EFFECTS OF COMPLETE FEED ON MILK PRODUCTION, COMPOSITION AND RUMEN METABOLISM OF DAIRY COWS

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ABSTRACT

A series of experiments were conducted with lactating dairy cows and fistulated non-lactating dairy cows to study the influence of feeding complete feed on milk production, composition and rumen metabolism.

In Experiment I, eight lactating cows were used in a latin square change-over design to compare three complete feeds containing corn silage, grass silage or ground hay with the conventional feeding regime containing long hay as the control. The roughage to concentrate ratio was 6:4.

The cows fed the corn silage mixture consumed less ($P<0.01$) dry matter and TDN than those fed grass silage, ground hay and long hay mixture. Total VFA from cows fed the ground hay mixture was higher ($P<0.05$) than those fed corn silage and long hay mixtures but not different with that of grass silage mixture. The molar percentage of acetic, butyric, valeric, branched-chain fatty acids (isobutyric and isovaleric) and molar proportion of acetic to propionic were not affected ($P<0.05$) by different sources of roughage. The corn silage mixture resulted in lower TDN, dry matter, crude protein and crude fibre digestibility values ($P<0.05$) than the other treatments. Blood glucose and urea concentrations were not affected by dietary treatments.

The mean daily milk yield of cows fed long hay and ground hay mixtures were higher ($P<0.05$) than those of grass silage mixture. No significant differences in mean daily milk yield ($P<0.05$) were observed among the cows fed long hay, ground hay and corn silage mixtures. However, the mean daily FCM yield was not affected ($P<0.05$) by the source of roughage. The percentages of milk fat, total solids, solids-not-fat,

protein, lactose and ash were not influenced ($P<0.05$) by the source of roughage.

In Experiment II, twelve lactating cows were used in a switch back design. The complete feed containing corn silage, grass silage or ground hay as well as the control containing long hay consisted of 40% roughage and 60% concentrate. Mean daily feed and TDN consumption were not affected ($P<0.05$) by the source of roughage. Total rumen VFA, molar percentage of VFA and molar proportion of acetic to propionic acid were not affected by the experimental treatments. There were no significant differences ($P<0.05$) in TDN, dry matter, crude protein and NFE digestibility among the experimental treatments.

The mean daily milk yield and FCM yield were not affected significantly ($P<0.05$) by the experimental treatments. The percentages of milk fat, total solids, solids-not-fat, protein, lactose and ash were not influenced ($P<0.05$) by the experimental treatments.

In Experiment III, four fistulated non-lactating dairy cows were used in 4 X 4 latin square design to study the effect of semipurified and conventional diets with and without diethylstilbestrol (DES) on digestibility, nitrogen retention, blood components and rumen metabolism. The feed intake for each cow was fixed at 10 kg/day using an automatic feeder device and each cow was fed 13-15 g in 2 min intervals.

The results indicated that the use of the automatic feeder resulted in the rumen of the cows having a virtually constant fermentation rate. Uniformity in composition of rumen samples were observed and nyctohemeral and day-to-day variations in concentrations of VFA, ammonia, pH and protozoa were removed.

The total VFA, molar percentage of VFA and pH were not influenced ($P<0.05$) by the experimental treatments. Rumen ammonia concentration was higher ($P<0.01$) for cows fed the semipurified diet when compared with those fed the conventional diet. Rumen ammonia concentration increased ($P<0.01$) when DES was included in the experimental diets. Neither diet nor DES affected the weight and dry matter percent of rumen digesta. Crude protein percent of rumen digesta was higher ($P<0.01$) for cows fed the conventional diet when compared with those fed the semipurified diet.

Inclusion of DES in the experimental diets had no specific effect on digestibility coefficients of nutrients consumed and TDN values. Also, there were no significant differences for digestibility coefficients of nutrients (except crude protein) and TDN among the experimental diets.

The cows fed the conventional diet had higher levels of blood glucose ($P<0.01$), blood hemoglobin ($P<0.05$) and hematocrit values ($P<0.01$) than those fed the semipurified diet. Inclusion of DES to the experimental diets resulted in an increase of blood glucose level ($P<0.01$), blood hemoglobin ($P<0.05$ in semipurified diet) and hematocrit values ($P<0.01$). Neither diet nor DES had an effect on ammonia and urea levels in the blood. No significant differences ($P<0.05$) were observed for free amino acids concentration in the plasma among the experimental treatments.

Diet and DES affected ($P<0.05$) lysine, proline, methionine and phenylalanine content of rumen protozoa. Neither diet nor DES affected the amino acids content of rumen bacteria. Aminoethylphosphonic content of protozoa and diaminopimelic content of bacteria were unaffected ($P<0.05$) by experimental treatments. However, total amino acid content of dry

protozoa was higher than that of dry bacteria.

Microbial content of rumen digesta as determined by gravimetric procedure and markers (aminoethylphosphonic and diaminopimelic) gave different values but similar trends in response to DES treatments. Total numbers of protozoa were significantly higher ($P<0.01$) for cows fed the conventional diet than those fed semipurified diet. The inclusion of DES in experimental diets resulted in a significant increase ($P<0.01$) in total number of protozoa and aided the retention and establishment of different ciliate protozoa species.

The inclusion of DES in experimental diets increased the level of protozoal and microbial amino acid fractions in rumen digesta. Rumen microorganisms synthesized about 9 times more amino acids than was found in the semipurified diet.

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INTRODUCTION

With increasing production capacity of dairy cows; improvements in milking techniques; and automated feeding, it has become imperative to seek a new feeding system. A recent innovation is that of mixing concentrates and roughages together into one complete feed. Such complete feeds have been called built-in roughage, blended, all concentrate and all-in-one feeds.

The results of feeding complete feeds to lactating cows have shown certain advantages such as reduced labor, controlled nutrient balance and controlled roughage to concentrate levels. The results of feeding complete feeds to lactating dairy cows have indicated slight increases, no change or slight decrease in milk yield and fat percentages and no significant change in feed intake when compared with that of those fed the roughage and concentrate portions of the ration separately.

The first part of this study was undertaken to determine the value of corn silage, grass silage and ground hay in complete feeds for lactating dairy cows as compared with a conventional system of feeding (long hay and concentrates fed separately) and study the influence of complete feed on milk yield and composition as well as on digestibility and rumen metabolism.

There has been considerable interest in the past few years in determining the quantity, quality and efficiency of protein synthesis by rumen microorganisms. Several investigations dealing with the importance of microbial protein can be found in the literature but few authors have studied the complex protein synthesis interrelationship between protozoa

and bacteria. Various researchers have also shown that urea can replace part or all of the nitrogen requirements of ruminants. Some studies suggest that diethylstilbestrol improves urea utilization in ruminants.

The second part of this study, in view of the improvement in urea utilization which can be achieved through the application of frequent feeding and inclusion of diethylstilbestrol in complete feeds, was, designed to study the effect of inclusion of diethylstilbestrol in semipurified and conventional diets on rumen metabolism and digestibility as well as on the quantity and quality of microbial biosynthesis of protein in the rumen of dairy cows.

REVIEW OF LITERATURE

Effects of Feeding Various Diets on Intake and Feed Utilization

Efficiency of conversion of feed into milk involves many genetic and environmental as well as physiological factors (80). However, the efficiency of conversion of feed energy into milk energy appears to depend on the variation of intake, digestion and metabolism (172,252,402,466). Various aspects of digestion influence food intake and utilization, all directly or indirectly influence the metabolic reactions in the ruminant.

High Level of Concentrate. Several symposiums and reviews are available concerning various aspects of high level of concentrates for dairy cows (36, 127,230,253,400,466). It seems that several factors can affect the voluntary intake of diets containing concentrates fed to ruminants (24,60,127,329,330). The voluntary intake may be increased to three or even to six times the amount required for maintenance by increasing the concentrate levels (24,325).

There have been few controlled studies to measure the effect of the level of concentrate in the diet on intake per se (252,342). Putnam & Loosli (391) found that apparent digestibility of dry matter was increased with increasing levels of concentrates in the diet. Others (42,66,248) noted no such effect. Most reports, in general, agree that crude fibre digestibility is decreased when the concentrate portion of the diet is elevated (42,267,287,391).

Eckles (154) concluded that digestion coefficients determined at

maintenance levels are not applicable to cows with high milk production. Reid (400) and Moe et al (325) pointed out a decrease in digestibility can be expected as feed consumption is elevated. Moe et al (325) observed a decrease in relative TDN value from 100 to 77% as the consumption level increased from maintenance to six times maintenance. Lassiter et al (267) studied the effect of intake on digestibility by dairy cows, using grain-hay ratio 20:80, 50:50, and 80:20 on a dry matter basis. Coefficients of digestibility of dry matter, protein and crude fibre increased as feeding level increased.

Bloom et al (65) observed that dry matter digestibility was higher when lactating cows were fed high concentrate ration (85%) than when fed a low concentrate ration (25%). The coefficients of digestibility of dry matter for high and low concentrate rations were 63.1% and 54.5% respectively. Brown (83) indicated a definite depression in apparent digestibility with increasing levels of intake of diets containing either 4:1 or 2:1 grain to hay ratios. He also had shown that it is not possible to predict the effect of grain levels on the depression in digestibility associated with level of feeding. This conclusion is in general agreement with that reviewed by other workers (24,170,322,402).

Blaxter & Wainman (62) concluded that metabolizable energy per unit of feed was unaffected by level of feeding with diets containing high levels of maize when the plane of nutrition of sheep and cattle was increased from slightly less than maintenance to twice that amount. The cause for this was that methane production decreased at high levels of intake of high maize diet. Flatt (170) found that digestibility of diets containing concentrates decreased slightly as the plane of nutrition increased. He also observed

that metabolizable energy remained fairly constant as the plane of nutrition was increased from maintenance to approximately three times maintenance. Thus, the depression of digestible energy would be of academic interest only, as ME was changed very little.

Physical Form of the Hay. In many experiments, grinding and pelleting was found to enhance voluntary intake of roughages (81,127). Miller (321) reported the pelleting of hay caused an increase in consumption compared with coarsely ground hay. In experiments with lactating cows, the feeding of ground or pelleted hay as the only feed resulted in increased dry matter intake when compared with long hay or chopped hay (252,409). Other workers concluded that pelleted forage was consumed more than the same forage in coarsely chopped or a long hay form (38,179,202,319). Meyer et al (320) suggested that fine grinding was probably the major factor causing the increased feed consumption of pelleted hay and that the pelleting process serves to put a fine dusty feed into a more palatable form.

It appears that two factors may be responsible for the increased intake of ground forage compared with non-ground forage. These two factors, rumen fill and rate of passage from the rumen, probably operate together in making more room available in the rumen for more feed (24,63, 106,259,331). Grinding and pelleting a hay diet also resulted in a faster passage through the rumen to the lower gut (23).

Minson (324) and Beardsley (38) have reviewed the effect of grinding or grinding and pelleting of forage on its digestibility. Alexandre et al (11) found that crude fibre digestibility was decreased and nitrogen-free extract increased by grinding or pelleting hay. In general, it appears that

grinding and/or pelleting of forage reduce dry matter, crude fibre and cellulose digestibility (38,61,91,259,324,331,398,405,479). However, grinding and/or pelleting forage do not appear to affect metabolizable and net energy values (60,61,173,319,324,331).

Silages. Many studies show that dry matter intake decreased as level of silage in the ration increased (74,85,86,414). Dry matter consumption is decreased for silage fed cows when compared with the same crop fed as hay (86,104,188,406,428,442,459). Dry matter intake was also decreased for the silage fed heifers (254,376,445,452,453). The causes of decreased dry matter intake of the higher moisture silages have not been clearly established. Many factors have been studied to determine their effect on dry matter intake. The results suggest that addition of water (206,453), pH levels (206), histamine levels (346), lactic acid and silage acids (454), and rumen retention time (476) had no effect on dry matter intake of silage as compared with hay. However, some studies (453) suggest that adding acetic, propionic, lactic and all three acids to the diet resulted in lower intake.

Several studies (188,406,476) have shown a slight decrease in the digestion coefficient of dry matter of grass silages when compared with the same roughages fed as hay. Owen & Howard (364) and Gordon et al (188) have shown that the digestibility of crude protein of silage was less than the same roughage fed as hay whereas, other workers (155,187,428) reported dry matter and energy digestibility to be about the same.

Braund et al (74) indicated that cows fed all corn silage consumed less dry matter than cows fed any level of hay. These data are in general agreement with other workers (85,256). Several researchers (94,103,105,

197,228,344,491) had studied the effect of stage of maturity on dry matter intake, and digestibility of corn silage. In general, dry matter intake was higher by cows fed more mature or lower moisture (50%-55%) corn silages as compared with cows fed immature or higher moisture (70%-75%) corn silages (94,105,197,228,344). Byers & Ormiston (105) suggested that the digestible energy concentration of corn silage is primarily a function of moisture content. However, Huber et al (228) found no significant difference in TDN due to maturity of silage (25.4% soft, 30.3% medium and 33.3% hard dough stages).

Complete Feeds. At present, more attention is being focused on low roughage-high concentrate complete feeds for lactating dairy cows (148,240,293,335,390, 470). Alfalfa hay, grass hay, corn silage, ground corn cubs, rice hulls and cottonseed hulls, have been used in complete feeds as source of roughage (46,47,221,240,293,335,470).

No significant differences were observed in TDN and DE intake when three complete feeds containing alfalfa hay, cottonseed hulls and native grass hay as sources of roughage were compared with a conventional diet containing long hay (470). Benz (46) and Thurmon (456) reported total dry matter intake was similar for a complete feed and conventional diet made up of similar ingredients. While Olson (348) reported that cows consumed less complete feed containing hay and grain than when the ingredients were fed separately but there were no significant differences when TDN consumptions were compared. Girouard et al (185) observed that no significant differences were found in intakes of DE and TDN between the two dietary regimes. Average dry matter intake observed for complete feed containing hay was higher

than that for a conventional hay diet (390).

Derbyshire et al (148) noted that dry matter consumption was similar for an ensiled complete corn silage ration and fresh corn silage mixed with concentrate at the time of feeding. Johanson et al (240) found that total dry matter consumption was slightly higher for complete feed containing 40% corn silage than a complete feed containing 40% alfalfa and haylage. Braund et al (74) presented data indicating that cows fed a complete corn silage ration consumed less dry matter and produced more milk than cows fed a conventional hay-grain diet. They also observed that feeding a complete silage ration resulted in similar dry matter intake as feeding fresh corn silage and grain separately.

Effects of Feeding Various Diets on Milk Production

High Level of Concentrate. Increasing the grain in the diet as a means of increasing energy intake has been studied by several researchers. Most studies (52,87,88,89,101,102,107,116,157,195,287,313,334,338,349,417,446) have shown an increase in milk yield with increasing grain consumption. Other reports (71,172,218,391,413,497) have shown no significant increases in milk yield when the cows were fed more grain.

Variation in response of milk yields to concentrate levels are expected due to variation in roughage sources, method of feeding and production of the cows used. In most cases, roughage was fed ad libitum while the concentrate was fed in proportion to production (50,52,71,215,338, 417). Few reports are available in which high producing cows were fed ad libitum quantities of all parts of their diets (172,391). There are

numerous reports studying the effect of hay and concentrate in its different combination on milk yield and composition.

Bloom et al (66) studied the effect of varying concentrate to hay ratios (25:75, 45:55, 65:35 and 85:15) on milk production. The result was that milk production increased as the level of grain increased. In another report by Hotchkiss et al (223) found that milk production and milk protein content were significantly ($P<0.05$) increased by increasing concentrate levels, with no significant effect on milk fat percentage. The ratios of concentrate to hay fed were 25:75, 45:55, 65:35 and 85:15 (on net energy basis). Morris et al (334) studied the effect of 20, 40 and 80% of net energy from concentrate and the remainder from hay. They observed that 4% FCM increased when the net energy supplied by the concentrate increased. However, most of the reports, in general, showed that the increasing ratio of grain to hay resulted in an increase in milk production (66,157,223,313,334) while the results of other workers (172,218,287,391) suggest that high grain feeding is not always efficient for milk production.

Physical Form of the Hay. In recent years the use of a mixture of low roughage-high concentrate in complete feeds has received considerable attention for dairy cows. Early investigations by Powell (378,379,380) suggested that feeding finely ground roughage did not affect milk yield but decreased milk fat content and SNF. On the basis of the results, he postulated that there is a relationship between milk composition and rumen metabolism. King & Hemken (258) reported that feeding ground or pelleted hay and grain resulted in depression of milk yield as compared with long hay and grain. They also noted that pelleted hay and grain increased the protein content and SNF of the milk. These data are in general agreement

with those obtained by Hand (193).

Other workers (27,29,165,252,405,409,418) found that dairy cattle fed on ground or pelleted hay produce milk with lower milk fat content than the control cows fed on long hay. Recent study by Putnam & Davis (390) suggested that there was no depression in milk fat content of cows fed pelleted hay as compared with cows fed long hay.

A number of other studies (252,257,377,409,427) had compared chopped hay with long hay for feeding dairy cows. Their results indicated that there was no significant difference between cows fed chopped hay or cows fed long hay. They suggested that chopped hay was similar to long hay in its nutritive value for milk yield.

Silages. Interest in using corn silage and grass silage to produce milk has increased, for the following reasons:

1. Silage feeding is easily automated.
2. Silage can be readily incorporated in complete rations.
3. Silage can be fed in all silage feeding programs, and
4. Silage feeding offers economical storage.

There has been considerable interest in comparing corn silage and/or legume-grass silage with alfalfa hay (85,86,133,206,231,339,381,414). The results of Hillman et al (206), Conrad et al (133) and Gordon et al (188) indicate that cows fed all legume-grass silage consistently produce lower milk yield than cows fed hay. Many studies have indicated that cows fed grass silage consistently produce as much and sometimes more milk, than cows fed hay (86,104,187,339,381,406,428,442).

The use of corn silage for dairy cows has been vigorously sought during the past 20 years to study its effect on milk yield and composition. Many studies (85,155,198,199,201,256,414,474) have compared corn silage with alfalfa hay or with hay and corn silage combinations.

Braund et al (74) indicated that cows fed all corn silage produced more milk than cows fed any level of hay. These data are in general agreement with other workers (85,134,256) who suggested that an all corn silage program would maintain milk production.

White & Johnson (491) compared early maturity corn to late maturity corn silage. The milk production was similar when the cows were fed the silages on an equal dry matter basis. Byers & Ormiston (105) observed similar milk production for cows fed corn silage ensiled at 54.9% dry matter and cows fed corn silage ensiled at 32% dry matter when fed with hay. Bryant et al (94) found an improvement in milk production by increasing dry matter intake of corn silage by increasing DM % at time of ensiling.

Complete Feeds. Some studies indicated that feeding complete feeds to lactating dairy cows resulted in slight increases or no significant changes in milk yield (240,292,293,408,456). No significant differences were observed on milk production, FCM or milk constituents when comparing cows fed complete feeds with those fed under the conventional system of feeding the roughage and concentrate separately (470). The results of Thurmon (456) indicated that the cows fed concentrate and hay ad libitum produced 42.39 lb of milk while those fed the complete feed ad libitum produced 43.42 lb of milk daily with no significant differences between the two dietary systems. Variations in milk fat percentage or FCM yield were not statistically

significant when the cows fed complete hay were compared with those fed a conventional hay diet (390). Girouard et al (185) studied the effect of ad libitum feeding of a conventional ration (hay and concentrate fed separately) and complete feed containing chopped alfalfa hay. No significant differences were found in mean FCM production between the two feeding systems.

Braund et al (74) observed that cows fed complete corn silage feed produced more milk than cows fed conventional hay diets. Muller et al (335) concluded that lactating dairy cows can efficiently utilize a complete ration mixture of corn silage and concentrate with no significant difference in daily FCM yield.

Effects of Feeding Various Diets on Milk Composition

Milk Fat Contents. Milk composition is affected by both environmental and genetic factors (266,268,269,460). Several comprehensive reviews have summarized the nutritional factors affecting the fat and solids-not-fat content of milk (101,227,253,264,410,466). The nutritional factors affecting milk fat content have received considerable attention. Many theories have been proposed to explain the nutritional causes of increase or decrease in milk fat content. Rook (410) postulated that physical characteristics of rumen contents, types and number of rumen microorganisms and relative proportions of rumen VFA affect milk fat content. He suggested that a ratio of acetic to propionic acid of 3:1, or greater, will maintain fat test. Ratios of acetic to propionic of 1:1 or less, caused a decrease in milk fat content (165,167).

Van Soest (466) reviewed three theories for explaining the depression of milk fat. The first theory suggested by Tyznik (461) and supported by others (28,90,174), was that the deficiency in the amount of acetate supplied by rumen micororganism cause a decrease in milk fat content. High concentrate rations suppress the mobilization of fat from the tissues and thereby cause a decline in blood lipids required for milk fat synthesis (466). Numerous studies have indicated that high concentrate feeding resulted in reduced rumen acetate levels and milk fat depression (30,52,82,196,286,408,411). However, Brown et al (90) reported no effect of high concentrate on milk fat depression. Other observations (239,262,403) are not in agreement with this theory. Blood studies (291,420,469) show no significant drop in the level of blood acetic acid associated with low milk fat. Furthermore, there was no significant decrease in absolute concentration of rumen acetate of animals fed high concentrate diets (28, 420,466).

The second theory was postulated by Shaw & Knodt (419) and Van Soest & Allen (469) and states that a deficiency of beta-hydroxybutyric acid causes a depression in milk fat content. This theory is restricted by the fact that the relation between rumen acid ratios and low milk fat is not always consistent (90,200).

McClymont & Vallance (290) have postulated the third theory which suggests that the glucogenic response (endocrinological control) results in a decrease of milk fat. This theory is supported by many observations (19,350,492), however there is also an adverse argument to this theory (142,239,403).

The fatty acid composition of milk fat appears to be affected by the depression of milk fat. Balch & Rowland (25) reported a decrease in saponification number and in Reichert-Missel number (120,291,441). Iodine number has been decreased (28,441); the ratio of short-chain fatty acids decreased (28) and the unsaturation increased (28,258,441). Interestingly, Kunsman and Keeney (263) found a decrease in C_{12} - C_{18} saturated fatty acids and an increase in the unsaturated fatty acids of milk fat when cows were fed their grain ration ad libitum.

Solids-Not-Fat. A number of reports have indicated that increases in the per cent solids-not-fat (SNF) resulted when the plane of energy intake is elevated (26,28,101,107,110,111,215,338,407). In contrast, other reports have indicated no significant effect in that respect (50,86,218).

Most of the increase in SNF with high energy consumption has generally occurred in the protein fraction (111,215,227,229,338,348,411). However, several researchers have reported a significant increase in per cent milk protein with no significant increase in SNF (52,71,73). In other studies, elevated energy intake promoted a higher SNF due to higher milk lactose (223,229,411,412). Rook & Line (411) noted an increase in milk lactose, but the magnitude of the increase was relatively less than that for protein. Also, they reported a significant decrease in non-protein nitrogen (NPN) fraction of the milk as the level of energy was increased.

Most reports show a decrease in SNF content as the level of energy is reduced under recommended levels (101,156,410). Milk protein and lactose were significantly depressed by underfeeding (223). Raising the energy intake above standard levels usually resulted in a smaller

increase in SNF content, (52,71,107,108,109,110,111,215,216,218,223,227,348, 410). Protein of the ration did not significantly affect SNF and true protein content (411) at any of the protein levels (70-160% of recommended levels). However, higher levels of dietary protein caused significant increases in the milk NPN. These data are in general agreement with those obtained by other workers (204,217,227,410).

The physical form of the ration affects SNF content (252,258). Protein and SNF content in the milk increased as the result of pelleting the ration (52,193,284). Jorgenson & Schultz (243) observed no change in SNF content in the milk as the result of pelleting the complete ration, the grain or the hay.

Effects of Feeding Various Diets on Rumen Metabolism

Rumen Volatile Fatty Acids. Since Phillipson & McAnally (369) and el-Shazly (422,423) demonstrated that rumen volatile fatty acids are mainly hydrolytic products of carbohydrate and protein fermentation, there have been many reports dealing with the effect of different diets on VFA concentration (31,35,126,143,421,451). The rumen VFA play an important role in ruminant metabolism because they serve as a main energy source for the ruminant (32,60,99). This finding stimulated researchers to study the relationship between VFA concentration, VFA proportions and animal performance as measured in terms of rate of gain, milk production, milk composition, and feed efficiency (36,60,466). The predominant rumen volatile fatty acids are acetic, propionic, isobutyric, butyric, valeric and iso-valeric (20).

Total VFA concentration in the rumen is affected by many factors such as food intake (35,496), frequency of feeding (189,192,261,396,415), time of sampling (35,163,404,487,496), and type of ration (31,158,251,393). The molar proportion of acetic to propionic acid is usually highest when feeding only long hay as compared with long hay plus concentrate, pelleted hay, and ground hay (20,165,168,243,447). Increasing the proportion of concentrate in the ration caused a reduction in the molar proportion of acetic to propionic acid in the rumen (20,29,31,35,138,139,157,168,172,245, 392,447,485).

It is well established that drastic changes in the diet cause a marked change in microbial populations (183,343,361,483). Also, it is known that the concentration and proportion of VFA in the rumen are a function of microbial activity and absorption from the rumen (233). Therefore, it is difficult to make comparisons among experiments because experimental conditions will vary.

Rumen pH. Rumen pH varies with diet, time after feeding, sampling location and CO_2 saturation (35,79,192,242,265,333,368,397,404,436,458). The lowest pH values and the greatest range in pH were obtained from the rumen of animals which were fed rations containing high concentrate (9,31,79,404).

Data published by Kay (251) indicate that normal range of pH of the rumen was 5.5 to 7.3. Briggs et al (79) reported normal pH of the rumen ranged from 5.0 to 7.5 and rumen pH values never fell below 4.35 as compared to 4.3 reported by Agrawala et al (9) on a purified diet.

Degradation of Nitrogen Compounds. Much attention has been paid to nitrogen metabolism in ruminants by numerous scientists. Several excellent reviews and monographs (33,54,76,112,207,232,233,251,271,272,300,310,351, 367,401,457,475) have been written on various aspects of nitrogen metabolism in ruminants.

The major sources of nitrogen in the rumen are dietary nitrogen, salivary nitrogen and nitrogen influx across the rumen wall. The nitrogen compounds in the rumen are proteins, peptides, amino acids, and non-protein nitrogen (NPN) compounds.

Dietary proteins are degraded in the rumen to varying degrees. el-Shazly (422) was one of the first to show protein degradation by rumen microorganisms leading to production of branched-chain fatty acids and ammonia. The same author (423) reported the deamination of amino acids by rumen microorganisms and suggested the possibility of a Stickland type of reaction. The degradation of protein and deamination of amino acids by mixed rumen microorganisms also have been studied by several scientists (146,273,275,276,283,432,484). Both bacteria and protozoa were found to have proteolytic activity (2,3,55,56,57,58,59,96,97). Optimal pH for proteolytic activity was between 6 to 7 for both bacteria and protozoa (3,55,57). Dietary NPN as well as recirculated urea from saliva (224,272, 295,296,437,438) and urea influx from the blood across the rumen wall (125, 181,182,225,246,431) are converted to ammonia and carbon dioxide (41,365, 473,474). Ammonia formed from metabolism of nitrogen compounds in the rumen is incorporated into microbial protein (95,233) or is absorbed through the rumen wall (137,180,208,209,272,295). The residual dietary proteins, microbial proteins and NPN are continuously passing from the rumen to the lower alimentary tract.

Synthesis of Microbial Protein. From the papers reviewed in the preceding sections it could be observed that the degradation of protein and non-protein nitrogen result in the presence of peptides, amino acids and ammonia in addition to VFA, CO_2 and other compounds. The complete biochemical pathways involving these compounds in the synthesis of microbial protein, have not been completely explored. However, ammonia appears to be a main source of nitrogen for protein synthesis by rumen microorganisms (37,95,100,233,270, 297,372).

The data from earlier papers (151,285) demonstrated the synthesis of ten essential amino acids in the rumen of sheep and goats fed purified diets containing urea as the sole source of nitrogen. Additional evidence from several workers (2,95,97,98,233,370) indicates that ammonia is an essential nutrient for most of the rumen bacteria. Also, it appears that the presence of ammonia is an essential nutrient of both cellulolytic and amylolytic rumen microorganisms (6,7,40,41,115,280,425).

Some major factors influencing the utilization of ammonia are the availability of carbon skeletons and energy to rumen microorganisms (14,39, 207,219,277,284,300,363,464,465) and rumen ammonia level. With respect to synthesis of amino acids by rumen microorganisms, they appear to require carbon from carbohydrate (2,97,219,309); keto acids (300,363); isovaleric acid (15); acetate; branched and straight chain VFA (12,13,14,16,207,219,233). Thus it appears that rumen microorganisms require a certain specific carbon skeleton to synthesize certain amino acids.

Various workers reported that inorganic sulfur can be synthesized to sulfur-containing amino acids (18,64,161,162,274). There is some evidence indicating that the requirement of sulfur-containing amino acids

can be provided to the host by rumen microorganisms (129,130,131,178,455). However, Conrad et al (129,130) reported that daily methionine synthesis in cows eating alfalfa varied between 31 and 59 mg/kg of body weight. Conrad et al (129) indicated that methionine synthesis increased as the level of dry matter and protein intake increased. Conrad et al (131) reported 6-15 g of methionine synthesis occurred in the rumen of dairy cows.

Many estimations of protein synthesis by rumen microorganisms have been made using different methods (58,67,160,190,191,233,294,298,307, 477,489). Gray et al (190) estimated that 11 g microbial protein was synthesized for each 100 g of fermentable carbohydrate. Hogan & Weston (210) indicated that microbial protein yield was approximately 15 g per 100 g of organic matter utilized in the rumen. These values agree with that estimated by Walker (477) and Bloomfield et al (67).

The quantitative relationships between microbial protein and rumen fermentation also has been discussed by Hungate (233). Hungate (233) concluded that microbial protoplasmic synthesis from the diets in the rumen is significantly lower than that under aerobic conditions. Only 10% of energy from rumen fermentation is used in synthesizing microbial cell bodies. Hungate (233) and Gray et al (190) estimated that for each 100 g of carbohydrate fermented, there is about 1.1 g of microbial cells. Recent estimates (128,478) suggested that 1.3 to 1.6 g of microbial nitrogen was synthesized per 100 g of feed fermented. From in vitro experiments, Bloomfield et al (67) found one gram nitrogen fixed for each 55 g carbohydrate fermented. Protein synthesized by rumen microorganisms appear to be a main source of amino acids for host protein synthesis. This conclusion is

especially true when non-protein nitrogen makes up a large proportion of dietary nitrogen. Therefore, the total host protein synthesis depends to some degree upon the magnitude of microbial protein synthesis in the rumen.

Fractionation studies of total rumen content (58,488) suggest that dietary nitrogen as a proportion of rumen nitrogen decreased after feeding while microbial nitrogen increased. The extent of dietary protein degradation in the rumen depends on the solubility of the protein. McDonald & Hall (298) indicated that 90% of the nitrogen of casein was incorporated into microbial proteins, while only 40% of less soluble zein was converted to microbial protein (294). Weller et al (489) estimated that 63-81% of nitrogen of the solids in the rumen was microbial nitrogen, while Blackburn & Hubson (58) reported 47-77% of the rumen nitrogen was in the form of microbial protein. Others (191,488,489) indicated that more than 50% of rumen nitrogen was in the form of microbial nitrogen. However, polynucleotide nitrogen represented 14-18% of the microbial nitrogen or 5-13% of ingesta (159). These data are in general agreement with those reported by Abdo et al (1).

Composition and Quality of Microbial Protein. Smith & Baker (434) reported that mixed rumen microorganisms on a dry matter basis had about 36% crude protein while Abdo et al (1) found the crude protein of mixed rumen microorganisms to be from 38-48%. The somewhat variable results of crude protein of rumen bacteria and protozoa are possibly associated with differences in chemical technique; the time of sampling after feeding; contamination with food particles; and differences due to feeding regime. Several workers (1,49,241,308,311,486) have determined the

protein content of bacteria and the results range from 35-77%. While protein content of protozoa have been reported from 24-55% (49,233,241, 308,486). Weller (486) and Hungate (233) reported that crude protein content of rumen bacteria and protozoa were not affected by rations.

Bacterial, protozoal and undegraded dietary proteins reaching the alimentary tract form the available protein for the host. If dietary non-protein nitrogen is increased, rumen microorganism proteins will make up a larger proportion of amino acids supplied to the host. Therefore, there has been considerable interest in determining amino acid composition of both bacteria and protozoa (1,49,212,318,388,484,486). Quality of microbial protein has been estimated by determining the amino acid composition of bacterial and protozoal protein and by determining the nutritive value of microbial proteins. Nutritive value has been determined by feeding microbial preparations to laboratory animals.

Generally, amino acid composition of bacterial protein was remarkably constant, as was the amino acid content of protozoal protein for different rations (48,318,351,388,486). Therefore, the amino acids reaching the alimentary tract in microbial form appear to be constant.

It is apparent that phenylalanine, lysine, leucine, isoleucine, glutamic acid and aspartic acid concentration in protozoal protein are slightly higher than in bacterial protein (48,214,318,388,486).

Ciliatin, 2-aminoethylphosphonic acid, has been found in protozoal protein. This amino acid is not characteristic of bacteria (5,220). Diaminopimelic acid, is found in bacteria but is not present in protozoal, animal or plant protein (388,424,489). It is possible these two amino acids could be used as markers to quantify protozoal and bacterial protein

available to the host.

The nutritive value of the rumen microorganisms can be estimated in terms of biological value, true digestibility and net protein utilization. Determinations of biological value and net protein utilization of rumen protozoa and bacteria by laboratory animals have yielded somewhat variable results (241,308,399,462,494). Bergen et al (48) estimated the biological value using protein quality index (10) based on enzymatic digestibility. Purser & Buechler (388) calculated the biological value using the index of Oser (362).

Generally, the rumen protozoa and bacteria protein are good sources of amino acids for rats compared with casein and egg protein. Protozoal protein is slightly superior in biological value than bacterial protein (241,308,388). However, the true protein digestibility of protozoa is greater than that of bacteria and thus net protein utilization (BVX True digestibility) of bacteria is lower than protozoa. The respective true digestibility, biological value and net protein utilization values were 74,81 and 60 for bacterial protein utilization and 90, 80, and 73 for protozoal protein (308). These values have been confirmed by other workers (48,241,399).

Some workers (4,260) found better nitrogen retention and protein digestibility for faunated lambs compared with defaunated lambs, which indicates a favourable effect of the rumen protozoa on nitrogen utilization by the host.

Factors Influencing the Population of Rumen Microorganisms. The available information on the complex interrelationship in the rumen is far too meager

to make accurate predictions about the relation between microbial population and diet. Many factors influence the rumen microorganisms quantitatively and qualitatively. A variety of factors such as diet (176,183,233,328,361, 386,395,429,482,494), seasonal variation (177,326,341), day to day fluctuation (72,341,387,494), diurnal variation related to time after feeding (327,328,341,385,387,394,482,483), species variation (153,340), geographic location (122), and feed processing (113,121) have been reported to affect the populations and numbers of rumen microorganisms. It has also been shown that certain types of protozoa are incompatable (153) and that protozoa and bacteria are in competition with each other (183,233,472). Thus, the concentration of bacteria is higher in defaunated than in faunated animals (233,482).

The effect of dietary NPN manipulations on microbial population in the rumen has not been established. There is little evidence on the influence of dietary protein quality on bacterial or protozoal protein quality (176). Supplying a part or all of dietary nitrogen as urea, causes a high ammonia concentration in the rumen within one to one and a half hours after feeding (114,358). Thus ruminal pH may be relatively higher one to two hours after non-protein nitrogen is consumed when compared with other protein sources (358). Recent studies (352,359) indicated that high ruminal ammonia level appears to reduce the salivary flow rate.

Purified diets appear to decrease pH of the rumen which may cause decreased protozoa numbers (387,483). At a low ruminal pH (5.4 to 6.0), transfer of ammonia through the rumen wall is much less rapid and more of the ruminal ammonia is trapped for microbial protein synthesis (209). Feeding of semipurified diets caused the disappearance of protozoa and an

increase in bacterial number in ruminal contents of sheep (183). This data is in general agreement with that obtained from cows, calves, and steers fed protein free diets (429,472). Grinding or pelleting of diets resulted in a reduction or disappearance of ciliate protozoa (113,121) whereas diethylstilbestrol feeding appeared to prevent the disappearance of protozoa from the rumen (121).

The lower performance of ruminants fed purified diets (34,124, 171,299,315,352,357,360) may be explained partly on the absence of protozoa (389) because protozoal protein has a higher protein utilization value compared to bacteria (486). This is supported by the observation of increased nitrogen retention in faunated lambs when compared with defaunated lambs (4).

Effects of Various Diets on Free Amino Acids of Blood Plasma

Free amino acid pattern of blood plasma has been recently used in an attempt to explain the poor performance of ruminants fed purified diets (175,353,354,356,357,374,389,416,448,449,472).

Some workers report that the plasma amino acid pattern of monogastric animals is influenced by the amino acid pattern of dietary protein (383,435). However, several factors may influence the plasma amino acid concentration in monogastric animals such as absorption, tissue utilization, liver catabolism , anabolism, amino acid pool and amino acid imbalance (213,336).

The influence of dietary protein sources upon plasma amino acid patterns in ruminants is more complex than in monogastric animals. Data is

lacking as to whether plasma amino acid pattern of ruminants reflects the amino acids present in the lower gut for absorption which are not directly proportional to the dietary protein because the mixture of amino acids in the intestine is a combination of dietary protein, degraded dietary protein and microbial protein. Also there is resynthesis of ammonia into amino acids in rumen mucosa (222,302,303).

PART ONE

Effects of Complete Feeds Containing Corn Silage, Grass Silage or Ground Hay on Milk Production, Composition and Rumen Metabolism

MATERIALS AND METHODS

EXPERIMENT I

Rations. Eight lactating cows, past peak production (four weeks after parturition), but not past midgestation at the end of the experiment, were used to study the effect of three complete feeds on milk production and composition. A Latin square change over design (Appendix I, table 1) was used (169). The experiment was started on November 23, 1966. Each experimental period consisted of fourteen days of adjustment and fourteen days of comparison. The cows were assigned at random within each block and treatment. The experimental treatments consisted of three complete feed and a control ration (Table 1). The various treatments, on a dry matter basis, were as follows:

1) A mixture of 60% corn silage and 40% concentrates. The concentrates were added at the time of ensiling.
2) A mixture of 60% alfalfa grass silage and 40% of concentrates. The concentrates were added at the time of ensiling.
3) A mixture of 60% ground alfalfa hay (0 - 1.5 cm) and 40% concentrates.
4) Long alfalfa hay and concentrates fed at a ratio of 60:40 to serve as control ration.

The chemical composition of experimental feeds is presented in Table 2. Direct cut corn (20%DM) was cut, chopped (2 cm) and loaded into wagons with cutter bar forage harvesters. Wilted alfalfa (44% DM) was cut with

Table 1. Ingredient composition of experimental diets on dry matter basis (Expt. I).

Ingredients	Corn silage mix. kg	Grass silage mix. kg	Ground hay mix. kg	Long hay + conc. kg
Roughage	595	600	600	600
Barley	386	396	396	396
Urea	9	-	-	-
Trace mineralized salt	2	2	2	2
Rock phosphate	4	2	2	2
Calcium carbonate	4	-	-	-
Total (Kg)	1000	1000	1000	1000

Table 2. Chemical composition of experimental diets as fed to the cows (Expt. I).

Treatments	Dry matter %	Crude protein %	Crude fiber %	Ether extract %	Ash %	NFE %
Corn silage mix.	28.32	3.51	5.10	0.78	1.66	17.27
Grass silage mix.	55.48	9.47	13.78	1.44	4.32	26.47
Ground hay mix.	90.76	12.22	12.76	2.26	4.60	58.92
Long hay + conc.	91.32	13.56	19.77	2.20	6.12	49.67

a cutter bar, chopped (2 cm) and loaded into wagons with a field forage harvester. Alfalfa hay and alfalfa silage were cut from the same field at the same time. Empty and loaded wagons were weighed on a platform scale and the total weight of each load of roughage was determined. The concentrate mixture was spread on top of the roughage just prior to blowing into the silo. The blower pipe was adjusted to deliver the silage mixture into the centre of the silo. The corn and alfalfa grass silage complete feeds were stored in two concrete stave silos (4.8 x 15.0 m). The complete feed in each silo was leveled, trampled and covered with plastic. The top plastic was weighted with approximately one ton of the same roughage which was not used in the experimental treatment.

The cows were housed in a stanchion barn and fed individually. The residual feed was weighed daily and daily feed intake was recorded. Stanchions were equipped with automatic watering cups. Wood shavings were used for bedding to prevent any additional roughage consumption.

Milk Production and Composition. The cows were milked twice daily and production recorded. Milk composition (fat, SNF, protein, lactose, ash) was determined twice weekly during the last fourteen days of each period on a combined milk sample of a consecutive night and morning's milking. A composite sample of the above 4 p.m. and 4 a.m. milkings, adjusted for weight of the milking, was made for each cow for each period. The composite samples were kept frozen until analyzed for milk fatty acids by gas liquid chromatography.

Digestibility Trials. Fifteen grams of chromic oxide pellet were administered orally at 7 a.m. and 3 p.m. during the last ten days of each period. Chromic oxide pellets were made from paper containing 5% moisture and 32.2% chromic oxide on a dry matter basis. Rectal samples of feces were taken at 9 a.m. and 5 p.m. during the last five days. During the digestibility trials, daily samples of feed were taken at random and daily feed intake recorded.

Rumen and Blood Samples. For studying the effect of the experimental feeds on the rumen volatile fatty acids, samples of rumen fluid were obtained on the last two days of each experimental period approximately three to four hours after the morning feeding. The samples were obtained by using a stomach tube and a vacuum pump. The samples of rumen fluid were strained through two layers of cheese cloth, and preserved by addition of two drops of saturated mercury chloride and kept frozen until further analysis. Samples of blood were taken from the jugular and mammary veins on the last two days of each experimental period approximately three to four hours after morning feeding for blood glucose and urea analysis.

EXPERIMENT II

Rations. Twelve lactating cows, (Appendix 1, table 4) past their peak of lactations but not past midgestation at the end of the experiment, were assigned at random to a switchback design (288).

Two blocks of four cows were placed on experiment on February 7, 1968 and a third block was placed on the experiment on March 7, 1968. The cows were fed three complete feeds and a control ration (Table 3) ad libitum.

The experimental treatments were as follows:

1) A mixture of 40% corn silage and 60% concentrates. Fifty-six percent of the grain was added at ensiling time and 44% was mixed at the time of feeding.
2) A mixture of 40% grass silage and 60% concentrates. Also, 56% of the grain was added at ensiling time and 44% was mixed at the time of feeding.
3) A mixture of 40% ground hay and 60% concentrates.
4) Long alfalfa-grass hay and concentrates were fed at a ratio of 40:60 respectively to serve as the control ration.

The chemical composition of the experimental feeds is presented in Table 4. The corn (25% DM) and grass alfalfa (29% DM) forage were treated and stored at ensiling time in a similar manner as those in Experiment I. Each experimental period consisted of 14 days of adjustment and 14 days of comparison. The cows were managed in a similar manner to those in Experiment I. Milk samples, milk data, feed intake data, digestibility

Table 3. Ingredient composition of experimental diets on dry matter basis (Expt. II).

Ingredients	Corn silage mix. kg	Grass silage mix. kg	Ground hay mix. kg	Long hay + conc. kg
Roughage	395.2	395.2	400.0	400.0
Barley	571.2	576.2	580.8	556.8
Molasses	--	--	--	24.0
Trace mineralized salt	9.0	7.8	6.0	6.0
Rock phosphate	9.0	7.8	6.0	6.0
Sulfur	3.0	--	--	--
Calcium carbonate	4.2	--	--	--
Urea (at ensiling time)	6.8	9.2	--	--
Urea (at feed time)	1.6	3.4	7.2	7.2
Total (Kg)	1000.0	1000.0	1000.0	1000.0

Table 4. Chemical composition of experimental diets as fed to the cows (Expt. II).

Treatments	Dry matter %	Crude protein %	Crude fiber %	Ether extract %	Ash %	NFE %
Corn silage mix.	59.34	6.42	9.95	1.11	2.89	39.02
Grass silage mix.	55.56	8.92	9.40	2.01	3.20	32.02
Ground hay mix.	91.55	12.27	9.02	2.53	5.17	62.73
Long hay + conc.	90.60	11.74	13.84	2.59	4.91	57.62

trials, samples of rumen fluid and samples of blood were obtained and collected following the same procedure and timing as indicated in Experiment I.

The same methods and procedures of analysis were used for Experiments I and II. The percentage of milk fat was determined by the Gerber Method (184). The solids-not-fat were determined by using the plastic beads method of Golding (186).

Total solids, lactose and ash of the milk were determined according to the AOAC Method (22). The milk protein was determined by the Orange G dye binding method (21). Samples of milk fat were extracted to determine the fatty acids of milk as described by Lincoln (278). The following procedure was used for transesterification, formation of fatty acid methyl or butyl esters, of milk fatty acids (316,317). Milk fat (0.150 g) was dissolved in 4 ml boron triflouride-butanol in a screw-cap test tube. The air in the tube was replaced by nitrogen gas. The reaction mixture was placed in water bath at 65 to 70^{0} C for 10 min with the cap on loosely. Then the cap was screwed on tightly. The tubes were placed in an oven at 65 to 70^{0} C for 16 h (overnight). After cooling, 5 ml of distilled water was added and the fatty esters were extracted with 10 ml pentane (Skelly solve F). The sample was shaken vigorously by hand for one to two minutes. After separation the pentane layer was removed and dried over anhydrous sodium sulfate. In case of butyl-ester formation, the pentane layer was washed three times with 50 ml of distilled water. The tubes were kept in a refrigerator with the caps screwed on tightly for subsequent GLC analysis. A standard methylester mixture KC (Applied Science Laboratory, Inc. State College, PA. 16801) was used to establish the linearity of

response in relation to the quality of individual fatty acid ester. The area for each ester was determined by the methods of Keulemans (255) and Pecsok (366). The peak area of each ester, as a percent of total peak areas was determined, which is related to weight percent. Peak identities were determined by retention time relative to the retention time for the myristate ester (17,433). Butyl esters were used for short fatty acids because of possible losses of volatile methyl esters during extraction, evaporation and sublimation (140,234,433).

The gas liquid chromatograph was a F.M. Laboratory Chromatograph model 700 with thermal conductivity detector with a filament current of 250 ma. The apparatus employed a 180 by 0.318 cm (6' - 1/8") OD stainless steel column (20% diethylene glycol succinate on chromosorb P, 60/80 mesh) and oven temperature of 195^{o} C. Helium was used as a carrier gas with flow rate of 40 ml/min.

Volatile fatty acids of the rumen fluid were determined by the method of Erwin et al (166). Five milliliters of strained rumen fluid were added to 1 ml of 25% metaphosphoric acid, mixed, allowed to stand for 30 min and centrifuged at 1500 XG for 10 min. The supernatant was analyzed for VFA by gas liquid chromatograph (Burnell) with a flame-ionization detector. The apparatus employed a 180 X 0.318 cm OD (6' X 1/8") stainless steel column (20% N.P.G.S. and 2% H_3SO_4 on gas-chrom R, 60/80 mesh, conditioned 17 h at 200^{o} C). The temperature of the column bath was 175 to 183^{o} C. Helium (27 psig) was used as the carrier gas with a flow rate of 20 ml in 30 sec plus hydrogen (26 psig) with flow rate of 20 ml in 14 sec plus air (20 psig) with a flow rate of 20 ml in 3.2 sec. The gas liquid chromatograph was attached to a Honeywell recorder.

Samples of blood were analyzed for blood glucose by the anthrone method (332) and for urea by the method of Conway (135). The proximate composition of the rations fed and feces was determined by A.O.A.C. methods (22). Chromic oxide in feces samples was determined by atomic absorption spectrophotometry according to the Williams et al (495) procedure.

Statistical Analysis. The data was statistically analyzed according to Federer (169) with Experiment I and Lucas (288) with Experiment II and differences between means tested by Duncan's multiple range test (152). Statistical computer programs were designed to perform analysis of variance for Experiment I and II. They were written for IBM 360 using FORTRAN IV.

RESULTS

EXPERIMENT I

Feed and TDN Consumption. Mean of daily feed consumption of the corn silage mixture was significantly lower ($P<0.01$) than the grass silage, ground hay and long hay mixtures (Table 5). There was no significant difference among grass silage, ground hay and long hay mixtures in daily feed consumption.

The TDN consumption was calculated from the results of digestibility trials and feed consumption. The cows fed the corn silage mixture consumed less ($P<0.01$) TDN than those fed grass silage, ground hay and long hay mixtures (Table 5). No significant differences ($P<0.05$) among grass silage, ground hay and long hay were observed.

Ruminal VFA and pH. Total ruminal VFA of cows fed the ground hay mixture tended to be higher than other treatments (Table 6). Total VFA from cows fed the ground hay mixture was significantly higher ($P<0.05$) than those fed the corn silage or the long hay mixtures but not significantly different from grass silage.

The molar percentage of acetic, butyric and valeric acids were not affected significantly ($P<0.05$) by different sources of roughage (Table 6). However the molar percentage of acetic acid was somewhat lower for the ground hay treatment compared with the grass silage treatment. Branched-chain fatty acid, isobutyric and isovaleric, were not affected significantly

Table 5. Effect of complete feed on mean of daily feed and TDN consumption (Expt. I).

Treatments	Dry matter	TDN
	Kg	Kg
Corn silage mix.	10.8[A]	5.3[A]
Grass silage mix.	17.1[B]	10.0[B]
Ground hay mix.	15.8[B]	9.6[B]
Long hay + conc.	16.0[B]	10.3[B]

A,B Treatment means within a column not sharing a common superscript are significantly ($P<0.01$) different.

Table 6. Effect of complete feed on mean of total and molar percentage of VFA in ruminal fluid (Expt. I).

Treatments	Total VFA	C_2	C_3	iso-C_4	C_4	C_5	iso-C_5
	mmoles 100 ml	molar %					
Corn silage mix.	8.89[b]	60.30	23.15[a,b]	0.42	14.40	1.26	0.47
Grass silage mix.	10.29[a,b]	66.01	19.43[b]	0.50	11.88	1.44	0.74
Ground hay mix.	12.74[a]	59.43	27.91[a]	0.27	10.08	1.45	1.13
Long hay + conc.	9.59[b]	63.12	21.81[a,b]	0.32	12.26	1.74	0.75

a,b Treatment means within a column not sharing a common superscript are significantly ($P<0.05$) different.

($P<0.05$) by experimental treatments. The cows fed ground hay had higher ruminal molar percentage of propionic acid when compared with cows fed grass silage ($P<0.05$).

The molar proportion of acetic to propionic acid in ruminal fluid (Table 7) was not significantly ($P<0.05$) affected by the source of roughage. However, the C_2/C_3 ratio was somewhat lower for the ground hay treatment compared with that of grass silage or the long hay treatment.

Ruminal pH was affected by the source of roughage (Table 7). The ruminal pH of cows consuming corn silage and grass silage mixtures was significantly ($P<0.05$) higher than that of the ground hay mixture but not different from the long hay mixture.

Digestibility Trials. Long hay, ground hay and grass silage mixtures resulted in higher TDN and dry matter digestibility values ($P<0.05$) than the corn silage mixture (Table 8). There was no significant difference ($P<0.05$) in digestibility of crude protein between ground hay and long hay mixtures (Table 8). The crude protein digestibility of grass silage was significantly lower ($P<0.05$) than that of the ground and long hay mixtures. The digestibility of the crude protein of corn silage was significantly lower ($P<0.05$) than other treatments. The digestibility of the ether extract of cows receiving corn silage was significantly higher ($P<0.05$) than those obtained from grass silage, ground hay and long hay mixtures.

The digestibility of crude fibre was not significantly ($P<0.05$) affected by dietary treatments. However, the corn silage and ground hay fibre appeared to be lower in digestibility than the other treatments. The NFE digestibility of corn silage was significantly lower ($P<0.05$) than

Table 7. Effect of complete feed on mean of molar proportion of acetic to propionic acid and pH in ruminal fluid (Expt. I).

Treatments	C_2/C_3	pH
Corn silage mix.	2.8	6.9^{a}
Grass silage mix.	3.5	6.8^{a}
Ground hay mix.	2.4	6.1^{b}
Long hay + conc.	3.2	$6.4^{a,b}$

a,b Treatment means within a column not sharing a common superscript are significantly ($P<0.05$) different.

Table 8. Effect of complete feed on mean of total digestible nutrients (TDN) and digestible nutrients of experimental diets (Expt. I).

Treatments	TDN	Dry matter	Crude protein	Ether extract	Crude fiber	NFE
	%	%	%	%	%	%
Corn silage mix.	50.06^{b}	43.64^{b}	42.76^{c}	64.87^{a}	29.03	59.17^{c}
Grass silage mix.	59.85^{a}	59.85^{a}	54.71^{b}	55.11^{b}	49.26	70.41^{b}
Ground hay mix.	61.44^{a}	61.44^{a}	69.01^{a}	$57.19^{a,b}$	27.87	76.23^{a}
Long hay + conc.	62.44^{a}	62.01^{a}	62.89^{a}	53.39^{b}	34.87	$73.76^{a,b}$

a,b Treatment means within a column not sharing a common superscript are significantly ($P<0.05$) different.

the other three treatments. The NFE digestibility of grass silage was significantly lower ($P<0.05$) than the ground hay mixture. There was no significant difference between the NFE digestibility of ground hay and long hay mixtures.

Glucose and Urea Levels in Blood. There were no significant differences among treatment means for blood glucose levels from either the jugular vein or the mammary vein (Table 9). Also no significant differences were observed among the treatments in blood urea levels (Table 9).

Milk Production and Composition. The mean daily milk yield for cows fed long hay and ground hay mixtures were higher ($P<0.05$) than those fed the grass silage mixture (Table 10). The differences among long hay, ground hay and corn silage mixtures were not statistically significant ($P<0.05$). The mean daily FCM yield among the treatments was not affected significantly ($P<0.05$) by the source of the roughage (Table 10), however the trends were similar to mean daily milk yield. Statistical analysis of milk fat percentage indicated that the differences among all treatments were not significant ($P<0.05$). A slightly higher percent of milk fat was obtained from cows fed the grass silage mixture than the other three mixtures (Table 11). However, the differences were not significant.

In milk fat, palmitate made up the largest molar percent fatty acids. Butyrate, myristate and oleate also form a major proportion of the total fatty acids (Table 12). Feeding the corn silage mixture appeared to cause a small increase of molar percent of short and medium-chain fatty acids (C_4 to C_{12}) with a reciprocal decrease in C_{18} fatty acids (Table 12).

Table 9. Effect of complete feed on mean of glucose and urea levels, mg/100 ml, in the blood (Expt. I).

Treatments	Glucose		Urea	
	Jugular	Mammary	Jugular	Mammary
Corn silage mix.	28.62	25.66	23.94	20.50
Grass silage mix.	24.58	25.04	25.94	23.25
Ground hay mix.	22.96	21.70	25.13	24.57
Long hay + conc.	23.88	23.83	32.06	28.70

Table 10. Effect of complete feed on mean of daily milk yield (Expt. I).

Treatments	Milk yield	FCM
	Kg	Kg
Corn silage mix.	16.14[a,b]	15.42
Grass silage mix.	14.54[b]	14.30
Ground hay mix.	16.89[a]	15.60
Long hay + conc.	18.03[a]	16.75

a,b Treatment means within a column not sharing a common superscript are significantly ($P<0.05$) different.

Table 11. Effect of complete feed on mean of milk composition (Expt. I).

Treatments	Total solids	SNF	Fat	Protein	Lactose	Ash
	%	%	%	%	%	%
Corn silage mix.	12.93	9.25	3.68	3.41	5.02	0.82
Grass silage mix.	12.92	9.11	3.81	3.31	4.99	0.81
Ground hay mix.	12.78	9.19	3.59	3.47	4.87	0.85
Long hay + conc.	12.82	9.23	3.59	3.42	5.00	0.81

Table 12. Effect of complete feed on mean of fatty acid composition of the milk fat (Expt. I).

Fatty acids		Treatments: Corn silage	Grass silage	Ground hay	Long hay
		molar %			
Butyrate,	4:0	14.92	14.22	14.84	14.73
Caproate,	6:0	5.91	5.62	5.77	5.70
Caprylate,	8:0	2.77	2.46	2.68	2.73
Caprate,	10:0	5.05^{B} A,B,C,b,c,	4.32^{A} A,a	5.30^{B} B,b	5.17^{B} A,B,b
Laurate,	12:0	5.16	4.41	5.51	5.34
Myristate,	14:0	13.26	12.56	13.22	13.48
Palmitate,	16:0	30.59	39.59	29.99	31.48
Stearate,	18:0	6.00 A,B,b,c	6.15 A,a	5.54 A,B,a,b,	5.27 B,b,c
Oleate,	18:1	14.72	17.63	15.12	14.35
Linoleate,	18:2	1.62	2.04	2.03	1.75

A,B,C Treatment means within a row not sharing a common superscript are significantly ($P<0.01$) different.

a,b,c Treatment means within a row not sharing a common superscript are significantly ($P<0.05$) different.

The cows fed corn silage had a significant decrease in laurate when compared with those fed either ground hay ($P<0.01$) or long hay ($P<0.05$). The feeding of grass silage resulted in a significant ($P<0.01$) depression in the molar percent of caprylate and laurate with a reciprocal increase in oleate when compared with the other three treatments. Feeding grass silage mixture appears to decrease molar percent of myristate and increase the molar percent of stearate when compared with corn silage, ground hay and long hay mixtures.

The total solids and solids-not-fat were not affected significantly ($P<0.05$) by the source of roughage. Also, there were no significant differences among the treatments for milk protein, lactose or ash percentage.

EXPERIMENT II

Feed and TDN Consumption. Dry matter consumption by cows tended to be greater for the grass silage mixture when compared with that of the other three treatments (Table 13). However, the differences among treatments were not significant ($P<0.05$).

The cows fed grass silage appeared to consume more TDN than those fed corn silage, ground hay or long hay. However, there was no significant difference ($P<0.05$) among the experimental treatments.

Ruminal VFA and pH. Total ruminal VFA of cows fed the long hay mixture tended to be higher than corn silage, grass silage and ground hay treatments (Table 14). Total VFA from cows fed the ground hay mixture was somewhat lower than those fed the long hay mixture. However, the differences among the treatments were not significant ($P<0.05$). The cows fed long hay had higher ($P<0.01$) ruminal molar percentage of acetic acid when compared with those fed corn silage and grass silage mixture with no significant differences with ground hay. The molar percentagesof propionic, butyric and valeric were not affected significantly ($P<0.05$) by experimental treatments. However, butyrate tended to be higher for the silage mixtures. Branched-chain fatty acids, isobutyric and isovaleric, also were not affected significantly ($P<0.05$). The molar proportion of acetic to propionic acid in ruminal fluid (Table 15) was not significantly ($P<0.05$) affected by the experimental treatments. However, the acetic to propionic ratio was somewhat lower for the grass silage and ground hay.

Table 13. Effect of complete feed on mean of daily feed and TDN consumption (Expt. II).

Treatments	Dry matter	TDN
	Kg	Kg
Corn silage mix.	13.8	9.2
Grass silage mix.	16.6	9.8
Ground hay mix.	14.2	9.0
Long hay + conc.	13.6	8.1

Table 14. Effect of complete feed on mean of total and molar percentage of VFA in ruminal fluid (Expt. II).

Treatments	Total VFA	C_2	C_3	iso-C_4	C_4	C_5	iso-C_5
	mmole/ 100 ml	molar %					
Corn silage mix.	10.93	63.2^{B}	20.2	0.7	13.2	1.2	1.3
Grass silage mix.	10.97	61.3^{B}	21.3	0.9	13.0	2.1	1.4
Ground hay mix.	10.28	64.7^{A,B}	21.4	0.6	10.9	1.4	1.0
Long hay + conc.	12.72	67.5^{A}	20.0	0.5	10.1	1.2	1.1

A,B Treatment means within a column not sharing a common superscript are significantly ($P<0.01$) different.

Table 15. Effect of complete feed on mean of molar proportion of acetic to propionic acid and pH in ruminal fluid (Expt. II).

Treatments	C_2/C_3	pH
Corn silage mix.	3.3	6.8
Grass silage mix.	3.1	6.7
Ground hay mix.	3.1	6.5
Long hay + conc.	3.4	6.2

Table 16. Effect of complete feed on mean of total digestible nutrients and digestible nutrients of experimental diets (Expt. II).

Treatments	TDN	Dry matter	Crude protein	Crude fiber	Ether extract	NFE
Corn silage mix.	65.83	66.62	57.40	51.43	29.66[a]	75.88
Grass silage mix.	59.07	68.95	67.28	49.35	72.74[b]	75.81
Ground hay mix.	63.47	67.95	61.90	--	63.79[b]	80.28
Long hay + conc.	59.60	63.19	56.29	26.94	60.48[b]	74.99

a,b Treatment means within a column not sharing a common superscript are significantly ($P<0.05$) different.

Ruminal pH was not significantly ($P<0.05$) affected by the source of roughage. However, the ruminal pH was somewhat higher for the corn silage and grass silage treatments compared with that of ground hay and long hay treatments (Table 15).

Digestibility Trials. Mean total digestible nutrients were not affected significantly ($P<0.05$) by experimental treatment. However, TDN was somewhat higher for the corn silage and ground hay treatments compared with that of grass silage and long hay treatments (Table 16). There was no significant difference ($P<0.05$) in dry matter digestibility between experimental treatments. There were no significant differences ($P<0.05$) in crude protein, and NFE digestibility among the experimental treatments. The digestibility of ether extract of corn silage was significantly lower ($P<0.05$) than that of grass silage, ground hay and long hay mixtures. The crude fibre digestibility of ground hay mixture was near zero.

Glucose and Urea Levels in Blood. The mean of blood glucose level was not affected significantly ($P<0.05$) by experimental treatments. Also, there were no significant differences among treatment means for blood urea levels (Table 17).

Milk Production and Composition. Feeding the experimental treatments had no significant ($P<0.05$) effect on mean daily milk yield and FCM (Table 18). Mean daily milk yield of cows fed grass silage was somewhat higher than those fed corn silage, ground hay and long hay mixtures, however, this difference was reduced when FCM was compared.

Table 17. Effect of complete feed on mean of glucose and urea levels, mg/100 ml, in the blood (Expt. II).

Treatments	Glucose	Urea
Corn silage mix.	48.33	30.30
Grass silage mix.	48.03	30.15
Ground hay mix.	48.11	29.61
Long hay + conc.	48.08	29.86

Table 18. Effect of complete feed on mean of daily milk yield (Expt. II).

Treatments	Milk yield	FCM
	Kg	Kg
Corn silage mix.	18.6	19.1
Grass silage mix.	20.2	18.8
Ground hay mix.	18.8	18.9
Long hay + conc.	18.7	18.2

No significant differences ($P<0.05$) were observed among the mean percentage of milk protein and lactose of the experimental treatments. Percentage of ash were similar among the four treatments with no significant differences ($P<0.05$).

Fatty acid composition of milk fat was not significantly ($P<0.05$) affected by the treatments with the exception of stearic. Molar percent of palmitate form a major proportion of total fatty acids of the milk (Table 20) of cows fed the experimental treatments. Also, it was observed that molar percent of butyrate, myristate and oleate were somewhat higher than other fatty acids of milk fat in the four treatments.

Stearate was significantly higher for corn silage treatment than grass silage ($P<0.05$), ground hay and long hay ($P<0.01$) treatments. Molar percent of stearate was significantly higher ($P<0.05$) for grass silage treatment than for long hay treatment.

The total solids and solids-not-fat were not affected significantly ($P<0.05$) by the source of roughage (Table 19). A slightly higher percent of milk fat was obtained from corn silage and ground hay mixtures than grass silage and long hay mixtures; however, the differences among the experimental treatments were not significant ($P<0.05$).

Health and Body Weight. The overall health and outward appearance of the cows seemed quite good throughout Experiment I and II for all treatments. The level of grain (40% or 60%) in the feed did not cause any harmful effects. No allergic swelling, lamenesa or digestive disorders were observed.

Table 19. Effect of complete feed on mean of milk composition (Expt. II).

Treatments	Total solids	SNF	Fat	Protein	Lactose	Ash
	%	%	%	%	%	%
Corn silage mix.	13.54	9.44	4.10	3.17	5.46	0.81
Grass silage mix.	13.43	9.86	3.57	3.26	5.79	0.81
Ground hay mix.	13.08	8.98	4.10	3.19	4.98	0.81
Long hay + conc.	13.40	9.59	3.80	3.48	5.32	0.79

Table 20. Effect of complete feed on mean of fatty acid composition of the milk fat (Expt. II).

Fatty acids		Corn silage	Grass silage	Ground hay	Long hay
		molar %			
Butyrate,	4:0	13.25	14.06	13.63	13.71
Caproate,	6:0	6.03	5.97	5.93	5.90
Caprylate,	8:0	2.91	2.92	2.83	2.87
Caprate,	10:0	5.19	5.72	5.05	5.40
Laurate,	12:0	5.49	5.66	5.29	5.14
Myristate,	14:0	13.15	13.11	12.84	13.35
Palmitate,	16:0	31.00	28.77	29.15	31.59
Stearate,	18:0	6.85 A,a	5.91 A,B,b	5.35 B,b,c	5.30 B,c
Oleate,	18:1	14.47	15.67	17.08	14.20
Linoleate,	18:2	1.81	2.21	2.72	2.21

A,B Treatment means within a row not sharing a common superscript are significantly ($P<0.01$) different.

a,b,c Treatment means within a row not sharing a common superscript are significantly ($P<0.05$) different.

Table 21. Effect of complete feed on mean of daily body weight gain (Expt. I & II).

Treatments	Experiment I Kg	Experiment II Kg
Corn silage mix.	0.14	0.56
Grass silage mix.	0.41	0.37
Ground hay mix.	0.23	0.19
Long hay + conc.	0.57	0.48

The average initial weight was 590 and 570 kg for Experiment I and II respectively. The cows which were fed the corn mixture lost weight during Experiment I and II compared with those fed the grass silage, ground hay and long hay mixtures (Table 21). On the other hand, feeding the long hay mixture appeared to cause a larger increase in body weight gain of the cows than those receiving the grass silage or the ground hay mixture. Furthermore, the cows fed grass silage tended to gain more weight when compared with cows fed the ground hay mixture.

DISCUSSION

Feed and TDN Consumption. In Experiment I, feed consumption of the cows fed the complete feed containing corn silage were significantly ($P<0.01$) lower than those fed any of the other three treatments, and consequently there was a lower TDN consumption (Table 5). There were no significant differences in the feed and TDN consumption by the cows fed long hay, ground hay and grass silage mixtures. Some reports (84,85,86,414), have shown that cows fed corn silage consumed less dry matter when compared with cows fed alfalfa hay. However, the information on complete ensiled feeds based on corn silage fed to lactating dairy cows is limited (74, 147,314).

In Experiment II, it was observed that feed and TDN consumption of cows fed the complete feeds, containing corn silage, grass silage, ground hay were not significantly ($P<0.05$) different than those fed long hay and concentrate. It is possible that the differences in feed consumption noted in Experiment I would be decreased as the levels of forage decreased in Experiment II. Also dry matter content of corn silage in Experiment II was higher (59.34%) when compared with that (28.32%) of Experiment I. Also silage in Experiment I contained some moldy material which was not present in Experiment II.

Voluntary intake is positively correlated with dry matter content of corn, sorghum and alfalfa silage within certain ranges (188,228,481). Voluntary intake of corn silage dry matter apparently increases in direct proportion to dry matter content of the corn silage, up to about 35%. Above 35% dry matter, dry matter intake seems to level off.

A positive correlation between dry matter intake and silage dry matter content has also been noted with alfalfa silage in the range of 20 to 50 percent dry matter (188). Correspondingly, Huber et al (228) found that when corn silage dry matter increased from 25 to 30 to 33 percent, voluntary intake of these silages by cows increased from 1.95 to 2.13 to 2.31 kg DM per 100 kg of body weight, respectively. However, the exact nature of the factors responsible for the increase in voluntary dry matter intake with increasing dry matter content has not been established (24,132).

Ruminal VFA. In Experiment I, significant ($P<0.05$) differences were found among treatments for total ruminal VFA. Total VFA from cows fed complete feed containing ground hay was significantly higher ($P<0.05$) than those fed complete feed containing corn silage and control treatment, long hay, with no significant differences with grass silage (Table 6). No significant ($P<0.05$) differences were observed among treatments for all molar percentages of VFA studied except propionic acid ($P<0.05$). No apparent effect of treatment on branched-chain isomers of butyric and valeric acids was noted.

In Experiment II, no significant differences ($P<0.05$) were noted among the experimental treatments for total ruminal VFA and all molar percentages of VFA studied (Table 14) except acetic ($P<0.01$). The branched-chain isomers of butyric and valeric also were not affected by the treatments.

The mean ruminal VFA was significantly ($P<0.05$) affected by the treatments of Experiment I while no significant ($P<0.05$) differences were

observed among treatments of Experiment II for ruminal VFA (Table 15). It is difficult to make comparison between present experiments and other experiments because experimental conditions vary as to food intake, time of sampling and type of ration (35,251,404,393). There is no clear relationship between SNF, protein and lactose content in the milk and rumen VFA. However, it has been shown that rumen propionate increases on high-grain, low roughage rations (31,229,469). Higher propionate production in the rumen on high-grain ration may control degradation and synthesis of amino acid in the liver (227). Higher levels of alpha amino nitrogen in blood plasma of cows fed a high-grain compared with a low-grain ration have been observed (411). Powell (379) suggested that there was a relationship between milk composition and rumen metabolism. Stoddard et al (441) and Balch et al (30) have shown that milk fat depression was related to increased propionic acid and reduced acetic acid in the rumen. A relationship has been observed between gross efficiency of milk production and acetic:propionic ratios. Most studies (138,139,172, 391) have suggested that maximum gross efficiency of milk production occurs when ruminal acetic to propionic ratio is in the range of 2.5 and 3.0. Similar observations have been reported by Blaxter (60) and reviewed by Van Soest (466).

Digestibility. Significant ($P<0.05$) differences were observed among the treatments of Experiment I for TDN and all digestible nutrients studied (Table 8) except crude fibre digestibility. Mean of TDN, dry matter, crude protein, nitrogen-free extract were significantly ($P<0.05$) lower for cows fed complete feed containing corn silage than those fed the other

three treatments. A slight decrease in TDN, dry matter, crude protein and nitrogen-free extract digestibility was observed for cows fed complete feed containing grass silage when compared with those fed the long hay mixture. However, these differences were not significant ($P<0.05$). Similar observations have been reported by other workers (188,406,476).

No significant ($P<0.05$) differences were noted among the treatments of Experiment II for TDN, dry matter, crude protein, and nitrogen-free extract digestibility while ether extract digestibility was significantly ($P<0.05$) lower for cows fed complete feed containing corn silage than those fed the other three treatments. The crude fibre digestibility for cows fed complete feed containing ground hay was near zero. This result might partially be explained on the basis that grinding the hay resulted in a decrease in crude fibre digestibility (11,38,324), in addition to grinding; increasing the concentrate portion in the diet may result in a decrease in crude fibre digestibility (42,267,287).

In the present study (Experiment I and II), crude fibre digestibility was decreased and nitrogen-free extract increased for cows fed complete feed containing ground hay as compared with those fed long hay. Similar observations were reported by Alexander et al (11).

Blood Glucose and Urea. The concentration of glucose in venous blood was unaffected ($P<0.05$) by the diets fed in Experiments I and II. It was also observed that the concentration of urea in venous blood was not significantly influenced by the diets fed in Experiments I and II. Balch et al (28) observed that the level of blood glucose was not influenced by restricted roughage high concentrate diets. However, several reports have

shown increased blood glucose resulting from feeding higher levels of energy (227,244,411). Huber & Boman (227) reported that within a range of 40 to 75 mg/100 ml blood glucose had little effect on milk lactose. McClymont (290) suggested that increased levels of blood glucose suppressed the liberation,removal,of fatty acids from adipose tissue, thereby reducing plasma free fatty acids available for milk fat synthesis.

Milk Production and Composition. The results of Experiment I, as well as those of Experiment II, show that no significant ($P<0.05$) differences were observed between cows fed complete feed containing corn silage and those fed conventional long hay diet. Fat-corrected milk production was not significantly ($P<0.05$) different between the above treatments. A similar trend can be observed in the investigations by Brown et al (84,85), in which all-corn silage and all hay were compared as roughage for lactating cows. Their data showed that cows fed all-silage produced a slightly higher milk yield than cows fed all-hay in two complete lactations. Slight differences in milk production were observed when cows were fed all-corn silage compared with those fed all-hay feeds (198,465). Other workers (74,147,314) observed that milk production of cows fed the ensiled complete rations was equal to that of cows fed similar rations of corn silage with unfermented concentrate added at the time of feeding. Muller et al (335) found that the complete ration of corn silage with roughage to concentrate ratio of 68:32 maintained 15.3 kg FCM production remarkably well during an 18-week trial. Derbyshire & Gordon (147) noted that a complete corn silage ration (concentrate to roughage 60:40 ratio) was near the minimum amount that would supply the requirement of a 590 kg cow

producing 27 kg fat-corrected milk. In the present study, no significant differences were observed between cows fed complete feed containing corn silage and those fed complete feed containing grass silage for milk yield and fat-corrected milk production. Similar observations were noted by Murdock & Hodgson (337).

Milk yield was lower ($P<0.05$) for cows fed complete feed containing grass silage than those fed the conventional long hay diet (Table 10) harvested from the same field, whereas the fat-corrected milk production difference was not significant ($P<0.05$) (Experiment I). However, it was noted that there was some spoilage in the grass silage used in Experiment I. In Experiment II, the milk yield was higher for cows fed complete feed containing grass silage when compared with cows fed the conventional long hay diet harvested from the same field, but the difference was not significant ($P<0.05$). This difference was decreased when yield was expressed as FCM (Table 18). Several studies (75,86,104,187,205,281,381, 428,442) have indicated that cows fed all-grass silage produce as much or more milk than cows fed all-hay. Some reports (133,188,205) have shown that milk production was lower for the cows fed all-grass silage than those fed hay.

In Experiments I and II, no significant ($P<0.05$) differences were observed between cows fed complete feed containing ground hay and those fed long hay for mean of daily milk yield and fat-corrected milk. Other workers who fed ground hay rations have reported no change in milk production (23,185), a slight increase (252,409) or a decrease (70,164,244).

In the present studies, no significant differences were observed among the treatments of Experiments I and II for milk fat percent. However,

it has been a general observation that milk fat content was decreased as concentrate feeding was increased (52,82,253,466). Ronning (408) and Hawkins et al (196) observed a reduction in milk fat content when the proportion of dietary concentrate exceeded 30 or 35%, while other workers observed (87) that feeding grain had no significant effects on milk fat content when the grain consumption increased from about 11 to 17 to 36 lb per cow per day. Powell (379,378,380) found that feeding ground hay to lactating cows decreased milk fat content. The milk fat content was decreased when finely ground hay was included in a ration containing concentrates.

The composition of milk fat appears to be affected by the treatments of Experiments I and II. The levels of fatty acids in milk fat showed some uniform trends. Molar percent of palmitate made up the larger proportion of the fatty acids. Butyrate, myristate and oleate also form a major proportion of total fatty acids. This is in agreement with the result of other workers (90,433). Several workers have shown that low-roughage rations cause an increase in the iodine number with a decrease of Reichert-Missel value (28,30). Brown et al (90) and Shaw et al (421) did not find any alteration in the degree of unsaturation of the milk fat when the energy intake was maintained at or above recommended requirement.

In the present experiments, the content of lactose in the milk was unaffected by the treatments (Tables 11 and 19). This result could be expected because no significant differences were observed among treatments for the level of blood glucose. Linzell (279) reported in a review that the glucose and galactose (lactose molecule) are derived from

blood glucose. However, large changes in blood glucose were required to influence milk lactose (227,442).

In the present studies, no significant differences were observed among the treatments of Experiments I and II for solids not fat, and protein content of milk. Rook (410) suggested that feeding of grass silage was associated with a marked decrease in solids-not-fat content of milk. However, the physical form of the diet has a variable effect on milk protein, lactose and solids-not-fat (52,227,252,258).

PART TWO

Effects of Inclusion of Diethylstilbestrol (DES) to Semipurified and Conventional Complete Feeds on Rumen Metabolism, Digestibility, Nitrogen Retention, Rumen Microorganisms and Blood Component.

MATERIALS AND METHODS

EXPERIMENT III

Four mature, non-lactating dairy cows fitted with permanent rumen fistulae were used in a 4 X 4 latin square design. Each experimental period consisted of 14 days of adjustment and 14 days of comparison. The cows were placed in stanchions which were equipped with automatic watering cups. The experimental treatments consisted of feeding semipurified and conventional pelleted diets with and without 8 mg/day of diethylstilbestrol (DES) (Appendix IV, table 1). Feed intake was fixed at 10 kg per day for each cow to limit the effect of level of dry matter intake on rumen content. A steady state was established in the rumen of cows through 2 minute interval feeding using an automatic feeder device. Each automatic feeder supplied two cows (Figure 1). Feed was spread evenly on the two-ply rubber conveyor belts which move towards the centre of the unit, dropping feed into the respective manger in front of each cow. Each conveyor is 152 cm long by 41 cm wide and is mounted on steel rollers driven by a series of V belt pulleys. The drive for the pulley system is from a 1/3 H.P. (1725 rpm) electric motor through a 400:1 reducer-drive gear box. The motor is controlled by a 10-minute repeat cycle timer (Intermatic, International Register Co., Chicago) that can be switched on for 5-second periods, one or more times in each 10 minute cycle. The application of this feeding technique is described under the following title: "Effect of Continuous Feeding on the Composition of Rumen Digesta" (Appendix III).

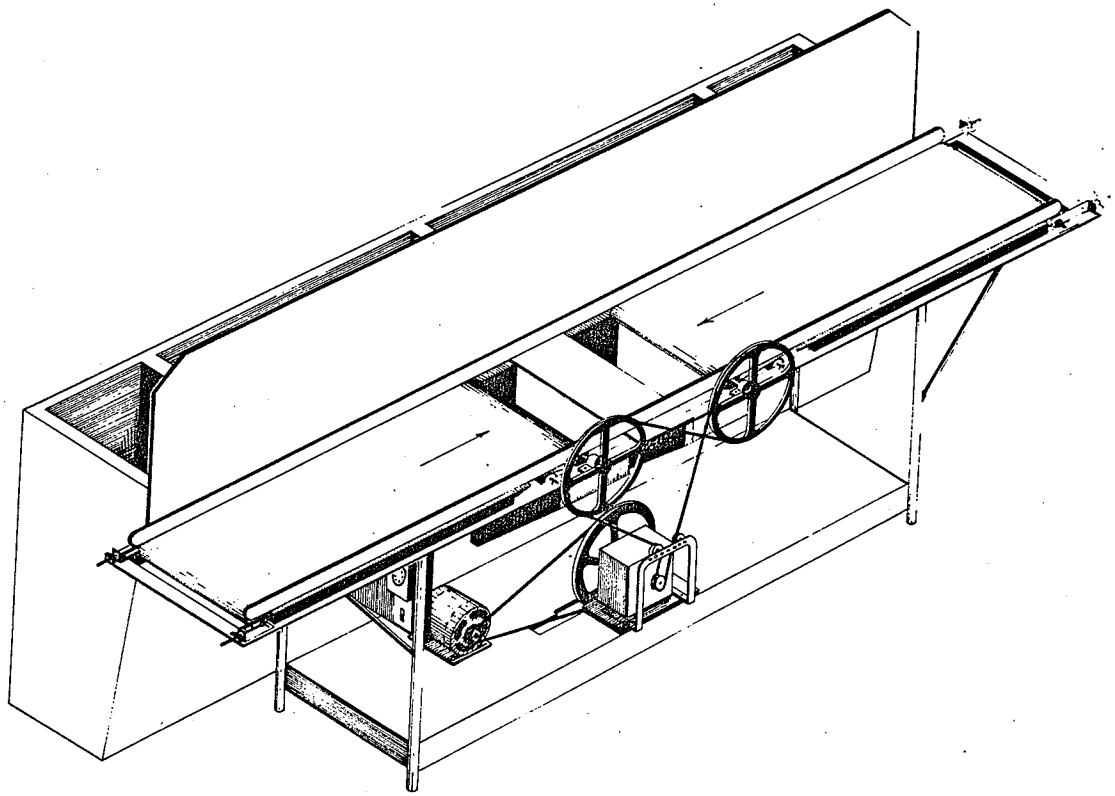

Figure 1. The automatic feeder.

About 500 ml of rumen contents were obtained from each cow and mixed together for cross-innoculations on the first and third day of each adjustment period to eliminate residual effect of the treatment on rumen microorganisms and to present all kinds of microorganisms to each animal at the beginning of each period. Thus any change in microbial population should be due to treatment effect.

Ruminal VFA, pH and Ammonia. During the first and fourth day of each comparison period, samples of rumen digesta were collected every 2 - 4 h for 24 h. The rumen fluid was strained through two layers of cheesecloth. After straining, the pH was determined. The rumen fluid was preserved with $HgCl_2$ for subsequent volatile fatty acid analyses. Volatile fatty acids were quantitatively determined as previously described under experiments I and II. Ruminal ammonia was immediately determined after collection by the method of Conway (135).

Since most of the chemical determinations were made on a concentration basis, ruminal fill was measured on the last day of each comparison period. The rumen of each cow was manually emptied through the fistula. Weight of digesta was recorded then mixed thoroughly, sampled and the digesta returned. Dry matter content of the digesta was determined. Dried samples of rumen digesta were ground and kept for analyses (crude protein, amino acid and lignin).

Digestibility Trials and Nitrogen Balance. Feces and urine were collected for 4 - 5 days (6 - 10th day of the comparison period). The fecal samples were used in determining digestion coefficients for dry matter, protein,

fibre, cellulose, ether extract, nitrogen free extract and total digestible nutrients. The proximate constituents of the diets and feces were determined according to A.O.A.C. (22) procedures. Ammonia from Kjeldahl distillations were collected in 20 ml of 2% boric acid and indicator mixture (bromocresol green + methyl red) and titrated with 0.1 N HCl. Cellulose was estimated by the method of Crampton & Maynard (141) and lignin was determined according to the Van Soest & Wine (467) procedure.

Urine was collected by using catheters. Concentrated sulfuric acid was added to collection bottles to prevent ammonia losses. Nitrogen in the urine was determined by the micro-Kjeldahl technique according to Chibnall et al (119) using the microdistillation apparatus of Markham (312).

Microbial Studies.

A. Counting and Identification of Ciliate Protozoa. Counting and identification of ciliate protozoa were carried out during the comparison period at 3-day intervals as described under the following title "Effect of Dietary Diethylstilbestrol on Populations and Concentrations of Ciliate Protozoa in Dairy Cattle" (Appendix IV).

B. Amino Acid Composition. Protozoa and bacteria and rumen content were collected and analyzed for amino acid composition as described under the following technical note: "Separation and Identification of Amino Acids in Rumen Microorganisms" (Appendix V).

Blood Studies. Samples of blood were collected from the jugular vein into test tubes treated with anticoagulant (potassium oxalate) in the form of a thin dried film over the inside surface. Blood glucose was determined as

described under experiment I and II and total hemoglobin was determined by using a Coleman "25" Photo-Hemoglobinometer (Coleman Instruments, Inc., Illinois, U.S.A.).

Hematocrit , red cell volume percent, was determined by using a Model CR micro-capillary reader (International Equipment Company, Mass.).

Preparation of Blood Plasma for Amino Acid Analysis. The protein of blood plasma was removed by precipitation with tungstic acid followed by centrifugation. One volume of 10% sodium tungstate solution was added to each volume of blood plasma followed by one volume of 0.66 N sulfuric acid while shaking. The mixture was then left standing for 10 min and then centrifuged at 1500 XG for 20 min. The protein-free supernatent of blood plasma, was freeze dried. The dry protein-free blood plasma was dissolved in a standard volume of pH 2.2 sodium citrate buffer (Appendix II, table 4).

The free amino acid composition of blood plasma was determined by using a Beckman Model 116 amino acid analyzer with norleucine as an internal standard (44,45 and Appendix II, table 5).

RESULTS

Ruminal VFA, pH and Ammonia. The chemical composition of feed ingredients are presented in Table 22. The diets containing DES resulted in a slight increase in total volatile fatty acid concentration (Table 23). However, no significant ($P<0.05$) differences were observed among the experimental diets for total VFA concentration and molar percentage. The mean values of pH were not affected significantly ($P<0.05$) by various treatments (Table 23).

Rumen ammonia concentration was higher for cows fed the semipurified diets compared with those fed conventional diets ($P<0.01$). Rumen fluid from the cows fed the semipurified diet with DES contained significantly higher ($P<0.01$) ammonia concentrations than cows fed the semipurified diet without DES. Rumen fluid from cows fed the conventional diet without DES contained 0.56 mmole/l of ruminal ammonia while cows fed the conventional diets with DES contained 2.43 mmole/l of ruminal ammonia.

Rumen Fill. The weight of rumen digesta was somewhat greater for cows fed semipurified diets when compared with cows fed the conventional diets (Table 24); however, the differences were not significant ($P<0.05$).

There was no significant difference ($P<0.05$) in dry matter percent of rumen digesta of cows fed the experimental diets. Crude protein percent of rumen digesta was higher ($P<0.01$) for cows fed conventional diets when compared with cows fed semipurified diets.

Inclusion of DES in the semipurified diet results in a higher crude protein percent of rumen digesta ($P<0.05$) whereas inclusion of DES in

Table 22. Chemical composition of the experimental diets (Expt. III).

Items	Semipurified	Conventional
	%	%
Dry matter	90.95	88.95
Crude protein	13.20	13.35
Total amino acid	1.02	11.04
Crude fiber	17.82	14.58
Cellulose	16.97	13.89
Ether extract	1.02	1.26
NFE	51.83	51.90
Ash	7.08	7.86
Lignin	4.06	3.93
Acid-detergent fiber	23.79	17.55

Table 23. Ruminal characteristics of dairy cows fed experimental diets (Expt. III).

Items	Semipurified		Conventional	
	No DES	DES	No DES	DES
Total VFA mmole/l	186.63	188.96	196.56	214.18
Acetic, molar %	66.38	65.17	67.53	65.46
Propionic, molar %	20.76	19.81	16.82	22.25
Isobutyric, molar %	0.28	0.20	0.30	0.46
Butyric, molar %	11.43	14.22	14.45	11.30
Valeric, molar %	0.58	0.45	0.66	0.15
Isovaleric, molar %	0.57	0.15	0.24	0.38
pH	5.78	5.70	5.55	5.98
Ammonia, mmole/l	32.82^{B}	55.65^{A}	$0.56^{C,b}$	$2.43^{C,a}$

A,B,C Treatment means within a row not sharing a common superscript are significantly ($P<0.01$) different.

a,b Treatment means within a row not sharing a common superscript are significantly ($P<0.05$) different.

Table 24. Rumen digesta data of dairy cows fed the experimental diets (Expt. III).

Items	Semipurified		Conventional	
	No DES	DES	No DES	DES
Rumen digesta, (Kg)	34.99	35.25	30.06	32.18
Dry matter, (%)	10.30	12.37	10.33	10.20
Dry matter, (Kg)	3.60	4.36	3.11	3.28
Crude protein, % DM	17.00^{B,c}	19.10^{B,d}	24.10^{A}	24.70^{A}
Total amino acid, % DM*	15.17	17.10	21.75	22.65
Lignin, % DM	9.69	9.05	8.01	8.96

* Pooled sample, not analyzed statistically.

A,B Treatment means within a row not sharing a common superscript are significantly ($P<0.01$) different.

c,d Treatment means within a row not sharing a common superscript are significantly ($P<0.05$) different.

conventional diet had no significant effect on crude protein percent of rumen digesta.

Digestibility Trials and Nitrogen Balance. Digestibility coefficients and TDN values for the experimental diets are presented in Table 25. From data thus presented, it seems that inclusion of DES in the experimental diets had no specific effect on digestibility coefficients of nutrients consumed and also on TDN values. Also, it could be noted that there were no significant ($P<0.05$) differences between semipurified and conventional diets on TDN values. Crude protein digestibility obtained for cows fed semipurified diet without DES was significantly higher ($P<0.05$) than those obtained from cows fed conventional diets without DES. Dry matter, crude fibre, and cellulose digestibilities obtained for cows fed the conventional diets were higher than those obtained for cows fed semipurified diets but, the differences were not significant ($P<0.05$).

Mean nitrogen retention, expressed as a percentage of intake increased significantly ($P<0.01$) when DES was included in the experimental diets (Table 26). There was a significant ($P<0.01$) decrease in urinary nitrogen losses with a consequent increase in nitrogen retention by inclusion of DES in the diets. This was true for both experimental diets. However, the reduction in urinary nitrogen losses due to the inclusion of DES was greater with semipurified diets than with conventional diets. When the cows were fed a semipurified diet, the fecal nitrogen losses were not significantly ($P<0.05$) affected by experimental treatments.

Blood Components. The level of blood glucose for cows fed the conventional

Table 25. Apparent digestibility data of dairy cows fed the experimental diets (Expt. III).

Items	Semipurified		Conventional	
	No DES	DES	No DES	DES
Dry matter digestibility, %	75.08	72.76	79.68	81.92
Crude protein digestibility, %	83.88[a]	80.69[a,b]	72.45[b]	76.51[a,b]
Ether extract digestibility, %	75.21	72.91	70.33	71.42
Crude fiber digestibility, %	53.38	51.02	60.06	61.01
NFE digestibility, %	82.55	81.42	85.43	86.76
Cellulose digestibility, %	53.56	52.61	63.58	61.53
TDN	64.97	63.62	64.76	66.16

a,b Treatment means within a row not sharing a common superscript are significantly ($P<0.05$) different.

Table 26. Nitrogen retention data of dairy cows fed the experimental diets (Expt. III).

Items	Semipurified		Conventional	
	No DES	DES	No DES	DES
N- intake, g/day	211.2	211.2	212.0	212.0
N- feces %, intake	15.11 ^{A,a}	22.71 ^{B,b}	26.86	24.53 ^{B,b}
N- urine %, intake	62.28	27.25	43.52^{c}	29.97
N- retention %, intake	22.60^{B}	50.04^{A}	29.63^{B}	48.00^{A}

A,B Treatment means within a row not sharing a common superscript are significantly (P<0.01) different.
a,b,c Treatment means within a row not sharing a common superscript are significantly (P<0.05) different.

Table 27. Blood components of the dairy cows fed the experimental diets (Expt. III).

Items	Semipurified		Conventional	
	No DES	DES	No DES	DES
Glucose, mg %	45.17^{C}	47.36^{B}	47.94^{B}	53.58^{A}
Hemoglobin, g %	12.05 ^{b,c}	13.83^{a}	13.23 ^{a,b}	14.08^{a}
Hematocrit	36.95^{C}	39.78^{B}	39.73^{B}	42.33^{A}
Ammonia umole %	29.97	28.83	25.90	28.74
Urea umole %	24.12	19.78	20.06	18.36

A,B,C Treatment means within a row not sharing a common superscript are significantly (P<0.01) different.
a,b,c Treatment means within a row not sharing a common superscript are significantly (P<0.05) different.

diet was significantly higher ($P<0.01$) when compared with cows fed the semipurified diet. Diethylstilbestrol also increased ($P<0.01$) blood glucose level for cows receiving both diets. There was no significant difference between cows fed the semipurified diet with DES and those fed the conventional diet without DES.

The cows fed conventional diets had higher blood hemoglobin values than cows fed semipurified diets but the only significant difference ($P<0.05$) was detected between cows fed conventional diet with DES and cows fed semipurified diet without DES (Table 27). The inclusion of DES in the experimental diets resulted in an increase of blood hemoglobin values; however, the difference was significant ($P<0.05$) for only the semipurified diet.

Hematocrit values were affected by the type of diet and the inclusion of DES. The inclusion of DES in the experimental diets caused a significant ($P<0.01$) increase in hematocrit values. It is also shown that hematocrit values for cows fed the conventional diet without DES were significantly higher ($P<0.01$) than those fed semipurified diet without DES. Also there was a significant difference ($P<0.01$) in hematocrit values between semipurified diet and conventional diet when DES was included in the diet.

Ammonia levels in blood were not significantly affected by treatments. There was no significant difference in urea concentration of blood from cows fed the experimental diets (Table 27).

Free Amino Acids of Blood Plasma. The concentration of the free amino acids and other ninhydrin-positive compounds in the blood plasma of the cows is

presented in Table 28. No significant ($P<0.05$) differences were observed among the treatments for total concentration of the free amino acids in the plasma. The content of lysine, histidine and glycine tended to be lower in the plasma of cows fed conventional diets than in those fed semipurified diets. However, no significant ($P<0.05$) differences were observed. The concentration of methionine and ornithine tended to be higher in the plasma of cows fed the conventional diets when compared with those fed the semipurified diets. No significant ($P<0.05$) differences were noted between nitrogen sources for concentration of all amino acids studied in the plasma.

Blood plasma concentration of isoleucine, leucine, valine, phenylalanine, alanine, tyrosine, cystine and proline were all found to be higher for cows fed experimental diets containing DES than those without DES. However, the differences were not significant ($P<0.05$). The concentration of taurine, was also increased ($P<0.10$) by inclusion of DES in the experimental diets.

Amino Acid Composition of Protozoa and Bacteria. No significant ($P<0.05$) differences were noted among the experimental treatments for amino acid contents of rumen protozoa with the exception of lysine, proline, methionine and phenylalanine (Table 29). The inclusion of DES in conventional diets appeared to cause an increase in lysine and proline content of rumen protozoa when compared with values obtained from cows fed the semipurified diet without DES. Phenylalanine content of protozoa obtained from cows fed the conventional diet with DES was significantly ($P<0.05$) higher than that obtained from cows fed the semipurified diets.

Table 28. Plasma free amino acid concentrations of cows fed experimental diets (Expt. III).

Amino Acid	Semipurified		Conventional	
	No DES	DES	No DES	DES
	umole/Liter			
Isoleucine	82.4	95.1	95.2	98.2
Leucine	89.8	110.2	115.7	139.5
Lysine	56.0	57.2	38.8	35.8
Valine	168.1	205.2	199.8	223.2
Phenylalanine	32.3	38.6	36.8	44.0
Threonine	103.3	83.4	81.4	88.3
Methionine	14.5	13.6	20.0	15.3
Histidine	32.9	27.8	20.8	22.1
Arginine	21.4	40.4	37.0	35.1
Glycine	249.0	317.9	244.6	219.5
Glutamic acid	120.4	95.4	116.8	122.3
Alanine	172.9	180.4	155.9	183.7
Tyrosine	30.1	31.6	28.8	34.9
Aspartic acid	22.7	19.7	17.6	29.9
Cystine	15.0	21.9	14.3	22.5
Ornithine	27.6	25.9	39.1	33.4
Proline	48.5	53.4	46.7	58.9
Taurine	34.5	68.9	48.8	66.4

Table 29. Amino acid composition of protozoal fraction from dairy cows fed the experimental diets (Expt. III).

Amino Acid*	Semipurified		Conventional	
	No DES	DES	No DES	DES
Lysine	6.35[b]	7.45[a,b]	8.89[a,b]	10.04[a]
Histidine	1.29	1.62	1.44	1.46
Arginine	3.31	3.67	3.24	3.59
Aspartic acid	10.88	11.66	12.05	12.37
Threonine	4.52	4.56	4.37	4.11
Serine	3.53	3.86	3.74	3.55
Glutamic acid	18.02	18.08	18.63	18.46
Proline	2.86[b]	3.31[a,b]	3.78[a,b]	3.96[a]
Glycine	4.96	4.88	4.34	4.38
Alanine	6.35	7.07	4.84	5.57
Cystine	3.00	2.14	1.97	2.26
Valine	5.40	5.73	4.74	4.77
Methionine	2.03[a,b]	2.52[a]	1.13[b]	1.14[b]
Isoleucine	5.44	5.28	5.81	5.38
Leucine	6.61	6.59	6.78	6.87
Tyrosine	4.10	4.49	4.49	4.54
Phenylalanine	4.45[b]	4.31[b]	4.90[a,b]	4.97[a]
Aminoethylphosphonic	0.62	0.54	0.49	0.43
Total (% of DM)	33.61	37.67	40.41	38.92

* Gram amino acid per 100 g amino acid.
a,b Treatment means within a row not sharing a common superscript are significantly ($P<0.05$) different.

Table 30. Amino acid composition of bacterial fraction from dairy cows fed the experimental diets (Expt. III).

Amino Acid*	Results of amino acid analyses			
	Semipurified		Conventional	
	No DES	DES	No DES	DES
Lysine	7.05	7.19	6.74	9.51
Histidine	3.01	2.90	3.21	3.55
Arginine	3.93	3.63	3.67	3.77
Aspartic acid	11.91	12.18	12.54	11.59
Threonine	5.50	5.54	5.28	5.19
Serine	4.19	4.23	4.20	3.95
Glutamic acid	12.60	12.37	11.67	11.93
Proline	3.34	3.10	3.25	3.39
Glycine	5.34	5.09	5.00	5.01
Alanine	7.29	7.03	6.86	7.15
Cystine	3.12	3.02	3.20	3.20
Valine	5.27	5.59	5.27	5.65
Methionine	4.39	4.68	4.37	3.91
Isoleucine	4.96	4.98	5.43	5.16
Leucine	6.24	6.69	7.03	6.80
Tyrosine	5.68	6.28	6.56	7.65
Phenylalanine	4.65	4.40	4.84	4.73
Diaminopimelic acid	1.58	1.38	1.18	0.95
Total (% of DM)	23.66	23.43	27.57	19.08

* Gram amino acid per 100 g amino acid.

Methionine content of protozoa from cows fed the semipurified diet with DES was significantly higher than that obtained from cows fed conventional diets. Aminoethylphosphonic acid content of protozoa was unaffected ($P<0.05$) by experimental treatments. No significant ($P<0.05$) differences were observed among the treatments for total amino acid contents of protozoa.

Neither the amino acid content nor the diaminopimelic acid content of rumen bacteria were affected ($P<0.05$) by the experimental treatment. (Table 30).

Lysine and glutamic acid content of protozoa were higher than that of the bacteria, whereas, histidine, threonine, serine, cystine and methionine content of bacteria were higher than that of protozoa. Total amino acid content of protozoa on dry matter basis is higher than that of bacteria (Table 30). No significant ($P<0.05$) differences were observed among the treatments for total amino acid contents of protozoa or bacteria. However, statistical comparison between protozoal and bacterial amino acid fractions could not be made with the present experimental design used.

Microbial content of rumen digesta as determined by markers (aminoethylphosphonic and diaminopimelic) and gravimetric methods resulted in different values but similar trends in response to DES treatment (Table 31). Neither DES nor diets significantly ($P<0.05$) affected the amount of bacterial dry matter in rumen digesta as determined by the gravimetric method, while DES increased the amount of protozoal dry matter in rumen digesta from cows fed the conventional diets ($P<0.01$) and the semipurified diet ($P<0.05$).

Protozoal dry matter obtained from the rumen of cows fed the conventional diet was significantly ($P<0.01$) higher than that obtained from

Table 31. Percentage of digesta as protozoa and bacteria

Items	Semipurified		Conventional	
	No DES	DES	No DES	DES
Marker*(W/W)	%	%	%	%
Protozoa	.8	2.3	1.4	2.9
Bacteria	1.0	1.7	2.2	3.0
Gravimetric (W/V)				
Protozoa	^{B,c}.5	^{B,b}.9	^{B,a}1.2	1.9^{A}
Bacteria	.7	.9	.7	.9

a,b,c Treatment means within a row not sharing a common superscript are significantly ($P<0.05$) different.

A,B Treatment means within a row not sharing a common superscript are significantly ($P<0.01$) different.

* Pooled samples, not analyzed statistically.

cows fed the semipurified diet (Table 31).

Microbial amino acid synthesis was estimated by using aminoethylphosphonic and diaminopimelic acids as markers for protozoa and bacteria, respectively, The total amino acid content of rumen digesta was higher for cows fed conventional diets than those fed semipurified diets (Table 32).

The calculated microbial fractions made up between 31.7 to 74.4% of the total amino acids in the rumen (Table 32). The protozoal fraction made up 16.13 and 40.08% of the total rumen amino acids for cows fed semipurified diet without and with DES respectively. Values of 25.51 and 48.84% total rumen amino acids were obtained for the protozoal fraction for cows fed conventional diets without and with DES, respectively.

The inclusion of DES in the semipurified diet increased the level of protozoal amino acid in dry rumen contents by 148.48% and 85.99% for total microbial fractions. While the inclusion of DES in the conventional diet increased total amino acids in dry rumen contents by 91.45% and 40.92% for protozoal and total microbial fraction, respectively. The inclusion of DES in experimental diets had no effect on the bacterial amino acid fraction (Table 32).

Quantitative estimates of total amino acids passing to the lower gut were determined by using lignin ratio method (233). Total amino acids reaching the lower gut of cows fed semipurified diets were less than that of cows fed the conventional diets (Table 33). Inclusion of DES in experimental diets increased the amino acids reaching the lower gut from 880 to 941 g/day for the semipurified diet and from 1109 to 1268 g/day for the conventional diet.

Table 32. Fractions of amino acids in the rumen of dairy cows fed the experimental diets (Expt. III).

Items	Semipurified				Conventional			
	No DES		DES		No DES		DES	
	I	II	I	II	I	II	I	II
Rumen digesta-AA	15.17	100.00	17.10	100.00	21.75	100.00	22.65	100.00
Protozoal-AA	2.45	16.13	6.85	40.08	5.55	25.51	11.06	48.84
Bacterial-AA	2.37	15.63	3.25	18.99	5.94	27.29	5.77	25.57
Microbial-AA	4.82	31.76	10.10	59.07	11.49	52.80	16.83	74.41
Unspecified-AA	10.35	68.24	7.00	40.93	10.26	47.20	5.82	25.69

I - Amino acid content of dry matter of rumen digesta.
II - As a percent of total AA in the rumen.

Table 33. Calculated amount of amino acid passing to lower gut, g/day.

Items	Semipurified		Conventional	
	No DES	DES	No DES	DES
Protozoal-AA	142	377	283	619
Bacterial-AA	138	179	303	323
Microbial-AA	280	556	586	942
Unspecified-AA	600	385	523	326
Total AA	880	941	1109	1268

When amino acids reaching the lower gut are expressed as a percentage of dietary nitrogen, DES caused an increase from 67 to 71% when the semipurified diet was fed and from 83 to 95% when conventional diet was fed. Expressing amino acids reaching the lower gut as a percentage of dietary amino acids, DES caused a 6.93% increase (from 880 to 941%) when semipurified was fed and 13.86% increase (from 101 to 115%) when the conventional diet was fed.

DISCUSSION

There have been a great number of studies on the effect of DES, either orally or as subcutaneous implants, on the performance of growing and finishing ruminants. Research results indicate that DES has a stimulating effect on the rate of gain and feed efficiency of ruminants. However, the stimulating effect of DES reported by various workers was not to the same degree in every case. Some plants, alfalfa and soybean, have been shown to possess estrogenic activity (51,117,118,371,480) which may contribute to variations in performance of ruminants receiving DES (117,118,347). Little is known concerning the mode of DES action in the ruminants. However, there is evidence that stilbestrol treatment stimulates the production of growth hormones, adrenal androgen hormones and thyroid hormones (123,271,289,382,426,444).

The results of the present study (Table 23) indicate that neither the source of diet nor the DES affected the total quantity or ratio of volatile fatty acids in the ruminal fluids of cows fed the experimental diets. Adeyanju et al (8) reported that stilbestrol did not exert an appreciable influence upon ration energy available for metabolism. The concentration of branched-chain butyric and valeric acids in the rumen (Table 23) were not significantly ($P<0.05$) affected when the semipurified diet was fed. This is in contrast to results observed by Bruggemann et al (93) and Ingalls et al (237). They observed that the concentration of branched-chain fatty acids (C_4, C_5) were reduced in the rumen when urea was fed. Differing results were reported with regard to the concentration and molar proportion of volatile fatty

acids (40,77,78,92,93,136,175,440). Some workers (77,136) observed that feeding urea to the ruminant would increase the concentration of total VFA. Stewart et al (440) reported that feeding urea to the cattle increased the production of butyric acid and decreased valeric acid in the rumen. No significant changes in the molar proportion of propionic and acetic were observed by Bruggemann et al (93) while Briggs et al (78) reported a significant increase in acetic acid with reduction of propionic acid when urea was included in the diets. Some differences may be explained on the basis that concentration and molar proportion of the rumen volatile fatty acids during urea feeding are more likely to be affected by the type of carbohydrate which was used for replacing the energy present in the proteins (439).

In the present experiment, neither the type of the diet nor DES significantly ($P<0.05$) affected the ruminal pH. The low ruminal pH (5.4 - 6.0) may be beneficial to cows fed the experimental diets by decreasing nitrogen absorption. Hogan (209) indicated that transfer of ammonia through the rumen wall is much less rapid at low pH (5.4 - 6.0) and more of the ammonia was used in the synthesis of microbial protein. However, Coombe & Tribe (136) reported higher pH values in the rumen of cattle fed urea in place of protein, while Briggs et al (77) observed the opposite effect.

In the present study, rumen ammonia concentration was significantly ($P<0.01$) higher for cows fed the semipurified diet when compared with those fed the conventional diet. This difference would be expected because the rate of urea hydrolysis is almost four times faster than microbial fixation (68). Ammonia levels in the rumen were increased when DES was included in the experimental diets. Warner (482) reported

that ammonia arises from endogenous nitrogen metabolism of protozoa. Increased ammonia level in the present experiment appeared to be associated with increasing number of protozoa.

Also, some microorganisms may digest and ferment other rumen microbes and nongrowing organisms with release of ammonia (233). Ammonia concentration in the rumen increases just after feeding then falls rapidly within 90 min (471). Furthermore, the ammonia concentration in the rumen is less when the animals are frequently fed (233, Appendix III, "4"). This is in agreement with the results of ammonia concentration in the rumen of cows fed experimental diets (Expt. III) when compared with ammonia concentration from animals fed infrequently (233,471, Appendix III, "4"). The uniform presentation of small quantities of feed (14 g/2 min) prevented fluctuation in concentration of VFA, ammonia, pH and protozoa in the rumen. Presumably, the uniformity in composition of rumen content improves feed utilization. This result could explain the increasing weight gains (Appendix IV, "22,28"), better nitrogen retention (Appendix III, "4") and increasing number of protozoa (Appendix IV, "28") in the frequently fed animals.

From digestibility trials, diethylstilbestrol had no specific effect on the coefficients of digestibility (DM, crude protein, fibre, cellulose and NFE) and total digestible nutrients. Similar results were reported by other workers (8,43,236,490). Diethylstilbestrol significantly ($P<0.01$) increased the nitrogen retention regardless of the dietary source of nitrogen. Similar results were obtained from balance trials with lambs (43,123,235,250,304,305.) Research results (43,235,250) indicated that DES improved non-protein nitrogen utilization.

Diethylstilbestrol causes a reduction in the time required by the ruminant to adapt its metabolism to the use of high levels of non-protein nitrogen (249,304,305).

The present experiment demonstrates that the blood glucose level for cows fed the experimental diets with DES was significantly ($P<0.01$) higher than that for cows fed the experimental diets without DES. This result is in agreement with the findings of Davis et al (144) and Preston & Burroughs (382) but not in agreement with those obtained by Whanger et al (490). Oltjen et al (337) reported that blood glucose level was higher for conventional diets than for semipurified diets.

The cows fed experimental diets containing DES have higher blood hemoglobin and hematocrite values than do the cows fed experimental diets without DES. Lower hematocrit values have been reported in sheep (493) and heifers (149) treated with DES.

Neither DES nor the diet had a significant effect on blood levels of ammonia and urea. Similar results were obtained by other workers (175,345,490). Little et al (280), in agreement with Blackburn & Hobson (56), reported that loss of nitrogen from the rumen due to rapid release of ammonia from a readily soluble nitrogen source may be particularly important when considering the utilization of non-protein nitrogen. In studying the factors related to urea utilization in lambs, McLaren et al (301) demonstrated that utilization of non-protein nitrogen in semipurified diets was related to both its energy level and the length of time the lambs were fed the diet. Hogan & Phillipson (211) have suggested that nitrogen absorption from the digestive tract in the ruminant was more or

less continuous. One might expect the most rapid uptake of free plasma amino acids of tissues to occur shortly after feeding when volatile fatty acid production is at a maximal level. It was, therefore, expected under the present experimental conditions that nitrogen utilization would be more efficient due to a steady state provided by continuous steady feed intake (Appendix III).

There was some response to diethylstilbestrol treatment on the free amino acid patterns in the blood of cows fed experimental diets (Table 28). However, neither DES nor the source of nitrogen influenced significantly ($P<0.05$) free amino acid patterns in the blood. Similar results were obtained from studies on cows (371), steers (345) and sheep (144,490), but are in disagreement with results obtained by other workers who used steers (353).

The lack of relationship between the concentration of free amino acid in the plasma and dietary amino acid intake in the present experiment was expected because of degradation of dietary protein to non-protein nitrogen and resynthesis into microbial protein.

Several factors may influence plasma amino acid concentrations (213,336,389,416,449) and there are other factors still unknown. However, various methods for determining limiting amino acids (145,282,306) have been used for monogastric animals. Absorption of amino acids from the alimentary tract occurred at different times (389). This may be important in ruminants since it has been demonstrated that amino acid metabolism of cows (53) and sheep (150) tissues was similar to the monogastric.

Theurer et al (450) indicated that plasma amino acid concentration in ruminant jugular samples collected 12 hours postprandial may be

representative of the relative concentrations in portal samples. However, the significance of the plasma amino acid pattern reflect a combination of dietary protein amino acid and microbial protein amino acid. In addition to that, differential amino acid absorption from digestive tract, liver catabolism, tissue metabolism and amino acid pool effect plasma on amino acid levels. It also seems that amino acid synthesis from ammonia in rumen mucosa (222) may interfere with the plasma amino acid patterns in the ruminant. Though plasma-free amino acid concentrations are extremely difficult (if not impossible) to interpret (238), it would appear to be too early to discard them as criteria for evaluation of amino acid nutritional status.

Microbial protein may influence plasma amino acid patterns in the ruminant. Thus, quantitative amino acids of protozoa and bacteria are important to determine microbial protein quality. However, little information is known about amino acid content of protozoa and bacteria in the rumen of cattle as affected by different diets.

The experimental results indicate that the amino acid content of protozoa was high as compared with bacteria. These results are in agreement with those obtained by Johnson et al (241). The concentration of lysine and glutamic acid were higher in protozoa than in bacteria. A similar result has been previously reported in sheep by many investigators (48,212,318, 388,486).

In the present experiments, neither DES nor diet influence amino acid composition of rumen bacteria. These results support the previous conclusion that there was very little difference for rumen bacterial protein composition even under widely differing dietary regimes (1,48,212,

318,486). A constant amino acid composition was observed with 22 strains representing 9 species of rumen bacteria grown in pure culture and in a non-selective medium (388). Nitrogen content of bacteria may be varied but the range of amino acid composition was rather small (388, 486).

The experimental diets significantly ($P<0.01$) influenced amino acid composition of the protozoal fraction. Lysine, proline and phenylalanine contents were significantly ($P<0.01$) higher for protozoa obtained from rumen of cows fed the conventional diet than those from cows fed the semipurified diet, whereas methionine content of protozoa obtained from cows fed the semipurified diet was significantly ($P<0.01$) higher than that of the conventional diet. Variation in amino acid composition, therefore, may be expected for protozoal fraction since present results indicate that diet and DES influences the population and concentration of ciliate protozoa (Appendix IV). Previous studies (212,373) confirm this finding and the explanation for this difference due to species differences in protozoal amino acid composition. However, mixed protozoal fraction may mask these species differences (48,318,486).

In the present study, total numbers of protozoa were significantly ($P<0.01$) higher for cows fed the conventional diet than those fed the semipurified diet. In addition, the feeding of DES resulted in a significant ($P<0.01$) increase in total numbers of protozoa. In the present study, 72% to 92% of total numbers of protozoa were attributable to entodinia. In a previous study by Harmeyer & Hill (194), it was observed that 90% of the total number of protozoa was made up by entodinia while 50% to 70% of cell volume of protozoa was attributable to larger, less

numerous species. This observation suggested that for the present results the larger species represented the major quantity of protozoal cell mass in experimental diets. It was also noticed that significant variations may occur in the protozoa size due to their nutrition and physiological state (340,483). Thus it would be more valid to make the comparison on the basis of both numbers and mass of organisms. The relative mass contributed by organisms might reflect more their share in metabolic activity and protein contribution to the host than numbers. The inclusion of DES in experimental diets increased significantly ($P<0.01$) protozoal dry matter in rumen digesta. The protozoal amino acid fraction in the rumen as determined by aminoethylphosphonic acid was affected by dietary nitrogen source and DES treatment. The bacterial amino acid fraction as determined by diaminopimelic acid was affected by dietary nitrogen source but apparently unaffected by DES treatment. The microbial amino acid fraction made up between 31 to 75% of the total amino acids in the rumen digesta. These data are in agreement with the results reported by other workers (191,233,294,489). The lower net protein utilization of bacterial protein (60%) compared with protozoal protein (73%) obtained by McNaught (308) suggests that the proportion changes in bacterial and protozoal protein fractions will change the quantity and quality of protein available to the host.

The quantities of total amino acid reaching the lower gut of cows fed the semipurified diet were less than that of cows fed the conventional diet. Inclusion of DES in experimental diets caused an increase of amino acid content reaching the lower gut. The results of the present experiments support the conclusion that quantity of amino acid reaching

the lower gut was lower with the high-urea diet due to nitrogen losses through rumen absorption of ammonia (375), while the ammonia absorbed through the rumen wall could be synthesized efficiently (222,302,303,323).

These results also indicate that significant quantities of nitrogen were absorbed through the rumen wall with the semipurified diet. When quantities of amino acids reaching the lower gut were expressed as percent of dietary nitrogen, the values of 71% and 67% were obtained when the cows were fed the semipurified diet with and without DES, respectively compared with 83% and 95% when the cows were fed the conventional diet with and without DES, respectively. However, the accuracy of using lignin as a marker and the possibility of endogenous nitrogen entering the rumen could influence the values reported above (69,203,224,247,431,438,463).

The results of the present experiment support the hypothesis that rumen microbes synthesize a large variety of proteins in appreciable quantities. Digesta reaching the lower gut contain 9.41 to 8.80 times more amino acids than was found in the semipurified diet with and without DES, respectively. Similar results were observed by Loosli et al (285) who reported that rumen material contained 9 - 20 times more amino acids than was in a purified diet containing urea as the sole source of nitrogen.

SUMMARY AND CONCLUSIONS

Lactating dairy cows were used to compare the use of three roughages (corn silage, grass silage and ground hay) in complete feed with long hay (control) and their effect on milk production, composition and rumen metabolism.

In experiment I, eight cows were used in a latin square change-over design. The complete feeds as well as the control consisted of 60% roughage and 40% concentrate. The cows fed the corn silage mixture consumed less ($P<0.01$) dry matter and TDN than those fed grass silage, ground hay and long hay mixture. Total VFA from cows fed ground hay mixture was higher ($P<0.05$) than those fed corn silage and long hay mixtures but not different with that of grass silage mixture. The molar percentage of acetic, butyric, valeric, branched-chain fatty acids (isobutyric and isovaleric) and molar proportion of acetic to propionic were not affected ($P<0.05$) by different sources of roughage. The corn silage mixture resulted in lower TDN, dry matter, crude protein and crude fibre digestibility values ($P<0.05$) than the other treatments. Blood glucose and urea concentrations were not affected by dietary treatments.

The mean daily milk yield of cows fed long hay and ground hay mixtures were higher ($P<0.05$) than those of grass silage mixture. No significant differences in mean daily milk yield ($P<0.05$) were observed among the cows fed long hay, ground hay and corn silage mixtures. Also the mean daily FCM yield was not affected ($P<0.05$) by the source of roughage. The percentages of milk fat, total solids, solids-not-fat, protein, lactose and ash were not influenced ($P<0.05$) by the source of roughage.

In experiment II, twelve cows were used in switch back design. The complete feeds as well as the control consisted of 40% roughage and 60% concentrate. Mean daily feed and TDN consumption were not affected ($P<0.05$) by the source of roughage. Total rumen VFA, molar percentage of VFA and molar proportion of acetic to propionic acid were not affected by the experimental treatments. There were no significant differences ($P<0.05$) in TDN, dry matter, crude protein and NFE digestibility among the experimental treatments.

The mean daily milk yield and FCM yield were not affected significantly ($P<0.05$) by the experimental treatments. The percentages of milk fat, total solids, solids-not-fat, protein, lactose and ash were not influenced ($P<0.05$) by the experimental treatments.

In experiment III, four fistulated non-lactating dairy cows were used in a 4x4 latin square design to study the effect of semipurified and conventional diets with and without diethylstilbestrol (DES) on digestibility, nitrogen retention, blood components and rumen metabolism. The feed intake for each cow was fixed at 10 kg/day using an automatic feeder device and each cow was fed 13-15 g in 2 min intervals.

The results indicated that the use of the automatic feeder resulted in the rumen of the cows having a virtually constant fermentation rate. Uniformity in composition of rumen samples was observed and nyctohemeral and day to day variations in concentrations of VFA, ammonia, pH and protozoa were removed.

The total VFA, molar percentage of VFA and pH were not influenced ($P<0.05$) by the experimental treatments. Rumen ammonia concentration was higher ($P<0.01$) for cows fed semipurified diet when compared with those

fed the conventional diet. Rumen ammonia concentration increased ($P<0.01$) when DES was included in the experimental diets. Neither diet nor DES affected the weight and dry matter percent of rumen digesta. Crude protein percent of rumen digesta was higher ($P<0.01$) for cows fed the conventional diet when compared with those fed the semipurified diet.

Inclusion of DES in the experimental diets had no specific effect on digestibility coefficients of nutrients consumed and TDN values. Also, there were no significant differences for digestibility coefficients of nutrients (except crude protein) and TDN among the experimental diets.

The cows fed the conventional diet had higher levels of blood glucose ($P<0.01$), blood hemoglobin ($P<0.05$) and hematocrit values ($P<0.01$) than those fed the semipurified diet. Inclusion of DES to the experimental diets resulted in an increase of blood glucose level ($P<0.01$), blood hemoglobin ($P<0.05$ in semipurified diet) and hematocrit values ($P<0.01$). Neither diet nor DES had an effect on ammonia and urea levels in the blood. No significant differences ($P<0.05$) were observed for free amino acids concentration in the plasma among the experimental treatments.

Diet and DES affected ($P<0.05$) lysine, proline, methionine and phenylalanine content of rumen protozoa. Neither diet nor DES affected the amino acid content of rumen bacteria. Aminoethylphosphonic content of protozoa and diaminopimelic content of bacteria were unaffected ($P<0.05$) by experimental treatments. However, total amino acid content of dry protozoa was higher than that of dry bacteria.

Microbial content of rumen digesta as determined by the gravimetric procedure and markers (aminoethylphosphonic and diaminopimelic) gave different values but similar trends in response to DES treatments. Total

numbers of protozoa were significantly higher ($P<0.01$) for cows fed conventional diet than those fed semipurified diet. The inclusion of DES in experimental diets resulted in a significant increase ($P<0.01$) in total number of protozoa and aided the retention and establishment of different ciliate protozoa species.

The inclusion of DES in experimental diets increased the level of protozoal and microbial amino acid fractions in rumen digesta. Rumen microorganisms synthesized about 9 times more amino acids than was found in the semipurified diet.

Under the conditions of the present experiments, the following conclusions seem justified:

1. Corn silage, grass silage and ground hay can be successfully incorporated into complete feed for lactating dairy cows.
2. The mean daily FCM yield and composition was not affected by the source of roughage and feeding regime.
3. Nyctohemeral and day-to-day variations in concentration of constituents of rumen digesta, VFA, ammonia, pH and protozoa were removed by feeding at 2 min intervals using an automatic feeder.
4. The frequent feeding apparently resulted in an increased daily intake of the semipurified diet.
5. Inclusion of DES in the experimental diets improved nitrogen utilization.
6. Inclusion of DES in the experimental diets resulted in an increase of microbial protein synthesis in the rumen due largely to the protozoa.

7. Microbial amino acid data indicate that aminoethylphosphonic and diaminopimelic acid could be used as markers to measure changes in protozoal and bacterial populations in the rumen.

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APPENDICES

Appendix I

Table 1. Latin square change-over design and treatment sequence (Expt. I).

Periods	Duration (days)	Treatment sequence							
		Block I				Block II			
		C.Gem 1	B.V.Myra 2	Princess 3	Reliance 4	Snow Puff 5	T.Lenora 6	Frendale 7	Lois 8
Preliminary	14								
Period I									
Adjustment	14								
Comparison	14	1	4	2	3	1	2	4	3
Period II									
Adjustment	14								
Comparison	14	2	3	1	4	3	4	1	2
Period III									
Adjustment	14								
Comparison	14	4	1	3	2	2	1	3	4
Period IV									
Adjustment	14								
Comparison	14	3	2	4	1	4	3	2	1

Treatment 1, Corn silage mix.; Treatment 2, Grass silage mix.; Treatment 3, Ground hay mix. and Treatment 4, Long hay + conc. (control).

Appendix I

Table 2. Data on some criteria measured on cows fed complete feeds (Expt. I).

Periods / cows no.	1	2	3	4	5	6	7	8
	Dry matter intake per day (lb)							
I	10.762	19.844	16.144	20.340	12.418	20.864	15.623	16.378
II	17.419	14.223	13.248	18.777	15.358	20.033	7.414	14.302
III	14.729	10.706	17.281	18.634	19.332	11.517	15.798	14.414
IV	8.487	14.404	8.793	11.353	15.743	18.334	15.464	8.590
	TDN consumed per day (lb)							
I	6.415	13.262	9.218	12.662	5.894	11.753	8.102	10.416
II	10.035	9.160	7.073	12.079	7.599	10.970	3.220	7.889
III	13.708	5.311	10.835	11.536	11.856	6.462	9.999	9.737
IV	4.691	8.079	4.995	4.687	9.490	11.666	9.628	3.237
	Acetic to propionic ratio							
I	3.679	1.477	3.479	3.132	2.633	2.587	1.656	3.252
II	4.541	1.350	3.258	3.091	1.203	4.515	3.516	4.368
III	4.219	1.227	2.303	2.975	3.985	2.396	1.483	3.571
IV	4.312	2.928	3.697	2.968	3.449	2.261	3.088	2.619

Appendix I - Table 2 continued

Periods / cows no.	1	2	3	4	5	6	7	8
	FCM (lb/day)							
I	38.37	46.02	35.02	45.07	42.17	40.16	41.66	40.83
II	32.45	45.05	37.28	38.60	37.58	45.96	35.80	30.65
III	31.52	30.52	31.56	40.42	30.34	28.60	29.44	31.75
IV	16.08	21.41	31.66	30.43	28.25	29.58	21.80	28.76
	Milk fat %							
I	4.36	2.46	3.6	4.27	3.83	2.95	2.66	3.69
II	3.98	3.38	3.85	4.	3.48	3.80	3.28	3.5
III	3.9	3.2	3.65	4.95	3.93	2.75	3.	3.8
IV	4.63	3.5	4.28	4.33	3.85	2.65	4.03	3.85
	Solids-not-fat %							
I	8.69	8.91	9.58	9.41	9.20	9.14	8.95	9.58
II	9.18	8.33	9.54	9.53	9.10	9.05	9.15	9.54
III	9.23	8.98	9.48	8.92	8.88	9.54	8.85	9.44
IV	8.25	8.94	9.64	9.42	9.09	9.42	8.70	9.45

Appendix I - Table 2 continued

Periods / cows no.	1	2	3	4	5	6	7	8
	Lactose %							
I	4.67	4.91	5.32	4.91	5.15	5.15	5.14	5.10
II	4.75	5.27	5.36	5.09	5.09	4.86	4.92	5.31
III	4.83	4.91	5.00	4.72	4.91	5.21	4.35	5.08
IV	4.13	5.02	5.12	4.89	4.99	5.11	4.72	5.05
	Milk protein %							
I	3.29	3.26	3.48	3.67	3.34	3.25	3.11	3.71
II	3.57	3.24	3.34	3.58	3.20	3.36	3.36	3.40
III	3.54	3.27	3.65	3.34	3.19	3.46	3.57	3.54
IV	3.31	3.09	3.66	3.65	3.28	3.43	3.15	3.56
	Milk minerals %							
I	0.73	0.74	0.78	0.83	0.71	0.74	0.70	0.77
II	0.86	0.82	0.84	0.86	0.81	0.83	0.87	0.83
III	0.86	0.80	0.83	0.81	0.78	0.87	0.93	0.82
IV	0.91	0.83	0.86	0.88	0.82	0.88	0.83	0.87

Appendix I

Table 3 . Analysis of variance (Expt. I)

Source of variation	DF	Mean squares: DM consumed	TDN consumed	C_2/C_3	FCM	SNF	Fat %	Protein %	Lactose %	Minerals %
Period	3	63.70	44.51	1.80	39.05	0.07	0.08	0.04	0.04	0.002
Cows	7	13.31	3.88	1.04	29.97	0.36	0.99	0.08	0.16	0.001
Treatments	3	63.70**	44.51**	1.80	39.05	0.07	0.08	0.04	0.04	0.003
Expt. error	18	4.98	3.67	0.49	15.10	0.07	0.15	0.02	0.04	0.001
Total	31									
Coefficient of variation		14.98	21.76	23.62	11.36	2.89	10.90	3.91	3.79	3.72
Standard error		2.23	1.91	0.70	3.89	0.26	0.40	0.13	0.19	0.03
S.E. difference between means		1.12	0.96	0.35	1.94	0.13	0.20	0.07	0.09	0.02

** Significant ($P<0.01$).

Appendix I

Table 4. Switchback design and treatment sequence (Expt. II).

Periods	Duration (days)	Treatment Sequence Block I				Block II				Block III			
		Elsie 9	Pactrice 10	Fusion 11	Della 12	Venus 13	Sol 14	Queen 15	R.Visigoth 16	Kathleen 17	Joan 18	Lyons 19	A.Visigoth 20
Preliminary	14												
Period I													
Adjustment	14												
Comparison	14	1	2	3	4	1	2	3	4	1	2	3	4
Period II													
Adjustment	14												
Comparison	14	2	3	4	1	3	4	1	2	4	1	2	3
Period III													
Adjustment	14												
Comparison	14	1	2	3	4	1	2	3	4	1	2	3	4

Treatment 1, Corn silage mix.; Treatment 2, Grass silage mix.; Treatment 3, Ground hay mix. and Treatment 4, Long hay + conc. (control).

Appendix I

Table 5. Data on some criteria measured on cows fed complete feeds (Expt. II).

Periods / cows no. 9	10	11	12	13	14	15	16	17	18	19	20
TDN consumed daily (Kg).											
I	10.64	8.91	9.23	8.51	9.75	10.88	8.13	6.50	8.83	9.13	10.17	8.57
II	11.32	8.43	8.16	9.75	8.77	8.75	8.75	9.26	7.64	7.74	9.52	8.57
III	10.08	9.56	9.02	9.02	8.93	8.11	8.22	7.22	9.69	9.65	9.98	8.23
Acetic to propionic acid ratio												
I	2.81	4.20	3.70	3.23	2.63	2.68	4.21	2.34	4.03	3.17	2.55	3.18
II	2.20	3.41	3.83	4.08	3.24	3.98	2.77	1.90	2.96	2.41	2.92	3.15
III	2.91	4.85	4.53	3.95	3.97	3.07	3.36	2.80	2.37	2.01	2.95	3.97
FCM												
I	66.70	36.28	49.99	51.79	35.98	35.34	44.89	38.80	45.20	60.78	48.87	60.09
II	54.66	32.16	40.59	47.28	34.24	24.70	43.74	41.17	36.96	54.83	40.81	43.87
III	49.03	26.66	33.40	32.98	25.43	25.22	41.83	32.41	27.33	43.82	40.63	39.54

Appendix I - Table 5 continued

Periods / cows no.	9	10	11	12	13	14	15	16	17	18	19	20
	Milk fat %											
I	3.95	3.75	2.95	3.60	3.50	3.45	3.65	4.00	4.00	3.90	4.40	4.50
II	3.30	4.60	3.40	4.20	3.80	3.50	3.70	3.90	3.70	4.50	4.40	4.65
III	4.20	4.50	4.00	3.80	3.10	3.80	3.60	3.90	3.10	3.20	5.30	4.40
	Milk protein %											
I	3.18	3.51	3.22	3.19	3.22	3.53	3.21	3.34	2.94	3.36	3.44	3.53
II	3.22	2.88	3.22	2.84	3.59	3.53	3.18	2.54	3.18	3.36	3.58	3.37
III	3.22	3.47	2.98	3.44	3.59	3.62	3.34	3.47	2.99	3.07	3.29	3.27
	Milk minerals %											
I	0.80	0.85	0.80	0.75	0.82	0.71	0.79	0.72	0.80	0.80	0.90	0.81
II	0.77	0.84	0.78	0.83	0.82	0.81	0.79	0.81	0.80	0.80	0.80	0.83
III	0.81	0.82	0.80	0.80	0.82	0.83	0.80	0.78	0.82	0.83	0.77	0.83
	Milk total solids %											
I	13.09	12.92	11.52	12.31	12.81	12.80	12.39	12.85	12.49	13.27	13.80	13.72
II	13.70	13.64	12.28	13.87	13.49	13.09	12.98	13.41	12.90	15.18	16.79	14.42
III	12.94	13.86	12.03	14.22	14.21	13.93	13.95	14.92	11.53	12.00	14.01	13.72

Appendix I

Table 6 . Analysis of variance (Expt. II).

Source of variation	DF	Mean squares: TDN consumed	C_2/C_3	FCM	Fat %	Protein %	Minerals %	Total solids
Blocks	2	1.08	5.07	5.02	0.02	0.11	0.00089	2.71
Treatments	3	2.72	2.89	4.62	0.35	0.11	0.00059	0.21
Expt. error	6	1.29	1.45	14.75	0.08	0.062	0.00067	0.34
Total	11							
Standard error		.22	.74	2.35	0.18	0.15	0.016	0.36
Grand mean		9.02	7.09	41.33	3.89	3.28	0.80	13.36

Appendix II

Table 1 . Latin square design and treatment sequence (Expt. III).

Periods	Duration (days)	Treatment sequence			
		O.Gertude 21	S.Pina 22	L.Jamie 23	L.Frily 24
Period I					
Adjustment	14				
Comparison	14	4	2	1	3
Period II					
Adjustment	14				
Comparison	14	3	1	2	4
Period III					
Adjustment	14				
Comparison	14	1	3	4	2
Period IV					
Adjustment	14				
Comparison	14	2	4	3	1

Treatment 1, Semipurified diet; Treatment 2, Semipurified diet + DES; Treatment 3, Conventional diet and Treatment 4, Conventional diet + DES.

Appendix II

Table 2. Data on some criteria measured on cows fed semipurified and conventional diets (Expt. III).

Periods/cows no.	21	22	23	24
	TDN of experimental diet			
I	64.07	66.26	65.13	65.09
II	64.19	62.64	57.72	66.60
III	66.22	58.86	64.15	62.78
IV	67.72	69.83	70.91	65.89
	N-Retention % intake			
I	43.17	48.89	28.12	27.15
II	12.33	11.01	44.81	42.74
III	19.55	29.58	48.56	58.13
IV	48.31	57.52	49.46	31.72
	Rumen content total (Kg)			
I	35.90	37.40	36.80	36.50
II	22.35	29.15	29.60	34.80
III	40.00	37.40	35.00	41.00
IV	33.00	23.00	24.00	34.00
	Rumen DM %			
I	10.60	13.57	9.91	10.74
II	10.41	13.29	12.32	8.19
III	7.94	11.33	9.12	11.27
IV	12.30	12.89	8.85	10.04

Appendix II - Table 2 continued

Periods/cows no.	21	22	23	24
	VFA concentration (u Mole/100 ml)			
I	21.828	20.348	18.037	27.031
II	15.749	27.136	18.954	22.724
III	15.145	21.235	21.209	19.498
IV	16.784	19.911	14.603	14.335
	Protozoa number/ml $X10^4$			
I	175.00	8.00	4.80	23.60
II	47.00	2.40	3.30	160.00
III	.10	35.00	105.20	2.20
IV	1.95	140.00	88.00	0.52
	Total amino acid of protozoa (% DM)			
I	47.01	35.89	33.61	52.83
II	40.41	33.69	37.24	37.99
III	32.60	39.91	36.31	38.58
IV	38.95	34.37	28.50	34.55
	Aminothylphosphonic acid protozoal % AA			
I	0.20	0.18	0.47	0.15
II	0.28	0.47	0.52	0.24
III	0.83	0.69	0.73	0.58
IV	0.88	0.53	0.84	0.69

Appendix II - Table 2 continued

Periods/cows no.	21	22	23	24
	Total amino acid of bacterial (%DM)			
I	27.22	35.92	29.94	37.21
II	26.13	23.28	23.19	15.27
III	19.13	31.32	22.70	22.89
IV	19.71	19.38	25.33	30.58
	Diaminopimelic acid % bacterial AA			
I	0.81	1.22	1.26	1.49
II	1.29	1.63	1.43	0.81
III	1.49	0.78	1.30	1.89
IV	0.98	0.88	1.14	1.93

Appendix II

Table 3. Analysis of variance (Expt. III).

Source of variation	DF	Mean squares									
		TDN	NR	RC	RDM	VFA	PN	AAP	AEP	AAB	DAP
Rows	3	29.00	245.00	104.45	1.41	23.39	376.98	47.13	0.23	90.35	0.02
Columns	3	1.23	104.34 **	22.77	6.97	20.15	81.52	43.07	0.04	14.55	0.14
Treatments	3	4.38	735.00	24.35	4.38	6.22	17965.20 **	34.06	0.03	52.34	0.29
Expt. error	6	11.94	24.49	10.15	1.37	13.58	626.21	21.22	0.01	16.30	0.10
Total	15										
C.V.		5.33	13.17	9.62	10.83	18.75	50.26	12.23	15.43	15.79	25.64
S.E.		3.46	4.95	3.19	1.17	3.68	25.03	4.61	0.08	4.03	0.32
S.E.M.		2.44	3.50	2.25	0.83	2.61	17.70	3.26	0.06	2.86	0.23

DF, Degrees of freedom; TDN, Total digestible nutrients; NR, N- retention % of intake; VFA, Total volatile fatty acid conc. in rumen fluid (u mole/100 ml); PN, Protozoa numbers per ml X 10^4; AAP, Total amino acid % of protozoal dry matter; AEP, Aminoethylphosphonic % of AA; AAB, Total amino acid % of bacterial DM; DAP, Diaminopimelic % of AA; **, Significant ($P<0.01$); C.V., Coefficient of variation; S.E., Standard error and S.E.M., S.E. of difference between two means.

Appendix II

Table 4. Sodium citrate buffers.

	pH				
	2.2	3.28	4.25	5.28	6.30
Sodium concentration (N)	0.20	0.20	0.20	.38	0.40
Sodium citrate .$2H_2O$ (g)	78.43	78.43	78.43	137.26	156.87
Concentrated HCl (ml)	66.00	49.3	33.50	26.0	26.0
Thiodiglycol (ml)	20.00	20.00	20.00	20.00	20.00
Brij-35 solution (ml)	8.00	8.00	8.00	8.00	8.00
Caprylic acid (ml)	.40	.40	.40	.40	.40
Final volume (l)	4.00	4.00	4.00	4.00	4.00

Appendix II

Table 5. Typical amino acid analysis schedule for a Beckman Model 116 amino acid analyzer.

	Basics amino acids	Acidic and neutral amino acid	Blood plasma amino acids*
Column size	0.9X23cm	0.9X69cm	.9X69cm
Resin type (Beckman)	PA-35	UR-30	UR-30
Column flow rate	68ml/hr	68ml/hr	68ml/hr
Column back pressure	40 psi	130 psi	130 psi
First buffer	5.25	3.25	3.25
Second buffer	n/a	4.30	4.30
Third buffer	n/a	n/a	6.30
change buffer to 4.30	n/a	85 min	85 min
change buffer to 6.30	n/a	n/a	138 min
Temperature	55.5^{o} C	55.5^{o} C	55.5^{o} C

* Except arginine, can be eluded by means of basic (short) column.

Appendix III

EFFECT OF CONTINUOUS FEEDING ON THE COMPOSITION OF RUMEN DIGESTA

Nyctohemeral and day to day variations in volumes and concentrations of constituents of rumen digesta pose major difficulties in the assessment of overall average rates of breakdown and synthesis of nutrients in the rumen. Experiments were planned to study protein metabolism in the rumen of cattle on diets which, in some cases, contained urea as virtually the sole source of nitrogen.

In the present experiments automatic feeders were used to try to eliminate variations in rumen contents and also to try to maximize intakes of the rather unpalatable diets.

The feed intake for each of the four fistulated dairy cows was fixed at 10 kg/head/day. The feeders were so set that about 14 g of pelleted feed dropped from the conveyors into the respective mangers at 2-min intervals. The room was lighted continuously and the cows quickly adapted to the regime so that they licked up the feed immediately it was presented, whether they were standing or lying down. Two cows were given a semi-purified diet based on wheat straw, starch and glucose with 4.6% urea as the major nitrogen source. The other cows were given a conventional type of diet containing the same levels of nitrogen and energy but consisting primarily of grass-legume hay, barley and soybean meal. Diethylstilbestrol was included in one of each of the main types of diet (3).

On the 14th day after the cows were placed on the respective diets, 10 samples of rumen contents were taken from the ventral sac of each cow, generally at 2-hour intervals (Fig. 1). The cows continued on the same diets and 14 days later a further, similar series of eight samples was taken at 2-hour intervals. About 8 hours after the last of these samplings, the rumen contents were removed through the fistula and mixed thoroughly; samples were taken and the digesta returned. Immediately following this the cows were changed to different diets and six further samples of rumen contents were taken at 2-hour intervals. The samples were analyzed for ammonia, total volatile fatty acids, pH and protozoal numbers.

The uniformity in composition of rumen contents samples from one of the cows is illustrated in Fig. 1, which also serves to show the sampling schedules. The final sample, which was of mixed rumen contents, was similar to the ventral sac samples taken previously. There were differences, due to diet, in the absolute values for the various parameters (3). The variabilities among replicates were examined by calculating coefficients of variation (standard deviation × 100)/mean, which proved to be gratifyingly small (Table 1).

These results indicate that the use of the automatic feeders resulted in the rumens of these cows becoming virtually constant rate fermentation systems. In these circumstances, inferences on the total metabolic activity of the rumen contents could be made using a quite simple schedule for sampling and analysis of rumen contents. Fig. 2 illustrates the remarkable rapidity with which rumen ammonia concentration became stabilized at new levels following changes in diet.

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Appendix III

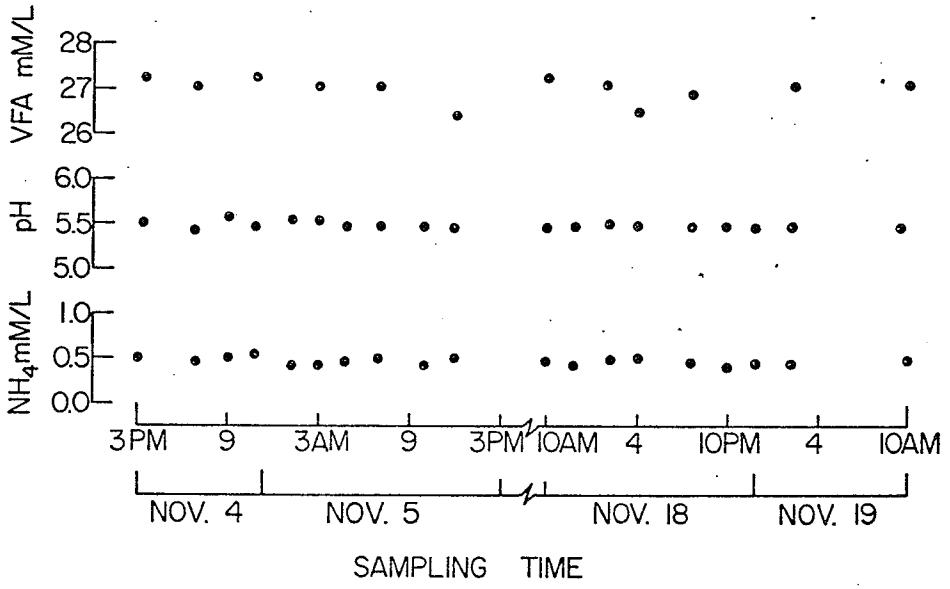

FIG. 1. Nycthemeral and day to day variations in concentration of rumen VFA, pH and ammonia for a cow fed at 2-min intervals.

Table 1. Coefficients of variation (%) of rumen content samples

	No. of samples	Diet*			
		SP	SPS	H	HS
Rumen pH	19	2.25	2.27	1.29	3.09
Rumen VFA concentration	12	0.25	6.94	1.14	3.40
Rumen ammonia concentration	19	0.17	0.62	0.80	1.81
Total protozoa numbers	10	3.71	2.00	5.29	2.86

*SP: semipurified diet; SPS: with stilbestrol; H: hay diet; HS with stilbestrol

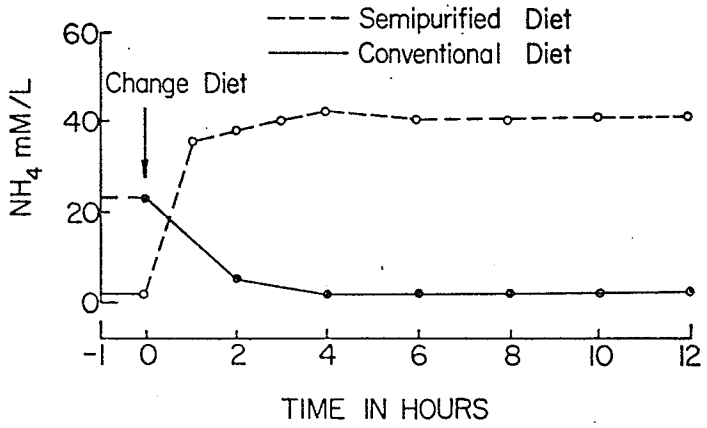

FIG. 2. Rumen ammonia concentrations following changes in diets for cows fed at 2-min intervals.

Appendix III

Increased frequency of feeding usually results in increased feed intake of conventional diets (4), but this effect was dramatic in the present experiments. Prior to use of the automatic feeder, the highest daily intake of the semi-purified diets, offered *ad libitum* to the cows, was about 3 kg per day. Intakes of 10 kg per day of these diets were attained in 2 days, and maintained for 28 days using the feeder.

Presumably, the uniform presentation of small quantities of feed prevented fluctuation in concentrations of whatever metabolites are concerned in regulating voluntary food consumption (1). This may be because the rumen microorganisms, which according to Hungate (2) are normally in a substrate-limiting environment, are maintained in the log phase of growth. If this is the case, then it is reasonable to suggest that high average turnover rates can be maintained without the metabolites reaching levels which trigger satiation mechanisms.

The application of this feeding technique, together with analysis for metabolite concentrations both within the rumen and in systemic body fluids, should be useful for investigating factors controlling hunger and satiation mechanisms in ruminants, as well as the dynamics of metabolism.

Acknowledgement is due to Carl Heinrichs, equipment foreman, for construction of the automatic feeder.

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Appendix IV

EFFECT OF DIETARY DIETHYLSTILBESTROL ON POPULATIONS AND CONCENTRATIONS OF CILIATE PROTOZOA IN DAIRY CATTLE

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ABSTRACT

Two experimental diets with or without diethylstilbestrol (DES) were fed to four fistulated dairy cows in a 4 × 4 Latin square design. A continuous feeding system was used to give each cow 13–15 g of diet every two minutes. The continuous feeding appeared to remove the usual variation in the numbers of protozoa in the rumen content. This facilitated the sampling for studies of the effect of the diet on the concentrations and varieties of protozoa. Numbers of protozoa among days were similar with no significant differences. Total protozoa numbers were significantly higher ($P < 0.01$) for cows fed natural diets than for cows fed semi-purified diets. In addition, the feeding of DES resulted in a significant increase in total protozoa numbers. The inclusion of DES in the experimental diets aided the retention and establishment of different ciliate protozoa.

INTRODUCTION

Over a century ago, Stein (32) suggested that ciliate protozoa played an important role in ruminant metabolism. More recently, their importance has been indicated by several workers (1, 6, 15). Several investigators (8, 27, 34, 35) have studied the effect of dietary manipulation on ciliate protozoa concentration.

Some reports indicated that diethylstilbestrol (DES) increased the rate of gain, feed efficiency and nitrogen retention in ruminants (7, 13). The mechanism by which these beneficial effects come about has not been established, nor has the effect of DES administration on ciliate protozoa been thoroughly investigated (5).

The purpose of the present investigation was to determine the effect of feeding DES in natural and semi-purified diets on ciliate protozoa in dairy cows.

MATERIALS AND METHODS

Two experimental diets (natural and semi-purified), with or without diethylstilbestrol (8 mg/day), were fed to four fistulated dairy cows in a 4 × 4 Latin square design (Table 1). Feed intake was fixed at 10 kg/day for each cow to limit the effect of level of dry matter intake on concentration of protozoa (6, 35). A continuous feeding apparatus (12) was used to feed each cow 13–15 g of diet at 2-min intervals to minimize diurnal changes in concentration of protozoa in response to feeding (22, 25, 35). Each experimental period was 4 weeks. The first 2 weeks of each period were allowed for adjustment. About 500 ml of rumen contents were obtained from each cow and mixed together for cross-inoculations on the 1st and 3rd day of each period. In the first two sampling periods the rumen samples were taken from the ventral sac at 4-hr intervals for 24 hr to determine any change in numbers of protozoa. The rumens were emptied at the end of each period.

Counting and identification of ciliate protozoa were conducted during the last 2 weeks of each period. Rumen samples were obtained at 3-day intervals from the ventral sac. Protozoa were identified on fresh rumen samples; a duplicate sample was fixed in 10% (v/v) formalin within 5 min after collection and kept at 4–6 C for further study. Microtechnical methods summarized by Lubinsky (21) were used in the study of these protozoa.

Counting of ciliate protozoa was based on the method of Naga and el-Shazly (23). The rumen fluid was strained through two layers of cheesecloth. Five ml

Appendix IV

Table 1. Composition and protein content of experimental diets

Ingredient	Percent composition			
	Semi-purified		Natural	
	No DES	DES	No DES	DES
Wheat straw	38.0	38.0		
Glucose	15.7	15.7		
Corn starch	27.0	27.0		
Soybean oil	2.0	2.0		
Alphacel*	5.0	5.0		
Urea	4.6	4.6		
Mineral mixture†	4.5	4.5		
Choline chloride 50%	0.2	0.2		
Molasses	3.0	3.0	3.0	3.0
Diethylstilbestrol	−	+	−	+
Vitamins A+D+E‡	+	+	−	−
Alfalfa hay			50.0	50.0
Barley			42.0	42.0
Soybean			3.0	3.0
Trace minerals			2.0	2.0
Crude protein§	13.2	13.2	13.4	13.4

*Solka floc BNB-20, Brown Co., New Hampshire.
†Mixture of the following salts as percentages: $CaHPO_4$, 48.94; K_2CO_3, 31.55; $MgSO_4$, 10.75; NaCl, 7.43; $FeSO_4$, 0.91; $Na_2B_4O_7$, 0.19; $ZnSO_4$, 0.07; $MnSO_4$, 0.10; $CuSO_4$, 0.02; KI, 0.3; MoO_3, 0.0008; and $CoCl_2$, 0.0003.
‡Supplied per ton (metric) of diet: Vit. A, 22 g (250,000 IU/g); Vit. D, 50 g (100,000 IU/g); and Vit. E, 2 g (100,000 IU/g).
§Determined by chemical analysis.

of strained rumen fluid in triplicate were diluted to 25 ml with 10% (v/v) formal saline containing a few drops of acid methylgreen to stain macronuclei of protozoa. After vigorous agitation to prevent sedimentation, five 0.1-ml samples were pipetted on to different microscope slides and spread carefully under a cover glass (22 × 40 mm). Differential counting of protozoa was made on 40 fields per slide for each of the three replicate dilutions. Protozoa numbers per 0.1 ml were calculated as follows: Total number of fields per slide × average count per field × dilution factor. The coefficient of variation among the five slides from each of the three replicate dilutions was less than 5%. The identification of genera and species of ciliate protozoa was based mainly on the papers of Kofoid and MacLennan (16, 17, 18), Kofoid and Christenson (19) and Lubinsky (20).

RESULTS AND DISCUSSION

With continuous feeding, there was no significant ($P < 0.05$) nyctohemeral variation in concentration of ciliate protozoa. This is in agreement with findings of other workers (22, 28) who reported that more frequent feeding reduced the diurnal fluctuation in concentration of protozoa. These workers also found increased numbers of protozoa with more frequent feeding. The numbers of protozoa found in the present experiment (Table 2) are higher than those indicated for cattle under normal feeding systems (10, 24, 31, 34). The results indicated that there were no significant differences in numbers of protozoa between samples from the ventral sac and samples representing total rumen contents. Protozoa numbers among days were not significantly different ($P < 0.05$). The coefficient of variation was 5.30%, which is lower than values reported by other workers (3, 23, 27).

Appendix IV

Table 2. Total numbers and percentage generic composition of ciliate protozoa in cows on the experimental diets

Items	Semi-purified		Natural	
	No DES	DES	No DES	DES
Total numbers of protozoa × 10^4/ml	2.0 *C*	3.9 *C*	48.4 *B*	145.1 *A*
Isotricha spp. %	1	3	2	3
Dasytricha spp. %	3	5	4	4
Entodinium spp. %	90 *a*	78 *b*	87 *a*	72 *c*
Ostracodinium spp. %	4	8	2	5
Polyplastron spp. %	1	2	4	7
Ophryoscolex spp. %	1	4	1	9

A,B,C Treatment means within a row not showing the same letter are significantly ($P<0.01$) different.
a,b,c Treatment means within a row not showing the same letter are significantly ($P<0.05$) different.

Total protozoa numbers were significantly higher ($P < 0.01$) for cows fed the natural diet than for cows fed the semi-purified diet (Table 2). Other investigators observed that rumen protozoa disappeared (8, 34) or decreased in numbers (24, 31) when sheep, steers or cows were fed semi-purified or purified diets. From earlier studies by Purser and Moir (26) and Quinn (29), it appears that protozoa are sensitive to low pH. However, in the present study there was no significant difference in rumen pH between the treatments (11). Some workers (27, 36) indicated an increase in concentration of protozoa when urea was added to the ration in place of gluten and linseed. Bacteria isolated from steers fed purified diets containing urea were negative for H_2S production (31) which has a toxic effect on ruminal ciliate protozoa (30). These results would suggest that the present difference in numbers of protozoa between the semi-purified diet and the natural diet was not due to the presence of urea. The decrease in the numbers of protozoa associated with the semi-purified diet might have resulted from rupture of cells caused by excessive ingestion of starch and glucose (9, 30). Other nutritional and physiological factors could have an effect on numbers of protozoa (30).

Adding DES to the natural diet caused a significant ($P < 0.01$) increase in total protozoa numbers. Also, total protozoa numbers were increased by the addition of DES to the semi-purified diet (Table 2). Increasing protozoa numbers in response to DES were previously observed *in vitro* and *in vivo* by Christiansen (4, 5). Estrogens are known to increase the biosynthesis of protein, lipids, glycogen, ribonucleic and nucleic acids (14, 33). The apparent effect of DES on numbers of protozoa might be explained on the basis of its estrogenic activity.

Two genera of Isotrichidae and four genera of Ophryoscolecidae (Table 2) were observed in rumen samples. The relative abundance of different ciliate protozoa was affected by type of diet (Table 2). Also, it was noted that *Entodinium* was the predominant genus in all treatments, a phenomenon which has been observed by various workers (2, 23, 24, 26, 32).

The inclusion of DES in the experimental diets caused a significant ($P < 0.05$) decrease in relative abundance of *Entodinium*. Furthermore, the inclusion of DES caused a marked increase in *Isotricha, Stracodinium, Polyplastron* and *Ophryoscolex*. Diethylstilbestrol treatment appeared to increase the relative abundance of *Dasytricha* in semi-purified diet treatments, with no effect on the same genus in the natural diet treatments.

Appendix IV

By using cross-inoculation, ciliate protozoa species present in the inoculum of all four cows at the beginning of each period had an equal chance to become established. Therefore, the presence or disappearance of some species of ciliate protozoa should be due to dietary effects. There were differences in the establishment of different species of ciliate protozoa in the rumen of cows fed different diets (Table 3). *Entodinium nanellum* and *E. simplex* were the predominant species in rumen samples of the four experimental diets.

Table 3. Occurrence of ciliate protozoa in the rumen of cows on the experimental diets*

Species	Semi-purified		Natural	
	No DES	DES	No DES	DES
Istricha intestinalis	+	+	+	+
I. prostoma	−	+	−	+
Dasytricha ruminantium	+	+	+	+
Entodinium bursa	−	+	+	+
E. caudatum	−	+	+	+
E. nanellum	+++	+++	+++	+++
E. simples	+++	+++	+++	+++
E. longinucleatum	+	+	+	+
E. biconcavum	−	+	−	+
E. minimum	−	−	−	+
Ostracodinium gracile	+	+	+	+
O. obtusum	−	+	−	+
O. mammosum	+	+	−	+
Polyplastron multivesiculatum	+	+	+	+
Ophryoscolex purkynjei	+	+	+	+
O. caudatus	−	+	−	+

*Plus (+) indicates present, minus (−) indicates not present.

Isotricha intestinalis, Dasytricha ruminantium, Entodinium longinucleatum, Ostracodinium gracile, Polyplastron multivesiculatum and *Ophryoscolex purkynjei* were observed with all treatments. Inclusion of diethylstilbestrol in the experimental diets prevented the disappearance of *Isotricha prostoma, Entodinium biconcavum, Ostracodinium obtusum,* and *Ophryoscolex caudatus* with both diets. Within the semi-purified diet treatments, *Entodinium bursa* and *E. caudatum* were observed only in cows receiving DES. Within the natural diet treatments, the inclusion of DES stimulated the establishment of *Entodinium minimum* and *Ostracodinium mammosum.* However, *E. minimum* was not established in cows receiving the semi-purified diet with or without DES.

Inclusion of DES, in general, aided the retention and establishment of ciliate protozoa and apparently prevented the disappearance of some protozoa species in the rumen of cattle receiving the experimental diets.

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Appendix IV

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Appendix V

SEPARATION AND IDENTIFICATION OF AMINO ACIDS PRESENT IN RUMEN MICROORGANISMS

Two amino acids have been found in rumen bacteria and protozoa that are absent from protein in feedstuffs normally consumed by the ruminant. The amino acid, 2-aminoethylphosphonic acid (AEP) is found in rumen ciliate protozoa (1,7) and in ciliate Tetrahymena pyriformis(9) but not in rumen bacteria or plant tissue. Rumen bacteria contain 2, 6-diaminopimelic acid (DAP) which is not found in ciliate protozoa or plant tissue (15).

Several workers (1,13,14,15) have suggested the use of AEP and DAP as markers to measure changes in the protozoal and bacterial population in the rumen. Methionine and DAP have similar elution patterns (5,16) using fraction collector. The present paper describes a method to resolve the problem of separation of DAP and methionine by ion exchange chromatography and identification of other ninhydrin reactive compounds which were observed in protozoa and rumen content hydrolysates.

Rumen contents, protozoa and bacteria were obtained from four fistulated dairy cows fed semi-purified and natural diets with and without diethylstilbestrol (8). Samples of rumen fluid were strained through two layers of cheese cloth into a thermos flask and fractionation started within 5-10 min after collection. Protozoa were obtained by gravimetric technique

based on methods used by Oxford (12) and Abou Akkada and Howard (2). Strained rumen fluid (1 liter) was placed in a 3 liter separatory funnel and diluted one to one (v/v) with an acetate-phosphate buffer ($NaC_2H_3O_2$, 2.15g; KH_2PO_4, 0.35g; K_2HPO_4, 1.00g; NaCl, 5g and $MgSO_4$, 0.12g made up to 1 liter with distilled water).

Carbondioxide was bubbled into the diluted ruminal fluid for one min and the mixture was then incubated at 39-40°C for one hr. The protozoa when settled to the bottom were transfered to a 500 ml separatory funnel containing acetate-phosphate buffer and incubated for an additional hour. The protozoa were then transfered to 200 ml centrifuge bottles containing 100-150 ml acetate-phosphate buffer and immediately centrifuged at 200 x G for 10 min. Precipitated protozoa were resuspended in a small volume of the acetate-phosphate buffer and spread on glass plates to dry at 39°C in a forced air drying oven. Before drying, each protozoal preparation was examined microscopically for feed contamination.

The bacterial fraction was obtained by differential centrifugation (11). The strained ruminal fluid was poured into 200 ml centrifuge bottles and centrifuged at 1000 x G for 10 min to remove protozoa and food debris. The supernatant was then recentrifuged at 40,000 x G for 20 min in 50 ml centrifuge tubes. The precipitant of bacterial residue was twice resuspended in 40 ml of acetate-phosphate buffer and centrifuged at 40,000x G for 20 min. The bacterial precipitate was resuspended in small

volumes of acetate-phosphate buffer and spread on glass plates to dry at 39°C in a forced air oven. The dried protozoal and bacterial residues were ground into a powder and stored in the freezer for subsequent analysis.

Dried samples (50 mg) of the ground diets, rumen contents, protozoa and bacteria were hydrolyzed under reduced pressure in 10 ml of 3N HCl in a sealed flask at 121°C for 15 hours (4). The hydrolysate was evaporated to dryness at 40°C under reduced pressure and washed with pH 2.2 (0.20N) sodium citrate buffer. The buffer residue solution was filtered and adjusted to a final volume of 50 ml. Amino acid analysis were determined on 0.5 ml of hydrolysate using the acid - neutral column on the Beckman model 116 amino acid analyzer (3). Amino acids were eluted from the ion exchange column with sodium citrate buffer, pH 3.28 (0.20N), 4.25 (0.20N) and 6.25 (0.40N) at zero, 85, and 138 minutes, respectively. The sodium citrate buffer pH 6.25 was applied in order to elute AEP and other amino acids which are more basic than phenylalanine. In order to locate peak-time of DAP and AEP, 0.5 ml of a standard solution containing 0.2 μ mole of each (Grade A, Calbiochem, Los Angeles, Calf.) was placed on the column with the amino acid calibration mixture (type 1, Beckman, Polo Alto, Calf.).

Chromatogram peak-time of DAP and methionine was similar at 134 minutes using the above conditions (Table 1). The

interference of DAP elution pattern with that of other amino acids was previously observed by other workers (5,16). Diaminopimelic acid has been measured (13,14,16) by reducing the pH until only the DAP reacted with ninhydrin. The modern amino acid analyzer system (Beckman 116) is not adaptable to this procedure. Therefore, other means must be employed to distinguish DAP from methionine. Through convertion of methionine to methionine sulfone by performic acid (6) it was possible to distinguish between DAP and methionine.

Fresh performic acid was prepared by mixing 1.0 ml 30% H_2O_2 and 9.0 ml 88% formic acid and left at room temperature for one hour, cooled in an ice bath and used immediately. Performic acid (2 ml) was added to the protein sample which was placed in a cooled ignition tube (2 x 20 cm) in an ice bath. The oxidation reaction was allowed to proceed for 20 hours in the ice bath then excess performic acid was reduced by the addition of 0.30 ml 48% H Br. Bromine released was removed by evaporation under reduced pressure at 40°C to dryness using a rotary evaporator. The residue was subjected to hydrolysis, as described above.

Methionine sulfone is eluted with a peak-time of 50 minutes thus not interfering with other amino acid elution times. At the same time, cystine is oxidized to cysteic acid with a peak-time of 19 minutes as compared with 106 minutes for cystine. These amino acids are readily measured quantitatively by analysis

of the unknown vs an oxidized standard amino acid mixture containing cystine and methionine.

Several unknown peaks were observed on chromatograms obtained with the hydrolysates of protozoa and rumen contents. Ninhydrin reactive substances were eluted with peak-times of 17, 27, 179, 182, 188 and 192 minutes (Table 1). In order to identify some of the unknown substances 0.1 μ mole each of 2-amino 3-phosphonopropionic, 2-amino 4-phosphonobutyric, DL-O-phosphoserine and 4-aminobutyric acid were placed on the column. The first unknown amino acid has an identical elution peak-time with 2-amino 3-phosphonopropionic acid at 17 minutes (Table 1). This amino acid had been detected in the ciliate Tetrahymena pyriformis (10). The second unknown amino acid is in identical location with DL-O-phosphoserine at 27 minutes. The unknown peak occurring at 182 minutes has an elution peak-time similar to that of 4-aminobutyric acid (Table 1).

The 2-amino 4-phosphonobutyric acid is eluted with a peak-time of 22 minutes and was not present in either protozoa or rumen contents. The amino acids with elution peak-time of 179, 188 and 192 minutes have not been identified.

Application of the above technique should be useful for detecting DAP as well as distinguishing between DAP and methionine in rumen bacteria and rumen contents when using amino acid analyzers. The method provides a quantitative analysis

for methionine and cystine (6).

The occurrence of 2-amino 3-phosphonopropionic acid DL-Ophosphoserine and 4-aminobutyric acid in the hydrolysate of ciliate protozoa and rumen contents suggests their possible use to measure protozoal populations. Furthermore, the determination of 2-amino 3-phosphonopropionic acid and DL-O-phosphoserine would take only 27 minutes compared to 185 for AEP, however, the amounts present are small. Further studies with protozoa are required to test the validity of using these amino acids as markers of protozoal population.

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Table 1. Amino acids present in diets, protozoa, bacteria and rumen contents.

Amino acid	Chromatograms Peak-time (min)	Diets	Protozoa	Bacteria	Rumen contents
2-amino-3-phosphonopropionic	17	-	+	-	+
Cysteic acid	19				
DL-O-phosphoserine	27	-	+	-	+
Aspartic acid	46	+	+	+	+
Methionine sulfone	50				
Threonine	54	+	+	+	+
Serine	57	+	+	+	+
Glutamic acid	65	+	+	+	+
Proline	72	+	+	+	+
Glycine	89	+	+	+	+
Alanine	94	+	+	+	+
Cystine	106	+	+	+	+
Valine	119	+	+	+	+
2,6-diaminopimelic	134	-	-	+	+
Methionine	134	+	+	+	+
Isoleucine	138	+	+	+	+
Leucine	141	+	+	+	+
Tyrosine	164	+	+	+	+
Phenylalanine	169	+	+	+	+
Unknown	179	-	+	-	+
4-aminobutyric	182	-	+	-	+
2-aminoethylphosphonic	185	-	+	-	+
Unknown	188	-	+	-	+
Unknown	192				

(+) indicates present, (-) indicates not present.

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