	6.04	2.28

a, b, c Means for each trait with different superscripts significantly different (P<.05).

Average pH of the cecum and stomach was almost identical for all groups (Table 8). The stomach pH was much more acidic than those pH's observed in other herbivores. This agreed with the observations of Smith (1965).

There was at least one death recorded in every group with the exception of the 74 percent alfalfa group where there was no mortality (Table 5). The groups fed diets containing 0 and 10 percent alfalfa with ADF levels of 3.8 and 6.6 percent, respectively had the highest mortality rates, excluding the 30 percent group. This agreed with Davidson and Spreadbury (1975), who observed that with less than six percent fiber, diarrhea occurred. Cheeke and Patton (1978) also observed higher mortality in groups fed 2.7 or 5.2 percent crude fiber.

This indicated that a higher fiber diet may have actually been beneficial in preventing mortality. The 30 percent alfalfa group lost seven rabbits. As mentioned earlier, rabbits of the same group were housed in close proximity to one another. The deaths may have been disease related, yet the diagnosis for cause of death was different for all but two rabbits. In a later study the same 30 percent alfalfa diet was fed to a group of ten rabbits, and only one death was reported. The studies were identical, with the exception of housing. The rabbits of the same group were not housed close together.

All rabbits dying during the entire study had various diagnoses. Several rabbits were diagnosed as having enteritis complex. The symptoms were stomach filled with water and gas and the small intestine contained a thick brown mucus, and thin brownish colored fluid in the abdominal cavity. Other rabbits had nephritis, pneumonia, impaction, or enterotoxemia. Enterotoxemia was characterized by such things as a positive result for a toxin produced by Cl. perfringens Type E, small intestine filled with gas and water, and a cecum distended with gas and fluid. Patton et al. (1978) also observed the presence of the toxin produced by Cl. perfringens Type E in incidences of enterotoxemia.

### Experiment 2

The average daily gains in groups fed 20 percent alfalfa and barley and 30 percent alfalfa and barley or corn were significantly ( $P < .05$ ) higher than average daily gains in both 74 percent alfalfa groups (Table 9).

Spreadbury and Davidson (1978) also found when comparing diets containing either 14 or 24 percent crude fiber that the 14 percent diet resulted in higher weight gains. The average daily gains in groups fed 20 percent alfalfa and corn or 50 percent alfalfa and corn or barley were not significantly different from average daily gains of any of the other groups but were closer in

TABLE 9. PERFORMANCE OF RABBITS. EXPERIMENT 2

Group	Treatment	Daily gain (g)	Daily feed intake (g)	Feed Conversion	Mortality	Daily DE intake (Kcal) <sup>d</sup>
1	20% alfalfa + corn	40.7 <sup>ab</sup> ± 2.1	111.1 <sup>b</sup> ± 5.6	2.8 <sup>c</sup> ± .1	2	369.6
2	20% alfalfa + barley	43.0 <sup>a</sup> ± 1.7	116.8 <sup>b</sup> ± 8.4	2.7 <sup>c</sup> ± .1	1	350.0
3	30% alfalfa + corn	42.3 <sup>a</sup> ± 1.5	112.6 <sup>b</sup> ± 4.7	2.6 <sup>c</sup> ± .1	1	355.8
4	30% alfalfa + barley	43.5 <sup>a</sup> ± 1.7	125.6 <sup>ab</sup> ± 3.3	2.9 <sup>c</sup> ± .05	1	355.4
5	50% alfalfa + corn	40.0 <sup>ab</sup> ± 3.0	129.5 <sup>ab</sup> ± 9.1	3.3 <sup>b</sup> ± .1	2	361.0
6	50% alfalfa + barley	39.1 <sup>ab</sup> ± 1.2	129.9 <sup>ab</sup> ± 4.4	3.3 <sup>b</sup> ± .1	2	319.3
7	74% alfalfa	35.8 <sup>b</sup> ± 1.4	138.0 <sup>a</sup> ± 4.8	3.9 <sup>a</sup> ± .1	0	323.2
8	74% alfalfa + flavor	36.0 <sup>b</sup> ± 2.0	138.6 <sup>a</sup> ± 3.3	3.9 <sup>a</sup> ± .2	0	324.6

<sup>a, b, c</sup> Means for each performance trait with different superscripts significantly different (P < .05).

<sup>d</sup> Calculated using digestible energy values in Table 10.

value to each other. When the groups fed the same levels of alfalfa, but substituting either corn or barley, were compared, it was found there was no significant difference in average daily gains. There was also no significant difference in average daily gains between the 74 percent alfalfa group with or without flavoring.

The average daily intakes tended to increase as the alfalfa level in the diets increased (Table 9). The 74% percent alfalfa groups had significantly higher ( $P < .05$ ) feed intakes than did the 20 percent alfalfa and corn or barley and 30 percent alfalfa and corn groups (Table 9). The increase in feed intake with an increase in alfalfa was due to the decrease in energy in the higher fiber diets (Table 10). The increase in feed intake was enough to ensure approximately the same average daily digestible energy intake (Table 9) as was true in experiment 1. There were no significant differences in intakes when diets with the same level of alfalfa, but with either corn or barley, were compared.

The feed conversion was better ( $P < .05$ ) in the 20 percent and 30 percent alfalfa levels with corn or barley. The poorest feed conversions were in the 74 percent alfalfa groups (Table 9).

This study again showed that an increase in fiber content (Table 6) in the diet up to 50 percent alfalfa with 19 percent ADF did not significantly decrease average

TABLE 10. DIET ANALYSIS. EXPERIMENT 2

Treatment	Crude protein (%)	Acid detergent fiber (%)	Digestible <sup>a</sup> energy (Kcal/kg)
20% alfalfa + corn	16.8	9.5	3327
20% alfalfa + barley	18.3	10.9	2997
30% alfalfa + corn	17.5	12.6	3160
30% alfalfa + barley	18.6	13.2	2830
50% alfalfa + corn	19.4	19.7	2788
50% alfalfa + barley	19.7	18.6	2458
74% alfalfa	21.2	24.8	2342
74% alfalfa + flavor	20.7	25.3	2342

<sup>a</sup>Calculated assuming 1800, 3660, 2500, 4000, 7600, and 3330 Kcal/kg for alfalfa, corn, molasses, soybean meal, corn oil and barley, respectively.

in the no-till treatment apparently completely stopped. However, in the bare and stubble fallow treatments there continued to be a net increase in nitrate-nitrogen. Corresponding with this same period was a rapid decrease in surface soil moisture. The reason for the large difference between the no-till and the other treatments may be due to the accumulation of organic materials close to the soil surface in the former and the mixing in the lower surface due to tillage in the latter two fallow treatments. Although the bare and stubble fallow treatments showed significant increases in nitrate-nitrogen, the bare fallow treatment had a significantly higher accumulation during this period. This is probably the result of a higher moisture content in the zone of organic material accumulation because of the moldboard plow action of burying the residues. The bare, stubble and no-till treatments accumulated 6.42, 4.03 and -0.75 ppm nitrate-nitrogen, respectively, during this period. These results show again the effects of different tillage systems in the time and manner of organic matter mineralization.

A rapid increase of nitrification due to rewetting of the surface soil occurred in the no-till and stubble mulch treatments after the September rains. The lack of a similar increase in the bare fallow treatment could be due to the low organic matter content near to the soil surface since the precipitation only rewet the upper (0-15 cm) portion.

By contrast, in the 15-30 cm soil profile, there were no significant differences in nitrate-nitrogen, either in treatments or sampling dates through the July 3 period. There were steady increases in nitrate-nitrogen throughout this period, but were not large enough to be significant. However, between the July 3 and 19 sampling periods

the rabbits were positive for Rotavirus. Patton et al. (1978) also found the presence of the toxin produced by Cl. perfringens Type E in rabbits with enterotoxemia. Another rabbit was diagnosed as having fecal necrotizing cecitis. One rabbit was too autolyzed to be examined.

### Experiment 3

There were no significant differences in weight gains when comparing the ad libitum pelleted diet, ad libitum pellets and greens, 75 g pellets and greens ad libitum, and 50 g pellets and greens ad libitum groups (Table 11). The weight gain in the 100 percent greens group was significantly lower than all other groups, except the 25 g pellets and greens ad libitum group. The growth results indicated that supplementation with greens did not adversely affect weight gains, except when less than 50 g of pellets was available to the animal. These results disagreed with those of Kirton et al. (1971), who found weight gains in the group fed 100 percent pasture to be less than half of that in the group fed pasture and five percent barley.

The ad libitum pellet group had the highest feed intake (Table 11). When greens were added to the ad libitum pelleted diet the pellet intake decreased significantly. Limiting the pellet intake to 75 g a day resulted in an actual pellet intake of 66.6 g per day

TABLE 11. PERFORMANCE OF RABBITS. EXPERIMENT 3

Group	Treatment	Daily gain (g)	Daily pellet intake (g)	Feed Conversion	Mortality
1	<u>Ad libitum</u> pellets	36.8 <sup>a</sup> ±1.9	127.0 <sup>a</sup> ±6.2	3.4 <sup>a</sup> ±.04	1
2	<u>Ad libitum</u> pellets and greens	35.4 <sup>a</sup> ±3.8	79.7 <sup>b</sup> ±6.2	2.6 <sup>b</sup> ±.4	1
3	75 g pellets and greens	37.2 <sup>a</sup> ±1.6	66.6 <sup>c</sup> ±2.0	1.8 <sup>c</sup> ±.1	2
4	50 g pellets and greens	31.4 <sup>ab</sup> ±1.4	46.8 <sup>d</sup> ±.8	1.4 <sup>cd</sup> ±.09	1
5	25 g pellets and greens	26.0 <sup>bc</sup> ±1.0	24.9 <sup>e</sup> ±.1	1.0 <sup>d</sup> ±.05	2
6	<u>ad libitum</u> greens	25.2 <sup>c</sup> ±1.1	0 <sup>f</sup>	--- <sup>g</sup>	1

a,b,c,d,e,f Means for each performance trait with different superscripts significantly different (P<.05).

<sup>g</sup>No feed conversion calculated for this group since only greens were eaten.

Table 16. Nitrogen mineralization potential by depth for three fallow tillage treatments in 1980.

Treatments	Depth	Nitrogen Mineralization Potential			
		Rep. 1	Rep. 2	Rep. 3	Mean
	cm	----- ppm -----			
Bare Fallow	0-15	68.98	59.78	62.42	63.74
	15-30	33.87	49.45	45.48	42.93
Stubble Mulch	0-15	70.21	72.80	67.61	70.21
	15-30	46.85	31.15	59.82	45.94
No-Till	0-15	75.40	45.48	59.82	60.23
	15-30	41.66	49.45	39.06	43.39

TABLE 12. MEAT ANALYSIS. EXPERIMENT 3

Group	Treatment	Dressing %	Crude Protein %	Ether Extract %
1	<u>Ad libitum</u> pellets	46.9 <sup>a</sup> ±.9	77.3 <sup>c</sup> ±1.6	14.1 <sup>a</sup> ±1.4
3	75 g pellets	47.7 <sup>a</sup> ±.6	81.2 <sup>b</sup> ± .9	11.3 <sup>a</sup> ±1.2
6	<u>Ad libitum</u> greens	46.0 <sup>a</sup> ±1.4	85.9 <sup>a</sup> ± .8	4.9 <sup>b</sup> ± .6

a, b, c Means for each trait with different superscripts significantly different (P<.05).

vegetable wastes ad libitum, which again disagreed with these results. The dressing percentages observed in this study agreed with the dressing percentages (46 to 49 percent) of rabbits 8 to 12 weeks of age fed 100 percent Purina Rabbit Chow (Chen et al., 1978).

The results indicated that the carcass quality in the 75 g pellets plus greens groups was competitive with the ad libitum pellet group.

There was at least one death in each group (Table 11). The rabbit which died in the 100 percent greens group died two days after the study began, which probably indicated that it was sick before the study began. Most of the rabbits were diagnosed as having enterotoxemia with symptoms such as small intestine filled with watery green substance, cecum filled with brown watery substance, petechial hemorrhages in the cecum, and positive results for toxin.

#### Experiment 4

The digestibility of fiber was maximal ( $P < .05$ ) in the group fed sodium hydroxide treated millrun (Table 13). Even without the sodium hydroxide treatment, the fiber digestibility in the millrun fed group was significantly higher than for the diets containing alfalfa or corn cobs. Millrun was the only feedstuff which showed an increase in fiber digestibility when treated with sodium hydroxide.

TABLE 13. DIGESTIBILITY AND DIET ANALYSIS. EXPERIMENT 4

Treatment	% ADF digestibility	% ADF in diet	% CP in diet	Gross Energy in diet (Kcal)	% CWC in diet
20% alfalfa untreated	33.6 <sup>c</sup> ±2.4	12.0			
20% alfalfa NaOH treated	25.4 <sup>cde</sup> ±5.2	12.0			
40% alfalfa untreated	26.2 <sup>cd</sup> ±1.3	18.2			
40% alfalfa NaOH treated	27.8 <sup>cd</sup> ±1.0	19.8			
60% alfalfa untreated	16.8 <sup>e</sup> ±2.3	24.1			
60% alfalfa NaOH treated	21.8 <sup>de</sup> ±.6	26.8			
40% corn cobs untreated	21.6 <sup>de</sup> ±3.9	23.2			
40% corn cobs NaOH treated	18.6 <sup>de</sup> ±2.2	24.1			
40% millrun untreated	47.1 <sup>b</sup> ±2.1	9.4	16.8	4383	17.9
40% millrun NaOH treated	66.7 <sup>a</sup> ±4.1	13.0	17.2	4329	30.2

a, b, c, d, e ADF digestibility means with different superscripts significantly different (P<.05).

The digestibility of fiber decreased as the untreated alfalfa increased from 20 to 60 percent in the diet (Table 13). The fiber in the untreated 40 percent alfalfa diet was as digestible as that in the untreated 20 percent alfalfa diet. The treated and untreated corn cob diets had significantly lower fiber digestibilities when compared to the untreated 20 percent alfalfa group. The fiber digestibilities in all of the sodium hydroxide treated feeds, except millrun, were not significantly different from each other. With the exception of the millrun, these results disagreed with Swick et al. (1978) who found fiber digestibility to increase when feces were treated with sodium hydroxide.

The 40 percent sodium hydroxide treated corn cob or alfalfa diets had a one percent higher ADF value than the untreated corn cob or 40 percent alfalfa diets (Table 13). The 60 percent treated alfalfa diet had an ADF value 2.7 percent higher than the 60 percent untreated diet, while the treated millrun had an ADF that was 3.6 percent higher than the untreated millrun. It is possible that the sodium hydroxide may have formed an insoluble complex with some compound in the feedstuffs resulting in higher ADF values in the treated groups.

The digestibility of crude protein, CWC, and energy was also calculated in the millrun groups since the fiber digestibility was significantly higher in these

groups. The crude protein and energy digestibilities in the sodium hydroxide treated millrun were not significantly different ( $P < .05$ ) from the untreated group (Table 14). There was a significant increase in the CWC digestibility in the treated millrun group.

When CWCs were done on both millrun diets, the treated millrun resulted in a CWC value approximately two times greater than the untreated diet (Table 13). This higher CWC value could have accounted for the higher digestibility in that group. Again the higher CWC value may have been an artifact due to an insoluble complex occurring between the sodium hydroxide and some component of the feedstuff. Since the fiber and CWC were more digestible in the treated millrun, the digestible energy should have also increased in this diet, which it did not. This supports the idea that there was a reaction which occurred between the sodium hydroxide and some constituent of the millrun causing spurious high values for the ADF and CWC of the treated millrun.

There was a tendency for mold growth to occur in the sodium hydroxide treated feeds. To alleviate this problem, the treated feedstuffs had to be stirred several times during the drying process which required extra labor. There was also a feed spillage problem in both of the groups fed corn cobs. The ground corn cobs were coarse, which probably decreased palatability.

TABLE 14. MILLRUN DIGESTIBILITY. EXPERIMENT 4

Treatment	% Digestibility			
	ADF	CP	CWC	Energy
40% millrun untreated	47.1 <sup>b</sup> ±2.1	82.4 <sup>a</sup> ±1.0	30.5 <sup>b</sup> ±2.6	81.8 <sup>a</sup> ±.9
40% millrun NaOH treated	66.7 <sup>a</sup> ±4.1	79.8 <sup>a</sup> ±2.6	62.2 <sup>a</sup> ±2.6	82.4 <sup>a</sup> ±1.6

a,b Means for each measure with different superscripts significantly different (P<.05).

## V. CONCLUSIONS

This research indicates alfalfa can directly replace up to 79.7 percent of corn in the diet without adversely affecting weight gain and dressing percentages. At this level of alfalfa, 60 percent, the ADF level was 20.1 percent. Even when alfalfa replaced barley there was not any significant difference in growth rates in diets with 20, 30, or 50 percent alfalfa with ADF values of 9.5 to 18.6. Both experiments showed that a balanced diet containing 50 to 60 percent alfalfa could actually have the same growth results as a more costly high grain diet, resulting in lower feed costs for the producer.

The 74 percent alfalfa group (ADF, 24.5) showed growth rates similar to all other groups in experiment 1, but in experiment 2 the growth rate was significantly lower in the 74 percent group. This possibly indicates that there is an area between 60 and 74 percent alfalfa level where the growth rate begins to diminish.

The average daily intake increased as the fiber level in the diet increased and the energy level decreased. Even at the 74 percent alfalfa level the rabbits were able to consume enough feed to compensate for lower energy availability. The gut capacity was not surpassed and the average daily gain was not adversely affected.

As the fiber level in the diet increased the mortality decreased, thus the lowest mortality was in the 74 percent alfalfa groups. This indicated a possible beneficial effect of higher fiber in the diet.

When pellets were supplemented with fresh greens, the rabbits fed 50 or 75 g of pellets gained as much weight as those fed pellets only. The rabbits given free choice of greens and pellets actually consumed one-third less pellets than did the group fed ad libitum pellets. Maximum daily pellet consumption when greens were given was 66 to 75 g.

Green supplementation would be feasible in an area where vegetable wastes are abundant. This would not be practical in a large rabbitry if greens are not readily available, due to the intensive labor required to gather the greens. A small rabbit operation would also be an ideal situation for greens supplementation.

An attempt was also made to improve the digestibility of fiber in alfalfa, millrun, and corn cobs via sodium hydroxide treatment. It was found that alkali treatment only improved fiber digestibility in the millrun group, but this may have been an artifact. If fiber digestibility had actually improved in the treated millrun group, there should have also been an increase in digestible energy, which there was not. The treated diet, when analyzed for ADF content, had a much higher

value than the untreated diet. It would seem, if sodium hydroxide solubilized hemicellulose, that the ADF value should have been the same or even less for the untreated diet than for the treated diet. These observations indicate that perhaps an insoluble complex formed between the sodium hydroxide and some plant component.

## VI. SUGGESTIONS FOR FURTHER RESEARCH

This research indicated several areas where more experimentation could be done. A follow-up digestibility study should be done on the high fiber diets used in experiments 1 and 2. It would also be worthwhile to compare high and low fiber diets which are not iso-nitrogenous. In order to maintain a low mortality rate but possibly increase body weight, diets including higher fiber levels plus animal fat should be investigated.

The greens supplementation study indicated a greens preference trial needs to be performed. Many other types of greens not used in this study could also be used. Since the fresh greens expose the rabbit to parasites, it would be advantageous to sample rabbits for parasites.

Since the sodium hydroxide did not increase fiber digestibility in this research, other feed treatments such as steam and ammonia could be tried. A follow-up on the sodium hydroxide treated millrun needs to be done to ascertain the reason the ADF and CWC values were high.

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