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Effects of Carbohydrate Feeding Levels on Roughage Digestion in Dairy Cattle

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PURPOSE OF INVESTIGATION

Molasses has long been recognized by stockmen as an appetizer and a valuable aid in increasing palatability of low quality roughages. Patterson and Outwater (1907) report the first recorded use of molasses as a stock feed was in 1811. Since the turn of the 20th century, many workers have investigated the role of molasses in livestock nutrition. Literature reveals considerable variation in actual effect of molasses in the ration. Its value as an appetizer and dressing for poor quality roughages is accepted. In addition, there is no question but that molasses is a valuable and often a cheap source of energy and minerals. However, there is much difference of opinion as to the effect of molasses on digestibility of the rest of the nutrients in the ration.

Morrison (1948) states that experimental work done by numerous workers seems to indicate that molasses increases palatability but not digestibility of hay. Most, but not all, of the protein experimental work indicates that the low protein value of molasses decreases digestion of the other protein in the feed. Other results show that molasses is beneficial in providing micro-organisms with a source of energy for converting non-protein nitrogen to protein. Divergent results also are reported on other food nutrients, although the literature indicates this may be due to an incomplete understanding of the proper amount of molasses to feed with different types of rations.

While the presence of micro-organisms in the digestive tract of the rumen has long been accepted, many of the functions of these unicellular organisms are not understood. Their role in the breakdown of fibrous portions of the rumen ingesta is acknowledged as is the synthesis of amino acids and vitamins. However, little is known about nutrient requirements of the organisms as they occur in the rumen or even the specific function of many of those present. Only meager information is available on the numbers of micro-organisms present in different types of rations. Virtually nothing has been done to compare quantitative and qualitative population with digestibility of the ration.

This experiment has been set up with the express purpose of establishing, if possible, practical molasses feeding levels for dairy cattle using practical farm rations. By feeding various amounts of molasses under controlled feeding conditions it is possible to establish the effect of molasses under such conditions. Many times, economic factors may make the feeding of higher levels of molasses advisable even though the overall digestibility of the ration is decreased.

By comparing bacterial and protozoa population with various coefficients of digestibilities obtained, some light may be shed on the proper balance of the various feed nutrients for the most efficent action of these rumen micro-organisms.

While results of this experiment will not give specific information for each individual stockman to use, due to the difference in rations, the facts established should aid in arriving at the best type of ration and supplement to use under various feeding conditions.

PREVIOUS WORK

The modern-day concept of dairy cattle nutrition embraces not only digestibility of the ration but also the role of micro-organisms of the rumen in their breakdown of foodstuffs. A summary of work done by other investigators follows:

Molasses as a Source of Carbohydrates

According to Morrison (1948), the average analysis, in percent, of molasses is: *total dry matter* 74, *total digestible nutrients* 54, *protein* 2.9, *sugars* 55, *nitrogen-free extract* 62.1, *and mineral matter* 9.0. High sugar content makes molasses an extremely good source of energy; however, the T. D. N. value is only two-thirds that of whole corn. The crude protein is of low nutritive value and is virtually indigestible. Depending on how the molasses has been fed, it has been credited with being worth up to 85 percent of the value of corn in cattle feeding. Generally, molasses is used as a top-dressing for poor quality hay to increase consumption. In this instance it may be worth as much or more than corn, according to Morrison (1948).

The petroleum industry recently entered the alcohol manufacturing field and now produces most of the alcohol needed (Doyle, 1952). According to Doyle (1952), the nation's sugar industry has to find a new outlet for an annual output of 200 to 267 million gallons of molasses formerly used in making ethyl alcohol. Flour and Feed (anon., 1952) market reports showed a drop from 18.5 cents per gallon in July, 1952, to 10 cents per gallon in November of the same year. Morrison (1952) remarked that the current price of molasses made it a very attractive buy for livestock feed.

Effect of Molasses Feeding Level on Digestibility of Ration

The value of molasses as a source of energy in the ration of the ruminant has long been recognized. Lindsey and associates (1907,

1909) fed molasses at the rate of 10 to 15 percent of the total dry matter with hay and found no depression of dry matter digestibility. No mention was made as to whether the digestible nutrients in the molasses were considered in making their observations. When they increased the molasses to 20 percent of the dry matter, a 4.5 percent depression in digestibility of the hay was observed.

Lindsey and Smith (1909) explained that the digestibility depression noted on high level molasses feeding appeared to depend upon the character of the feed (that is, there was less digestibility depression on an all hay ration than on hay and concentrate), the amount of molasses fed, and the individuality and condition of the cow. They observed in later trials that the inclusion of molasses at the rate of 20 percent of the dry matter caused a decrease of 6 percent in digestibility of hay. However, their results do not show definitely that the depression of digestion can be credited to molasses feeding.

Patterson and Outwater (1907) conducted a series of digestion trials exploring the effect of blackstrap molasses on the digestibility of a standard grain and hay ration. Their results indicate that molasses exerted a beneficial influence and generally increased digestibility of the ration. They emphasized the value of molasses as an appetizer and in making the feed more palatable.

Brintnall (1921) tried replacing corn grain pound for pound with blackstrap molasses in the ration of dairy cows. He fed from 2 to 5 pounds of molasses daily and found molasses fully equivalent to corn grain in production of milk and butterfat and in maintenance of body weight. This information regarding butterfat content conflicts with the report of Lindsey *et al.* (1907), who stated that the molasses ration used in their work seemed to produce milk with slightly less butterfat and solids-not-fat than did a corn-meal ration. This change in milk composition was not significant. It does not agree with later experiments which show that feed has little or no effect on milk composition (Cobble and Herman, 1951).

Williams (1925) fed molasses at rates of 15 and 25 percent of concentrates in a ration. The milk production curve seemed to follow that of a normal lactation. He assumed that nutrients in molasses were all digested so they were not added as a part of the feed consumed. This makes interpretation of the results somewhat difficult. His data show that molasses had no uniform effect on digestibility of the different nutrients, although in six of eight comparisons the percent digestibility of protein was reduced by 7 to 10 percent apparently as the result of the molasses.

Gerlaugh (1930) found that molasses was an excellent appetizer and that it did increase feed consumption. However, as the amount fed exceeded 2 pounds daily for fattening calves, the feed value of molasses decreased. Snell (1935), of the Louisiana Station, studied blackstrap molasses and corn-soybean silage for fattening steers. He observed that when molasses was added to a dry ration it had no significant effect upon digestibility of crude protein, ether extract, or crude fiber. When molasses was added to silage rations, digestibility of crude protein was lowered 12 percent, while ether extract, carbohydrate and ash digestibility were increased 20, 2 and 14 percent, respectively.

These findings were confirmed by Bray *et al.* (1945), who made further observations on this subject. They found that while the conventional sprinkling of small amounts of molasses on roughage encouraged greater intake, even more roughage was consumed when molasses was placed on the concentrate. They also observed that molasses appeared to give best results when fed at the rate of 4 pounds daily, but that it could be fed up to 8 pounds daily with good results. Bray and his associates were working with three-year-old steers.

Briggs and Keller (1940) ran a series of trials where large amounts of blackstrap molasses were included in rations of lambs. The digestion coefficient of crude protein was decreased from 78.9 to 72.0 percent in the alfalfa-corn ration, and from 77.8 to 67.7 in the oats-alfalfa ration. The digestion of fat was decreased from 55.8 to 30 percent, while the average coefficient of digestibility of crude fiber was changed little in any of the trials. Nitrogen-free extract digestion was down 2 percent in one oats-alfalfa ration. Lambs used in this experiment digested fat and crude fiber in the oats-alfalfa hay ration more completely than the smaller amounts of fat and fiber in a corn-alfalfa hay ration.

Effect of Carbohydrates on Protein Digestibility

Mitchell, Hamilton, and Haines (1940) report that addition of glucose to a basal ration decreases digestibility of insoluble carbohydrates (as measured by crude fiber digestion) that depend on the fermentative action of micro-organisms of the paunch for their digestion. Amount of decrease was from 39.94 to 29.74 percent. They concluded that 50 percent of the metabolizable energy of the glucose supplements to a basal ration is wasted as a result of the increment in heat production thus induced.

Hamilton (1942) investigated the effect of added glucose upon digestibility of protein and crude fiber in rations for sheep. He reported that this supplementation increased the apparent digestibility of dry matter from 65.4 to 67.7 percent, nitrogen-free extract from 76.4 to 79.7 percent and total carbohydrates from 68.9 to 71.9 percent. Decreases were noted from 61.9 to 54.1 percent in total nitrogen and from 43.8 to 31.9 percent in crude fiber. No significant effect was noted on the apparent digestibility of ether extract or in changes of gross energy. He concludes that there was no effect of glucose on the true digestion of total nitrogen since the apparent decrease obtained in digestibility could be entirely accounted for by the estimated increase in metabolic nitrogen in feces of the sheep on the sugar ration. Another significant observation was that the marked decrease in digestibility of crude fiber when sugar was present was apparently due to preference of micro-organisms in the paunch for sugar.

Swift *et al.* (1947) investigated the effect of supplementing the ruminant ration with extra amounts of one of the constituents of a normal ration. Nutrients used were crude fiber, ether extract, protein, urea, and carbohydrates. Only crude fiber addition made a substantial change. Crude fiber was supplied principally in the form of oat straw while the other supplements were provided as practically pure compounds. Corn oil in small amounts increased digestibility of each feed constituent from 1 to 2 percent. In larger amounts, however, corn oil produced a decrease in digestibility of all constituents.

Effect of Carbohydrates on Digestibility of Cellulose

Louw *et al.* (1947) were aware that the ability of sheep in South Africa to digest cellulose in a basal ration of veld hay was soon impaired if the animals were kept on the hay for too long a time. They fed 20, 50, and 85 gram-levels of grain concentrate mixture daily and noted no improvement in digestion of the cellulose of the basal ration.

Clark and Quin (1951) working with sheep in the same general area as Louw (1947) discovered that the addition of molasses alone to the hay had no demonstrable beneficial effect as far as weight gain was concerned. However, upon adding ammonium nitrate to the hay-molasses combination there was a slightly increased intake of hay and less rapid loss of weight. It should be noted that the sheep were not receiving a maintenance ration.

They concluded molasses and urea improved feeding value of the hay. Along with other work included in this review, their experience seems to indicate that the increased energy source supplied by molasses and nitrogen furnished by urea provide for greater bacterial activity, resulting in increased cellulose digestion (Burroughs *et al.*, 1950; Johnson *et al.*, 1942).

Burroughs *et al.*, (1949b) fed cornstarch with corncobs and alfalfa hay and discovered that the average percent digestibility of corncob dry matter decreased with each increment increase in starch. Further questions on the action of carbohydrates on roughage digestion are supplied by their statement that cornstarch did not appreciably influence alfalfa hay digestion.

The same men, working with Arias (1951) and using the artificial rumen technique, found that energy sources such as molasses, starch, dextrose, and others increased cellulose digestion, which resulted in a similar increase in urea utilization. Since they were using actual rumen samples in the artificial rumen it appears that rumen microorganisms need some energy source until their cellulose digestion power develops and that additional sugars seem to keep them so well supplied that they reduce their activities, which results in lowered cellulose digestion.

They report Hale (1940) as estimating that 85 percent of the digestible cellulose of hay disappears from the rumen of the cow within 48 hours after feeding. Gray (1947) also is reported as showing that 70 percent of the digestible cellulose is fermented in the rumen. These observations are timely in view of the previously mentioned conclusions of Clark and Quin (1951). They report additional studies which indicate that molasses or starch added to the diet of sheep reduced the digestibility of cellulose. These experiments strongly suggest that the composition of the diet affects the nature of the rumen population (Louw and Van der Wath, 1943; Hamilton, 1942; Johnson *et al.*, 1942).

Effect of Carbohydrates on Digestibility of Crude Fiber

In 1934, Mangold reported that fiber digestion was affected by all other ingredients in the ration and that its digestibility is decreased when easily soluble carbohydrates are fed. He also reported that, as believed by others, this effect is associated with the nutritive ratio of the ration and that the addition of protein to the ration decreases fiber digestion. Naturally, consideration must be given the effect of stage of development of the plant on the digestion of the crude fiber fraction.

Nordfeldt *et al.*, (1950), in Hawaii, were concerned with the problem of exceptionally high crude fiber content in the main roughages in their region. They used Holstein cows in their investigations, feeding them chopped napier grass with supplementation of minerals and carotene. The T. D. N. and D. C. P. were kept constant with crude fiber as the only variable factor. The amount of crude fiber in the ration exerted a definite influence on milk yield with the best milk production obtained when the crude fiber was less than 16 percent of the ration.

Relationship Between Carbohydrate and Nitrogen Levels

Johnson *et al.* (1942) studied the relative efficiency of urea as a protein substitute in the ration of ruminants. They used a basal ration containing 20 percent corn molasses. The urea was generally added to the basal ration since it is not a primary source of energy. Soybean meal was also used as a nitrogen supplement and when used replaced part of the corn molasses. In order to vary the amount of molasses and urea to equal the same energy content of molasses and soybean oil meal mixture, cornstarch and wood flour were used to replace part of the molasses. When the two nitrogen sources replaced 60 percent of the corn molasses the apparent digestibility of the nitrogen was 62.8 percent for the soybean meal ration and 64.5 percent for the urea ration.

Increase in cellulose digestion at equal levels of molasses and urea substantiated observations of Clark and Quin (1951). This confirms some previous reports that, in ruminants, supplements of soluble sugars depress digestibility of fibrous constituents of the basal feeds, probably by diverting bacterial action from the less to the more fermentable carbohydrates. However, as noted, this supplementation of molasses did enhance the utilization in digestion of urea nitrogen.

Mills and associates (1944) also were interested in the value of urea in the growth of dairy heifer calves, with corn or cane molasses supplying the readily available carbohydrates. With timothy hay as the sole ingredient of a basal ration, utilization of urea was very low with a rumen protein level of only 7.5 percent one hour after feeding. When corn molasses was added, urea was fairly well utilized up to a level of 9.5 to 10.0 percent. When starch was added in addition to the molasses, urea utilization was still somewhat higher at 10.75 to 11.0 percent, indicating that starch might be a more desirable energy source than the highly soluble molasses. This latter ration also seemed to promote more rapid growth in calves with an average difference of about 0.75 pounds daily.

Burroughs *et al.* (1949a) showed that when ground corn cobs were fed in the absence of starch, the protein requirement for efficient utilization of this roughage was very low — possibly as little as 4 percent. They remark that while feeding rations containing grain in addition to roughage may result in levels of protein sufficient to meet body protein needs, such rations may not be high enough in protein to maintain efficient roughage utilization due to increased carbohydrate level.

Later work by Burroughs *et al.* (1950d) reports the same results, adding that as the protein requirements increase the grain or starch requirements also increase. They conclude that apparently the protein requirement for roughage digestion becomes the protein requirement for rumen bacterial growth.

Watson *et al.* (1947) observed that there were two ways of varying the nutritive ratio: (1) Adding pure substances such as casein or starch. (2) Varying the proportions of concentrates and roughages.

They selected the second method, using common livestock rations. They concluded that, for rations containing a protein concentrate, a carbohydrate concentrate, and a roughage, the nutritive ratio does not influence the digestibility as long as it remains between the approximate range of 1:2 to 1:9.

Culbertson (1950) reports that ammoniated molasses may be used as a protein replacement for up to one-half of the protein needs, since it also supplies the readily fermentable carbohydrates necessary for efficient utilization of the non-protein-nitrogen of the feedstuff. He reports that the body weight gains and feed consumed indicate that cane molasses was not beneficial from the standpoint of stimulating appetite or gain. It did give excellent results in this form in replacing protein.

Tillman and Kidwell (1951) used ammoniated condensed distillers molasses solubles as a feed for beef cattle. They reported a somewhat more efficient use of the entire ration as the trial progressed, which caused them to speculate that this gradual change might be due to a change in the rumen microflora.

Knodt *et al.* (1951) also fed ammoniated cane molasses and ammoniated condensed distillers molasses solubles to dairy cattle. While they were palatable they apparently were of no value to calves under 12 weeks or at least until the rumen began to function.

One of the more encouraging projects regarding the influence of molasses in digestion is reported by Burroughs and co-workers (1951b). Three different amounts of molasses ash from 2, 4 and 8 grams of molasses were used in flasks containing cellulose, two parts cellulose and one part starch, and equal parts of starch and cellulose, respectively. Nine grams of filter paper were used as a source of cellulose. Two grams of molasses were also added to 9 grams of filter paper as a source of cellulose. *Cellulose digestion and urea utilization were increased significantly by molasses and by molasses ash.*

Addition of sugar to the molasses ash showed similar results and better than sugar alone. They have shown in the artificial rumen that the amount of ash needed for efficient cellulose digestion and urea utilization is dependent upon the amount of available energy in the fermentation flask, rather than being specifically related to the amount of cellulose present. They also demonstrated that addition of starch indicates a definite need for more molasses.

Morrison (1952) notes that in New Jersey investigations where cane molasses replaced one-half the total digestible nutrients in the grain allowance, there was a highly significant decrease in milk yield. Wisconsin workers showed more favorable results for molasses.

Normal Microflora of the Paunch

Hastings (1944) has provided one of the early reviews on the significance of bacteria and protozoa in the bovine rumen. He remarks that it is hard to determine how the organisms actually break down fibrous portions of the rumen ingesta, although it is known that it occurs as the result of enzymes produced by the micro-organisms. It is possible that some protozoa rub or bite off pieces of the plant fibers, although others are known to actually devour the fibrous portion converting it to starches and sugars and gradually accumulating it in the cell. He notes that cellulose splitting ability of protozoa is questioned but that both protozoa and bacteria terminate as food for the host animal. He indicates that changes in rations should not change the flora and that 10 percent of the volume of the rumen at peak digestion conceivably might be bacteria and protozoa.

Baker (1942), using freshly slaughtered cattle, isolated protozoa of the families *Ophryoscolecidae* and *Isotrichidae*. He also reported rather extensive iodophile microflora, at least five of which were large and sufficiently distinct to easily distinguish. They were:

(1) A colorless, spore-forming *Oscillarium*.
(2) A giant *Spirillium*.
(3) Large *Sarcina* packets.
(4) An unidentified navicular organism forming rosette-shaped associations of five to 30 units.
(5) Coccoid chains of two to eight units.

A later report by Baker (1943) contends that during passage through the alimentary canal the micro-organism population is eliminated, the protozoa by peptic and tryptic digestion, and the iodophile microflora by (1) digestion by protozoa, (2) autolysis, and (3) action of digestive enzymes. Thus the products of microbial synthesis are held to be assimilated by the host animal.

In the same paper, Baker (1943) presents a more complete account of the rumen population. He has observed both iodophile and aniodophile microflora of fixed and free type. Specifically isolated iodophile cocci, diplococci, and short and long chain cocci were seen. Also, curved vibrionic types and short slender non-sporing rods were reported. The aniodophile microflora include vast numbers of minute cocci and rods and in addition, *Spirochaetes, Fusobacteria* and *Selonomanas* were identified.

Ration Effect on Micro-organism Population

Pounden and Hibbs (1948a) discovered a rather definite relationship between the type of ration and the species of micro-organisms.

A breakdown of the hay flora showed two distinct groups. Group I was made up of quite large Gram-positive coccoids in closely knit pairs. Group II included large, square-ended, Gram-positive rods; very large Gram-negative cigar-shaped rods; and smaller Gram-negative short rods in fours and multiples of four. The grain flora was also placed in two groups. Group I was composed of medium sized comparatively thin Gram-positive rods. Group II included Gram-positive rods resembling coliform organisms and other packets resembling Baker's (1942) *Sarcina* packets. They noted that rumen cud inoculations in young calves established only those organisms peculiar to the type of ration fed, which is in direct contrast to Baker's (1943) report.

Subsequent work by Pounden and Hibbs (1948b) showed that moderate numbers of protozoa and hay flora accompanied the ingestion of hay without grain. When moderate quantities of grain were fed with hay, masses of protozoa along with fairly numerous flora of the two hay groups of bacteria were observed. Similar concentrations of protozoa, accompanied by rather limited numbers of organisms of the varieties observed to associate with grain consumption, occurred with the ingestion of approximately equal quantities of hay and grain. When the ration consisted of almost all grain, the number of protozoa was quite small while the grain flora bacteria was greatly increased. No organisms of the hay group were observed. A general observation indicated that a ratio of grain to hay of 3 to 1 resulted in no indication of the hay group micro-organisms being present, while the grain flora micro-organisms occurred on the 3 to 2 grain to hay ratio.

Later work by Pounden *et al.* (1950) reviewed the type of micro-organisms present in other parts of the digestive tract. Their observations indicate that some organisms undergo rapid disintegration shortly after encountering abomasal fluids, while others hold their form until they reach the posterior parts of the digestive tract. Still others pass through the animal. This latter type might become of value to the host through pre-digestion by protozoa. Gall (1948, 1951) also noted a relationship between the ration and the type of flora.

Ration Effect on Numbers of Rumen Micro-organisms

Work by Bortree *et al.* (1946) shows that there is a rapid increase in the numbers of these organisms present in the rumen within two hours after the animals have been fed and that these counts are increased for several hours and then gradually return to the range observed prior to feeding. While the bacteria counts were influenced somewhat by roughage quality, the changes were not marked. Also, when the animals were changed to pasture the changes in the counts were not great. Of considerable significance is the observation that 3 pounds of glucose added to the usual ration of hay increased bacterial counts 100 percent over those of hay alone. However, the addition of starch under similar conditions produced results which were only slightly different from those obtained with hay alone.

Burroughs *et al.* (1950e) report results which indicate the presence of inorganic nutrients in good quality alfalfa hay or meal which are essential to rumen micro-organisms involved directly in roughage digestion.

Digestion by Micro-organisms

Walker (1952) notes that ingested feed lies in the rumen for attack by micro-organisms before digestive juices of the lower digestive tract have access to the ingesta. Bacteria use sugar and soluble protein in the feed to live and multiply. As they develop, they secrete enzymes which dissolve starch and cellulose and use them for nutrients — and keep multiplying.

Gall *et al.* (1949b) in contrast to the reports of Pounden *et al.* (1948a, 1948b) and in agreement with Baker (1943) concluded that the bacteria seen in the rumen of both cattle and sheep are generally similar regardless of season, ration, or species.

In cultural work, they split the types of organisms into fast and slow growing groups, both of which were present in all rations. The fast growing organisms were glucose fermenting and were largely coccoid with some small rods. They postulated that the fast growing organisms, because they break down glucose, utilized starch and other soluble carbohydrates in the rumen. They thought the slower growing organisms which were observed to clump cotton might be the cellulose digestors. These slow growing organisms also are quite similar, morphologically, to types found attached to plant fibers on the Gram stains. In addition to their cultural observations, they report an average bacterial count of 50 billion per gram of fresh rumen contents on winter feed, while pasture gave a considerably higher average of 96.1 billion for cattle.

Burroughs *et al.* (1950d), in studying the influence of casein upon roughage digestion, made morphological examinations similar to those of Gall. Since there was considerable variation in type of ration fed, the bacterial counts varied directly with the type of ration. This led the authors to think that protein supplies some nutrient needed by rumen bacteria, which has been shown to be nitrogen.

Moir and Williams (1951) obtained comparable results from protein supplements in sheep. Later work by Burroughs *et al.* (1951a) showed that nitrogenous requirements of rumen micro-organisms were relatively simple in nature, essentially involving ammonia but not involving the more complex forms of nitrogen such as amino acids.

McNaught and Smith (1947), in reporting rumen bacteria activity in the utilization of non-protein nitrogen, indicate that it has only one-half the value of the same amount of protein nitrogen. They caution that the deliberate use of non-protein compounds in rations will be successful only when the available feed stuffs are rich in starch and poor in protein and when the protein is only sparingly soluble.

Wegner *et al.* (1940) set up an *in vitro* experiment to test the hypothesis that inorganic nitrogen utilization in ruminants is done via rumen bacteria. Carbohydrate studies were first made using corn molasses, cerelose (common glucose), starch, and cellulose. The sources of carbohydrate, with the exception of cellulose, all supported bacterial growth as measured by the ammonium nitrogen disappearance. Cellulose failed as an energy source for the organisms used, probably because of its slowness in becoming available.

Van der Wath (1948) showed that in sheep receiving a regular supply of starch, the bacterial disintegration of starch granules commenced five hours after ingestion and was completed within 18 to 20 hours. In basal diets not including starch, but where starch was added through a rumen fistula, the digestion commenced seven hours after ingestion and took much longer to complete. There seemed to be a much greater bacterial development in sheep receiving regular starch diets. Organisms observed in the rumen were quite similar to those reported by Baker (1942) and Hastings (1944).

Conrad *et al.* (1950) found that when roughage constituted the entire dry feed, rumen cud inoculations aided in providing microorganisms which digested cellulose somewhat more efficiently than in uninoculated calves. These inoculations also seemed to stimulate earlier hay consumption. Baker (1939) points out that the ease of attack on cellulose by micro-organisms depends on the absence of encrusting substances.

Louw and Van der Wath (1943), South Africa, examined the influence of small supplements of protein-rich and carbohydrate-rich feedstuffs on the bacterial count of the rumen and on the digestibility of the cellulose in a ration of winter veld grass containing approximately 3 percent protein. Then the carbohydrate-rich supplement was gradually increased to determine the effect on microbiotic decomposition of cellulose in the winter ration.

Even though the bacterial population in the rumen was increased, there was no improvement noted in the cellulose digestion of this poor quality roughage source. In fact, a progressive decrease in cellulose digestion was noticed as maize supplementation increased. They also noted that protein supplementation is futile in poor roughage of this type unless the energy source is increased comparably. In addition they observed that 50 to 100 grams of maize per day favored bacterial growth but heavier supplementation tended to reverse this trend.

Hungate (1944, 1947) succeeded in locating and observing some types of organisms capable of digesting cellulose. One type was morphologically a cocci. He observed both Gram-positive and Gram-negative cocci. The other type was rod shaped. Six strains of this rod were observed and five were isolated in pure culture. He had previously reported the isolation of a cellulose digesting anaerobic organism called *Clostridium cellobiopams*.

Cowles and Rettger (1931) isolated another species called *Clostridium cellulosolvens*. Sijpestijn (1951) has two cellulose-decomposing bacterium from the rumen of sheep and cattle. They are from the strain of *Ruminococcus flavefaciens* and are Gram-positive, strictly anaerobic streptococci which attack only cellulose and cellobiose. Strain D from the rumen of the cow occurs singly, in pairs, or short chains, while strain S from sheep occurs primarily in a long chain form. Strain D also grows on glucose agar, while strain S does not.

Burroughs *et al.* (1950a) report a close correlation between bacterial population and cellulose digestion, showing that bacteria can break down cellulose when favorable growth conditions are kept. Burroughs *et al* (1950c) report that rumen micro-organisms have nutritional requirements which must be fulfilled if they are to digest roughages and cellulose properly. These nutrients seem to be sources of energy, protein or nitrogen in some form, vitamins synthesized by the rumen micro-organisms, and inorganic elements believed to be integral constituents in enzymes or enzyme systems.

Still another experiment by Burroughs *et al.* (1950b) discloses that cellulose digestion by micro-organisms in an artificial rumen is influenced by cereal grains and protein-rich feeds.

Recent studies on cellulose digestion by rumen micro-organisms are the *in vitro* work on antibiotic effect by Wasserman *et al.* (1952). Their results show that in the concentration used, penicillin stimulated cellulolytic rumen micro-organisms at the lower concentrations, neomycin was stimulatory at all concentrations, streptomycin was slightly stimulatory in the lowest concentration, while chloromycetin adversely affected the micro-organisms. This effect is accomplished by the antibiotics inhibiting the growth and development of micro-organisms not compatible with the cellulolytic types.

MATERIALS AND METHODS

The effect on digestibility of roughages by varying the carbohydrate intake of dairy cows was studied as follows: (1) determination of the coefficient of digestibilty of nutrients in rations supplemented with varying amounts of molasses, and (2) determination of the number of bacteria and protozoa at various molasses feeding levels on different types of roughages.

Experimental Design

Five separate series of digestion trials or phases were conducted. The first three phases were set up on a 4 x 4 randomized block design with each of the four trials in each phase running for 14 days. This made a 56-day experimental period for each of the three phases. Phases IV and V were composed of three trials of 14 days each, making two 42-day experimental periods.

Experimental Animals

Animals were selected from the Missouri Station milking herd. Those selected for each phase were in the last stage of their lactation or were dry. All animals had completed or were completing one or more lactations. While it was not always possible to obtain animals of the same breed for each phase of the experimental work, it was felt that use of the randomized block design would eliminate variation due to breed differences.

The animals are identified in the following table by phase, breed, and herd number.

Phase I	Phase II	Phase III	Phase IV	Phase V
Jerseys	Jerseys	Jerseys	Guernseys	Jerseys
558	518	564	229	544
560	510	557	228	545
		994	219	564
Guernseys	Holsteins	562	221	557
205	314		493	556
206	317			

Body Weights

Each animal was weighed just before the evening feeding on the first three days of the first experimental period and on the last three days of that and each subsequent period. These weights were then averaged to determine any gain or loss of weight during each two week feeding trial.

Feedstuffs

Blackstrap molasses was obtained on the open market. The grain concentrate was the normal ration given to milking animals in the Missouri Station dairy herd. However, it was mixed especially for these experimental trials and in an amount sufficient to last through each phase. The grain ration was composed of shelled corn, 650 pounds; oats, 650 pounds; bran, 350 pounds, soybean oil meal, 300 pounds; steamed bone meal, 20 pounds; and salt, 20 pounds. Using Morrison's (1948) standards, this gave a digestible protein content of 13.24 percent and a total digestible nutrient content of 71.99 percent.

The variable constituent of the total ration was the roughage. Stemmy alfalfa hay of ordinary quality was purchased on the open market for use in Phase I. A leafy, green lespedeza-timothy hay mixture of good quality, grown on the University Department of Animal Husbandry farm, was used in Phase II. Leafy, green alfalfa hay of good quality, mixed with about 10 percent bromegrass, was used in Phase III. The alfalfa hay used in Phase IV had somewhat less leaf and color than the hay used in Phase III and was not mixed with brome. A somewhat brown alfalfa hay of average quality was fed in Phase V. All of the alfalfa hay was purchased on the open market as ungraded hay. The poor hay season in 1952 prevented use of roughages of top quality, although the hay used in Trial III was good.

Mixing and Feeding the Ration

All roughage was chopped into 2- to 3-inch lengths by running it through a hammer-mill. Samples for analyses were taken as the chopped hay emerged from the blower. The chopped hay was then sacked into large beet-pulp sacks for conveyance to the experimental barn. Each sack approximated the amount needed for one feeding for all experimental animals.

Rations were fed in equal portions twice daily. Hay was weighed into large 30 gallon cans equipped with lids. The molasses was then weighed and diluted with enough hot water to make complete evacuation of the molasses container possible. This was poured over the hay which absorbed most of the mixture. At feeding time any molasses left in the container was poured over the hay in the manger. Grain was placed in five gallon feeding tubs. Seven and one-half gm. of chromium oxide was added to each ration by sprinkling it over the grain.

If any significant amount of the ration was not consumed, the refuse was removed, dried, weighed and analyzed to determine the actual amount of each feed nutrient consumed.

Water was available at all times in drinking cups in the stalls. In addition, the animals were turned into a dry lot for exercise between 8:00 a.m. and 12:00 o'clock each day where water and salt were available.

Changes in rations between trials were made abruptly. Animals were fed at 6 a.m. and 3 p.m. daily.

Collection and Handling of Excreta

Fecal collections were made under the system described by Moore (1952). Since the chromium oxide method of determining digestibility was used, total fecal collections were not necessary. A 10-day preliminary period and four-day collection period made up each trial. The fecal collections were made between the hours of 1:00 and 3:00 p.m. on each of the four collection days. Each passage of feces was collected as it fell from the cow into a scoop shovel where it was thoroughly mixed with a large kitchen mixing spoon. "Grab samples" were then obtained from the mixed collection. One large sample was placed in a plastic freezer bag ordinarily used in packaging turkeys for freezing. A smaller sample was placed in a quart-sized plastic bag for nitrogen analyses. All passages of feces from each cow during the two-hour collection period and for each of the four collection days were sampled in this manner and placed in the same container. In the event any animal failed to defecate naturally during this period she was induced to do so.

At the end of each collection period the fecal samples were immediately placed in a deep freeze, where they were frozen. At the start of the next day's collection period, samples were removed from the deep freeze and the additional fecal samples obtained were added to the frozen sample. After the final collection period the composite sample was kept frozen until it was dried for analyses.

Methods of Analyses

Frozen composite fecal samples were allowed to thaw out overnight in the plastic containers. They were then transferred to Pyrex trays for drying. Drying was done in a Freas drying oven at 50° C. with frequent stirring. Usually 24 to 36 hours were necessary for complete drying, after which the dried samples were allowed to reach an air dry condition in the cooled oven. They were then placed in plastic bags and conveyed to the analytical laboratory where they were ground through a Wiley mill, thoroughly mixed, and sampled for analysis. Duplicate samples of all feeds and feces were kept.

All feed and fecal samples were analyzed in the Agricultural Chemistry laboratory. Analyses were made of moisture, nitrogen, ash, crude fiber, and ether extract according to the methods of the Association of Official Agricultural Chemists (1950). The nitrogen-free extract was determined by difference.

Nitrogen determinations in triplicate on both wet and oven dried fecal samples for Phase I and Phase II were made. The results were so close that determinations on the wet samples were discontinued. All of the other determinations were made in duplicate. Cellulose was determined by the method of Crampton and Maynard (1938).

Determination of Coefficients Of Apparent Digestibility

The coefficients of digestibility were determined in the manner described by Gehrke *et al.* (1950)), using the chromium oxide indicator method. The complete equation for the calculation of digestibility using this method is given below:

$$\text{Digestibility} = 100 - 100 \; \frac{(\%Cr_2O_3 \text{ in Feed})}{\%Cr_2O_3 \text{ in Feces}} \times \frac{(\% \text{ of nutrient in Feces})}{\% \text{ of nutrient in Feed}}$$

Collection of Rumen Samples

The rumen samples were obtained and handled in the manner described by Gall *et al.* (1949a), with some modifications. A smooth rumen tube with a ⅝ inch lumen, rounded off at one end, was used. It was carefully inserted smooth end first, via a speculum tied in the cow's mouth, into the rumen. The tube was then blown out to prevent any saliva contamination. In addition, blowing on the tube caused a bubbling noise in the rumen which gave assurance that it was in the correct location. The exterior end of the tube was fastened to a glass tube inserted through a rubber cork into the top of a two liter suction flask. The vacuum was provided by running a smaller rubber tube from the side arm of the suction flask to an adjustable stall cock on the milking machine vacuum line. To prevent undue clogging of the rumen tube, a constant in and out motion was applied to the tube during the collection. Occasionally it was necessary for the operator to remove the tube from the suction flask and blow some solid rumen content back into the rumen. The method described provided a quick and easy way of obtaining both liquid and solid rumen samples. Samples were taken between 2:30 and 3:00 p.m. on the last collection day of each trial.

Analyses Made of Rumen Samples

Immediately after the rumen sample was obtained, it was taken to the laboratory where it was filtered through a double layer of cheese cloth. The solid portion was obtained by squeezing out all of the liquid possible by hand. The liquid portion was tested for pH with a Beckman model G-glass-electrode potentiometer within 15 to 20 minutes of collection time. Dry matter was obtained by drying samples of the solid portion at 75° C. for 24 hours.

Ten gm. of the solid material were weighed and placed in a bottle in 90 ml. of distilled water. The bottle was stoppered and shaken vigorouslv for three minutes. A further dilution of 10^{-3} was made in 99 ml. distilled water. This method is reported by Gall *et al.* (1947) as giving counts slightly less but comparable to those obtained from fistulated animals.

Making and Counting Slides

Gram stains were made from the 1:10 dilution of the rumen contents and were examined microscopically. The various morphological types of organisms were observed and their presence recorded. Nigrosine slides for counting the bacteria present were made in the manner described by Gall *et al.* (1949a, 1947), with slight variations. The method in brief follows: 0.01 ml. of the diluted sample was transferred by Breed pipette to the center of a marked 4 cm. square on a clean slide and a 3 mm. loop of nigrosine stain was placed on the drop. Complete mixing was effected, using the loop to stir and spread the material in a thin film evenly over the slide. Rocking the slide from side to side to effect an even color and to allow slight evaporation proved helpful. The slide was then placed on a flat surface hot plate heated to 600° F. Drying took only two to three seconds at most. The slide was then removed from the hot plate, marked and placed in a glass slide box to await counting.

The nigrosine stain was a saturated methyl alcohol solution of water-soluble nigrosine. It was found that the addition of two drops of saffranin to 10 ml. of the nigrosine solution gave a somewhat more even stain. A careful attempt was made to cover all portions of the slide evenly to prevent shrinkage or ridges of dye.

Gram stains of each sample were examined before any slide was counted to establish the morphological characteristics of each organism observed. In order to establish as nearly uniform conditions as possible in counting, 20 fields were counted on each of four slides made from each sample. The first field counted was in the upper left hand corner of the slide and successive fields were counted in a diagonal line running to the lower right hand corner. Any field having much debris or a large light or dark cracked area was discarded. Chains or pairs were counted as one and each distinct type of organism was counted separately in an attempt to obtain qualitative as well as quantitative data on the rumen population.

The count per gm. or ml. of material was calculated by multiplying the average count obtained from the 20 fields with a factor obtained as follows: The factor obtained by calibrating the microscope is multiplied by the dilution (1,000) of the sample; this value is multiplied by 4, representing the square centimeter area of the slide. The factor used in this experiment was 2.3 billion.

Method for Counting Protozoa

One hundred ml. samples of the liquid portion of the rumen content were transferred to a screw-top bottle and 5 ml. of formalin added immediately to preserve the sample. Two ml. of this material was transferred to a watch glass where two drops of methylene blue stain was mixed with it. A drop of this stained sample was then placed under each side of the cover slip on a haemocytometer counting chamber. Four fields were counted from each of four chambers, making a total of 16 fields counted. The total count per ml. was arrived at by multiplying the average count by the factor obtained by calibrating the microscope, using the low-power lens.

It was found that this method gave effective results if the stained sample could be counted in less than five minutes. At that time the protozoa were beginning to absorb the stain, making it impossible to differentiate between live and dead organisms. Only those protozoa resembling ciliates were counted.

EXPERIMENTAL RESULTS

Comparisons of Different Levels of Carbohydrate Feeding on Digestibility of Various Kinds and Qualities of Roughage

Since the grain ration was mixed separately for each trial, there was some slight variation in the average nutrient content. Molasses was purchased on the open market and some variation in its nutrient content was observed. Average percentage composition of the hay, grain, and molasses fed in each phase is shown in Table 1. Since there is so much variation in the apparent digestibility of ether extract and ash as observed by Maynard (1951), they will not be included in the narrative of results but will be included in the tables.

Phase I: A Bleached Stemmy Alfalfa Hay. The average coefficients of apparent digestibility obtained are shown in Table 2. Although the alfalfa hay fed was rather bleached and stemmy it contained 14 percent crude protein. There was virtually no change in apparent digestibility of crude protein at 1 and 2-pound molasses levels, compared with Ration 1, which did not include molasses. When the molasses level fed daily was increased to 4 pounds there was a drop in digestibility from 67.57 percent at the 2-pound molasses level to 65.71 percent at the 4-pound molasses level or a decrease of less than 3 percent. This is not in keeping with observations of other experimental workers reported earlier in this paper. A rather different picture was observed in studying the effect of molasses on digestion of crude fiber. Apparent digesti-

TABLE 1--AVERAGE PERCENTAGE COMPOSITION OF RATIONS FED DURING EACH PHASE

Phase	Feed	Dry Matter	Crude Protein	Crude Fiber	Ether Extract	Ash	Nitrogen-free Extract	Cellulose
I	Alfalfa Hay	86.90	14.00	33.42	0.97	5.63	32.83	33.08
	Grain	87.82	15.75	7.13	3.22	5.72	56.00	6.83
	Molasses	74.07	2.94			7.30		
II	Timothy-lespedeza Hay	89.46	7.81	32.29	1.71	5.05	42.60	30.50
	Grain	87.76	17.43	6.60	3.84	5.73	54.16	6.39
	Molasses	75.70	2.63			6.16	66.91	
III	Alfalfa-brome Hay	93.11	13.00	35.94	1.24	6.66	36.27	31.97
	Grain	90.37	16.94	7.11	3.38	6.38	56.56	7.23
	Molasses	72.84	3.00			8.55	67.29	
IV	Alfalfa Hay	90.95	14.69	31.89	1.30	7.10	35.97	28.12
	Grain	90.36	16.56	6.93	2.85	5.44	58.58	6.59
	Molasses	74.37	2.31			7.95	64.11	
V	Alfalfa Hay	92.68	14.56	32.31	1.10	7.90	36.81	28.37
	Grain	88.65	18.12	6.55	3.65	6.05	54.28	6.47
	Molasses	72.44	2.88			9.67	49.89	

TABLE 2--AVERAGE DIGESTION COEFFICIENTS* AND BACTERIAL COUNTS AT VARYING LEVELS OF MOLASSES FEEDING WITH THE ALFALFA HAY FED IN PHASE I

Ration Number	Lb. Molasses in Daily Ration	Coefficients of Apparent Digestibility in Percent							Bacteria Count**
		Dry Matter	Protein	Crude Fiber	Ether Extract	Ash	Nitrogen-free Extract	Cellulose	
1	0	53.39	67.30	43.51	47.55	24.75	70.09	59.96	67
2	1	61.25	67.80	48.59	47.28	28.55	75.03	63.58	81
3	2	65.26	67.57	52.23	48.87	38.60	77.35	64.96	93
4	4	62.54	65.71	42.21	52.31	41.81	77.42	59.91	106

* A 4 x 4 randomized block design was used, furnishing four animal experimental periods for each ration.

** In billions, per gram of wet rumen ingesta.

bility of crude fiber where no molasses was fed was found to be 43.51 percent; at the 1 pound daily molasses feeding level, 48.59 percent; and at the 2 pound molasses level, 52.23 percent or an increase of nearly 16 percent, apparently as the result of including 2 pounds of molasses in the ration. However, when the molasses level was increased to 4 pounds daily, the average apparent digestibility was only 42.21 percent, a decrease of about 19 percent from the 2 pound level and nearly 3 percent below that obtained with Ration 1, which included no molasses.

Similar results to those with crude fiber were obtained in digestion of cellulose. Virtually the same apparent digestibility, or 59.96 compared with 59.91 percent, was obtained with Ration 1 containing no molasses as with Ration 4 containing 4 pounds of molasses. However, when the molasses feeding level was 1 and 2 pounds, in Rations 2 and 3, the apparent digestibility was 63.58 and 64.96 percent, respectively.

As expected, there was a gradual but well defined increase in apparent digestibility of nitrogen-free extract content of the rations, as shown in Table 2. This is in keeping with the increased percentage of N. F. E. in rations including molasses.

Apparent digestibility of dry matter content increased rather sharply from 53.39 to 61.25 percent when 1 pound of molasses was fed, and increased again to 65.26 percent when the molasses level was increased to 2 pounds daily. When the molasses level reached 4 pounds daily there was a decrease of about 4 percent to 62.54 percent, largely due to decrease in the apparent digestibility of cellulose and crude fiber at this molasses level.

Average composition of rations in this phase is shown in Table 3. There is a gradual decrease in percentage composition of all nutrients

TABLE 3--AVERAGE COMPOSITION OF RATIONS FED IN PHASE I

Ration Number	Lb. Molasses	Percentage Composition: Molasses	Crude Protein	Crude Fiber	Nitrogen-free Extract	Ash	Cellu-lose	Dry Matter	Ether Extract
1	0	0	14.57	26.34	51.89	5.66	26.22	86.96	1.55
2	1	4.41	13.94	25.24	53.57	5.67	25.67	86.17	1.43
3	2	8.15	13.47	25.25	54.15	5.74	24.58	85.91	1.36
4	4	16.50	12.57	22.02	58.02	5.86	21.92	83.85	1.28

except ash and nitrogen-free extract as the percent molasses in the ration increases. This is due to the rather high mineral and sugar content of the molasses. Animals used in this phase consumed all of the rations readily. While there seemed to be a bit of hesitancy at a change from dry hay to that covered with molasses and the same with the reverse, the ration was always consumed with the exception of some stems which were recovered, weighed, analyzed and deducted from total nutrient intake.

Chromium oxide, which was added to the ration, was not objected to by any of the animals. Since all cows were at the end of their lactations and with calf, there was no difficulty encountered with heat periods nor did any animal give indication of digestive disturbances. It is rather interesting that all fecal samples were normal with only a very mild difference in moisture content of the feces.

Phase II: A Good Quality, Leafy, Green Mixed Timothy-lespedeza Hay. All four animals ate well, with Holstein cow 314 having an especially large appetite. Holstein cow 317 injured her right rear leg at the start of Trial IV and did not consume as much hay as she had during the other three trials. Fortunately, she was on Ration 1, with no molasses at that time. Nevertheless, the apparent digestibility of this ration was below the average on the other three cows receiving the same ration. Since she seemed perfectly normal otherwise, the digestion coefficients were included in the averages for Ration 1.

Coefficients of apparent digestibility obtained in Phase II are listed in Table 4. The average percentage composition of the rations is shown in Table 5. As was the case in Phase I, the average percentage composition of all nutrients except ash and nitrogen-free extract decreased with increasing levels of molasses.

TABLE 4--AVERAGE DIGESTION COEFFICIENTS* AND BACTERIAL COUNTS AT VARYING LEVELS OF MOLASSES FEEDING WITH THE TIMOTHY-LESPEDEZA HAY FED IN PHASE II

Lb. Molasses in Daily' Ration	Coefficients of Apparent Digestibility in Percent							
	Dry Matter	Crude Protein	Crude Fiber	Ether Extract	Ash	Nitrogen-free Extract	Cellulose	Bacteria Count**
0	54.41	56.88	50.61	68.46	75.80	61.96	54.61	63
1	55.92	56.54	51.62	66.24	57.95	63.64	55.53	77
2	57.89	57.58	47.38	66.17	36.24	68.20	54.67	84
4	63.70	57.98	54.44	68.89	45.69	73.77	58.34	120

* A 4 x 4 randomized block design was used, furnishing four animal experimental periods for each ration.
** In billions per gram of wet rumen ingesta.

TABLE 5--AVERAGE COMPOSITION OF RATIONS FED IN PHASE II

Ration Number	Lb. Molasses	Percentage Composition							
		Molasses	Crude Protein	Crude Fiber	Nitrogen-free Extract	Ash	Cellulose	Dry Matter	Ether Extract
1	0	0	10.23	25.84	56.45	5.23	24.49	88.98	2.25
2	1	3.89	9.82	24.79	58.04	5.23	23.46	88.40	2.14
3	2	9.29	9.43	23.00	59.79	5.24	22.07	87.74	2.07
4	4	16.55	8.95	21.14	62.16	5.29	20.55	86.84	1.89

Somewhat different results were obtained in the apparent digestibility of the various feed nutrients when timothy-lespedeza hay was fed. Crude protein digestibility was practically the same at the 1-pound molasses level as that obtained on Ration 1, which had no molasses. Similarly, the apparent digestibility of protein was the same at the 2 and 4-pound molasses levels, being 56.88, 56.54, 57.58 and 57.98, respectively. Crude fiber digestibility increased from 50.61 percent with no molasses to 51.62 percent at the 1-pound molasses level. When 2 pounds of molasses was fed the apparent digestibility dropped to 47.38 percent, a decrease of 8 percent. However, as the molasses feeding level was increased to four pounds daily, the apparent digestibility rose to 54.44 percent, an increase of nearly 13 percent.

Apparent digestibility of cellulose followed the same trend as crude fiber, increasing from 54.61 to 55.53 percent at the no-molasses and 1-pound molasses feeding levels. It then dropped to 54.67 percent at the 2-pound molasses level and rose to 58.34 percent on Ration 4, which contained 4 pounds of molasses daily.

The differences in digestibility between timothy-lespedeza hay and alfalfa hay are rather unusual, although the lower protein content of the timothy-lespedeza hay might acçount for the uniform digestion of the nutrient. The same explanation could apply to crude fiber and cellulose, although it is difficult to understand the decrease in digestibility noted on Ration 3.

Apparent digestibility of nitrogen-free extract and dry matter increased at increasing levels of molasses. Since this phase was conducted during the hot months of June and July, 1952, it was found necessary to turn the experimental animals into a dry lot during the night. Whether the high temperature of that period had any effect on the digestibility of the rations is not known. There was a difference in the amount of exercise obtained since the animals seemed rather restless and desirous of joining the rest of the herd in the pasture. Certainly the conditions were not as controlled as was possible in the other phases of the experimental work.

Phase III: Good Quality, Green Alfalfa-brome Hay. The hay was readily consumed with no weigh-back except for a slight amount during Phase I. Cow 994 was the only animal used in this phase that was not with calf. The one time she was in heat seemed to have no effect on her appetite or general behavior. She got excited while rumen samples were being obtained but, since they were obtained at the end of each collection period, this was not considered to be a factor of importance. Other animals used were normal in every respect.

Observations of Morrison (1951, 1952) that molasses was of questionable value in high quality roughage rations might be worth consideration in reviewing results obtained in this phase. The coefficients of apparent digestibility are shown in Table 6 and the average percentage composition of the rations is shown in Table 7. There was a slight increase from 68.74 to 69.26 percent when 1 pound of molasses was added to the ration daily. When the molasses was increased to 2 pounds daily there was a decrease of over 4 percent to 66.27 percent. When 4 pounds of molasses was included in the ration, a decline to 61.08 percent was

TABLE 6--AVERAGE DIGESTION COEFFICIENTS* AND BACTERIAL COUNTS AT VARYING LEVELS OF MOLASSES FEEDING WITH THE ALFALFA-BROME HAY FED IN PHASE III

Lb. Molasses in Daily Ration	Coefficients of Apparent Digestibility in Percent							
	Dry Matter	Crude Protein	Crude Fiber	Ether Extract	Ash	Nitrogen-free Extract	Cellulose	Bacteria Count**
0	61.31	68.74	48.31	50.54	35.14	70.14	59.45	69
1	60.65	69.26	46.14	45.70	43.55	70.29	58.21	84
2	62.11	66.27	47.49	48.38	40.48	71.38	57.76	98
4	59.97	61.08	41.25	39.58	41.68	73.86	54.59	104

* A 4 x 4 randomized block design was used, furnishing four animal experimental periods for each ration.

** In billions, per gram of wet rumen ingesta.

TABLE 7--AVERAGE COMPOSITION OF RATIONS FED IN PHASE III

Ration Number	Lb. Molasses	Percentage Composition							
		Molasses	Crude Protein	Crude Fiber	Nitrogen-free Extract	Ash	Cellulose	Dry Matter	Ether Extract
1	0	0	13.85	29.79	48.13	6.54	26.68	93.12	1.70
2	1	3.98	13.45	28.83	49.41	6.70	25.80	92.42	1.62
3.	2	7.93	12.99	27.36	51.32	6.76	24.52	91.62	1.57
4	4	14.24	12.26	25.70	54.36	6.90	23.02	90.42	1.44

observed, representing a decrease of nearly 8 percent. Somewhat the same trend was noted with crude fiber, where the digestibility was 48.31 percent on the molasses-free ration. However, in this case the apparent digestibility dropped to 46.14 percent on Ration 2 containing 1 pound of molasses daily, but rose to 47.49 percent when 2 pounds of molasses was included in the ration. The same general decrease was observed in protein digestibility obtained with crude fiber when 4 pounds of molasses was fed daily. At this molasses feeding level the apparent digestibility was only 61.08 percent.

The apparent digestibility of cellulose showed a gradual but consistent decrease as the molasses level increased. While the trends in digestibility of these nutrients were different from those obtained in the previous two phases, they were not unusually large and were obtained on a different quality roughage. Digestibility of nitrogen-free extract increased gradually from 70.14 with no molasses to 73.86 percent when 4 pounds of molasses was fed daily. Digestibility of the dry matter content decreased from 61.31 percent with no molasses to only 60.65 percent at the 1-pound molasses level, then increased to 62.11 percent with 2 pounds of molasses, and decreased again to 59.97 percent when 4 pounds of molasses was fed. It should be noted that although the quality of the roughage used in Phase III seemed to be much higher than that in Phase I, there is little difference in average percentage composition of the rations. Apparently the brome content of the hay used in Phase III had some effect. There was, however, less response, either positive or negative, to the addition of molasses to the alfalfa-brome hay.

Phase IV: Medium Quality Alfalfa Hay. All cows were placed on the control ration of alfalfa hay and 5 pounds of the usual grain mix daily. No molasses was fed during Trial I. In Trial II, cow A continued to receive the control ration while cows B, C, D, and E received 2, 4, 6, and 8 pounds of molasses daily in addition to hay and grain. The same rations were fed in Trial III to study the effect of continued molasses feeding. Average composition of all rations fed is shown in Table 9. Due to an error in grinding the roughage and a subsequent shortage of hay, a slightly different composition hay was fed in Trial I than in Trials II and III. The average coefficients of apparent digestibility are shown in Table 8.

TABLE 8--DIGESTION COEFFICIENTS AND BACTERIAL COUNTS AT VARYING LEVELS OF MOLASSES FEEDING WITH THE ALFALFA HAY FED IN PHASE IV

Trial	Cow	Lb. Molasses Fed Daily	Coefficients of Apparent Digestibility in Percent: Dry Matter	Crude Protein	Crude Fiber	Ether Extract	Ash	Nitrogen-free Extract	Cellulose	Bacteria Count*
I	A	0	65.60	70.31	51.15	50.12	53.22	75.64	64.22	62
	B	0	65.91	72.26	52.70	56.18	55.15	73.78	62.39	61
	C	0	65.87	73.69	50.61	57.47	57.81	74.14	62.62	57
	D	0	59.84	68.65	42.63	46.67	50.72	69.74	57.88	56
	E	0	62.99	69.01	49.78	45.32	53.87	71.20	60.28	57
II	A**	0	60.42	74.12	41.41	48.40	35.20	71.37	55.38	51
	B	2	69.02	76.40	52.50	59.58	42.77	80.41	64.30	69
	C	4	64.70	71.66	42.06	51.38	47.39	78.09	56.71	90
	D	6	63.85	69.04	41.69	64.30	50.90	77.19	53.28	106
	E	8	64.37	65.37	43.06	29.74	51.71	78.49	55.04	107
III	A**	0	54.68	72.02	27.84	41.48	31.36	68.72	43.24	57
	B	2	62.72	73.02	38.58	51.36	43.91	76.18	52.07	67
	C	4	64.88	71.97	39.98	47.49	43.39	77.90	52.25	86
	D	6	63.19	66.43	36.36	38.77	47.02	79.39	51.84	100
	E	8	64.79	66.75	37.73	29.91	50.45	80.67	50.45	111

* In billions, per gram of wet rumen ingesta.
** Cow A came down with an attack of mastitis at the start of trial II and this condition continued for the remainder of the experimental period.

At the start of Trial II, Cow A, receiving the control ration, suffered an attack of chronic mastitis which lasted throughout the experimental period. Apparently this was the cause of the considerable variation in apparent digestibility of feed nutrients in Ration 1. Crude protein digestibility increased from 70.31 percent in Trail I to 74.12 percent in

TABLE 9--COMPOSITION OF RATIONS FED IN PHASE IV

Trial	Cow	Lb. Molasses Fed Daily	Percentage Composition							
			Molasses	Crude Protein	Crude Fiber	Nitrogen-free Extract	Ash	Cellulose	Dry Matter	Ether Extract
I	A	0	0	13.32	30.48	47.92	6.74	27.19	92.59	1.54
	B	0	0	13.37	29.63	48.66	6.75	26.45	92.51	1.59
	C	0	0	13.36	29.78	48.53	6.75	26.58	92.53	1.58
	D	0	0	13.29	30.94	47.51	6.74	27.58	92.63	1.52
	E	0	0	13.04	31.01	47.70	6.74	27.66	92.64	1.51
II	A	0	0	16.20	27.08	49.36	5.76	23.97	90.83	1.60
	B	2	8.19	15.07	24.17	53.22	6.03	21.41	89.35	1.51
	C	4	14.62	14.25	22.67	55.49	6.20	20.28	88.19	1.39
	D	6	18.34	14.73	22.23	55.48	6.26	19.67	87.54	1.30
	E	8	21.33	13.42	21.76	57.26	6.33	19.25	87.00	1.23
III	A	0	0	16.12	26.09	50.30	5.83	23.11	90.81	1.66
	B	2	8.81	14.89	23.59	53.96	6.03	20.90	89.36	1.53
	C	4	14.62	14.13	22.67	55.70	6.11	20.07	91.03	1.39
	D	6	18.42	13.58	22.18	56.78	6.16	19.61	87.80	1.30
	E	8	23.09	13.00	20.92	58.60	6.26	18.52	87.04	1.22

Trial II, then dropped to 72.02 percent in Trial III. Crude fiber digestibility of 51.15 percent obtained in Trial I dropped to 41.41 in Trial II and again to 27.84 percent in Trial III. Apparent digestibility of the cellulose also decreased from 64.22 percent in Trial I to 55.38 percent and then to 43.24 percent in Trials II and III, respectively.

The same trend was observed in digestion of the rest of the feed nutrients, including nitrogen-free extract, which decreased from 75.64 percent in Trial I to 71.37 and 68.72 percent in Trials II and III. When 2 pounds of molasses was added to Ration 2 in Trials II and III, the digestibility of crude protein increased from the high level of 70.31 to 76.40 percent but decreased to 73.02 percent in Trial III. The apparent digestibility of crude fiber increased from 51.70 percent in Trial I to 52.50 percent in Trial II, then decreased rather sharply to 38.58 percent in Trial III. Cellulose digestibility continued to follow that of crude fiber, increasing from 62.39 to 64.30 percent in Trial II and down to 52.07 percent in Trial III. The effect of continued molasses feeding at the same level for four weeks also had the same effect on apparent digestibility of the other feed nutrients. Nitrogen-free extract digestibility increased from 73.78 percent in Trial I to 80.41 percent in Trial II, then decreased to 76.18 percent in Trial III. Digestibility of dry matter was 65.91, 69.02 and 62.72 percent for the same three periods.

Four pounds of molasses daily was added to Ration 3 during Trials II and III. This resulted in a decrease of apparent digestibility of crude protein from 73.69 percent on the control ration to 71.66 and 71.97 percent in Trials II and III, respectively. Digestibility of crude fiber decreased from 50.61 percent in Trial I to 42.06 and finally to 39.98 percent in Trials II and III. Cellulose digestion decreased from 62.62 to 56.71 and 52.26 percent during the same three trials. However, the apparent digestibility of the nitrogen-free extract increased from 74.14 percent in Trial I where no molasses was fed to 78.09 percent in Trial II, but decreased to 77.90 percent in Trial III. Apparent digestibility of dry matter content decreased from 65.87 percent in Trial I to 64.70 and 64.88 percent in Trials II and III.

The daily molasses feeding level was raised to 6 pounds daily in Ration 4 during Trials II and III. The digestibility of protein increased from 68.65 percent in Trial I to 69.04 percent in Trial II, then decreased to 66.43 percent in Trial III. Crude fiber digestibility decreased slightly from 42.63 percent in Trial I to 41.69 percent in Trial II and continued downward to 36.36 percent in Trial III. Digestibility of cellulose also decreased from 57.88 to 53.28 to 51.84 percent during Trials I, II and III, respectively. Nitrogen-free extract digestibility increased from 69.74 percent when no molasses was fed in Trial I to 77.19 and 79.39 percent when 6 pounds of molasses was fed daily. Dry matter content of this ration showed a digestion coefficient of 59.84 percent in Trial I where no molasses was fed, increasing to 63.85 percent in Trial II and 63.19 percent in Trial III.

When 8 pounds of molasses was added to the ration a fairly large decrease in crude protein digestibility from 69.01 percent in Trial I to 65.37 and 66.75 percent in Trials II and III was observed. A sharp decrease of crude fiber digestibility from 49.78 percent in Trial I to 43.06 percent in Trial II and 37.73 percent in Trial III occurred. Cellulose digestibility decreased from 60.20 percent down to 55.04 percent and finally 50.45 percent during the same three periods. However, the reverse was obtained in digestibility of nitrogen-free extract material and dry matter content. The nitrogen-free extract digestibility increased from 71.28 percent in Trial I to 78.49 percent in Trial II and 80.67 percent in Trial III. Digestibility of the dry matter increased from 62.99 to 64.37 and 64.79 percent in the three trial periods.

Phase V: Average Quality Brown Alfalfa Hay. Organization and conduct of this phase were the same as in Phase IV. At the start of Trial II, cow E, receiving 8 pounds of molasses, refused to eat the molasses-hay mixture at first so the preliminary feeding period for her was only five days, rather than the usual 10 days. This was the only instance throughout the investigation where an animal disliked the molasses-hay mixture. Once cow E started to consume her roughage regularly she seemed to relish the mixture. Average composition of all rations fed in Phase V is shown in Table 11.

TABLE 10--DIGESTION COEFFICIENTS AND BACTERIAL COUNTS AT VARYING LEVELS OF MOLASSES FEEDING WITH THE ALFALFA HAY FED IN PHASE V

Trial	Cow	Lb. Molasses Fed Daily	Coefficients of Apparent Digestibility in Percent: Dry Matter	Crude Protein	Crude Fiber	Ether Extract	Ash	Nitrogen-free Extract	Cellulose	Bacteria Count*
I	A	0	58.59	73.23	29.25	60.48	36.48	73.99	48.54	58
	B	0	62.61	75.10	39.38	64.90	52.81	74.84	54.26	62
	C	0	52.52	68.97	18.87	49.75	31.31	70.31	40.53	62
	D	0	55.61	68.84	35.35	27.16	45.26	68.24	49.14	63
	E	0	56.67	69.80	29.21	34.36	42.08	71.56	47.23	68
II	A	0	60.93	74.72	35.66	55.19	55.92	73.96	52.89	58
	B	2	57.57	68.59	29.80	29.42	49.41	74.17	46.15	85
	C	4	56.02	62.44	19.44	38.35	48.21	74.89	37.53	115
	D	6	56.77	57.58	26.47	28.90	46.27	75.19	42.64	117
	E	8	56.22	49.85	15.62	27.56	48.26	75.76	29.96	127
III	A	0	58.49	73.20	31.77	53.56	41.64	71.39	49.45	65
	B	2	64.28	73.31	38.67	53.16	56.19	78.33	53.16	76
	C	4	57.81	62.23	22.79	42.29	49.45	76.60	41.10	103
	D	6	58.38	62.50	27.65	32.70	49.43	77.52	41.15	104
	E	8	59.96	58.50	28.66	37.03	51.95	78.09	43.58	116

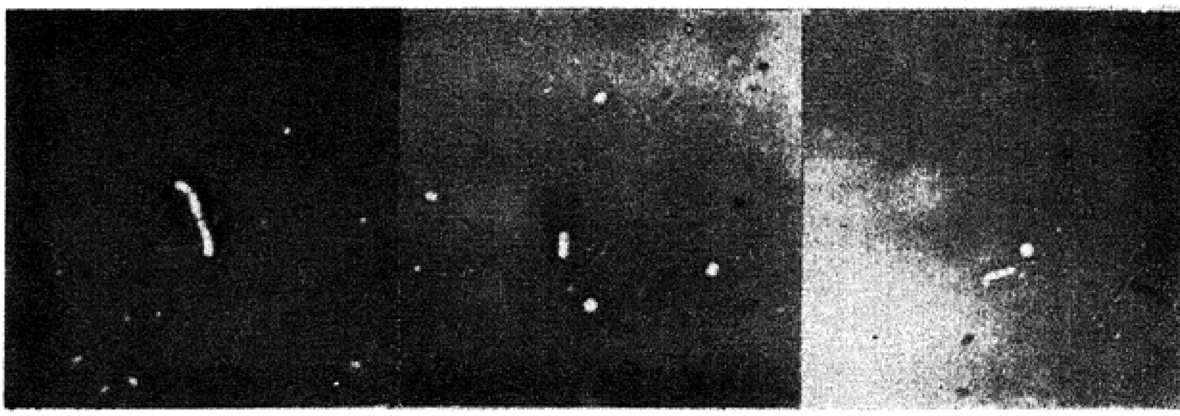
*In billions, per gram of wet rumen ingesta.

TABLE 11--COMPOSITION OF RATIONS FED IN PHASE V

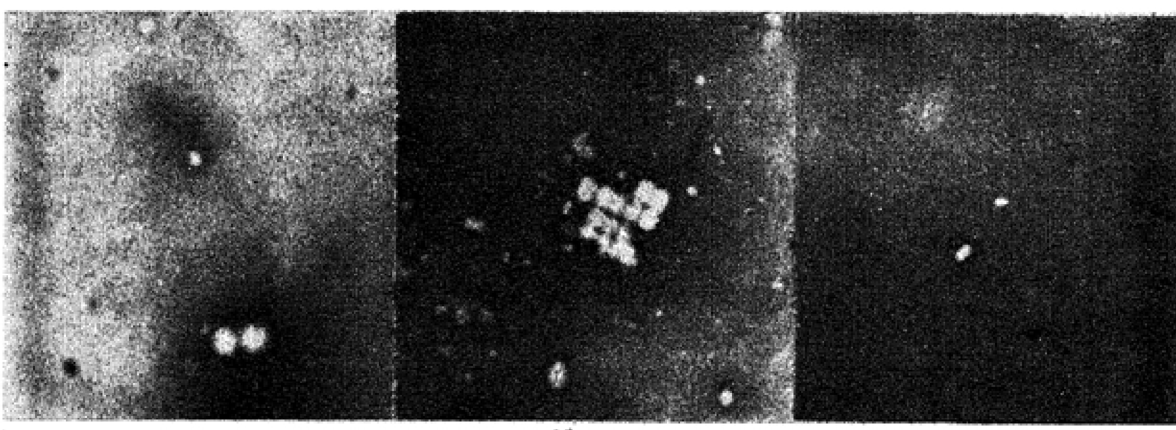
Trial	Ration Number	Lb. Molasses	Percentage Composition: Molasses	Crude Protein	Crude Fiber	Nitrogen-free Extract	Ash	Cellulose	Dry Matter	Ether Extract
I	1	0	0	15.59	24.81	50.44	7.36	22.05	91.52	1.80
	2	0	0	15.40	26.27	48.76	7.47	23.23	91.73	2.10
	3	0	0	15.60	25.20	50.00	7.45	22.40	91.29	1.75
	4	0	0	15.26	27.20	48.42	7.54	24.06	91.89	1.58
	5	0	0	15.35	26.94	48.56	7.56	23.84	93.23	1.59
II	1	0	0	15.62	25.78	49.36	7.49	22.88	91.66	1.75
	2	2	9.82	14.24	24.07	52.45	7.75	21.32	89.90	1.49
	3	4	19.24	13.12	21.13	56.44	7.93	18.74	88.01	1.38
	4	6	21.93	12.61	21.46	56.65	8.05	18.98	87.65	1.23
	5	8	35.19	11.08	16.41	63.15	8.20	14.54	84.85	1.16
III	1	0	0	15.62	25.84	49.31	7.49	22.93	91.67	1.74
	2	2	10.85	14.21	23.21	53.31	7.74	20.59	89.60	1.53
	3	4	17.67	13.24	22.05	55.42	7.93	19.54	88.39	1.36
	4	6	21.48	12.65	21.68	56.39	8.05	19.17	87.75	1.23
	5	8	26.73	11.65	19.46	59.80	7.97	17.21	84.52	1.12

There was more variation in digestion coefficients obtained in this trial than in Phase IV. Coefficients of apparent digestibility are shown in Table 10. There was a slight decrease in the digestibility of protein at the 2-pound molasses level, although this decrease was not as great in Trial III. At the higher molasses feeding levels a much greater decrease in protein digestibility was observed.

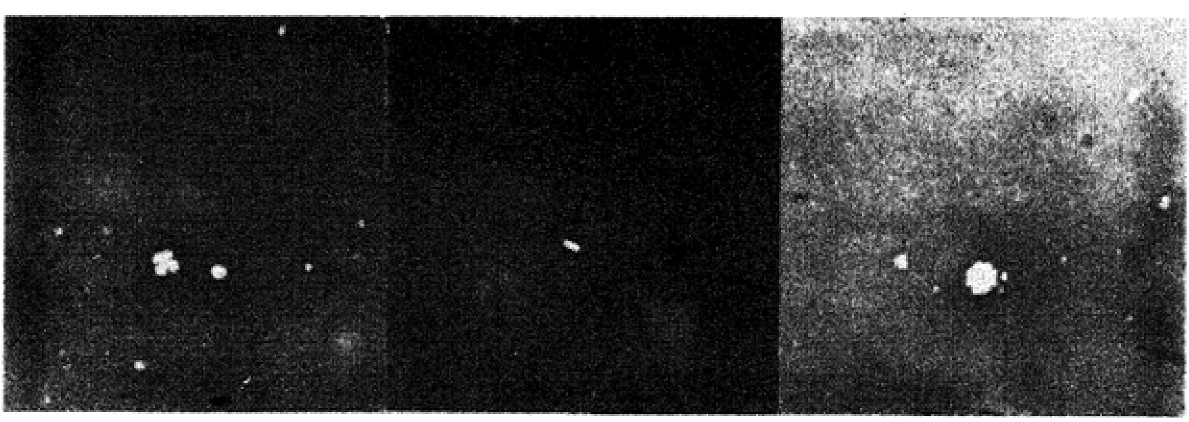
Considerable difficulty was encountered in the laboratory in obtaining repeatable results on the crude fiber fraction of fecal samples. There



Long chain cocci | Cigar-shaped rod and single cocci | Short chain cocci



Giant diplococci | Sarcina packet | Diplococci



Tetrad and short rod | Long rod | Wheel-shaped

Figure 1 — Micro-photographs of typical rumen micro-organisms observed in this investigation.

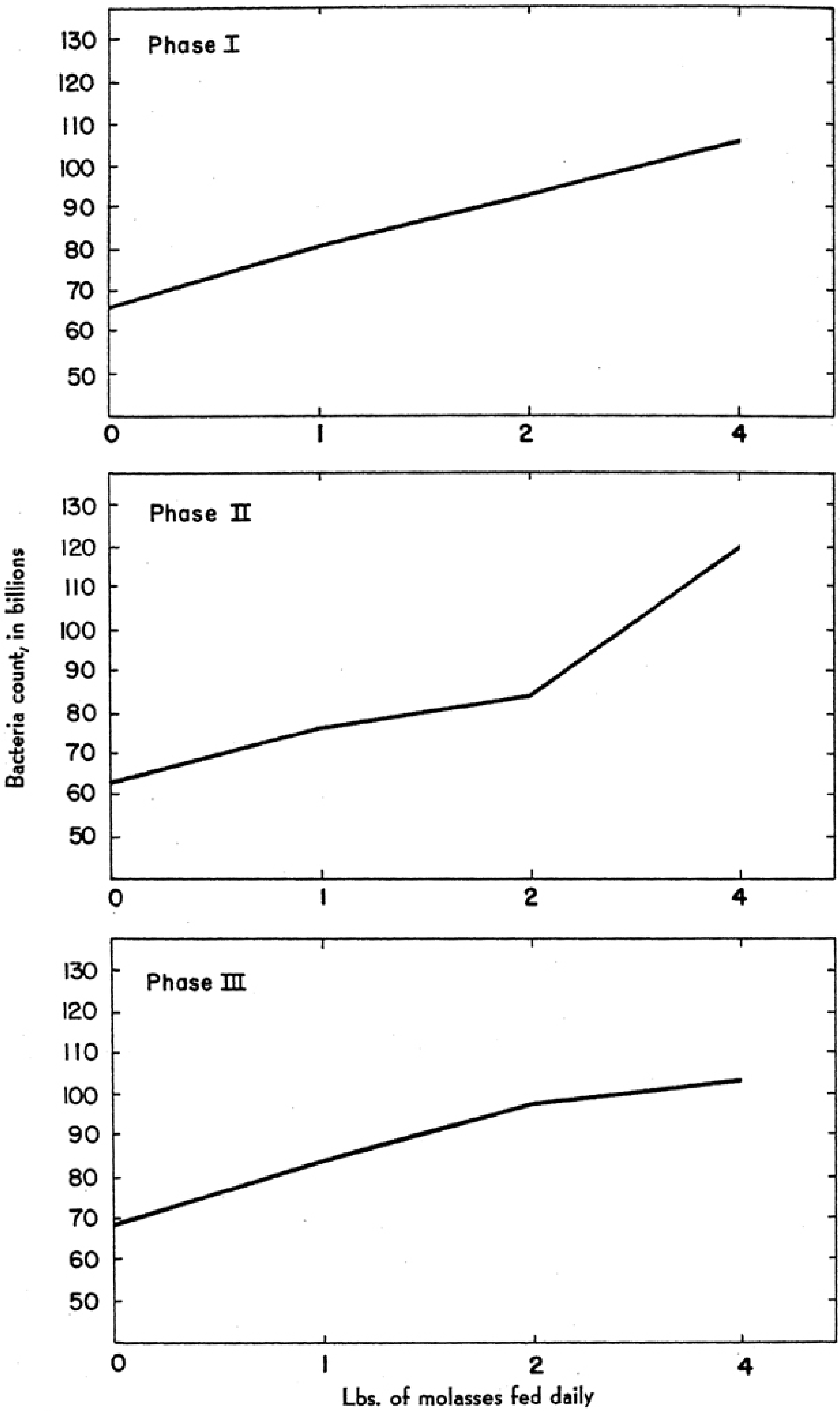


Figure 2 — The trend in bacterial population at the different levels of daily molasses feeding.

is an inclination to blame unusual variations in crude fiber digestibility obtained for these laboratory difficulties. However, it was discovered that digestibility of the cellulose followed essentially the same trends as did the crude fiber digestibility. It was found that the digestibility of crude fiber and cellulose was lower during Trial II than during Trial III. Since treatments were the same in both cases it is difficult to explain why such a variation occurred. Dry matter content and nitrogen-free extract increased in digestibility as the daily molasses feeding increased.

Bacterial Counts

Many different kinds and sizes of micro-organisms were observed in Gram stains of the rumen samples. After careful study of the morphological characteristics of the bacteria in the Gram stains, it was possible to identify the same bacteria in the nigrosine stains. While all organisms were counted, it was decided to list only the predominant organisms as observed by Baker (1942, 1943) and others. These were the various sized single cocci, diplococci, tetrads, short chain cocci, long chain cocci, short rod and long rod-shaped organisms together under each specific group name. In addition, numerous wheel-shaped organisms similar to the navicular organism described by Baker (1942) and occasional *Sarcina* packets, also reported by Baker (1943), were counted as were the large cigar-shaped organisms observed by Pounden and Hibbs (1948a). Photographs of these predominant organisms are shown in Figure 1. Trends in bacterial population at the different levels of molasses feeding are shown for the first three phases in Figure 2 and for Phases IV and V in Figure 3.

Phase I: Average bacterial counts obtained on the four different rations fed are shown in Table 12. The average count in billions obtained

TABLE 12--AVERAGE BACTERIAL COUNTS, IN BILLIONS, PER GRAM OF WET RUMEN INGESTA AT VARYING LEVELS OF MOLASSES FEEDING WITH THE ALFALFA HAY FED IN PHASE I

Ration Number	Lb. Molasses	Single cocci	Diplo-cocci	Tetrads	Short Chain Cocci	Long Chain Cocci	Short Rods	Long Rods	Sarcina Packets	Wheel-shaped	Cigar-shaped	Total Bacterial Count
1	0	50	2.5	.13	.09	.03	15	.05	0	.08	.43	67
2	1	60	1.9	.11	.17	.01	18	.02	0	.09	.43	81
3	2	71	1.5	.16	.12	.04	22	.03	0	.15	.46	93
4	4	75	1.8	.28	.15	.02	28	.02	0	.32	.46	106

per gram of wet rumen content is listed. While there were many different morphological types of organisms observed, only those most prevalent were counted. The method of counting used was comparative-

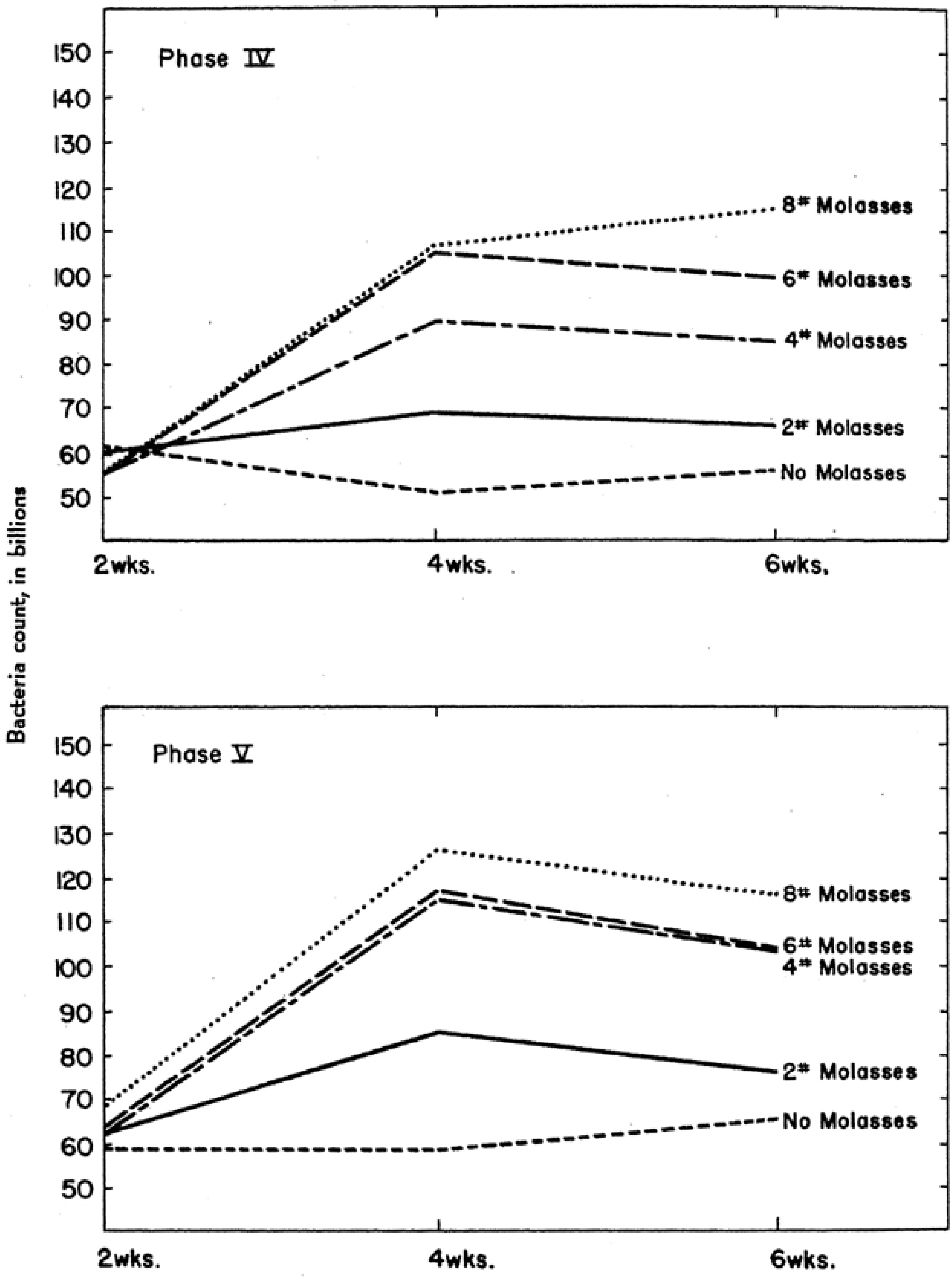


Figure 3 — The trend in bacterial population at the different levels of daily molasses feeding when no molasses was fed during the first two weeks of the trials.

ly simple but the actual counting process was very tedious and time-consuming. It is recognized that direct microscopic counts are not as accurate as plate counts, however, the work of Gall *et al.* (1949) indicates that there is a close relationship between the two techniques. It was felt that for this particular project the trends established would be of value in determining any relationship between digestibility of a ration and the bacterial population.

Since single cocci and short rod-shaped organisms were the predominant micro-organisms observed, some attention will be given them and the total bacterial counts in this paper. Gall *et al.* (1949b) has been reported previously in this paper as showing that there is little difference in the type of flora observed in Gram-stain studies although more cocci were found in high grain feeding. Since each animal received 5 pounds of grain daily in addition to three rations containing molasses, the results obtained in this and subsequent studies are not surprising. A single cocci count of 50 billion per gram of wet rumen ingesta was obtained from the ration containing no molasses. When the molasses level was raised to 1, 2, and 4 pounds the single cocci population was found to be 60, 71 and 75 billion, respectively. The short rod population was found to be 15, 18, 22 and 28 billion for the same four rations. The short rod population is of considerable importance in view of the cellulose-digesting, short rod organisms isolated by Hungate (1944, 1947) and Cowles and Rettger (1931). Hungate (1944, 1947) and Sijpestijn (1951) have also isolated cellulose-digesting cocci. Burroughs *et al.* (1950a, 1950b) has reported a close correlation between bacterial population and cellulose digestion under proper growth conditions.

The total bacterial count obtained on Ration 1, containing no molasses, was 67 billion. This is somewhat larger than that obtained by Gall *et al.* (1949). They found about 50 billion bacteria per gram of wet rumen content from winter rations and about 96 billion when the host animal was on pasture. They do not state how much, if any, grain was included in the winter ration. When one pound of molasses was added to the daily ration the total bacterial count rose to 81 billion and at 2 and 4 pounds of molasses the total count was 93 and 106 billion, respectively. This increase in bacterial count on the molasses supplemented rations corresponds to the results Bortree *et al.* (1946) obtained by the addition of glucose to a hay ration.

Phase II: Bacterial counts were quite similar to those obtained in Phase I. Complete average counts for all organisms counted are shown in Table 13. The single cocci count of 44 billion per gram of wet rumen content on the control ration, which contained no molasses, was slightly

TABLE 13--AVERAGE BACTERIAL COUNTS, IN BILLIONS, PER GRAM OF WET RUMEN INGESTA AT VARYING LEVELS OF MOLASSES FEEDING WITH THE TIMOTHY-LESPEDEZA HAY FED IN PHASE II

Ration Number	Lb. Molasses	Single cocci	Diplo-cocci	Tetrads	Short Chain Cocci	Long Chain Cocci	Short Rods	Long Rods	Sarcina Packets	Wheel-shaped	Cigar-shaped	Total Bacterial Count
1	0	44	6.6	.25	.15	.01	12	.30	.09	.03	.09	63
2	1	54	7.4	.27	.11	.07	15	.38	.05	.12	.13	77
3	2	62	5.4	.25	.08	.01	16	.43	.07	.08	.12	84
4	4	86	9.4	.14	.05	0	29	.02	.03	.13	.12	120

less than that obtained on the alfalfa hay ration. When molasses was introduced at the rate of 1 and 2 pounds daily, the single cocci count increased to 54 and 62 billion, respectively. At the 4-pound molasses level the single cocci count was 86 billion, nearly twice that on the ration which contained no molasses. The short rod counts followed the same general trend as the cocci counts, increasing from 12 billion on Ration 1 to 15, 16 and 29 billion on Rations 2, 3 and 4. This shows a rather unusual increase of over 80 percent when the molasses was increased from 2 to 4 pounds daily. The total bacterial count followed almost identically the trends of the single cocci, increasing from 63 billion bacteria on Ration 1 to 77 and 84 billions on Rations 2 and 3 and then nearly doubling the 63 billion count obtained on the control ration with a count of 120 billion on Ration 4 containing 4 pounds of molasses daily.

Phase III: A new sample of nigrosine stain was prepared for this phase but for some reason the slides were not staining properly and there was considerably more surface tension than had been observed previously. At the start of the second trial of Phase III a new slide cleaning technique reported by Bovey and Unger (1951) was used. It consisted of a solution of 25 gm. KOH and 15 gm. NH_4NO_3 in 650 ml. of distilled water. The slides were placed in this solution for 20 minutes and then allowed to soak for an hour in a solution of detergent and water. They were then rinsed in distilled water and placed in ethyl alcohol until used. This technique resulted in much more desirable slides with less bubbles and artifacts. While there was little difference in the total bacterial count and in most of the organisms counted there was a very slight decrease in the single cocci count, suggesting that it may have been impossible to discern between certain air bubbles on the slide and the cocci.

Bacterial counts in this phase were similar to those observed in the two previous phases, although there was not as great a population of single cocci and short rods at the 4-pound molasses level as had been observed before. The total average count of all the organisms counted is shown in Table 14. The single cocci count was 44 billion on the control ration and increased to 58, 63 and 71 billion as the daily molasses level increased to 1, 2, and 4 pounds daily. While the short rod count was higher than before on the control ration it increased to only 18 billion when 1 pound of molasses was added. Also, when the molasses level went to 2 and then to 4 pounds, the short rod count increased to only 19 and 22 billion, respectively. The total bacterial count of 69 billion obtained on Ration 1, which did not include molasses, was higher than had previously been observed. However, the counts of 84, 98 and 104 billion obtained at the 1-, 2- and 4-pound molasses levels were quite similar to the bacterial counts in Phases I and II.

Phase IV: The average total bacterial and protozoa counts are shown in Table 15. In this phase each animal was placed on a two-week prelim-

TABLE 14--AVERAGE BACTERIAL COUNTS, IN BILLIONS, PER GRAM OF WET RUMEN INGESTA AT VARYING LEVELS OF MOLASSES FEEDING WITH THE ALFALFA-BROME HAY FED IN PHASE III

Ration Number	Lb. Molasses	Single cocci	Diplococci	Tetrads	Short Chain Cocci	Long Chain Cocci	Short Rods	Long Rods	Sarcina Packets	Wheel-shaped	Cigar-shaped	Total Bacterial Count
1	0	44	6.2	.45	.47	.22	16	.51	.21	.14	.24	69
2	1	58	7.3	.35	.34	.30	18	.32	.13	.20	.23	84
3	2	63	8.3	.64	.35	.22	19	.38	.12	.15	.32	98
4	4	71	6.4	.62	.52	.23	22	.39	.12	.37	.21	104

TABLE 15--BACTERIAL COUNTS, IN BILLIONS, PER GRAM OF WET RUMEN INGESTA, AND PROTOZOA COUNTS, IN THOUSANDS, PER CC. LIQUID RUMEN CONTENT AT VARYING LEVELS OF MOLASSES FEEDING WITH THE ALFALFA HAY FED IN PHASE IV

Ration No.	Lb. Molasses	Single Cocci	Diplococci	Tetrads	Short Chain Cocci	Long Chain Cocci	Short Rods	Long Rods	Sarcina Packets	Wheel-shaped	Cigar-shaped	Total Count	Protozoa
1	0	44	5.2	.14	.32	0	11	.35	.07	.02	.07	62	272
2	0	43	5.4	.09	.21	.09	12	.35	.12	.07	.18	61	215
3	0	42	3.9	.07	.21	.12	10	.12	.02	.12	.09	57	240
4	0	41	4.7	.23	.07	.14	10	0	.02	0	.07	56	235
5	0	41	5.6	.18	.23	.07	10	.18	.02	0	.12	57	226
1	0	37	5.0	.09	.09	.12	8	.21	.02	.07	.09	51	284
2	2	52	5.9	.25	.30	.02	10	.30	.07	.14	.18	69	269
3	4	68	6.5	.58	.18	.12	13	.68	.23	.35	.12	90	155
4	6	79	8.9	.76	.12	.30	15	.41	.07	.44	.21	106	141
5	8	82	8.0	1.00	.18		14	.21	.09	.12	.30	107	317
1	0	41	5.1	.30	.16	.07	9	.32	.07	.09	.14	57	405
2	2	46	6.3	.30	.18	.07	12	.68	.07	.23	.14	67	154
3	4	65	5.1	.35	.51	.05	12	.28	.07	.23	.28	86	181
4	6	73	8.1	.55	.21	.14	17	.37	.09	.25	.14	100	172
5	8	84	9.2	.58	.23	.12	19	.46	.14	.35	.21	115	225

inary feeding period during which no molasses was fed. There was some variation in bacterial counts between animals observed during this first trial. The lowest total bacterial count of 56 billion was obtained from cow D on Ration 4. The count obtained from cows C and E on Rations 3 and 5 was 57 billion, while cow B had a total count of 61 billion and cow A 62 billion. The single cocci counts were somewhat closer between animals, being 44, 43, 42, 41 and 41 billion for cows A, B, C, D and E on Rations 1 through 5, respectively. The short rod count was 11 and 12 billion for cows A and B on Rations 1 and 2 and 10 billion for the other three animals. Protozoa count varied from 215 thousand in cow B to 226, 235, 240 and 272 thousand in cows E, D, C and A, respectively.

In the next two trials, when molasses was added to all except Ration 1, there was an increase in bacterial population of the rumen as shown in Table 15 and Figure 3. However, the counts obtained from cow A on the control ration decreased during Trial II. Cow B, after receiving 2 pounds of molasses daily, showed a single cocci increase after 43 billion to 52 billion at two weeks and then dropped to 46 billion at 4 weeks. The short rod count from the same animal remained at the 10 billion level at two weeks and then increased to 12 billion at four weeks. Total bacterial count followed the same trend as the single cocci by increasing from 61 billion to 69 billion after two weeks and then dropping to 67 billion at the end of four weeks. Protozoa counts increased from 215 thousand to 269 thousand in Trial II and decreased to 154 thousand in Trial III.

Cow C received 4 pounds of molasses daily and responded by yielding a total rumen bacterial count of 90 billion after two weeks of molasses and then dropping to 68 billion at the end of four weeks. Single cocci and short rod populations followed the same trend, first increasing from 42 and 10 billion to 68 and 13 billion and dropped again to 65 and 12 billion respectively. Protozoa count decreased from 240 thousand in Trial I to 155 thousand in Trail II and increased to 181 thousand in Trial III. Ration 4 included the addition of 6 pounds of molasses daily. After only two weeks on this ration the rumen content of cow D showed a total bacterial count of 106 billion, compared to 56 billion on a molasses-free diet. Protozoa count decreased from 235 thousand to 141 thousand during the same period. Single cocci and short rod counts increased from 41 to 79 and from 10 to 15 billion, respectively. After an additional two weeks of molasses at the same feeding level, total bacterial count had decreased to 100 billion and the single cocci count to 73 billion, while the protozoa count increased to 172 thousand. However, the short rod count increased from 15 to 17 billion per gram of wet rumen content.

Cow E, receiving Ration 5, was fed 8 pounds of molasses daily. The total bacterial count rose from 57 to 107 billion at the two-week level and continued to increase to 115 billion after four weeks of molasses. The same trend was observed with single cocci which increased from 41 to 82 to 84 billion and the short rod population which increased from 10 to 14 to 19 billion. Meanwhile, the protozoa count increased from 226 thousand to 317 thousand and then decreased to 225 thousand.

Phase V: The organization, conduct, and technique employed in this phase paralleled that of Phase IV. Total bacterial and protozoa counts are shown in Table 16. During the preliminary feeding period, or Trial

TABLE 16--BACTERIAL COUNTS, IN BILLIONS, PER GRAM OF WET RUMEN INGESTA, AND PROTOZOA COUNTS, IN THOUSANDS, PER CC. LIQUID RUMEN CONTENT AT VARYING LEVELS OF MOLASSES FEEDING WITH THE ALFALFA HAY FED IN PHASE V

Ration No.	Lb. Molasses	Single Cocci	Diplococci	Tetrads	Short Chain Cocci	Long Chain Cocci	Short Rods	Long Rods	Sarcina Packets	Wheel-shaped	Cigar-shaped	Total Count	Protozoa
1	0	43	4.9	.18	.18	.09	9	.2	0	.02	.18	58	336
2	0	43	6.4	.21	.07	.14	11	.14	.07	.02	.14	62	152
3	0	43	6.4	.14	.21	.09	11	.37	.02	.12	.09	62	152
4	0	44	6.6	.14	.09	.09	12	.21	0	.02	.18	63	314
5	0	46	7.5	.35	.09	.07	12	.30	0	0	.09	68	217
1	0	39	7.1	.23	.07	.14	12	.14	.23	.07	.09	58	307
2	2	61	7.4	.44	.21	.09	15	.55	.09	.14	.14	85	324
3	4	83	9.0	.69	.32	.07	21	.46	.02	.69	.25	115	119
4	6	87	9.2	.35	.25	.12	19	.25	.02	.32	.32	117	181
5	8	92	10.4	.83	.25	.09	22	.55	.07	.32	.23	127	373
1	0	44	6.4	.09	.12	.09	11	.21	.02	.09	.02	65	283
2	2	51	8.4	.32	.21	.14	15	.48	.07	.12	.14	76	195
3	4	68	8.5	.69	.30	.07	20	.51	.07	.30	.28	103	178
4	6	71	9.8	.64	.23	.18	21	.28	.09	.51	.28	104	299
5	8	80	9.3	.90	.23	.12	23	.64	.21	.87	.37	116	378

I, the total bacterial count varied from 58 billion per gram of wet rumen content from cow A on Ration 1 to 68 billion from cow E on Ration 5. The protozoa count varied from 152 thousand from cows B and C to 336 thousand from cow A. There was virtually no variation in average single cocci count among the five animals. Short rod counts varied from 9 billion from cow A to 12 billion from cows D and E.

When molasses was added to all rations, with the exception of Ration 1 fed to cow A, the bacterial counts increased considerably. The short rod count on Ration 1 was 9, 12 and 11 billion in Trials I, II and III, respectively. Single cocci counts for the same periods were 43, 39 and 44 billion. Total bacterial counts on Ration 1 were 58 billion in Trials I and II and 65 billion in Trial III, while the protozoa counts were 336, 307 and 283 thousand in the same three trials. When cow B, on Ration 2, was fed 2 pounds of molasses daily, her total bacterial count increased from 62 to 85 billion in Trial II but dropped to 76 billion in Trial III. Single cocci counts followed the same trend, while the short rod counts rose from 11 billion in Trial I to 15 billion in Trials II and III. Protozoa count increased from 152 thousand in Trial I to 324 thousand in Trial II and decreased again to 195 thousand in Trial III.

When the daily molasses feeding level was increased to 4 pounds daily for Ration 3 in Trials II and III, the total bacterial counts increased from 62 billion where molasses was not fed to 115 billion in Trial II, then decreased to 103 billion in Trial III. The same trend was observed in single cocci and short rod population, although there was a much greater variation in the former. Protozoa count decreased to 119 thousand in Trial II and increased to 178 thousand in Trial III.

At the 6-pound molasses level fed cow D on Ration 4 the total bacterial count increased from the 63 billion observed on the control ration to 117 billion in Trial II and then decreased to 104 billion in Trial III. The single cocci count also increased from 44 billion in Trial I to 87 billion in Trial II and then decreased to 71 billion in Trial III. However, the short rod population continued to increase from the 12 billion counted in Trial I to 19 billion in Trial II and 21 billion in Trial III. Protozoa count decreased from 314 thousand in Trial I to 181 thousand in Trial II and rose sharply to 299 thousand in Trial III.

Cow D, on Ration 5, received 8 pounds of molasses daily during Trials II and III. While cow D had the largest total bacterial count in Trial I, total count in Trial II increased from 68 billion to 127 billion, then decreased to 116 billion in Trial III. The same trend was observed in single cocci counts but the short rod counts increased from 12 to 22 to 23 in Trials I, II and III. Protozoa counts increased from 217 thousand in Trial I, to 373 thousand in Trial II, and increased slightly to 378 thousand in Trial III.

Bacterial Population and Digestibility

Phase I: A graphic relationship between total bacterial counts and apparent digestibility of protein, crude fiber, cellulose, and nitrogen-free extract is shown in Figure 4. A numerical comparison also is shown in Table 2. While there was a definite increase in bacterial count at each increase in pounds of molasses, digestion of crude fiber and cellulose did not follow this trend. It did follow the same trend to the 2-pound molasses level but decreased when 4 pounds of molasses was fed daily.

Digestibility of crude protein was virtually the same in Rations 1, 2 and 3 receiving 0, 1 and 2 pounds of molasses daily and decreased only slightly from 67.57 percent to 65.71 percent in Ration 4. An increase in nitrogen-free extract digestibility followed the increase in

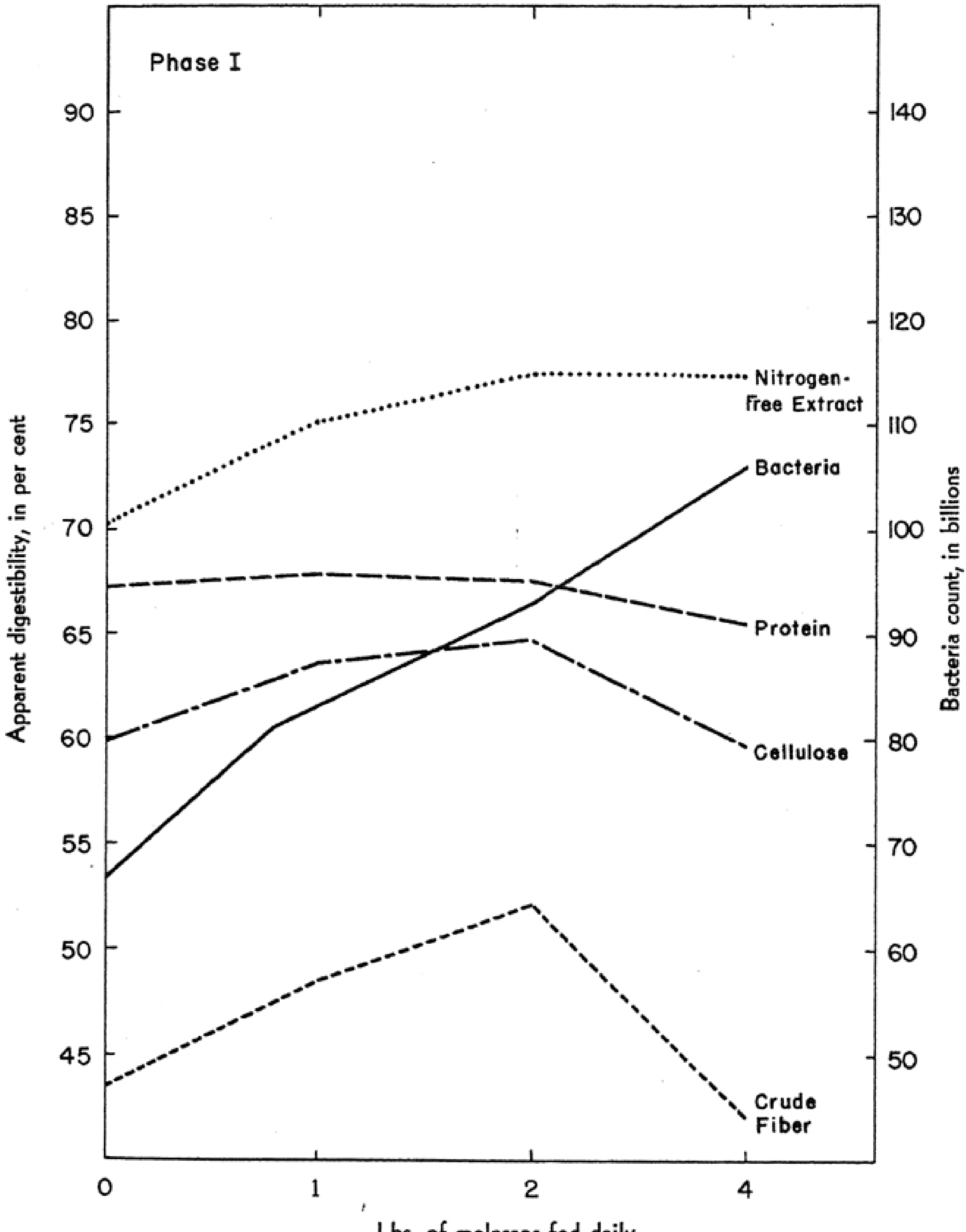


Figure 4 — The relationship between total bacterial count and the apparent digestibility of protein, crude fiber, cellulose and nitrogen-free extract at different levels of daily molasses feeding.

bacterial population. The relationship between cellulose digestion and the short rod population is shown in Figure 5.

Phase II: The same increase in bacterial counts obtained in Phase I prevailed in Phase II. However, on the timothy-lespedeza hay ration the crude protein digestibility was virtually the same when 1 pound of molasses was fed daily in Ration 2 as when no molasses was fed in Ration I. When the molasses feeding level was raised to 2 and 4 pounds daily in Rations 2 and 3, respectively, the protein digestibility increased from 56.54 percent in Ration 2 to 57.58 percent in Ration 3 and 57.98 percent in Ration 4. Crude fiber and cellulose digestibility increased when 1 pound of molasses was added to the ration. However, digestibility decreased when the daily molasses feeding level was 2 pounds and rose again to the highest percentage when the feeding level was 4 pounds. This trend in cellulose digestion did not follow the continuous increase in short rod bacterial counts as shown in Figure 5. A comparison of percent digestibility and bacterial counts is shown in Table 4 and Figure 6.

Phase III: Relationship between total bacterial counts and digestibility of feed nutrients is shown in Table 6 and Figure 7. Relationship between cellulose digestion and short rod population is shown in Figure 5. While bacterial counts showed the usual increase corresponding to higher molasses levels, digestibility of protein in this ration increased slightly at the 1-pound molasses feeding level, then decreased at the higher levels. Crude fiber and cellulose showed a negative relationship, decreasing as bacterial counts went up. Only nitrogen-free extract digestion paralleled bacterial count increases.

Phase IV: Relationship between bacterial counts and digestibility of rations is shown in Table 8. In this phase, it was discovered that bacterial counts increased during the first two weeks of Trial II where molasses feeding was started. When the same level of molasses was fed each animal for four weeks, the count decreased slightly at the eight-pound molasses feeding level and increased again in Trial III. The apparent digestibility of protein followed the same trend. When molasses exceeded 2 pounds daily, there was a progressive decrease in digestion after the molasses feeding was initiated and continued in Trials II and III. Crude fiber and cellulose digestion followed the trends of crude protein.

Nitrogen-free extract digestion followed bacterial trends at both 2- and 4-pound daily molasses feeding levels but, when 6 and 8 pounds were fed, the apparent digestibility of nitrogen-free extract increased in Trial II and Trial III, while total bacterial count dropped on Ration 4 in Trial II and increased on Ration 5 during the same period.

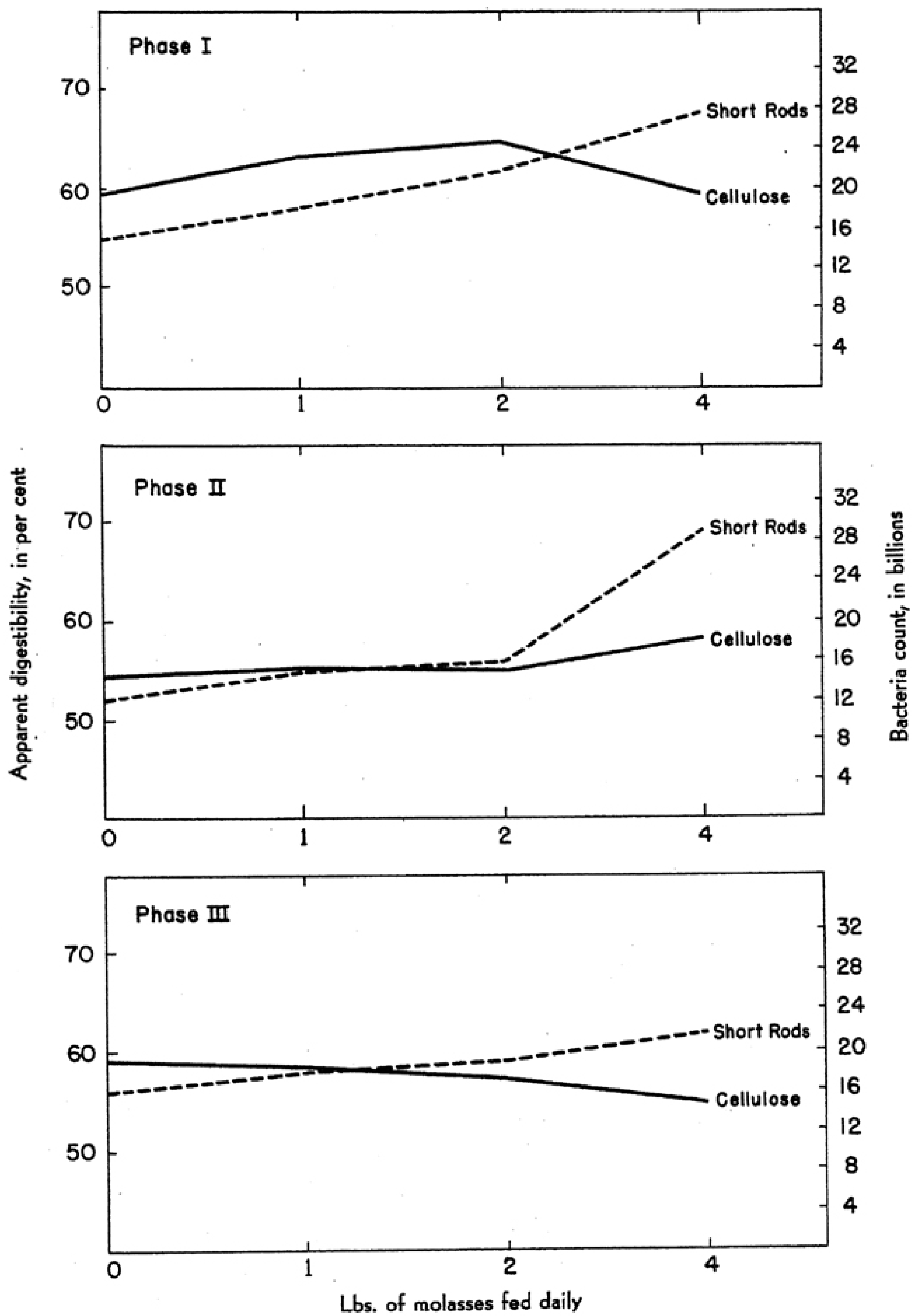


Figure 5 — Relation between short rod counts and cellulose digestion at various levels of molasses feeding.

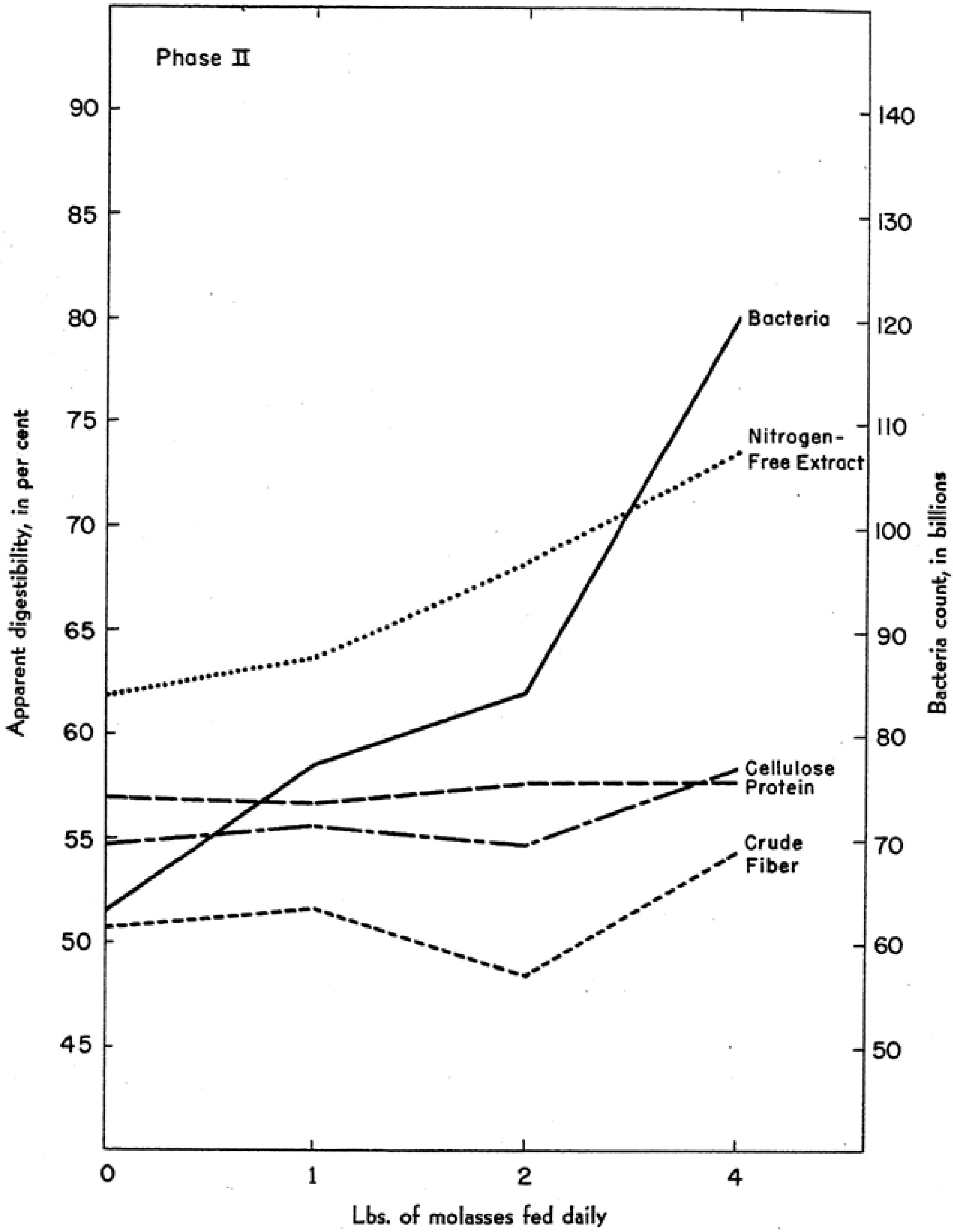


Figure 6 — The relationship between total bacterial count and apparent digestibility of protein, crude fiber, cellulose and nitrogen-free extract at different levels of molasses feeding.

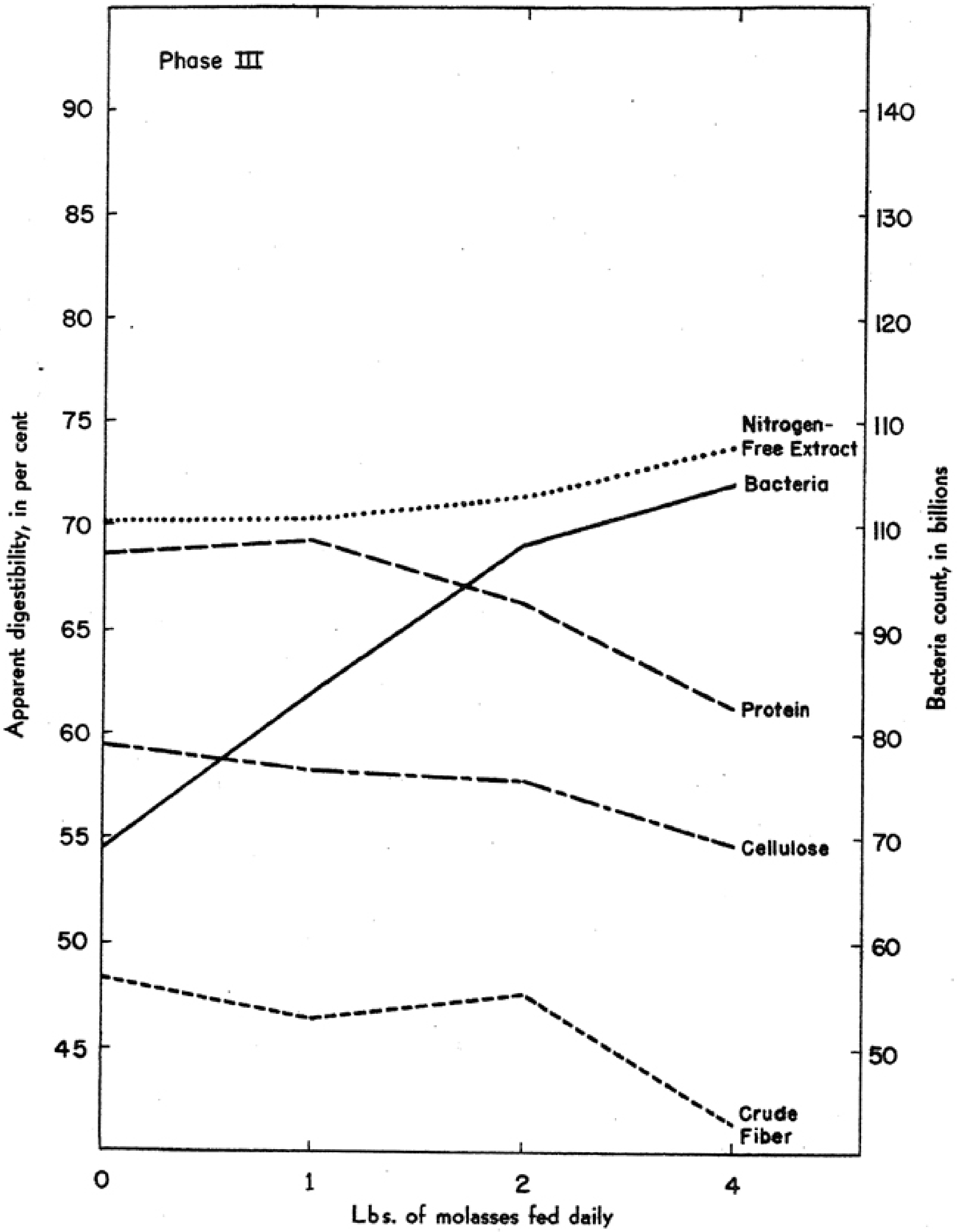


Figure 7 — Relationship between total bacterial count and apparent digestibility of protein, crude fiber, cellulose and nitrogen-free extract at different levels of molasses feeding.

Phase V: Relationship between bacterial counts and digestibility of rations is shown in Table 10. In Phase V, bacterial counts increased when molasses was added to rations during Trial II. When the same ration fed in Trial II was continued for an additional two weeks in Trial III, the total bacterial counts decreased slightly. Apparent digestibility of protein decreased during Trial II as the bacterial counts increased. Protein digestibility increased again in Trials II and III in all rations providing molasses, while the bacterial counts decreased. Digestibility of protein in this ration remained constant. Crude fiber digestibility followed the same trend noted in protein digestibility, except at the 4-pound molasses feeding level. The trend in digestibility of the cellulose fraction was essentially the same as for crude fiber and protein. However, apparent digestibility of the nitrogen-free extract fraction continued to increase slightly in Trial III, compared to Trial II, even though the bacterial counts decreased.

Body Weight Changes

Phase I: All animals used in this phase were nearing the end of lactation so there was little demand for feed consumed except for maintenance and development of the calf each was carrying. Results are shown in Table 17. The average weights obtained over a three-

TABLE 17--CHANGES IN BODY WEIGHT OF COWS AT VARYING LEVELS OF MOLASSES FED WITH THE ALFALFA HAY FED IN PHASE I

Trial	Cow Number	Ration Number	Lb. Molasses Fed Daily	Body Weight in Lb.		
				Start of Trial	End of Trial	Gain or Loss
I	206	1	0	937	926	+30
	205	2	1	1015	1037	+22
	560	3	2	892	903	+11
	558	4	4	890	918	+28
II	558	1	0	918	963	+45
	206	2	1	967	983	+16
	205	3	2	1037	1037	0
	560	4	4	903	919	+16
III	560	1	0	919	928	+ 9
	558	2	1	963	920	-43
	206	3	2	983	936	-47
	205	4	4	1037	993	-44
IV	205	1	0	993	1015	+22
	560	2	1	928	924	- 4
	558	3	2	920	942	+22
	206	4	4	936	970	+34

day period show that all animals maintained or increased their body weight except cows 558, 206 and 205 during Trial III, where rather substantial weight losses were observed for no apparent reason. During Trial IV, cow 560 lost 4 pounds.

Phase II: Animals in this phase either maintained or gained weight during the entire period, except during Trial II where slight losses were observed and during Trial III when cow 317 lost 13 pounds. Results are shown in Table 18.

TABLE 18--CHANGES IN BODY WEIGHT OF COWS AT VARYING LEVELS OF MOLASSES FED WITH THE TIMOTHY-LESPEDEZA HAY FED IN PHASE II

Trial	Cow Number	Ration Number	Lb. Molasses Fed Daily	Body Weight in Lb. Start of Trial	End of Trial	Gain or Loss
I	314	1	0	1337	1350	+13
	317	2	1	1312	1358	+46
	510	3	2	945	945	0
	518	4	4	936	965	+29
II	518	1	0	965	957	- 8
	413	2	1	1350	1350	0
	317	3	2	1358	1356	- 2
	510	4	4	945	940	- 5
III	510	1	0	940	940	0
	518	2	1	957	965	+ 8
	314	3	2	1350	1405	+55
	317	4	4	1356	1343	-13
IV	317	1	0	1343	1378	+35
	510	2	1	940	949	+ 9
	518	3	2	965	993	+28
	314	4	4	1405	1447	+42

TABLE 19--CHANGES IN BODY WEIGHT OF COWS AT VARYING LEVELS OF MOLASSES FED WITH THE ALFALFA-BROME HAY FED IN PHASE III

Trial	Cow Number	Ration Number	Lb. Molasses Fed Daily	Body Weight in Lb. Start of Trial	End of Trial	Gain or Loss
I	562	1	0	804	839	+35
	994	2	1	826	834	+ 8
	564	3	2	763	815	+32
	557	4	4	834	870	+36
II	557	1	0	870	870	0
	562	2	1	839	854	+15
	994	3	2	834	847	+13
	564	4	4	815	822	+ 7
III	564	1	0	822	783	-39
	557	2	1	870	875	+ 5
	562	3	2	854	843	-11
	994	4	4	847	850	+ 3
IV	994	1	0	850	843	- 7
	564	2	1	783	796	+13
	557	3	2	875	897	+22
	562	4	4	843	869	+26

Phase III: Results for this phase are shown in Table 19. Rather substantial body weight gains were observed during Trial I, while Trial II cow 557 receiving no molasses barely held her weight. Other animals receiving molasses in Trial II increased in body weight. During Trial III, cow 564 receiving no molasses lost 39 pounds and cow 562 receiving 2 pounds of molasses daily lost 11 pounds. Cow 994, fed ration 1 containing no molasses, lost 7 pounds in Trial IV. There was a net loss of weight on the ration containing no molasses in this phase.

Phase IV: There was considerable variation in body weight gains and losses during this trial as shown in Table 20. During Trial I where no molasses was fed, cows 229, 228 and 221 lost weight. Cow 219 barely maintained her weight, while 493 gained 37 pounds. When molasses was added as shown in Table 20, cows 228 and 229 continued to lose some weight while cow 221 gained 19 pounds and cow 493 gained 14 pounds. Again, cow 219 just maintained her weight. During Trial III, only cow 221, receiving 4 pounds of molasses, gained in body weight. The other animals suffered slight losses.

TABLE 20--CHANGES IN BODY WEIGHT OF COWS AT VARYING LEVELS OF MOLASSES FED WITH THE ALFALFA HAY FED IN PHASE IV

Trial	Cow Number	Ration Number	Lb. Molasses Fed Daily	Body Weight in Lb. Start of Trial	End of Trial	Gain or Loss
I	229	1	0	898	889	- 9
	228	2	0	855	846	- 9
	221	3	0	987	936	-51
	219	4	0	946	946	0
	493	5	0	1032	1069	+37
II	229	1	0	889	885	- 4
	228	2	2	846	842	- 4
	221	3	4	936	955	+19
	219	4	6	946	946	0
	493	5	8	1069	1083	+14
III	229	1	0	885	894	+ 9
	228	2	2	842	841	- 1
	221	3	4	955	951	- 4
	219	4	6	946	949	+ 3
	493	5	8	1083	1075	- 8

Phase V: Before the start of this phase the cows had been receiving alfalfa hay, silage and grain. Perhaps the change to an all hay roughage ration accounted for the large weight losses during Trial I, when no molasses was fed, as shown in Table 21. Cow 544 lost 107 pounds while the smallest weight loss was 43 pounds from cow 557. There was no further weight lost during the phase and cow 556 receiving 8 pounds of molasses daily during Trials II and III made the greatest gain.

TABLE 21--CHANGES IN BODY WEIGHT OF COWS AT VARYING LEVELS OF MOLASSES FED WITH THE ALFALFA HAY FED IN PHASE V

Trial	Cow Number	Ration Number	Lb. Molasses Fed Daily	Body Weight in Lb. Start of Trial	End of Trial	Gain or Loss
I	544	1	0	890	783	-107
	545	2	0	785	711	- 74
	564	3	0	822	746	- 76
	557	4	0	889	846	- 43
	556	5	0	969	906	- 63
II	544	1	0	783	828	+ 45
	545	2	2	711	747	+ 36
	564	3	4	746	790	+ 44
	557	4	6	846	897	+ 51
	556	5	8	906	980	+ 74
III	544	1	0	826	845	+ 19
	545	2	2	747	754	+ 7
	564	3	4	790	810	+ 20
	557	4	6	897	898	+ 1
	556	5	8	980	1022	+ 42

DISCUSSION

It has been shown that addition of molasses to a hay and grain ration may have a definite effect on digestibility. The degree and direction of this effect depend upon the type of roughage and on the amount of molasses fed.

While some investigations have indicated that molasses had little or no effect on digestibility of crude protein, most workers have shown that there is a decrease in protein digestibility, especially at high levels of molasses feeding. Morrison (1952) states that molasses does have value when included in a poor quality, high roughage diet where additional protein is supplied, both from the palatability standpoint and its use as a cheap source of energy. Results of this investigation indicate that the effect of molasses on protein digestibility depends on both the kind and the quality of roughages and on the molasses feeding level.

Addition of up to 2 pounds of molasses daily increased the digestibility of protein in rations used in these trals. When the daily molasses feeding level reached 4 pounds and the roughage fed was alfalfa hay, there was a slight depression in protein digestion. When the roughage was a timothy-lespedeza hay mixture, an increase in digestibility of protein was observed. When amounts of molasses exceeded 4 pounds daily, rather sharp decreases in protein digestion resulted. Part of this decrease may be explained by the observations of Hamilton (1942), who suggests that the decrease in nitrogen digestion obtained by addition of glucose to the ration of sheep can be accounted for by the estimated increase in metabolic nitrogen in feces.

There was considerable variability in results obtained on digestibility of crude fiber. Feeding of up to 2 pounds of molasses daily increased digestibility of the stemmy, high crude fiber alfalfa hay fed in Phase I. When the molasses allowance was increased to 4 pounds daily, digestibility of crude fiber was about the same as in the ration containing no molasses. There was little difference in digestion of crude fiber in the timothy-lespedeza ration up to the 4-pound daily feeding level, though a slight increase in digestibility was noted.

In feeding trials where a roughage of higher quality was fed, there was a slight decrease in digestibility of crude fiber when 1- and 2-pound daily allowances of molasses were fed. When the molasses feeding level exceeded 2 pounds, the decrease in crude fiber digestion was rather pronounced. Mangold (1934) has reported that crude fiber digestibility is decreased when easily soluble carbohydrates are fed. He also calls attention to the fact that consideration must be given to the effect of stage of plant development on digestion of the crude fiber fraction. Hamilton (1942) states that the decrease in digestibility of crude fiber when sugar is fed is due to preference of micro-organisms of the paunch for sugar. Nevertheless, these results show that 2 pounds of molasses daily does not decrease greatly the digestibility of crude fiber. In fact, it may increase the digestibility of crude fiber in certain roughages.

Digestibility of the cellulose fraction parallels that of crude fiber, although at the higher molasses feeding levels, the decrease is not nearly so pronounced as the decrease in crude fiber digestion. The work of Louw *et al.* (1947) and of other investigators indicates that increasing the nitrogen level of rations containing molasses will affect the magnitude of the decrease in digestibility of crude fiber and cellulose. This factor should be considered in undertaking future investigations of the value of molasses in the rations of ruminants.

In virtually all cases, digestibility of nitrogen-free extract increased as the level of molasses was increased, which confirms the work of Snell (1935) and others. Snell (1935) feels that the increased digestibility of N.F.E. is probably due to the presence of easily digested nutrients in the molasses, rather than any increase in digestibility of the N.F.E. in the non-molasses portion of the ration.

The study of rumen micro-organisms made in this investigation indicates a large and pronounced increase in bacterial population of the rumen as the daily molasses intake increases. This confirms work of Bortree *et al.* (1946). The increase in digestibility of the ration, when up to 2 pounds of molasses is fed daily, can logically be credited to increase in bacterial population at that point. Apparently the continued increase in numbers of bacteria and decrease in digestibility of crude fiber, cellulose and protein fractions at higher daily molasses feeding levels would suggest that bacteria are following the path of least resistance by feeding on the molasses. This confirms the hypothesis set up by other investigators.

Of considerable importance in this connection is the difference in degree of decrease in digestibility of crude fiber and cellulose. Hungate (1944, 1947), Cowles and Rettger (1931), and Sijpestijn (1951) have isolated short rod-shaped organisms capable of digesting cellulose. While the increased short rod count obtained in this investigation does not explain the decrease in digestibility of cellulose at the high daily molasses feeding levels, it probably does account for the smaller decrease in cellulose digestion when compared to crude fiber digestion. This is especially important when consideration is given to the large cellulose portion of most roughages. Burroughs *et al.* (1950a, 1950c) have reported a close correlation between bacterial population and cellulose digestion *in vitro* investigations. They show that bacterial nutritional requirements must first be fulfilled if they are to digest cellulose properly.

Population of single cocci in the rumen accounted for the largest part of the bacteria population observed in this investigation. The population of short rod types of bacteria was next in magnitude. Based on results of this and previous investigations, the role of short rod type bacteria should be given first consideration in future studies. While the type of investigation followed here does not provide the controlled condition of *in vitro* work, as far as bacteriological studies are concerned, the importance of working with the host animals cannot be overemphasized. It would appear that in the future some attention must be paid to supplying the particular nutritive needs of the bacteria in the rumen. This applies, especially, to the cellulose-digesting rod-shaped organisms.

It is reasonable to assume from results of this investigation that up to 2 pounds of molasses daily may be fed to dairy cows with good results. Probably 4 pounds of molasses may be fed with good results with all roughages except high quality hay. Amounts of molasses exceeding 4 pounds daily may be justified only if the price of molasses is very low. In that event, additional protein supplementation would seem to be advisable — at least until more is known about nutritive requirements of rumen micro-organisms.

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ABSTRACT

Digestion trials were run with dairy cattle to investigate effects of carbohydrate level (molasses) on digestibility of constituents of roughages. This investigation was divided into five phases with a different type or quality of roughage fed in each phase. The first three phases were set up on a 4 x 4 randomized block design using four dairy cows as experimental animals. Molasses feeding levels were 0, 1, 2, or 4 pounds daily per cow, depending on their position in the experimental design. In Phases V or VI, five cows were used and each was fed 0, 2, 4, 6, or 8 pounds of molasses daily.

A study also was made of types and numbers of micro-organisms normally found in the bovine rumen. Counting of protozoa was restricted to those resembling ciliates. No relationship could be observed between make-up of ration and number of protozoa counted. Bacterial numbers in the rumen increased as the amount of molasses fed daily was increased.

Increases in the apparent digestibility of crude protein, cellulose, and crude fiber were obtained when daily feeding levels of up to 2 pounds of molasses were fed. When the roughage was mixed timothy-lespedeza hay, 4 pounds of molasses fed daily decreased digestibility of crude protein, cellulose and crude fiber. Nitrogen-free extract digestion was increased with the increase in daily molasses feeding levels.

Apparently practical rations for dairy cattle could justifiably include up to 4 pounds of molasses daily per cow, with the exception that when high quality alfalfa is fed the 2-pound molasses level would be more desirable. In considering the amount of molasses to be fed daily per animal, appraisal must be made of the roughage quality as well as the price of molasses. Downward trends in molasses prices might justify feeding of 4 to 8 pounds of this carbohydrate daily as a source of energy, even to the extent of some sacrifice in digestibility of the roughage portion of the ration.

The bulletin is a report on Department of Dairy Husbandry research project number 139 entitled, "Milk Production".