CORN STEEP LIQUOR AND FERMENTED AMMONIATED CONDENSED WHEY AS PROTEIN SOURCES FOR LACTATING COWS AND YEARLING HEIFERS GRAZING WINTER NATIVE RANGE

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CHAPTER I

INTRODUCTION

Cattle are commonly maintained on dormant native range or other low-quality roughage throughout the winter. Provided forage is plentiful, protein is the nutrient most likely to be deficient in winter feeding programs utilizing low-quality roughages. Protein requirements for both the rumen and the animal must be met in order to maintain adequate roughage intake, growth rate, reproduction and milk production.

Dry concentrates or liquid supplements are usually the preferred methods for correcting protein deficiencies in cattle diets. The choice of a dry versus liquid supplement must be made on the basis of relative cost and animal performance. Liquid supplements are usually self fed and may reduce the labor involved in feeding. Many modern liquid supplements contain agro-industrial by-products of the sugar, paper and fermentation industries and are available at a low cost per ton. Consequently, feeding liquid protein supplements may potentially reduce production costs for many cattlemen.

Several researchers (Beeson et al., 1969; Gay and Vetter, 1967; Kercher and Paules, 1967; Perry et al., 1967; Rush and Totusek, 1976) have demonstrated that the physical form of a protein supplement does not significantly affect animal performance. A correctly formulated liquid supplement will elicit animal performance comparable to that obtained by a properly formulated dry supplement (Wornick, 1969). The

success of either supplement depends on the ability of the specific ingredients to meet the protein needs of the animal being fed.

Considerable data is available concerning the use of processed oil meal protein supplements and urea-molasses based liquid supplements by cattle on winter native range. However, limited information exists dealing with the utilization of agro-industrial by-products as protein sources. The purpose of this study was to evaluate two liquid by-product feed ingredients, corn steep liquor and fermented ammoniated condensed whey, as sources of crude protein for cattle grazing winter native range.

CHAPTER II

REVIEW OF LITERATURE

Liquid supplements have been used for many years to feed cattle. In the early 19th century, molasses was used in cattle feeds as a source of energy and to improve palatability (Coppock, 1969). In 1896, a British patent was issued for a product formed by the combination of ten parts by weight of phosphoric acid to 100 parts by weight of beet molasses (Wornick, 1969). Scotish workers, in 1943, dissolved urea in molasses and an equal volume of water in order to facilitate mixing urea into a complete ration for dairy cows (Owen, 1943). Since that time, various types of molasses and other liquid carriers have been used as vehicles for non-protein nitrogen and natural protein compounds.

Most of the early liquid protein supplements were mixtures of urea and molasses. These supplements have been fed to ruminant animals consuming low-quality roughages for many years with varying degrees of success. Several authors have demonstrated an increase in roughage intake and, as a result, some improvement in performance when a supplement of urea and molasses is fed.

Beames (1959) fed a molasses and urea mixture to two and three-year-old Hereford heifers consuming pasture hay. Molasses plus urea supplementation increased hay intakes by 38.8% ($P<.05$), while molasses alone had little effect on intake. Heifers supplemented with molasses plus urea maintained body weight for 16 weeks while heifers receiving no

supplement or molasses alone lost an average of 61.3 kg and 43.1 kg, respectively.

Williams et al. (1959) showed that 2.5 year old Corriedale wethers fed a ration of poor-quality oat straw supplemented with urea and molasses lost less body weight and ate more straw than wethers fed only oat straw. Dry matter intakes and live weights of sheep fed grass and clover hay, containing adequate protein, were not improved by supplementation with urea and molasses.

Clark and Quin (1951), Coombe and Tribe (1963) and Smith (1962) showed that supplementing low-quality roughage diets with urea and molasses resulted in an increased rate of cellulose digestion, an increased rate of food passage through the gut and an increase in dry matter intake. Coombe and Tribe (1963) further demonstrated that when dietary levels of urea were increased, the added nitrogen was practically all excreted in the urine and no additional increases in digestion and intake occurred. They concluded that a small supplement of urea improves nitrogen status and increases dry matter intake supplying more digestible energy to the animal resulting in reduced weight loss. However, Coombe and Tribe also noted that low-quality roughage plus urea and molasses did not result in the retention of high amounts of nitrogen.

With limited numbers of animals per treatment, inadequate experimental controls and sometimes questionable experimental procedures, much of the data demonstrating the positive effects of urea-based liquid supplements for ruminants consuming low-quality roughages is of limited value. Subsequent trials conducted in the United States have employed dry, natural protein concentrates as experimental controls. By using such techniques, the value of urea plus molasses liquid supplements can

be compared directly to dry, natural protein sources.

Rush et al. (1972) wintered 44 yearling heifers grazing winter range with a 35% crude protein, non-protein nitrogen-based liquid supplement or a 30% crude protein dry, natural protein supplement. Even though daily crude protein intakes were equal, heifers wintered on the non-protein nitrogen supplement lost an average of 10 kg more weight ($P<.01$) than those wintered on dry, natural protein supplement.

Clanton (1973) fed dry pregnant cows either no supplement (negative control), .45 kg/head/day of a 32% crude protein dry, natural protein supplement (positive control) or .5 kg/head/day of a 32% crude protein urea-based liquid supplement. Weight gains for cows consuming the negative control, positive control and liquid supplement were 20.4, 37.2 and 29.0 kg per head, respectively.

Rush and Totusek (1976) supplemented the diets of lactating, pregnant cows grazing native range with a 15% crude protein dry, natural protein supplement (negative control), a 30% crude protein dry, natural protein supplement (positive control), a 30% crude protein urea-based liquid supplement or a liquid supplement containing only molasses. Winter weight losses for cattle consuming the negative control, liquid urea-based supplement and the molasses supplement were similar. Cattle consuming the positive control lost less weight ($P<.10$) than those fed the other treatments.

McKee et al. (1977) compared a 16 and 18% crude protein, non-protein nitrogen-based liquid supplement with a 16% dry, natural protein supplement for dry pregnant cows grazing milo stubble. Cattle receiving the liquid supplements lost more weight than those consuming no supplement or the natural protein supplement control.

In general, the above trials demonstrate that urea in liquid supplements is poorly utilized by cattle consuming low-quality roughages. These results are in agreement with the conclusion drawn by Loosli and McDonald (1968). After an extensive review of the literature they concluded that "no evidence is available to support the contention that urea can be employed profitably with low-quality roughages in genuine pastoral conditions" (p. 77).

Modern liquid supplements contain a variety of ingredients, many of which are by-products of the sugar, paper or fermentation industries. Since urea and other non-protein nitrogen products have performed poorly with cattle fed roughages, some liquid supplement manufacturers have begun using these by-product ingredients as "all natural" sources of protein (Lusby and Armbruster, 1977). Data relative to the composition of many by-product liquid feed ingredients or their usefulness as protein sources for ruminants fed low-quality roughage is limited.

Lusby and Armbruster (1981) compared condensed molasses solubles (CMS), the residue from molasses that has been used in various fermentations to produce glutamic acid, citric acid, ethanol and other products, to cottonseed meal as a crude protein source for dry, pregnant Hereford cows grazing winter native range. Weight and body condition losses for cattle fed a negative control (.14 kg crude protein per head per day) and the CMS-based liquid supplement (.26 kg crude protein per head per day) were similar. Cattle fed a positive control cottonseed meal supplement (.29 kg crude protein per head per day) lost less weight ($P<.05$) and less body condition.

The remaining sections of this review deal with the manufacture and utilization of corn steep liquor and fermented ammoniated condensed whey

as sources of crude protein for ruminant animals.

Corn Steep Liquor

Corn steep liquor (CSL) or condensed, fermented corn extractives is a by-product of the corn wet-milling industry. The first step in the recovery of corn starch by the corn refining process is to condition the grain by steeping or soaking. The steeping process softens the corn kernel for grinding, dissolves protein that holds the starch granules together in the kernels and removes solubles from the germ. This step produces optimum milling and separation of the corn components (Inglett, 1970).

Steeping is accomplished in a series of wooden or stainless steel tanks. Typically, 26.5 to 53 l of a water sulfur dioxide solution is used for each 25 kg of corn. The acid water is transferred and recirculated through the tanks for 28 to 48 hours. Some lactic acid fermentation is allowed during the steeping process because it aids subsequent processing (Inglett, 1970). The liquor, which contains soluble portions of the corn kernel (Corn Wet-Milled Feed Products, 1975) and microbial cells is drained off the tanks and concentrated in vacuum evaporators (Inglett, 1970) to 30 to 60% solids (Gutcho, 1973). CSL contains up to 50% crude protein on a 100% dry matter basis (Inglett, 1970).

Woods et al. (1970) concluded that CSL is an effective source of supplemental protein for cattle fed growing and finishing rations. In five feedlot trials, Woods et al. (1969) compared CSL to soybean meal as protein sources for calves fed corn silage rations and for finishing cattle fed high grain rations. Average daily gains were similar for cattle fed soybean meal and CSL.

Lusby and Armbruster (1981) compared CSL to cottonseed meal as a protein source for dry, pregnant Hereford cows wintered on native range. Cattle fed a protein deficient, negative control supplement lost more weight ($P<.01$) and body condition than cows fed either a positive control cottonseed meal supplement or CSL. Weight and body condition losses were similar for cows fed the positive control and CSL.

Johnson et al. (1962) reported that CSL was superior to urea as a nitrogen source for steers fed a 90% roughage diet. Average daily gains were higher ($P<.05$) for cattle supplemented with CSL. In additional trials, they showed that CSL significantly increased dry matter, crude fiber and cellulose digestibilities in lambs when added to a 50% roughage ration.

Based on unpublished data, Johnson et al. (1962) stated that CSL is a rich source of "cellulolytic bacteria growth factors" as described by Bentley et al. (1955). Valeric and caproic acids, and to a lesser extent, iso-butyric and iso-valeric acids increased the rate of cellulose digestion and ammonia utilization by rumen microorganisms in vitro.

Liquid supplements composed of CSL and molasses contain amino acids (Lusby and Armbruster, 1981). Nolan et al. (1976) and Satter et al. (1979) have shown that amino acids are utilized by rumen bacteria. Amino acids increase rumen microbial protein synthesis when added to a diet containing urea as the sole nitrogen source (Maeng and Baldwin, 1976c). Dehority et al. (1957, 1958) and Potter et al. (1966) demonstrated that amino acids stimulate rumen cellulolytic activity in vitro. Proline is deaminated by rumen microorganisms to form valeric acid and has been shown to stimulate rumen cellulolytic activity (Dehority et al., 1958; El-Shazly, 1952; Potter et al., 1966).

Fermented Ammoniated Condensed Whey

Fermented ammoniated condensed whey (FACW) is manufactured from liquid whey, a by-product of the cheese industry. From 1964 to 1974, cheese sales in the United States increased 67% (Milk Facts, 1974) with a proportional rise in the amount of whey produced. In 1973, nearly half of the whey produced was disposed of as a waste product (Schinoethe, 1976). Strict anti-pollution regulations now limit the dumping of waste by-products, including whey, into the environment. Consequently, it may now be more profitable to produce FACW and other manufactured whey products.

Arnott et al. (1958) and Reddy et al. (1976) described in detail the manufacture of FACW. Sweet or acid whey is inoculated with Lactobacillus bulgaricus. Lactose in the whey is fermented to lactic acid while the pH of the solution is maintained at about 5.5 by continuously neutralizing the lactic acid formed with ammonia. The fermented ammoniated whey is then evaporated to about 60% solids (Juengst, 1979). Commercially-produced FACW contains 45% crude protein (Juengst, 1979). Approximately 80% of the total crude protein equivalent is from ammonium lactate, 10% from lactalbumin and 10% from microbial cells (Hawkins et al., 1979).

Data comparing FACW-based liquid supplements to dry, processed oil meal protein supplements for cattle consuming low-quality roughages is non-existant. Several experiments have been conducted, however, comparing FACW to soybean meal and urea as protein supplements for beef and dairy cattle fed corn silage rations.

McCullough et al. (1972) compared FACW to soybean meal and a molasses-urea mixture as protein sources for Hereford calves fed a 60% corn

silage, 40% concentrate ration. Calves receiving FACW achieved similar gains as those receiving soybean meal. Average daily gains were lower for cattle supplemented with urea.

Henderson et al. (1975) full-fed either an all silage or a 60% corn silage, 40% shelled corn ration supplemented with FACW, urea, soybean meal or ammonia-treated silage to steer calves. All sources of supplemental protein supported greater ($P<.01$) average daily gains than an unsupplemented control ration. Differences between sources of crude protein, however, were not significant.

Hawkins et al. (1979) reported that steer calves fed an all corn silage ration supplemented with either FACW or soybean meal consumed more dry matter and gained more weight per day ($P<.001$) than calves fed no supplement. Feeding soybean meal, however, resulted in higher ($P<.05$) dry matter intakes and average daily gains than did feeding FACW.

Based on four trials involving 240 steers, Crickenberger et al. (1981) concluded that dry matter intake, average daily gain and feed efficiency are comparable for cattle receiving FACW and soybean meal at isonitrogenous levels. In addition, both FACW and soybean meal tend to be superior to urea as a crude protein source for steer calves and yearlings fed diets containing corn silage.

Early studies with dairy cattle suggested that rations supplemented with FACW may be less palatable than those supplemented with soybean meal (Hazzard et al., 1958). Subsequent trials show that reduced palatability is not a problem when FACW is fed in well-mixed silage rations (Huber et al., 1976) or when FACW is dilluted with molasses (Kung et al., 1980) prior to feeding.

Kung et al. (1980) supplemented free choice corn silage diets with a 29% crude protein FACW or a 6.7% crude protein control liquid feed.

Holstein heifer calves receiving FACW gained more weight than those consuming the control supplement ($P<.001$). In a second trial, average daily gains were not significantly different for heifers supplemented with FACW or soybean meal.

Huber et al. (1976) fed 75 lactating Holstein cows corn silage-based rations supplemented with soybean meal, urea or FACW. Cattle fed protein-supplemented rations consumed more dry matter and maintained higher milk production ($P<.01$) than cattle fed unsupplemented control rations. Differences in milk production by cows receiving isonitrogenous levels of FACW, urea or soybean meal were not significant. The authors concluded that FACW was equal to soybean meal and urea in maintaining milk yields of cows fed these supplements to supply 27% of their dietary nitrogen in rations containing corn silage.

Ammonium lactate comprises approximately 80% of the crude protein equivalent in FACW (Crickenberger et al., 1977; Hawkins et al., 1979; Henderson et al., 1974 and 1975; Reddy et al., 1976). Dutrow et al. (1974) compared ammonium lactate and ammonium propionate to soybean meal as nitrogen supplements in corn-corn silage-based rations for lactating Holstein cows. Ammonium lactate and ammonium propionate supported similar milk yields as soybean meal.

The digestibilities of dry matter, organic matter, cellulose or crude protein were similar for Hereford steer calves fed 80% roughage diets supplemented with soybean meal, urea or the ammonium salts of volatile fatty acids. Steers fed soybean meal or ammonium salts also retained more of their dietary nitrogen than steers fed urea (Varner and Woods, 1971).

Belasco (1954) used *in vitro* rumen fermentation techniques to study

the utilization of several ammonium salts and urea by rumen microorganisms. The rate of nitrogen utilization from ammonium succinate and ammonium lactate was greater than that observed from urea, resulting in lower levels of free ammonia in the system. Belasco postulated that "the organic fragments of these salts enter into some biosynthetic process stimulatory to nitrogen fixation by rumen microflora" (p. 610).

Lactate is metabolized to volatile fatty acids by rumen microorganisms (Phillipson and McAnally, 1947; Woodman and Evans, 1938). Acetate is formed from lactate via pyruvate (Baldwin et al., 1962; Bruno and Moore, 1962; Jayasuriya and Hungate, 1959). Two equivalents of acetate then condense to form butyrate (Satter et al., 1967; Wood, 1962). Lactate is converted to propionate via succinate (Johns, 1951a, 1951b, 1951c) or via the acrylate pathway (Baldwin et al., 1962; Ladd and Walker, 1959 and 1965). Hoover et al. (1963) demonstrated that ^{14}C-labelled acetate, propionate and butyrate were rapidly incorporated into bacterial amino acids and protein.

Stangassinger and Giesecke (1980) postulated that the nitrogen from ammonium lactate remains in the rumen and is available for microbial protein synthesis longer than the nitrogen from urea. At any one time, over 99% of the nitrogen in ammonium lactate is in the ionized ammonium form and is not readily absorbed across the rumen wall at pH 5.8 to 7.2. When rumen pH is above 7.2, ammonium is rapidly converted to free ammonia and is absorbed across the rumen wall.

Crickenberger et al. (1977) showed that intraruminal infusions of FACW and ammonium lactate resulted in lower rumen pH values and plasma ammonia levels than isonitrogenous infusions of urea. Lewis (1960 and

Webb et al. (1972) reported lower rumen pH and reduced plasma ammonia levels in cattle fed ammonium salts.

CHAPTER III

MATERIALS AND METHODS

Sixty-one lactating, fall-calving first-calf Hereford heifers and 32 yearling Hereford heifers were allotted by weight to four treatments. The trial started November 20, 1980 and was terminated on February 12, 1981. During the 84-day period, all cattle grazed together in two pastures (100 hectares) of native tallgrass range in north central Oklahoma. The predominant forage species were little bluestem (Andropogan scoparius), switchgrass (Panicum virgatum), big bluestem (Andropogan gerardi) and Indian grass (Sorghastrum nutans).

Cattle were gathered from the pasture at 8 a.m., six days per week and fed their supplements individually in covered stalls. Corn steep liquor (CSL) and fermented ammoniated condensed whey (FACW) protein supplements used in this study were mixed at a commercial facility by NAMOLCO, Inc., Willow Grove, Pennsylvania. Cane molasses was included in each supplement at levels intended to equalize crude protein contents and palatability of CSL and FACW supplements. Sulfuric acid was added to each liquid supplement to lower pH and to prevent gelling. Five tons of each liquid supplement were delivered to the Range Cow Research Center and held in bulk storage tanks until feeding. Dry control supplements were manufactured at the Oklahoma State University feed mill. All supplements were formulated to be approximately isocaloric and to provide equal amounts of calcium, phosphorus and potassium. The composition of

each supplement is shown in Table I.

Cattle fed the negative control (NC) supplement received approximately one-half the crude protein that was supplied to cattle consuming the positive control (PC). Cattle on the liquid supplement treatments received cottonseed meal to supply equal crude protein as the NC. Corn steep liquor or FACW was then fed to supply enough crude protein to make up the difference between the NC and PC. Supplement treatments and crude protein intakes are presented in Table II.

The described treatments enable the direct comparison of CSL and FACW to cottonseed meal. Differences between performance of cattle fed the liquid supplements and those fed the NC were compared to differences in the performance of cattle fed NC and PC. Any differences between the performance of cattle fed the liquid supplements and those fed the positive control should reflect the relative value of CSL and FACW compared to cottonseed meal.

Samples of CSL and FACW were taken weekly from the emptying spout of the storage tanks and analyzed for crude protein by Kjeldahl total nitrogen determination and for dry matter by toluene distillation (A.O.A.C., 1975). Supplement refusals were weighed and recorded daily. Supplement intakes were adjusted weekly based on the laboratory analyses and supplement refusals to insure that the desired crude protein levels were being fed.

Cattle were visually scored for body condition (1 = very thin, 9 = very fat) after an overnight withdrawal of feed and water at the beginning and end of the trial. The condition scores used for each animal in the analysis represented the mean of three condition scores assigned by three individuals unfamiliar with the treatment allotments.

TABLE I

COMPOSITION OF PROTEIN SUPPLEMENTS FED TO LACTATING FIRST-CALF HEIFERS AND YEARLING HEIFERS

Ingredient[a]	International Reference No.	Negative Control	Positive Control	CSL	FACW
Cottonseed meal	5-07-872	11.5	66.7		
Corn, ground	4-02-931	41.8	30.6		
Sorghum, grain	4-04-383	41.8			
Dicalcium phosphate	6-01-080	3.2	.6		
Limestone	6-02-632	.7	2.2		
Potassium chloride		1.2			
Vitamin A (30,000 IU/g)		.1	.1	.3	.3
Trace mineral premix[b]		.1	.1	.1	.1
CSL				69.9	
FACW					37.2
Cane molasses	4-04-696			27.4	60.3
Sulfuric Acid				1.1	1.0
Analysis					
Dry matter		89.8	91.0	53.7	69.0
Crude protein		12.9	29.0	16.8	16.0
Total digestible nutrients		72.0	72.6	55.0	62.0
pH				4.2	5.0

[a]Percent composition, as fed basis.
[b]Ingredients in trace mineral premix, %: Zn, 16.0; Fe, 12.0; Mg, 3.0; Mn, 6.0; Cu, 1.0; Co, .3; I, .6; K, 1.0.

TABLE II

SUPPLEMENT AND CRUDE PROTEIN INTAKE BY LACTATING FIRST-CALF HEIFERS AND YEARLING HEIFERS

	Treatment							
	Lactating Heifers				Yearling Heifers			
Item	Negative Control	Positive Control	CSL	FACW	Negative Control	Positive Control	CSL	FACW
Crude Protein (CP) Intake (kg/head/day)								
Total	.31	.67	.68	.68	.10	.20	.20	.21
From dry supplement	.31	.67	.33	.33	.10	.20	.08	.08
From liquid supplement			.35	.35			.12	.13
Supplement Intake (kg/head/day as fed basis)								
12.9% CP dry supplement	2.4				.8			
29% CP dry supplement		2.3				.7		
41.0% CP cottonseed meal			.8	.8			.2	.2
16.8% CP CSL			2.1				.7	
16.0% CP FACW				2.2				.8

Cattle weights were taken initially and at 28-day intervals after an overnight shrink (16 hr). Calves were not separated from their dams prior to weighing. Cows and heifers were exposed to bulls from December 1 to February 1 and pregnancy was determined by rectal palpation approximately 60 days after the end of the breeding season.

Pregnancy rates were analyzed by the Chi-square procedure (Snedecor and Cochran, 1967). Analyses of variance were conducted and differences in treatment means were compared using orthogonal contrasts, for cow and heifer weight change, cow and heifer condition change and calf weight gain according to the procedures outlined by Snedecor and Cochran (1967) for a completely randomized design.

Rumen Fluid Analysis

Rumen liquor samples were obtained on day 49 of the trial from ten randomly selected, first-calf heifers from each treatment at 1 and 4 hr postfeeding via stomach tube by a modification of the technique disscibed by Raun and Burroughs (1962). Approximately half of a 3-m length of 1.6 cm inside-diameter tubing with several small holes drilled into the end was inserted through the esophagus and into the rumen. A hand-held, manually-operated, boat bilge pump was used to draw the liquor into the tube. An aliquat of rumen fluid was discharged directly into a beaker for immediate pH determination using a portable pH meter. The remaining liquor (150 ml) was discharged into plastic bottles containing 15 g of metaphosphoric acid to stop microbial activity. Samples were then frozen for subsequent laboratory analysis.

Ruminal ammonia concentration was determined by a modification of the colorimetric procedure of Chaney and Marbach (1962). Undiluted

rumen fluid (.02 ml) was added to 1.98 ml distilled water. Five ml of phenol reagent were added followed by addition of five ml sodium hypochlorite reagent prior to mixing. Samples were incubated at room temperature for 30 min prior to reading on a Gilford spectrophotometer (625 nm).

Rumen soluble carbohydrate concentration was determined by a modification of the colorimetric procedure described by Johnson et al. (1966) for the determination of carbohydrates in corn silage. Duplicate samples of .01, .05, .1 and .2 ml of undiluted rumen fluid were added to test tubes and diluted to 2.0 ml with distilled water. One ml of phenol reagent and 5 ml of concentrated sulfuric acid were added to each test tube. Samples were mixed and incubated at room temperature for 10 min followed by mixing and an additional incubation period of 20 min at 30°C. The absorbance was determined on a Gilford spectrophotometer (490 nm) and compared to a glucose standard curve. Twenty, 40, 60 and 80 µg of glucose per test tube were used to develop the standard curve.

Thirty-five ml of rumen fluid were centrifuged at 1000 X g for 10 min. Five ml of the supernatant were added to 1 ml, 25% meta-phosphoric acid plus 2.7537 g/liter 2-ethyl butyric acid (used as an internal standard). Samples were centrifuged at 25,000 X g for 20 min. Total volatile fatty acids, as well as the molar % acetate, propionate, isobutyrate, butyrate, iso-valerate, valerate and caproate were determined by gas chromatography as described by Erwin et al. (1961).

Analyses of variance were conducted and differences in treatment means were compared using the least significant difference procedure for rumen pH, the Student Newman Keuls multiple range test for rumen volatile fatty acids and orthogonal contrasts for rumen ammonia and

soluble carbohydrate levels according to the procedures outlined by Snedecor and Cochran (1967) for a completely randomized design.

In Vitro Ammonia Accumulation

Fermentation in vitro, as described by Stanton et al. (1981), was used to compare the rate of ammonia release from CSL and FACW to the rate of ammonia release from soybean meal (SBM), urea and ammonium lactate (AL). Inoculum from a ruminally fistulated steer (681 kg), allowed to consume prairie hay (IRN 1-02-187) ad libitum plus .9 kg of a 41% crude protein supplement (Table III) per day, was strained through four layers of cheesecloth and mixed 1:1 with buffer (McDougall, 1948). The buffer contained no nitrogen source. Triplicate samples of CSL, FACW, SBM, urea or ammonium lactate, each containing 20 mg nitrogen, were added to fermentation tubes. Thirty ml of inoculum were then added to each tube. At the end of 1, 2, 4 and 8 hr fermentation, 2 ml of 20% HCl were added to the appropriate tubes to stop microbial activity. Tubes were then centrifuged at 7,000 X g for 10 min at 0°C. The supernatant was removed and refrigerated until ammonia concentrations could be determined as previously described on .02 and .005 ml samples of undiluted fermentation fluid.

Data were subjected to analysis of variance and least significant differences between treatment means were calculated as outlined by Snedecor and Cochran (1967) for a completely randomized design with a factorial arrangement of treatments.

Liquid Supplement Analysis

In addition to the dry matter and crude protein determinations

TABLE III

COMPOSITION OF SUPPLEMENT FED
TO DONOR STEER

Ingredient	International Reference Number	Percent Composition (as fed basis)
Soybean meal	5-04-604	93
Molasses	4-04-696	3
Dicalcium phosphate	6-01-080	2
Trace mineralized salt[a]		2
Vitamin A[b]		

[a]Ingredients in TM salt, %: Zn, 16; Fe, 12; Mg, 3; Mn, 6; Cu, 1; Co, .3; I, .6; K, 1.
[b]Vitamin A, to add 26,000 IU/kg of supplement.

discussed above, the crude protein (nitrogen X 6.25) fractions of CSL and FACW were subjected to further analyses. Free ammonia nitrogen in each supplement was determined by magnesium oxide distillation as described by A.O.A.C. (1975). Long-chain polypeptide and protein nitrogen was determined by precipitation of 6 g of sample in 10 ml of 1.07 N H_2SO_4 and 5.0 ml of a 10% sodium tungstate solution. The solution was refrigerated overnight and centrifuged at 10,000 X g for 10 min. The precipitate was analyzed for total Kjeldahl nitrogen (A.O.A.C., 1975). A ninhydrin procedure was used to determine the amino acid nitrogen content of CSL and FACW.

Since the terminal amino group of peptides and proteins reacts with ninhydrin, long-chain polypeptides and proteins were precipitated from solution using sodium tungstate as described above. The supernatant from each sample was adjusted to approximately 9.0 with sodium hydroxide and because free ammonia also reacts with ninhydrin, the solutions were distilled for three hours to drive off all ammonia. Following distillation, the remaining portion of each solution was diluted to 500 ml with distilled water. Five, 10, 15, and 20 ml samples of the CSL solution and 15, 20, 25 and 30 ml samples of the FACW solution were diluted to 100 ml with distilled water for analysis.

Duplicate, .5 ml samples of each dilution were then added to test tubes containing 1.5 ml of ninhydrin reagent.[1] The tubes were then covered with a marble and heated in a boiling water bath for 20 min

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[1]The ninhydrin reagent was prepared by first dissolving 1.72 g citric acid, 3.48 g Na_3 citrate $2H_2O$ and 134.4 mg anhydrous $SnCl_2$ in 100 ml of distilled water. This solution was then mixed with 100 ml of ethylene glycol monomethyl ether containing 4 g dissolved ninhydrin.

followed by cooling to room temperature. Eight milliliters of a 50% aqueous n-propanol solution were rapidly added to each tube. After standing for 10 min, the absorbance was determined on a Gilford spectrophotometer (570 nm) and compared to a standard glycine solution.

CSL and FACW were analyzed for L (+) and D (-) lactic acid using enzymatic procedures as described by Sigma Technical Bulletin No. 826-UV, (1976).[2] Eight grams of each supplement were diluted to 500 ml with distilled water. Two milliliters of each solution were then added to test tubes containing 4.0 ml cold, 8% percholoric acid. Tubes were mixed vigorously for 30 seconds, kept cold for an additional 5 min and then centrifuged for 10 min at 3,000 X g to precipitate protein from the solution. Samples were read on a Gilford spectrophotometer (340 nm).

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[2]Sigma Chemical Company, P.O. Box 14508, St. Louis, Missouri 63178

CHAPTER IV

RESULTS AND DISCUSSION

Cattle Performance

The performance of the lactating, first-calf heifers is presented in Table IV. Heifers fed the negative control (NC) supplement lost more ($P<.005$) weight (54.4 kg) and body condition (1.6 units) than those fed the positive control (20.8 kg and .9 units). Differences in weight and condition losses between heifers fed the positive control (PC), CSL (25.0 kg and .8 units) and FACW (22.5 kg and .7 units) were not significant ($P>.05$).

Differences in conception rates between treatments were not statistically significant. The lower conception rate for lactating heifers fed the PC (60%) probably reflects low numbers per treatment.

Although differences in calf weight gain were not statistically significant, calves of the NC supplemented cattle gained less weight ($P<.25$) than calves whose dams received PC, CSL or FACW (31.7 versus 37.7, 34.9 and 36.0, respectively), indicating that milk production by cattle fed the NC supplement may have been reduced.

Bond et al. (1964) and Harris et al. (1962, 1965) demonstrated that Angus cows fed low protein diets produced less milk than cows fed cottonseed meal-based protein supplements. The data of Kropp et al. (1973) and Lusby (1974) demonstrate that the influence of supplemental feed on

TABLE IV

PERFORMANCE OF LACTATING, FIRST-CALF HEIFERS AND THEIR CALVES

Item	Treatment[a]			
	Negative Control	Positive Control	CSL	FACW
Number of pairs	15	15	15	16
Initial cow weight (kg)	404.1 ± 8.7	401.2 ± 7.6	401.2 ± 7.1	403.6 ± 9.5
Cow weight change (kg)	-54.4[b] ± 3.2	-20.8[c] ± 2.6	-25.0[c] ± 2.8	-22.5[c] ± 3.4
Initial body condition (1-9)	6.5 ± .1	6.4 ± .1	6.4 ± .1	6.4 ± .1
Body condition change	-1.6[b] ± .1	-.9[c] ± .1	-.8[c] ± .1	-.7[c] ± .1
Initial calf weight (kg)	50.0 ± 2.1	50.0 ± 1.6	53.9 ± 1.9	57.5 ± 2.9
Calf weight gain (kg)	31.7 ± 1.4	37.7 ± 3.7	34.9 ± 2.6	36.0 ± 1.4
Conception rate, percent	86	60	80	81

[a]Means ± standard error.
[bc]Means with different superscript letters differ (P<.005).

milk production is greater in cattle with a higher genetic potential to produce milk. The nutritional stress that the NC cows were subjected to, may not have been sufficient to drastically lower milk production in Hereford cattle.

Table V presents weight and body condition changes for the yearling heifers. Heifers receiving the NC supplement lost more ($P<.005$) weight than those consuming the PC (22.4 versus 4.8 kg). Weight gains by heifers supplemented with CSL and FACW were similar (8.0 and 4.2 kg, respectively) and greater ($P<.005$) than weight gains of heifers fed the PC (-4.8 kg). Changes in body condition were similar ($P>.05$) for heifers consuming the NC (-.9 units), PC (-.7 units), CSL (-.7 units) and FACW (-.8 units).

Differences in conception rates between treatments were not statistically significant. The lower conception rate for heifers fed the PC (67%) probably reflects low numbers per treatment.

Since all of the supplements were formulated to provide equal energy, vitamin A and essential minerals, greater weight and body condition losses by cattle fed the NC supplement indicate a protein deficiency was established in the NC cattle. Egan and Moir (1965) demonstrated that the nitrogen status of a ruminant governs the digestibility and intake of roughages of low nitrogen content. Protein supplementation improves a ruminant's nitrogen status, resulting in increased energy intake from roughage. Increased energy intake depresses fat mobilization and reduces weight loss.

Because only eight yearling heifers were used per treatment, improved weight gains of yearling heifers fed both liquid supplements compared to cottonseed meal may have been due to individual animal

TABLE V

PERFORMANCE OF YEARLING HEIFERS

Item	Treatment[a]			
	Negative Control	Positive Control	CSL	FACW
Number	8	8	8	8
Initial weight (kg)	353.6 ± 7.8	348.5 ± 9.2	348.6 ± 9.4	349.6 ± 9.8
Weight change (kg)	-22.4[b] ± 3.1	-4.8[c] ± 2.9	+8.0[d] ± 2.8	+4.2[d] ± 2.5
Initial body condition (1-9)	7.2 ± .1	7.0 ± .1	7.2 ± .1	7.1 ± .1
Body condition change	-.9 ± .1	-.7 ± .2	-.7 ± .1	-.8 ± .1
Conception rate, percent	83	67	100	100

[a]Means ± standard error.
[bcd]Means with different superscript letters differ (P<.005).

variation. Certainly, these results are too preliminary to suggest that CSL and FACW are superior to cottonseed meal. However, these data, plus similar weight and body condition losses observed with the lactating heifers fed the cottonseed meal-based PC and both liquid supplements suggest that CSL and FACW are effective sources of crude protein for cattle wintered on native range.

In a previous study at the Oklahoma Agricultural Experiment Station, Lusby and Armbruster (1981) reported that CSL effectively maintained the weight and body condition of dry, pregnant cows wintered on native range. Woods et al. (1970) concluded that CSL was as effective as soybean meal as a protein source for cattle fed growing and finishing rations.

Data comparing FACW to dry, natural protein supplements as a nitrogen source for cattle fed low-quality roughage is non-existant. Several experiments have shown that FACW is an effective nitrogen source for beef and dairy cattle fed corn silage-based rations (Crickenberger et al., 1981; Hawkins et al., 1979; Henderson et al., 1975; Huber et al., 1976; Kung et al., 1980; McCullough et al., 1972).

Rumen Fluid Analysis

The pH of rumen fluid obtained from cattle at one and four hours post-feeding is presented in Table VI. At one hour post-feeding, the ruminal pH of cattle fed the NC and PC supplements (7.07 and 7.24, respectively) were higher ($P<.05$) than the pH of rumen fluid from the CSL and FACW supplemented cattle (6.73 and 6.79, respectively). At four hours post-feeding the ruminal pH of the PC cattle was higher ($P<.05$) than that of the NC, CSL and FACW supplemented cattle (7.06 versus 6.83, 6.89 and 6.78, respectively).

TABLE VI

RUMINAL pH AND THE CONCENTRATION OF AMMONIA AND SOLUBLE CARBOHYDRATE IN RUMEN FLUID COLLECTED ONE AND FOUR HOURS POST-FEEDING

Item	Treatment[a]			
	Negative Control	Positive Control	CSL	FACW
Number of cows sampled	10	10	10	10
Rumen pH				
1 hr post-feeding	$7.07^{b} \pm .05$	$7.24^{b} \pm .04$	$6.73^{c} \pm .07$	$6.79^{c} \pm .07$
4 hr post-feeding	$6.83^{c} \pm .08$	$7.06^{b} \pm .05$	$6.89^{c} \pm .06$	$6.78^{c} \pm .07$
Rumen Ammonia (mg/dl)				
1 hr post-feeding	$8.4^{d} \pm 1.88$	$10.5^{d} \pm 1.30$	$18.9^{c} \pm .90$	$26.2^{b} \pm 2.18$
4 hr post-feeding	$3.8^{d} \pm .40$	$11.3^{c} \pm .61$	$14.8^{c} \pm 1.54$	$23.1^{b} \pm 2.44$
Rumen soluble carbohydrate (μmoles/l)				
1 hr post-feeding	$1865.6^{c} \pm 338.3$	$2268.9^{c} \pm 161.7$	$5016.1^{c} \pm 316.7$	$17271.7^{b} \pm 2679.4$
4 hr post-feeding	$2144.4^{c} \pm 194.4$	$1705.0^{c} \pm 208.3$	$2163.9^{c} \pm 122.2$	$3703.9^{b} \pm 213.3$

[a]Means ± standard error

[bcd]Means in the same row with different superscripts differ ($P<.05$).

The concentrations of ammonia in rumen fluid obtained from cattle at one and four hours post-feeding are also shown in Table VI. At one hour post-feeding, similar ($P>.05$) ammonia levels, 8.4 and 10.4 mg/dl, were observed for cattle fed the NC and PC supplements, respectively. Ammonia levels in rumen fluid from cattle fed CSL (18.9 mg/dl) were higher ($P<.05$) than ammonia levels in the NC and PC supplemented cattle. Rumen fluid from FACW supplemented cattle contained the highest ($P<.05$) ammonia levels (26.2 mg/dl). By four hours post-feeding, rumen ammonia levels in the NC cattle were lower ($P<.05$) than the levels observed in cattle fed PC, CSL and FACW (3.8 versus 11.3, 14.8 and 23.1 mg/dl, respectively). Rumen ammonia levels in the PC and CSL supplemented cattle were similar ($P>.05$) and remained significantly lower than the ammonia levels observed for the FACW-supplemented cattle.

Ammonia nitrogen can potentially supply 50-70% of the nitrogen in microbial protein (Hespell, 1979). Therefore, ammonia nitrogen concentrations must be sufficient to maintain microbial protein synthesis. Satter and Slyter (1974) postulated that maximal microbial growth *in vivo* is achieved with ammonia concentrations of 5 mg/dl. Hespell (1979) reported that maximal microbial growth rates *in vitro* could be achieved with ammonia concentrations of only .425 mg/dl. Hespell (1979) contended that improvements in cell yields or dry matter digestion observed *in vivo* with higher ammonia levels do not stem from direct ammonia effects on microbial growth. He proposed that any benefits seen are due to indirect effects such as increased rumen pH. Since ammonia levels observed in this study for all treatments except the NC at four hr post-feeding were substantially greater than the levels reported by Hespell (1979) and higher than the levels proposed by Satter and Slyter (1974),

it appears unlikely that microbial protein synthesis was limited by rumen ammonia concentration.

Ammonia levels in rumen fluid reflect the rate of ammonia release from dietary nitrogen sources. Theoretically, to support maximum microbial growth, ammonia release should parallel the slow rate of cellulose fermentation in the rumen of cattle consuming high roughage diets (Johnson, 1976). Excess ammonia, released more rapidly than the rate of cellulose digestion, is absorbed through the rumen wall into the blood stream and is ultimately excreted in the urine.

The relatively high ammonia levels (26.2 and 23.1 mg/dl at one and four hours post-feeding, respectively) observed in the rumen fluid of the FACW supplemented cattle may remain available to microbes in the rumen and not be absorbed into the blood stream rapidly. Absorption of ammonia from the rumen is retarded by lower pH (Johnson, 1976). Crickenberger et al. (1977) showed that intraruminal infusions of FACW and ammonium lactate resulted in reduced plasma ammonia levels and lower rumen pH values than isonitrogenous infusions of urea. Stangassinger and Giesecke (1980) reported that in the pH range of 5.8 to 7.2, over 99% of the ammonia in ammonium lactate is in the ionized ammonium form and cannot be absorbed across the rumen wall. Only 1% of the ammonia is unionized and available for absorption from the rumen at any one time.

The soluble carbohydrate concentration in ruminal fluid collected at one and four hours post-feeding (Table VI) partially reflects the percent molasses in each of the supplements. The FACW supplement fed in this trial contained 60.3% molasses (Table I) and resulted in greater ($P<.005$) soluble carbohydrate concentrations at both one and four hours post-feeding, 17,271.7 and 3703.9 μmoles/l, respectively. Rumen fluid

from CSL (27.4% molasses) supplemented cattle tended (nonsignificant) to have higher soluble carbohydrate levels than the fluid from NC and PC supplemented cattle at one hour (5016.1 versus 1865.6 and 2268.9 µmoles/l, respectively) and at four hours (2163.9 versus 2144.4 and 1704.0 µmoles/l, respectively) post-feeding.

Hespell (1979) postulated that the in vivo growth of rumen bacteria in the fluid phase may be limited by carbohydrate availability. Between 60 and 1000 µmoles of soluble carbohydrate per liter of rumen fluid is required by rumen bacteria to promote maximum cell growth (Hespell, 1969). Since the mean soluble carbohydrate concentration in rumen fluid from all treatments exceeded this range, microbial cell growth may not have been limited by carbohydrate availability at one or four hours post-feeding. However, the supply of available energy may become depleted at later times post-feeding. The major limitation of microbial protein synthesis may be energy availability (Johnson, 1976).

At one hour post-feeding, the high concentration of soluble carbohydrate (17,271.7 µmoles/l) paralleled the high concentration of ammonia (26.2 mg/dl) in the rumen fluid of cattle fed FACW. The synchronized availability of ammonia and energy may enhance microbial protein synthesis. However, Johnson (1976) stated that molasses may not be the best source of carbohydrate for stimulation of ammonia utilization.

Volatile fatty acid (VFA) concentrations in rumen fluid sampled at one and four hours post-feeding are presented in Tables VII and VIII, respectively. Total VFA concentrations at one hour post-feeding were 92.42, 95.53, 93.60 and 93.88 µmoles/ml of NC, PC, CSL and FACW, respectively. At four hours post-feeding, ruminal VFA levels in cattle fed the NC, PC, CSL and FACW were 93.37, 73.63, 92.92 and 88.33 µmoles/ml,

TABLE VII

VOLATILE FATTY ACID CONCENTRATION (MOLAR %) IN RUMEN FLUID COLLECTED ONE HOUR POST-FEEDING

Item	Treatments				SE[a]
	Negative Control	Positive Control	CSL	FACW	
Total VFA (μmoles/ml)	92.42	95.53	93.60	93.88	7.97
Acetate	71.90[b]	71.48[b]	69.87[bc]	68.77[c]	.74
Propionate	16.06[c]	16.21[c]	16.76[c]	19.38[b]	.63
Isobutyrate	1.07[b]	1.19[b]	1.13[b]	.28[c]	.14
Butyrate	9.24[c]	8.78[c]	10.74[b]	10.71[b]	.38
Isovalerate	1.07[b]	.99[b]	1.32[b]	.39[c]	.11
Valerate	.59[b]	.72[b]	.68[b]	.44[c]	.06
Caproate	.08	.07	.05	.02	.02
Acetate/Propionate	4.53[b]	4.32[b]	4.38[b]	3.62[c]	.18

[a]Standard error of the mean with 10 observations per treatment.
[bcd]Means in same row with different superscripts differ ($P<.05$).

TABLE VIII

VOLATILE FATTY ACID CONCENTRATION (MOLAR %) IN RUMEN FLUID COLLECTED FOUR HOURS POST-FEEDING

Item	Treatments				SE[a]
	Negative Control	Positive Control	CSL	FACW	
Total VFA (μmoles/ml)	93.37	73.63	92.92	83.33	6.71
Acetate	71.13[b]	71.76[b]	65.90[c]	61.05[d]	1.10
Propionate	15.15[c]	15.85[c]	15.31[c]	17.93[b]	.75
Isobutyrate	1.12[b]	1.23[b]	1.12[b]	.56[c]	.14
Butyrate	10.26[d]	9.36[d]	14.42[c]	18.80[b]	.53
Isovalerate	1.06[c]	.83[c]	1.52[b]	.42[d]	.11
Valerate	1.02	.85	1.34	1.15	.24
Caproate	.26[bc]	.12[cd]	.39[b]	.10[d]	.07
Acetate/Propionate	4.74[b]	4.65[b]	4.33[b]	3.54[c]	.22

[a]Standard error of the mean with 10 observations per treatment.
[bcd]Means in same row with different superscripts differ ($P<.05$).

respectively. Differences between treatment means were not significant ($P>.05$) suggesting that rumen fermentation was not limited by treatment.

Church (1976) suggested that normal VFA concentrations are within the range of 80-150 μmoles/ml depending upon the diet, the sampling technique and other factors such as rumen volume. High roughage, low protein diets result in lower VFA concentrations. Comparisons of total VFA production between experiments may not be valid because of the important effects of sampling technique and analytical procedure on total VFA concentration (Church, 1976).

At one hour post-feeding, the ratio of acetate to propionate in rumen fluid was lower ($P<.05$) for cattle fed FACW (3.62) than for cattle fed NC, PC and CSL (4.53, 4.32 and 4.38, respectively). At four hours post-feeding, the ratio of acetate to propionate in rumen fluid was significantly lower for cattle fed FACW (3.54) than for cattle fed NC, PC and CSL (4.74, 4.65 and 4.33, respectively). Lower acetate to propionate ratios are associated with reduced heat losses and as a result, higher net energy values. However, the causal relationship between high acetate to propionate levels and high heat increments is not clear (Church et al., 1974).

The molar concentrations of acetate, propionate, isobutyrate, butyrate, isovalerate, valerate and caproate did not differ ($P>.05$) between the NC and PC at one and four hours post-feeding, indicating that level of protein supplementation did not alter VFA fermentation. Supplementing cattle diets with either CSL or FACW, however, significantly affected the molar concentration of several VFA at both time periods.

Ruminal propionate levels were higher ($P<.05$) in cattle fed FACW than in those fed the NC, PC and CSL supplements (19.38 versus 16.06,

16.21 and 16.76 molar % at one hour post-feeding and 17.93 versus 15.15, 15.85 and 15.31 molar % at four hours post-feeding, respectively). Butyrate levels in rumen fluid of FACW supplemented cattle were significantly higher than the levels observed in the NC and PC rumen fluid (10.71 versus 9.24 and 8.78 molar % at one hour post-feeding and 18.80 versus 10.26 and 9.36 molar % at four hours post-feeding, respectively). The molar percent acetate was lower ($P<.05$) for the FACW treatment (68.77) than for the NC (71.90) and PC (71.48) at one hour post-feeding. At four hours post-feeding, ruminal acetate levels were lower ($P<.05$) in the FACW supplemented cattle than in the cattle fed NC or PC (61.05 versus 71.13 and 71.76 molar %, respectively). The effect of CSL supplementation on butyrate and acetate percentages followed a similar pattern. Rumen fluid of CSL supplemented cattle contained 10.74 molar % butyrate and 69.87 molar % acetate at one hour post-feeding and 14.42 molar % butyrate and 65.90 molar % acetate at four hours post-feeding. The observed changes in the levels of acetate, propionate and butyrate can be explained by the effects of lactic acid on rumen fermentation.

The CSL and FACW supplements used in this study contained 5.6 and 10.2% lactic acid on an as-fed basis, respectively (Table X). Lactate forms acetate via pyruvate (Baldwin et al., 1962; Bruno and Moore, 1962; Jayasuriya and Hungate, 1959). Two equivalents of acetate then condense to form butyrate (Satter et al., 1967; Wood, 1961). Lactate is also converted to propionate via succinate (Johns, 1951a, 1951b, 1951c) or via the acrylate pathway (Baldwin et al., 1962; Ladd and Walker, 1959 and 1965).

Volatile fatty acids are the main source of energy for the ruminant animal. Acetate, propionate and butyrate are all oxidized via the TCA

TABLE IX

IN VITRO AMMONIA CONCENTRATION
FROM NITROGEN SOURCES

Nitrogen Source	Time of Incubation (hr)			
	1	2	4	8
	mg NH_3-N/dl[a]			
SBM[i]	13.83 ± 1.43[b]	15.33 ± .34[bc]	19.89 ± .49[bc]	36.83 ± .60[d]
CSL	23.58 ± .27[c]	23.23 ± 1.25[c]	22.71 ± 1.55[bc]	36.00 ± 3.21[d]
FACW	42.59 ± 3.08[de]	44.51 ± 1.06[def]	50.13 ± 3.90[ef]	43.86 ± 2.02[def]
AL	71.11 ± 4.50[g]	71.47 ± 5.17[g]	69.16 ± 9.23[g]	62.92 ± 3.50[g]
Urea	24.38 ± 1.42[c]	53.00 ± 2.93[f]	71.82 ± 1.04[g]	85.33 ± .51[h]

[a]Means ± standard error with three tubes/treatment.
[bcdefgh]Means with different superscripts differ (P<.05).
[i]Nitrogen source by incubation time interaction (P<.005).

TABLE X

DRY MATTER, LACTIC ACID AND CRUDE PROTEIN COMPOSITION OF CSL AND FACW

Item	Supplement	
	CSL	FACW
Dry matter	53.74	68.95
Lactic acid[a]		
L(+) lactate	1.49	3.64
D(-) lactate	4.11	6.56
Total lactate	5.60	10.20
Crude protein (N X 6.25)[a]	16.85	16.00
Protein fractionation[b]		
NH_3-N	13.3	60.0
Tungstic acid precipitable N	29.6	13.2
Ninhydrin positive N	17.0	7.0
Undetermined N (includes short-chain polypeptides)	40.1	20.0

[a]Percent composition as fed basis.
[b]Percent of total nitrogen.

cycle to produce ATP. The relative efficiency of oxidation, however, differs for each VFA. The oxidation of one mole of acetate, propionate and butyrate yields 10, 18 and 26 moles of ATP, respectively (Church et al., 1974). Propionate serves as a major source of carbon for the net synthesis of glucose. One mole of glucose is oxidized to form 38 moles of ATP (Church et al., 1974). Increased levels of propionate and butyrate in the rumen fluid of FACW supplemented cattle may reflect a more efficient utilization of dietary energy.

At one hour post-feeding, the molar % isobutyrate, isovalerate, valerate and caproate in rumen fluid of cattle fed CSL, NC and PC were similar ($P>.05$). By four hours post-feeding, the molar concentrations of isovalerate and caproate were significantly higher ($P<.05$) in fluid from the CSL treatment. Although not statistically significant, ruminal valerate levels also tended to be higher for the CSL treatment at four hours post-feeding.

Lusby et al. (1982) reported free and total amino acid profiles of an identically formulated CSL supplement. In addition to several other amino acids, CSL contained proline, valine and leucine at concentrations of 3.0, 1.2 and 2.9% of dry matter, respectively. Within the rumen, proline is metabolized to valerate (Dehority et al., 1958; El-Shazly, 1952), valine is converted to isobutyrate and leucine forms isovalerate (Church et al., 1974). Ruminal metabolism of amino acids present in CSL may account for the observed increase in isovalerate, valerate and caproate levels.

Branched-chain VFA, including isobutyrate and isovalerate stimulate cellulose digestion in the rumen (Hespell, 1979). Bentley et al. (1955) showed that isobutyrate, isovalerate, caproate and especially valerate

stimulated celulose digestion in the rumen. Dehority et al. (1958) demonstrated that the intermediates of the metabolism of valine, proline and leucine had a similar effect on cellulose digestion. Johnson et al. (1962) observed significantly higher crude fiber, cellulose and dry matter digestibilities in lambs when CSL was added to a 50% roughage ration.

At one hour post-feeding, ruminal molar concentrations of isobutyrate, isovalerate and valerate were significantly lower ($P<.05$) for the FACW treatment than for the NC, PC and CSL treatments (.28, .39 and .44 versus 1.07, 1.07, and .59; 1.19, .99, and .72; 1.13, 1.32 and .68 molar %, respectively). At four hours post-feeding, ruminal fluid from the FACW treatment had significantly lower ($P<.05$) levels of isobutyrate and isovalerate than the fluid from the NC, PC and CSL treatments (.56 and .42 versus 1.12 and 1.06, 1.23 and .83, 1.12 and 1.52 molar %, respectively).

Supplementation with FACW generally resulted in lower ruminal levels of isobutyrate, isovalerate and valerate. This may simply reflect the lower amino acid content of FACW compared to CSL (Table X). However, cattle supplemented with FACW also received cottonseed meal to supply equal crude protein as the NC supplement. Therefore, if the amino acid content of FACW is low, both treatments, FACW and NC, should have supplied similar amounts of proline, valine and leucine. Altered VFA profiles suggest differences in the population of rumen microorganisms between cows fed the two supplements.

In Vitro Ammonia Accumulation

In vitro ammonia concentrations for soybean meal (SBM), CSL, FACW,

ammonium lactate (AL) and urea are presented in Table IX. Figure 1 graphically displays in vitro ammonia accumulation for the nitrogen sources after 1, 2, 4 and 8 hours incubation.

Each incubation tube contained approximately 62.5 mg added nitrogen per dl of fluid. The mean ammonia concentration of three blank tubes with no added nitrogen was 12.09 mg/dl. Assuming ammonia is 82% nitrogen, the maximum initial free ammonia concentration possible at time 0 was approximately 88.3 mg/dl.

The SBM incubation fluid had the lowest ($P<.05$) one hour ammonia concentration (13.83 mg/dl). The ammonia concentration in the CSL fermentation tubes at one hour was 23.58 mg/dl. The difference between the two, is a reflection of the free ammonia content of the CSL protein supplement (Table X). There were no significant differences ($P>.05$) between the rate of ammonia accumulation from CSL and SBM at 2, 4 and 8 hr incubation time (23.23, 22.71 and 36.00 versus 15.33, 19.89 and 36.83 mg/dl, respectively).

The concentration of ammonia in the urea fermentation tubes (24.38 mg/dl) at one hour was similar ($P>.05$) to the concentration of ammonia in the CSL tubes. However, ammonia accumulated rapidly in the urea system. The concentration of ammonia increased to 71.82 mg/dl (81.3% of proposed maximum) by 4 hours incubation and 85.3 mg/dl (96.7% of proposed maximum) by 8 hours incubation.

The levels of ammonia in the AL tubes were similar ($P>.05$) at 1, 2, 4 and 8 hr incubation (71.11, 71.47, 69.16 and 62.92 mg/dl, respectively). These figures represent 80.5, 80.9, 78.3 and 71.3% of the proposed 88.3 mg/dl maximum.

The ammonia concentration in the FACW tubes followed the same

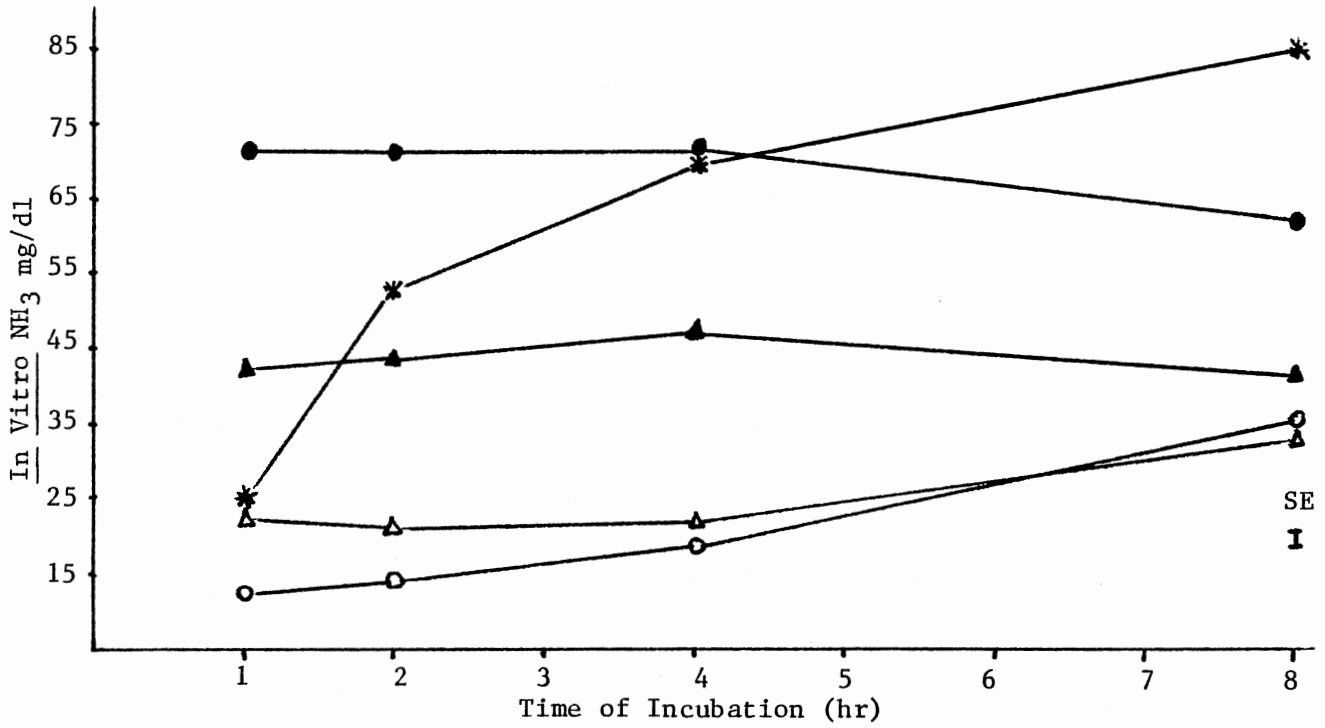

Figure 1. In Vitro Ammonia Concentrations of the Following Nigrogen Sources: SBM (-o-), CSL (-Δ-), FACW (-▲-), AL (-●-) and Urea (-*-)

pattern over time as the AL ammonia levels. Free ammonia levels in the FACW system (42.59, 44.51, 50.13 and 43.86 mg/dl) were similar ($P>.05$) at 1, 2, 4 and 8 hr incubation, respectively. The one hour ammonia concentration in the FACW tubes was approximately 60% of the one hour ammonia concentration in the AL tubes. This difference reflects the free ammonia content of the FACW supplement (Table X). By 8 hr incubation, the ammonia level in the FACW system was approximately 70% as high as the level in the AL system. The proportional increase of ammonia in the FACW system compared to the AL system, may be due to the gradual release of ammonia from natural protein in the FACW supplement.

Ammonia is apparently released gradually from SBM and CSL, presumably due to digestion by microbial proteases and the subsequent deamination of amino acids. Gradual ammonia release aids microbial protein synthesis when cellulose is the primary source of fermentable energy (Johnson, 1976).

Microbial urease rapidly hydrolyzes urea to ammonia. The ammonia in AL apparently establishes an immediate equilibrium between the disassociated free ammonium ion and the closely associated ammonium salt form according to pH of the system. If the free ammonia formed is removed from the rumen more rapidly than energy becomes available, microbial protein synthesis is depressed, resulting in reduced performance. Crickenberger et al. (1976) demonstrated that intraruminal infusions of FACW and AL resulted in lower ruminal pH and reduced plasma ammonia levels than isonitrogenous infusions of urea. The ammonia from FACW and AL may remain in the rumen longer and thus be available for microbial protein synthesis.

Liquid Supplement Analysis

The percent dry matter, lactic acid, crude protein and the composition of crude protein in CSL and FACW is presented in Table X. CSL and FACW contained 53.74 and 68.95% dry matter, respectively. The lactic acid content of CSL and FACW was 5.6 and 10.2%, respectively. As discussed earlier, feeding lactic acid alters rumen fermentation (Satter et al., 1967; Walker, 1968), resulting in a lower ($P<.05$) acetate to propionate ratio and a higher ($P<.05$) molar percent butyrate.

The CSL supplement used in this study contained 16.85% crude protein (N X 6.25) on an as-fed basis. Free ammonia nitrogen, tungstic acid precipitable nitrogen and free amino acid nitrogen (as determined by ninhydrin reaction) accounted for 13.3, 29.6 and 17.0% of the total nitrogen in CSL, respectively. Lusby et al. (1982) reported values of 6.3% for ammonia nitrogen, 35.0% for tungstic acid precipitable nitrogen and 24.0% for free amino acid nitrogen in an identically-formulated CSL protein supplement. Differences in the nitrogen fractionation data between these two supplements reflect the variability of by-product feed ingredients.

The FACW supplement contained 16.0% crude protein (N X 6.25). Sixty percent of the total nitrogen in FACW was found in the free ammonia form, while 13.2 and 7.0% was in tungstic acid precipitable nitrogen and free amino acid nitrogen, respectively. Pure FACW often contains 80% of the crude protein equivalent as ammonium lactate, 10% as lactalbumin and 10% as microbial cells (Hawkins et al., 1979). The FACW supplement used in the present study was diluted with molasses. Therefore, the nitrogen fractions observed in this study appear reasonable.

As discussed above, amino acids may be metabolized into rumen cellulolytic factors (Church et al., 1974; Dehority et al., 1958; El-Shazly, 1952; Potter et al., 1966). Amino acids may be directly incorporated into microbial protein (Nolan et al., 1976; Salter et al., 1979). Amino acids added to a diet containing urea as the sole nitrogen source increased rumen microbial protein yield (Maeng and Baldwin, 1976c).

In conclusion, CSL and FACW appear to be effective sources of crude protein for cattle grazing winter native range. Corn steep liquor appears to be a good source of natural protein and amino acids. Fermented ammoniated condensed whey is a high non-protein nitrogen (NPN) supplement. Historically, cattle grazing low-quality roughages have performed poorly when supplemented with NPN-molasses.

The ammonia in FACW may remain in the rumen longer due to the physical-chemical properties of ammonium lactate or the effects of pH on ammonia absorption from the rumen. Rumen microbial protein synthesis may be improved with FACW as compared to conventional NPN sources. Lactic acid is utilized by rumen microbes and appears to alter VFA fermentation. Reduced acetate to propionate ratios and the increased molar percentage of butyrate may improve the efficiency of energy utilization and reduce weight losses.

CHAPTER V

SUMMARY

Sixty-one lactating, fall-calving, first-calf Hereford heifers and 32 yearling Hereford heifers were allotted by weight to four treatments. During the 84-day trial, all cattle grazed together in two pastures (100 hectares) of native tallgrass range in north central Oklahoma. The predominant forage species were little bluestem (Andropogan scoprius), switchgrass (Panicum virgatum), big bluestem (Andropogan gerardi) and Indian grass (Sorghastrum nutans). Cattle were gathered from the pasture at 8 a.m., six days per week and fed their supplements individually in covered stalls.

Supplement treatments were: negative control (NC), positive control (PC), corn steep liquor (CSL) and fermented ammoniated condensed whey (FACW) to provide .31, .67, .68 and .68 kg crude protein (CP) per day, respectively to the lactating heifers and .10, .20, .20 and .21 kg CP per day, respectively, to the yearling heifers.

Cattle on the liquid supplement treatments received cottonseed meal to supply equal crude protein as the NC. Corn steep liquor or FACW was then fed to supply enough crude protein to make up the difference between the NC and PC. All supplements were formulated to be approximately isocaloric and to provide equal amounts of calcium, phosphorus and potassium.

Cattle were visually scored for body condition (1 = very thin,

9 = very fat) at the beginning and end of the trial and weights were taken initially and at 28-day intervals after an overnight shrink (16 hr). Calves were not removed from their dams prior to weighing. Cows and heifers were exposed to bulls from December 1 to February 1 and pregnancy was determined by rectal palpation approximately 60 days after the end of the breeding season.

Rumen liquor samples were obtained on day 49 of the trial from 10 randomly selected, first-calf heifers from each treatment at 1 and 4 hr post-feeding via stomach tube. Rumen fluid was analyzed immediately for pH. Ruminal ammonia, soluble carbohydrate and volatile fatty acid (VFA) concentrations were determined.

Fermentation in vitro was used to compare the rate of ammonia release from CSL and FACW to the rate of ammonia release from soybean meal (SBM), urea and ammonium lactate (AL).

Liquid supplements used in this study were analyzed for total nitrogen, ammonia nitrogen, amino acid nitrogen and long-chain polypeptide and protein nitrogen. Samples were also analyzed for dry matter and lactic acid concentrations.

Lactating heifers fed the NC supplement lost more ($P<.005$) weight (54.4 kg) and body condition (1.6 units) than those fed the PC (20.8 kg and .9 units). Differences in weight and condition losses between lactating heifers fed the PC, CSL (25.0 kg and .8 units) and FACW (22.5 kg and .7 units) were not significant ($P>.05$). Calves of the NC supplemented cattle tended ($P<.25$) to gain less weight than calves whose dams received PC, CSL or FACW (31.7 versus 37.7, 34.9 and 36.0 kg, respectively). Differences in conception rates between treatments were not statistically significant.

Yearling heifers receiving the NC supplement lost more ($P<.005$) weight than those consuming the PC (22.4 versus 4.8 kg). Weight gains by yearling heifers supplemented with CSL and FACW were similar (8.0 and 4.2 kg, respectively) and greater ($P<.005$) than weight gains of heifers fed the PC (-4.8 kg). Changes in body condition were similar ($P>.05$) for yearling heifers consuming the NC (-.9 units), PC (-.7 units), CSL (-.7 units) and FACW (-.8 units). Differences in conception rates between treatments were not statistically significant.

Lower ruminal pH was observed in cattle fed CSL and FACW than in cattle fed NC and PC. At one and four hours post-feeding, rumen ammonia levels were higher ($P<.05$) in FACW fed cattle than in CSL supplemented cattle. Rumen ammonia concentrations were higher ($P<.05$) in CSL supplemented cattle than in the NC or PC cattle at one hour post-feeding and similar ($P>.05$) to the ammonia level in the PC cattle at four hours post-feeding. Rumen fluid from cattle fed FACW contained higher ($P<.05$) soluble carbohydrate levels than the fluid from NC, PC and CSL supplemented cattle. Total ruminal VFA concentrations were similar ($P>.05$) for cattle fed NC, PC, CSL and FACW. Feeding FACW resulted in a lower ($P<.05$) acetate to propionate ratio than feeding NC, PC or CSL. Rumen fluid from FACW supplemented cattle contained lower ($P<.05$) acetate and higher ($P<.05$) propionate and butyrate levels than rumen fluid from NC or PC supplemented cattle. Feeding CSL resulted in higher ($P<.05$) butyrate and lower ($P<.05$) acetate levels than did feeding NC and PC. The molar concentrations of isovalerate and caproate were higher ($P<.05$) in rumen fluid from the CSL treatment.

Ammonia was apparently released gradually *in vitro* from SBM and CSL, presumably due to digestion by microbial proteases and the subsequent

deamination of amino acids. The concentration of ammonia in the urea system was low initially but rapidly increased due to the hydrolysis of urea to ammonia. The ammonia in AL and FACW apparently established an immediate equilibrium between the disassociated free ammonium ion and the closely associated ammonium salt form.

The CSL supplement fed in this study contained 53.74% dry matter, 5.6% lactic acid and 16.85% crude protein (N X 6.25). The nitrogen in CSL was composed of 13.3% ammonia nitrogen, 29.6% long-chain polypeptide nitrogen and 17.0% amino acid nitrogen. The FACW supplement contained 68.95% dry matter, 10.2% lactic acid and 16.0% crude protein (N X 6.25). The nitrogen in FACW was composed of 60.0% ammonia nitrogen, 13.2% long-chain polypeptide and protein nitrogen and 7.0% amino acid nitrogen.

Since all of the supplements were formulated to provide equal energy, vitamin A and essential minerals, greater weight and body condition losses by cattle fed the NC supplement indicate a protein deficiency was established in the NC cattle. Improved performance by yearling heifers fed either liquid, plus similar weight and condition losses by lactating heifers fed the PC, CSL and FACW, suggest that CSL and FACW are effective sources of crude protein for cattle wintered on native range. Corn steep liquor appears to be a good source of amino acids and natural proteins. Fermented ammoniated condensed whey is a high non-protein nitrogen (NPN) supplement.

The results of this study indicate that liquid supplements should be evaluated for the specific type of NPN present. The effects of NPN sources on rumen pH and VFA fermentation may influence the performance of cattle fed such supplements. Slower absorption of ammonia from the rumen may aid microbial protein synthesis and improve performance.

Altered VFA molar concentrations may improve the efficiency of energy utilization and reduce weight losses.

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APPENDIX

TABLE XI

ANALYSIS OF VARIANCE (PERFORMANCE OF LACTATING HEIFERS)

AOV Table for Heifer Weight Change

Source of Variation	df	Mean Squares	P Value
Total	60	----------	-------
Treatments	3	18,272.948	P<.005
NC vs (PC + CSL + FACW)/3	1	53,996.832	P<.005
PC vs (CSL + FACW)/2	1	278.784	P>.250
CSL vs FACW	1	212.268	P>.250
Error	57	678.677	------

AOV Table for Heifer Condition Change

Source of Variation	df	Mean Squares	P Value
Total	60	----------	------
Treatments	3	2.563	P<.005
NC vs (PC + CSL + FACW)/3	1	7.260	P<.005
PC vs (CSL + FACW)/2	1	.240	P>.100
CSL vs FACW	1	.127	P>.250
Error	57	.161	------

AOV Table for Calf Weight Gain

Source of Variation	df	Mean Squares	P Value
Total	60	----------	------
Treatments	3	465.883	P>.10
NC vs (PC + CSL + FACW)/3	1	1113.032	P>.10
PC vs (CSL + FACW)/2	1	238.144	P>.250
CSL vs FACW	1	42.483	P>.250
Error	57	435.411	------

TABLE XII

ANALYSIS OF VARIANCE (PERFORMANCE OF YEARLING HEIFERS)

AOV Table for Heifer Weight Change

Source of Variation	df	Mean Squares	P Value
Total	31	----------	------
Treatments	3	7,070.875	P<.005
NC vs (PC + CSL + FACW)/3	1	17,876.042	P<.005
PC vs (CSL + FACW)/2	1	3,057.940	P<.005
CSL vs FACW	1	280.228	P>.250
Error	28	315.723	------

AOV Table for Heifer Condition Change

Source of Variation	df	Mean Squares	P Value
Total	31	----------	------
Treatments	3	.0927	P>.250
NC vs (PC + CSL + FACW)/3	1	.2321	P>.100
PC vs (CSL + FACW)/2	1	.0480	P>.250
CSL vs FACW	1	.0002	P>.250
Error	28	.1256	------

TABLE XIII

CHI-SQUARE ANALYSIS FOR LACTATING HEIFER AND YEARLING HEIFER PREGNANCY DATA

Analysis of Lactating Heifer Data					
	Treatment				
	NC	PC	CSL	FACW	Total
Heifers Pregnant	12	9	12	13	45
Heifers not Pregnant	2	6	3	3	14
Total	14	15	15	16	59
χ^2 = 3.35 with 3 d.f. ns					
Analysis of Yearling Heifer Data					
	Treatment				
	NC	PC	CSL	FACW	Total
Heifers Pregnant	5	4	7	5	21
Heifers not Pregnant	1	2	0	0	3
Total	6	6	7	5	24
χ^2 = 4.57 with 3 d.f. ns					

TABLE XIV

ANALYSIS OF VARIANCE (RUMEN pH, ONE AND FOUR HOURS POST-FEEDING)

AOV Table for Rumen pH, One Hour Post-feeding			
Source of Variation	df	Mean Squares	P Value
Total	39	------	-----
Treatments	3	.5663	P<.005
Error	36	.0332	------

AOV Table for Rumen pH, Four Hours Post-feeding			
Source of Variation	df	Mean Squares	P Value
Total	38	------	------
Treatments	3	.1514	P<.05
Error	35	.04284	------

TABLE XV

ANALYSIS OF VARIANCE (RUMEN AMMONIA CONCENTRATION, 1 AND 4 HOURS POST-FEEDING)

AOV Table for Rumen Ammonia, 1 hour post-feeding			
Source of Variation	df	Mean Squares	P Value
Total	39	--------	------
Treatments	3	672.295	P<.005
NC vs (PC + CSL + FACW)/3	1	767.754	P<.005
PC vs (CSL + FACW)/2	1	966.652	P<.005
CSL vs FACW	1	268.132	P<.005
Error	36	26.591	------

AOV Table for Rumen Ammonia, 4 hours post-feeding			
Source of Variation	df	Mean Squares	P Value
Total	39	--------	------
Treatments	3	641.805	P<.005
NC vs (PC + CSL + FACW)/3	1	1188.811	P<.005
PC vs (CSL + FACW)/2	1	389.691	P<.005
CSL vs FACW	1	346.861	P<.005
Error	36	22.134	------

TABLE XVI

ANALYSIS OF VARIANCE (SOLUBLE CARBOHYDRATE CONCENTRATION, ONE AND FOUR HOURS POST-FEEDING)

AOV Table for One Hour Post-feeding

Source of Variation	df	Mean Squares	P Value
Total	39	----------	-------
Treatments	3	170,170.532	P<.005
(NC + PC)/2 vs (CSL + FACW)/2	1	266,930.244	P<.005
NC vs PC	1	263.393	P>.250
CSL vs FACW	1	243,319.594	P<.005
Error	36	6,009.825	-------

AOV Table for Four Hours Post-feeding

Source of Variation	df	Mean Squares	P Value
Total	39	----------	-------
Treatments	3	2,485.379	P<.005
(NC + PC)/2 vs (CSL + FACW)/2	1	3,301.489	P<.005
NC vs PC	1	313.315	P>.250
CSL vs FACW	1	3,841.438	P<.005
Error	36	114.686	------

TABLE XVII

ANALYSIS OF VARIANCE (TOTAL VFA CONCENTRATION, ONE AND FOUR HOURS POST-FEEDING)

AOV Table for One Hour Post-Feeding			
Source of Variation	df	Mean Squares	P Value
Total	39	----------	-------
Treatments	3	.00001129	P=.9967
Error	36	.00063539	-------

AOV Table for Four Hours Post-feeding			
Source of Variation	df	Mean Squares	P Value
Total	39	----------	-------
Treatments	3	.00050343	P=.3543
Error	36	.00045001	-------

TABLE XVIII

ANALYSIS OF VARIANCE (ACETATE TO PROPIONATE RATIO, ONE AND FOUR HOURS POST-FEEDING)

AOV Table for One Hour Post-Feeding			
Source of Variation	df	Mean Squares	P Value
Total	39	-------	-------
Treatments	3	1.6485	P=.0061
Error	36	.3393	-------

AOV Table for Four Hours Post-Feeding			
Source of Variation	df	Mean Squares	P Value
Total	39	-------	-------
Treatments	3	2.9775	P=.0017
Error	36	.4813	-------

TABLE XIX

ANALYSIS OF VARIANCE (MOLAR PERCENT ACETATE, ONE AND FOUR HOURS POST-FEEDING)

AOV Table for One Hour Post-feeding

Source of Variation	df	Mean Squares	P Value
Total	39	--------	-------
Treatments	3	21.0085	P=.0178
Error	36	5.4941	-------

AOV Table for Four Hours Post-feeding

Source of Variance	df	Mean Squares	P Value
Total	39	--------	-------
Treatments	3	251.3756	P=.0001
Error	36	12.1300	-------

TABLE XX

ANALYSIS OF VARIANCE (MOLAR PERCENT PROPIONATE, ONE AND FOUR HOURS POST-FEEDING)

AOV Table for One Hour Post-feeding

Source of Variation	df	Mean Squares	P Value
Total	39	-------	-------
Treatments	3	24.0522	P=.0019
Error	36	3.9812	-------

AOV Table for Four Hours Post-feeding

Source of Variation	df	Mean Squares	P Value
Total	39	-------	-------
Treatments	3	16.4054	P=.0468
Error	36	5.6051	-------

TABLE XXI

ANALYSIS OF VARIANCE (MOLAR PERCENT ISOBUTYRATE, ONE AND FOUR HOURS POST-FEEDING)

AOV Table for One Hour Post-feeding			
Source of Variance	df	Mean Squares	P Value
Total	39	-------	-------
Treatments	3	1.8370	P=.0001
Error	36	.2022	-------

AOV Table for Four Hours Post-feeding			
Source of Variance	df	Mean Squares	P Value
Total	39	------	-------
Treatments	3	.9018	P=.0096
Error	36	.2040	-------

TABLE XXII

ANALYSIS OF VARIANCE (MOLAR PERCENT BUTYRATE, ONE AND FOUR HOURS POST-FEEDING)

AOV Table for One Hour Post-feeding

Source of Variation	df	Mean Squares	P Value
Total	39	--------	-------
Treatments	3	10.1734	P=.0008
Error	36	1.4698	-------

AOV Table for Four Hours Post-feeding

Source of Variation	df	Mean Squares	P Value
Total	39	--------	-------
Treatments	3	187.2339	P=.0001
Error	36	2.7918	-------

TABLE XXIII

ANALYSIS OF VARIANCE (MOLAR PERCENT ISOVALERATE, ONE AND FOUR HOURS POST-FEEDING)

AOV Table for One Hour Post-feeding

Source of Variation	df	Mean Squares	P Value
Total	39	------	-------
Treatments	3	1.5327	P=.0001
Error	36	.1166	-------

AOV Table for Four Hours Post-feeding

Source of Variation	df	Mean Squares	P Value
Total	39	------	-------
Treatments	3	2.1404	P=.0001
Error	36	.1131	-------

TABLE XXIV

ANALYSIS OF VARIANCE (MOLAR PERCENT VALERATE, ONE AND FOUR HOURS POST-FEEDING)

AOV Table for One Hour Post-feeding			
Source of Variation	df	Mean Squares	P Value
Total	39	------	-------
Treatments	3	.1533	P=.0156
Error	36	.0388	-------

AOV Table for Four Hours Post-feeding			
Source of Variation	df	Mean Squares	P Value
Total	39	-----	-------
Treatments	3	.4238	P=.5359
Error	36	.5737	-------

TABLE XXV

ANALYSIS OF VARIANCE (MOLAR PERCENT CAPROATE, ONE AND FOUR HOURS POST-FEEDING)

AOV Table for One Hour Post-feeding

Source of Variation	df	Mean Squares	P Value
Total	39	------	-------
Treatments	3	.007191	P=.2135
Error	36	.004581	-------

AOV Table for Four Hours Post-feeding

Source of Variation	df	Mean Squares	P Value
Total	39	------	-------
Treatments	3	.180560	P=.0149
Error	36	.045218	-------

TABLE XXVI

ANALYSIS OF VARIANCE (*IN VITRO* AMMONIA ACCUMULATION)

AOV Table for Ammonia Accumulation			
Source of Variation	df	Mean Squares	P Value
Total	88	--------	-------
Nitrogen Source	5	7827.0238	P<.005
Incubation Time	4	539.0104	P<.005
Nitrogen X Time	20	378.7809	P<.005
Error	59	31.8467	-------

VITA

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Master of Science

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