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The object of this research was to study the effect of readily available carbohydrates (RAC) upon the utilization of urea in fattening rations and to determine the effect upon carcass characteristics that determine quality and yield grades. A 2 x 2 latin square designed feedlot trial comparing urea at 0.5% and 1.5% and molasses at 2.5% and 7.5% of the ration resulted in a significant interaction ($p < .01$) for rumen ammonia, acetic, valeric, and isovaleric acids, total VFA, and butyric acid ($p < .05$) concentrations. Urea and molasses additions significantly ($p < .01$) increased acetic and propionic acid levels but molasses decreased ($p < .05$) the molar % of acetic acid.

Carcass characteristics were not affected by the urea or molasses treatments. Individual VFA concentrations had a low correlation to carcass characteristics, accounting for less than 20% of the total variability.

In a standard metabolism trial comparing fattening rations containing 0% or 1.5% urea and varying levels of RAC from 42% to 53%, the apparent digestibility of the feed components was not significantly ($p > .1$) affected by substituting 1.5% urea for natural protein. The percent of absorbed N retained increased 0.90% for each 1% increase in RAC, yielding a regression equation, $y = 64.98 + 0.8134 (X-47)$. The percent N retained increased 0.82% for each 1% increase in RAC and can be expressed by the regression equation, $Y = 46.82 + .7471 (X-47)$. This would suggest as the RAC level of the ration increased urea is more efficiently incorporated into protein.

The Effect of Varying Levels of Readily Available Carbohydrates on Urea Utilization

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INTRODUCTION

The ability of ruminants to convert non-protein nitrogen into protein was demonstrated before the turn of the century by Weiske et al. (1879). Practical use of this knowledge has been applied by nutritionists for the last 25 years or so. The current annual feed use of urea is estimated at 200,000 tons in the United States. It is increasing annually due to advanced knowledge on the use of non-protein nitrogen and increased competition for protein from other species, including man.

When we consider the ruminant's ability to convert feed protein to animal protein, it becomes obvious that only by producing milk or using non-protein nitrogen are they going to be competitive with other domestic species. The ruminant's low reproductive efficiency, when compared to the multiple offspring of swine and poultry, also contributes to this competitive gap.

The world shortage of protein for man and non-ruminants will probably continue to increase. Since the world's population is expected to double by the year 2000, the price of protein is likely to rise to a level such that inefficient animals will be eliminated from competition. The ruminant will be left in competition only through

the use of non-protein nitrogen and their ability to use roughages that other animals and man can use only after considerable processing.

Within the last ten years the emphasis in urea research has shifted from the preliminary focus on proving urea could be used as protein replacement to study of the nutritive factors that affect the efficiency of urea utilization. The amount of urea that can be efficiently used depends upon the toxic level of urea, the quantity and availability of protein, the amount and kind of carbohydrates present in the ration, and probably other unknown factors.

Rumen microorganisms need an adequate source of carbon and energy to maintain populations of sufficient numbers to incorporate available rumen ammonia into cellular protein. Carbohydrates normally comprise the major portion of ruminant rations. Therefore, they contribute significantly to the efficient use of non-protein nitrogen by supplying carbon skeletons and energy for rumen microorganisms. It has been accepted for some time that readily available carbohydrates are superior to cellulose and other slowly fermented carbohydrates for microbial non-protein nitrogen utilization. The literature is lacking in reports discussing the level of readily available carbohydrates that will most efficiently support urea utilization.

This research was conducted to study the effect of varying the

level of readily available carbohydrates and the amount of nitrogen furnished by urea on production of rumen volatile fatty acids and ammonia, animal performance, and carcass characteristics of steers fed under practical feedlot conditions. A follow-up study was also conducted to examine the effect of varying levels of readily available carbohydrates in high concentrate rations on digestibility and nitrogen balance when urea was supplying approximately 30% of the total nitrogen.

REVIEW OF LITERATURE

The feeding of non-protein nitrogen has been extensively explored by researchers throughout the world. Stangel et al. (1963) have reviewed this mass of literature and the book edited by Briggs (1967) follows with an excellent review and summary on a variety of topics related to nutritional aspects of urea and related compounds. Other review articles on non-protein nitrogen and nitrogen utilization include those by Reid (1953), Hungate (1966), Blackburn (1965), Phillipson (1964), Waldo (1968), Oltjen (1969) as well as numerous other papers. Because of the large volume of literature on feeding non-protein nitrogen to ruminants, this review will discuss only nitrogen utilization and the interrelationships of carbohydrates in feeding non-protein nitrogen, primarily as urea.

In the ruminant, nitrogen metabolism cannot be considered separately from carbohydrate digestion. This interrelationship between nitrogen and carbohydrates can influence their utilization as well as feed intake. Excess soluble nitrogen produces high rumen ammonia levels which reduce feed intake.

Ammonia Production

Nitrogen enters the rumen from three avenues: (1) ingested food, (2) saliva, (3) recycled urea by diffusion from the blood. Urea

can pass directly from the blood into the rumen (Packett and Groves, 1965; Cocimano and Long, 1967). Houpt (1959) found that 52% of intravenously infused urea was not recovered in the body fluid or urine and assumed it had been synthesized into protein by rumen microorganisms. He calculated that the mean rate of absorption was 2.4 mM. of ammonia-N/hour for sheep and states that 16 times as much nitrogen passes directly from the blood to the rumen as saliva supplies. The primary path of entry is diffusion through the rumen epithelium (Houpt, 1959; and Moir and Harris, 1962), but blood urea can also enter the abomasum and intestines (LeBars, 1967). Urea which diffuses into the abomasum can be partially used by the animal (McDonald, 1948). The rumen epithelium has urease activity that converts urea to ammonia; Rahman and Decker (1966) postulate that the urease in the rumen epithelium originates from bacteria. Houpt and Houpt (1968) agree with this conclusion and suggest that some ruminal urease penetrates the rumen epithelium.

Small amounts of urea enter the rumen by saliva. The presence of salivary urea was demonstrated by McDonald (1948) and Lewis (1957) reported that salivary urea concentration increased when rumen ammonia levels increased. Somers (1961) showed that the nitrogen secreted from the parotids was fairly constant when the dietary nitrogen intake was above 10 gm. per day. This would amount to approximately 0.5 gm. per day; therefore, supplies of

nitrogen from this source are of minor importance.

The major source of nitrogen in the rumen is ingested food. It may be either protein-N or non-protein-nitrogen. The amount of the total nitrogen found as non-protein nitrogen (NPN) in feed is variable, ranging from 4-5% in mature corn to 60-70% in unwilted silage (Waldo, 1968). The protein-nitrogen content is also variable depending upon maturity, source, and availability. The availability or solubility of proteins is important as it affects the amount and rate of nitrogen released. The breakdown of proteins varied from 40% of zein (McDonald, 1954) to over 90% of casein (McDonald and Hall, 1957). Rumen amino acids are low because microbial deaminases rapidly break them down to ammonia and volatile fatty acids (el-Shazly, 1952; Lewis, 1955; Warner, 1956). Otagaki et al. (1955) using ^{14}C labeled casein in an in vitro study, found that labeled ^{14}C fatty acids and ammonia were released. Nitrates may also contribute to rumen ammonia levels although generally not in significant amounts (Lewis, 1951a, b).

With diets containing urea, the enzymatic degradation of urea by bacterial urease to rapidly produce ammonia and carbon dioxide is an important source of rumen ammonia. Pearson and Smith (1943) demonstrated with an in vitro system that a consistently high rumen urease level was present when 100 gm. of rumen contents converted 100 mg. urea per hour to ammonia. Jones et al. (1964) also

demonstrated a high level of urease activity; by using differential centrifugation they showed that the urease activity was associated with the cellular fraction of the rumen contents. They also found that all fractions of bacteria showed urease activity although heavier fractions exhibited the most activity. Rumen protozoa show little if any urease activity (Jones et al., 1964; Abou Akkada and Howard, 1962). Soejima et al. (1958) reported that protozoa may show urease activity after a three hour lag period, but this would appear to be of minor importance due to the high bacterial urease activity.

One of the problems in feeding urea or NPN is to obtain efficient use of ammonia. Unless optimum conditions are maintained, urea hydrolysis can occur four times faster than uptake of ammonia by rumen microorganisms and this results in a loss of ammonia from the rumen (Bloomfield et al., 1960). Ammonia is absorbed across the rumen wall and carried by way of the portal system to the liver where it is converted to urea. Usually, only small quantities of ammonia are found in the peripheral blood. When rumen ammonia levels exceed 60 mM. per 1. of contents, ammonia begins to appear in the peripheral blood (Lewis et al., 1957). Toxic symptoms occur when ammonia concentrations reach 0.6 to 2.4 mM. per 1. peripheral blood. These include respiratory difficulty, increased salivation, muscle tremors, incoordination, and death in severe cases (Dinning, 1948; Davis and Roberts, 1959; Davis and Roberts, 1954; Whitehair

et al., 1955). The physiological cause of death is generally considered to be a complex reaction by (1) direct toxic action of the ion, (2) disturbance of the acid-base balance, and (3) a change in the electrolite balance (Lewis, 1961). Papers by Hale and King (1955) and King and Hale (1955) showed similar symptoms when ammonium carbamate was injected orally or intravenously. Lewis (1960) suggests that ammonium carbamate is unstable in the rumen environment and that ammonia would be released. Visek (1968) supports Lewis's conclusions and discusses the effect of ammonia on energy metabolism in the brain.

Preventing toxicity can be accomplished by following proper feeding practices which include safe levels in feed, proper mixing, and regular feeding. Adaptation to urea feeding and supplying a "high" level of readily available carbohydrates in the ration are important in reducing rumen ammonia (Barth, 1962; McLaren et al., 1965; McDonald, 1952; Coombe and Tribe, 1958; Annison et al., 1954).

In summary, rumen ammonia concentration is a balance between utilization by rumen microorganisms, absorption across the rumen wall into the portal system, and passage down the gastrointestinal tract and the ammonia produced by fermentation of food, both protein and non-protein nitrogen, saliva, and urea recycling into the rumen. These factors contribute to rumen ammonia levels ranging

from 0 to 130 mg. % (Johns, 1955).

Carbohydrates

To achieve the most efficient utilization of nitrogen, an energy level has to be supplied that will support optimal microbial growth and supply carbon fragments for protein synthesis. Hart et al. (1938) showed that heifers receiving 12% protein equivalent from urea or casein made equivalent growth. The following year, Hart et al. (1939) demonstrated that the addition of readily available carbohydrate (RAC) improved the utilization of urea nitrogen. Since then much research has been conducted to determine the optimum conditions for urea utilization.

Readily available carbohydrates are required for the utilization of NPN and other carbohydrates present in the ration. The fibrous components, particularly, will not successfully serve this purpose. Wegner et al. (1940), using an in vitro system, demonstrated that corn molasses, dextrose and starch, but not cellulose, produced a reduction of inorganic nitrogen in the system. The recovered bacterial cell nitrogen indicated an increase in bacterial protein. Belasco (1956), using an in vitro technique, concluded that urea utilization was dependent upon the amount and type of carbohydrate present and starch was superior to cellulose in promoting urea utilization. Xylan and pectin promoted less urea utilization but had

no cellulolytic response. Mills et al. (1942) concluded that only when adequate RAC is present in the ration can urea be used at a maximum rate and efficiency. Reis and Reid (1959) supported this conclusion. Deyoe et al. (1968) found by passing urea and starch through a cooker-extruder that the product was comparable to soybean as a protein supplement, indicating improved N utilization as a result of the physical form and/or the cooking of the starch.

Aris et al. (1951), using starches, sugars and cellulose in an in vitro study, showed each carbohydrate source aided urea utilization. Small amounts of RAC increased cellulose digestion, but large amounts decreased it. He concluded that rations containing high cellulose content should not have high RAC levels (above 30%) because of lower cellulose digestion over an extended period. However, Chappel and Fontenot (1965), using purified diets, reported no significant differences in cellulose digestibility with rations containing 0-32% RAC. McDonald et al. (1965) showed that cellulose digestion was improved when urea replaced one third of the natural protein in diets containing 52% RAC. Owen (1967), summarizing the literature on carbohydrates and urea utilization, concludes that starch is superior to soluble sugars in promoting cellulose digestion but a mixture of sugars and starch is better than either by itself. When urea replaces approximately one third of the protein, cellulose digestion is increased over rations containing only natural protein.

Much evidence has been presented that starch is superior to soluble sugars in promoting ammonia utilization in the rumen (Mills et al., 1942; Pearson and Smith, 1943; Virtanen, 1966; Chalupa, 1968; and others). Starch remains in the rumen two to three times as long as glucose. Phillipson and McAnally (1942) showed that starch is fermented slower than sugars and the production of VFA in the rumen is prolonged. Therefore, by supplying a source of energy for growth and carbon fragments for protein synthesis over an extended period, starch supports a larger microbial population and more ammonia is utilized.

Very little is to be found in the literature on the quantity of RAC needed for the maximum utilization of urea nitrogen. Virtanen (1966) believes that approximately 1 kg. of RAC is needed for each 100 gm. of urea in an adapted cow with 70% of the RAC supplied by starch. When the starch dropped below this level, production fell. McLaren et al. (1965), using semipurified diets, found the retention of absorbed nitrogen to be improved approximately 2% for each 100 kcal. of RAC in the ration, and this was not affected by their adaptation to urea. Barth (1962) found a linear increase of approximately 60% in nitrogen retention when the RAC level was increased from 1,200 to 1,900 kcal. per day. Over this range, nitrogen retention was improved 3% for each 100 kcal. of RAC added to the ration. Barth concluded that this relationship would become curvilinear when

maximum nitrogen retention was obtained.

Volatile Fatty Acids

Depending upon the diet, 40-70% of the total digestible dry matter is broken down by rumen microorganisms (Hale et al., 1947; Phillips et al., 1960; Hinders and Owens, 1964). The major end products of carbohydrate fermentation in the rumen are VFA and gases--primarily methane and carbon dioxide (McNaught, 1951). The degradation of protein (amino acids) produces VFA and ammonia (el-Shazly, 1952; Annison, 1954). The VFA are used for microbial growth and protein synthesis in the rumen, or they are absorbed through the rumen wall and lower gastro-intestinal tract and used for cellular energy (Hungate, 1966).

Evidence suggests that VFA are essential for the growth of some rumen microorganisms (Allison et al., 1962; Bentley et al., 1954; Bentley et al., 1955; Bryant and Robinson, 1961; and others). Bryant and Robinson (1963) demonstrated that a majority of rumen bacteria require or can use ammonia nitrogen as well as VFA for the synthesis of their cellular components. Cline et al. (1966) found that short-chain VFA additions to diets containing urea increased apparent digestibility and retention of nitrogen and cellulose and dry matter digestibility. Hoover et al. (1963) determined that VFA were incorporated into bacterial protein and polysaccharides, and that

acetate was incorporated into protein at the highest rate. Hemsley and Moir (1963) suggest that additions of isobutyric, isovaleric and n-valeric acids not only stimulate protein synthesis but directly stimulate microbial growth.

Rumen bacteria, especially cellulolytic and other species that depend upon ammonia nitrogen, require branched-chain VFA for growth and protein synthesis (Wagner and Foster, 1963; Oltjen and Putnam, 1966). In the rumen, branched-chain VFA are used for synthesis of bacterial protein. Some examples are the synthesis of leucine from isovaleric acid (Allison et al., 1966), isoleucine from 2-methyl butyrate (Hungate, 1966), valine from isovaleric acid (Bryant and Robinson, 1963). Cline et al. (1966) demonstrated that isobutyric and isovaleric acid increased cellular nitrogen. Dehority et al. (1967) supports this work and segregates cellulolytic bacteria according to their VFA requirements. This requirement for certain branched-chain VFA may become a limiting factor in rations where substantial quantities of protein are replaced by non-protein nitrogen.

Purified Diets

Some interest has been shown in feeding diets with non-protein nitrogen as the only source of nitrogen. Loosli et al. (1949) and Duncan et al. (1953) demonstrated that rumen microorganisms synthesize all essential amino acids when animals were fed diets with

urea as the only nitrogen source. Rumen bacteria can generally use ammonia as the only source of nitrogen. Bryant (1961) found that 80% of the 44 strains of bacteria studied could use ammonia as a source of nitrogen, 26% could use only ammonia nitrogen, and 55% could use either ammonia or amino nitrogen.

Purified diets have been used extensively to study the utilization of urea vs. protein. With adequate RAC levels, production has been comparable in some cases (Virtanen, 1966). Most evidence indicates purified diets are 60% to 70% as efficient in sustaining growth when nitrogen is supplied as NPN instead of amino nitrogen (Loosli et al., 1949; Oltjen et al., 1962; and Clifford and Tillman, 1968). In some cases the addition of amino acids did not improve growth (Harbers et al., 1961; Oltjen et al., 1962; Clifford et al., 1967; and McDonald, 1966). Bunn and Matrone (1968) report improved growth with 5% alfalfa supplementation and Barth et al. (1954) reported growth responses to methionine and tryptophan. Briggs (1967) suggested this growth depression may be due to a shortage of branched-chain VFA. Cline et al. (1966) received a growth response to iso-valeric, iso-butyric and n-valeric acids. Byers (1968) reported a lowering in the quantity of some plasma amino acids when urea supplied above 45% of the nitrogen. This work suggests that certain amino acids are not synthesized in quantities to support growth comparable to natural protein, but this aspect certainly needs more investigation.

EXPERIMENTAL PROCEDURE

Feed Lot Trial

Thirty-seven choice quality commercial feeder steers weighing approximately 230 kg. were divided into three lots of nine steers each and one lot of ten steers. During an adjustment period of 30 days the steers were brought to full feed on complete pelleted rations. The composition of the rations is shown in Table 1. The steers were self-fed and had free access to feed and water throughout the trial. They were weighed every 14 days and the trial was terminated for each steer when a weight of approximately 478 kg. was reached.

All steers were slaughtered in the O.S.U. meats laboratory. Samples were taken from the rumen immediately upon removal from the carcass. The rumen fluid was centrifuged at 12,500 r.p.m. for 20 minutes, and the supernatant frozen until analyzed. Carcass information was obtained for each animal. Those characteristics which affect yield grade and quality grade were analyzed for this study.

Rumen samples were analyzed for ammonia-nitrogen content. The Van Slyke and Cullen modified method using 5 ml. of rumen liquor was used (Oser, 1965). Samples were analyzed immediately upon thawing to reduce ammonia loss.

Rumen liquor was prepared for VFA determination by adding

Table 1. Composition of rations used in the feed-lot trial.

Ingredients	Ration No. 1	2	3	4
	Composition			
	%	%	%	%
Alfalfa hay, ground	15	12.5	4.5	6.25
Grass hay, ground	7	10	16.25	16.5
Cottonseed meal	3	4.5	--	1
Mustard seed, ground	7	8	2.5	4
Wheat mill run	1	1	1	1
Barley, steam-rolled	36	23	40	30.75
Wheat, steam-rolled	27.5	31.5	30	29
Molasses	2.5	7.5	2.5	7.5
Urea	.5	.5	1.5	1.5
Tallow	.5	.5	1.25	2.5
Premix*				

*Premix supplies 20 mg./kg. of terrmaycin and 2200 I.U./kg of Vitamin A.

Estimated Analysis**

Digestible Protein (%)	10.0	10.0	10.0	10.0
Crude fiber (%)	10.16	9.92	10.03	10.08
Readily available carbohydrate (%)	50.4	47.2	50.4	49.6
Digestible energy Kcal./gm.	3.15	3.15	3.15	3.15

**Calculated from Morrisons Feeds and Feeding or other suitable sources of information. RAC values were analytical values.

1 ml. of metaphosphoric acid (25%) to 5 ml. of previously centrifuged rumen fluid. After standing for 30 minutes the solution was centrifuged at 12,500 r.p.m. for 20 minutes (Erwin et al., 1961). The supernatant was refrigerated until analyzed. VFA analysis was done using the procedure of Baumgardt (1964) with a Varian Aerograph gas chromatograph equipped with a hydrogen flame detector. The chromograph contained 20% neopentylglycol succinate, 2% H_3PO_4 on 60-80 mesh firebrick column 0.318 cm. in diameter and 1.525 m. in length. Operating conditions were nitrogen flow 26 ml./min., hydrogen flow 30 ml./min., oven temperature 140°C., detector temperature 200°C., injector temperature 185°C., and attenuation 32x. The sample size injected varied from 0.3-0.4 µl. of liquor depending on the peak height which most closely compared to that of the standard solution. Concentrations of individual acids and molar percents were calculated.

The statistical design was a 2 x 2 Latin square using an analysis of variance. Correlation coefficients were determined between carcass characteristics and VFA concentrations as described by Little and Hills (1966).

Metabolism Trial

Two metabolism trials were conducted with commercial wether lambs averaging 38 kg. and of uniform condition. The lambs

were placed in individual metabolism stalls similar to those described by Briggs and Gallup (1949). Because the lambs had primarily grazed poor quality pastures, a 28 day initial adjustment period was used. This consisted of slowly replacing good quality alfalfa hay with the test rations. The feeding level to be used during the trial was established at the amount of feed that would be consumed by all lambs, 750 gm./day, and this amount was fed five days preceding the trial. The lambs were consuming about 2% of their body weight. During the second trial a 14-day adjustment period was used following a switch-back of the rations. The same feed level was used for this trial.

The composition of the rations in this trial is presented in Table 2. The rations varied in the percentage of RAC between 40-53% and contained either none or 1.5% urea. Each ration contained approximately 14.5% crude fiber and were essentially isonitrogenous and presumed to be isocaloric. All rations were pelleted. The rations were prepared in the O.S.U. feed mill and mixed in one half ton or larger batches.

The rations were fed in equal amounts twice daily and the lambs had free access to water between feedings. Any "fines" left at the end of the trial were weighed and deducted from the feed consumed; the amount was less than 50 gm. in all cases. During each trial, representative samples of each ration were taken and proximate analyses were performed by A.O.A.C. (1960) procedures.

Table 2. Composition of rations used in metabolism trial.

Ingredients**	Ration No.					
	1	2	3	4	5	6
	Composition					
	%	%	%	%	%	%
Alfalfa, ground	17.5	17	10.5	10.5	20	13
Grass hay, ground	--	--	4.5	4.5	10	17
Beet pulp	15	20.75	10	11.5	10	16.5
Cottonseed meal	8.75	--	10	--	8	--
Mustard seed, ground	6.5	5.5	1	1.5	6	6
Wheat millrun	--	--	--	--	12.5	12.5
Barley, steam-rolled	31	41	20.5	30	25	25
Wheat, steam-rolled	11.25	3.75	34	31	--	--
Tallow	1.5	2	1	1	--	--
Molasses	7.5	7.5	7.5	7.5	7.5	7.5
Urea	--	1.5	--	1.5	--	1.5
Premix*	1	1	1	1	1	1

*Premix supplies trace mineral salt, dicalcium phosphate, 20 mg. Terrmaycin/kg., and 2200 I.U./kg. Vitamin A.

**All ingredients pelleted.

Determination of RAC was by the method of Friedemann et al. (1967). Energy was determined with a Parr Oxygen-bomb calorimeter. The chemical analyses of the rations are presented in Table 3.

Feces and urine were collected throughout a seven day period. Samples equal to 10% of the daily excreta were taken for analysis. To minimize urinary nitrogen losses, 10 ml. of 4 N sulfuric acid was placed in the urine collection bottles before each collection. Samples were kept under refrigeration until analyzed.

The feces and urine were analyzed for nitrogen by the Kjeldahl method (Oser, 1965). The feces were also subjected to a complete proximate analysis and energy determination. Some difficulty was experienced in getting the feces samples to burn completely in the bomb calorimeter. This problem was overcome by reducing the sample size to 0.5 gm. and oxygen pressure to 10 atmospheres.

The results were analyzed by an analysis of variance. Regression analysis equations were determined for nitrogen retention and crude fiber as described by Little and Hills (1966).

Table 3. Chemical analysis of rations used in metabolism trial.

Feed	% Dry matter	Percent composition of dry matter					Energy Kcal./gm
		Crude protein	Crude fiber	Either extract	Ash	RAC	
Ration 1	86.5	16.1	15.2	5.7	5.6	41.7	4.50
Ration 2	86.7	16.1	14.4	5.8	5.0	43.1	4.50
Ration 3	85.8	15.6	13.6	3.3	4.8	52.8	4.48
Ration 4	85.6	15.8	13.7	5.0	4.8	51.2	4.51
Ration 5	84.6	15.5	15.1	5.5	4.6	46.0	4.45
Ration 6	84.5	16.2	15.9	3.2	4.8	47.1	4.45

RESULTS AND DISCUSSION

The effect of RAC on the utilization of urea-containing diets will be discussed in two phases. The first phase is a feed-lot trial in which the levels of urea and molasses were varied; rumen VFA concentrations and ammonia levels were measured. Gain and carcass characteristics were also determined and correlated to rumen measurements. The second phase was a metabolism trial in which the level of RAC was varied in rations and apparent digestibility of the components of the various rations were measured.

Feed-Lot Trial

Rumen Ammonia

The mean values for rumen ammonia concentrations for each ration are presented in Table 4. The concentration of ammonia found in the rumen were on the order of 0.09-0.16 mg./ml.; these differences were not significant ($p > .1$) between rations due to the variability that existed between animals. These values are comparable to those reported by Oltjen and Davis (1965) and Oltjen and Putnam (1966) when they fed fattening rations.

The effect of urea and molasses on rumen ammonia concentrations are summarized in Appendix Table 1. The rumen ammonia concentrations were not significantly ($p > .05$) affected by increasing

Table 4. Mean values for rumen contents of steers in the feed-lot trial.

Item	Ration Number 1	2	3	4
VFA concentration, mM./100 ml.				
Acetic acid	7.76[a]	12.04[b]	13.09[b]	13.03[b]
Propionic acid	3.30[a]	7.56[b]	5.87[ab]	8.52[b]
Butyric acid	1.58[a]	2.25[b]	2.52[b]	2.18[b]
Valeric acid	0.15[a]	0.67[b]	0.82[b]	0.64[b]
Isovaleric acid	0.17[b]	0.15[b]	0.13[b]	0.00[a]
Total VFA	12.96[a]	22.67[b]	22.43[b]	24.37[b]
VFA molar %				
Acetic acid	61.3[a]	53.4[b]	58.0[b]	55.4[b]
Propionic acid	23.3[a]	33.2[b]	26.2[ab]	35.6[b]
Butyric acid	13.1	9.9	11.3	8.1
Valeric acid	1.0	2.9	3.8	0.9
Isovaleric acid	1.3	0.6	0.7	0.0
Rumen Ammonia NH_3-N/ml. rumen fluid, mg.				
	0.11	0.16	0.15	0.09

[a, b] Means on the same line within a subgroup bearing different subscript letters differ significantly ($p < .05$).

the amount of nitrogen supplied by urea. They were decreased significantly ($p < .01$) with an increase in molasses. There was also a significant interaction between the molasses and urea treatments.

Oltjen and Davis (1965) showed no difference in rumen ammonia levels between soybean meal or urea-containing all-concentrate diets. Little et al. (1968) supported these findings under feed-lot conditions. Many researchers disagree and find that urea increases the rumen ammonia levels, but such observations have been made with rations containing higher amounts of roughage (Coombe and Tribe, 1962; Freitag et al., 1968; Stallcup and Loper, 1958). Chalupa (1962) concluded that the solubility of the protein or NPN affects rumen ammonia levels and that soluble proteins may be hydrolyzed at rates comparable to urea, causing similar rumen ammonia levels.

Coombe and Tribe (1962) showed that 3% molasses lowered rumen ammonia levels when urea was added to a poor quality roughage. Mills et al. (1944) reported that molasses increased the ruminal protein levels. Bell et al. (1951) supported this finding. Gallup et al. (1954) also reported that molasses increased urea utilization.

This interaction is present because with 0.5% urea in the diet an increase in molasses from 2.5% to 7.5% caused an increase in rumen ammonia concentration. With 1.5% urea in the diet, a similar increase in molasses caused the opposite affect. Wegner et al. (1941); McNaught and Smith (1949); Reid (1953) and others have

shown when the level of protein in the ration exceeds 10% to 12%, urea is inefficiently used. When molasses replaces starch in the ration, ammonia nitrogen is not used as efficiently (Mills et al., 1944; Lewis and McDonald, 1958; Bell et al., 1951; and Bloomfield et al., 1958). Britton et al. (1968) report that additions of small amounts of molasses in semi-purified diets containing urea increased fiber digestibility and nitrogen retention.

In this experiment, the rations with 0.5% urea may have contained enough natural protein to meet the microorganisms' requirement for nitrogen. With these rations,an increase in molasses actually decreased the RAC level in the ration due to a decrease in the amount of cereal grains, possibly resulting in a microbial population that could not utilize ammonia-nitrogen as efficiently as the ration containing a higher RAC level. This resulted in an increase in rumen ammonia level from 0.11 to 0.16 mg./ml. of rumen contents. However with rations containing 1.5% urea, 30% of the protein equivalent, increasing the molasses changed the RAC level very little, 50.4 to 49.6%, but it reduced the rumen ammonia level from 0.15 to 0.09 mg./ml. of rumen contents. This may indicate that a balance more conducive to ammonia utilization by rumen microorganisms has been established between starch and soluble sugars. Bohman et al. (1954) and Owen (1967) conclude that a combination of molasses and starch is better than either individually in promoting

growth with urea supplemented rations.

Rumen Volatile Fatty Acids

In discussing VFA production the reader is cautioned that there are many variables that affect the concentrations and molar percentages of these acids. Rumen bacterial and protozoa populations are affected by the solubility of carbohydrates, the mixture of carbohydrates, the amount of protein, the solubility of the protein, the ammonia-nitrogen available, the energy level of the diet and many other factors. The microbial population sustained in the rumen determines the amount and proportion of VFA found in the rumen contents. Satter et al. (1967) reported on the effect of dietary changes and associated changes in rumen microorganisms when polysaccharides were added to the ration. They also noted an altered fermentation pattern of cellulose and hemicellulose. Church (1969) concluded that different diets support different microbial populations and that the end-product of different organisms differ, resulting in changes in VFA production.

The mean VFA and ammonia values of the rumen contents for each treatment group are presented in Table 4. Ration 1 is significantly lower in all VFA concentrations measured except isovaleric. The natural protein-containing diets produces higher concentrations of this acid ($p < .05$) than the urea-containing diets.

The total concentration of VFA was increased significantly ($p < .01$) as the amount of urea increased from 0.5% to 1.5% in the ration. There was also a significant increase ($p < .01$) in the concentration of acetic and propionic acids. The other VFA or the molar percentages were not significantly ($p > .1$) affected by the urea treatment. The statistical analysis is presented in Appendix Table 1.

As the amount of molasses increased in the ration, there was a significant increase ($p < .01$) in the concentration (mM. %) of acetic, propionic, and isovaleric acids. There was a significant decrease ($p < .01$) in the molar percentage of acetate and increase in the molar percentage of propionate. There was no statistically significant effect on the concentration or molar percentage of the other VFA measured. A significant ($p < .01$) interaction occurred between urea and molasses treatments and the concentration of acetic, butyric, valeric and isovaleric acids, and total VFA levels.

The concentration of VFA found in this work is considerably higher than the concentrations reported by many other workers using similar rations (Church, 1969; Oltjen and Davis, 1965). Rations 1, 2, 3, and 4 had total concentrations of 12.9, 22.6, 22.4 and 24.3 mM. % and most workers report concentrations ranging from 9 to 12 mM. % of rumen contents. These higher values were probably partly caused by the difference in sampling technique. Stomach tube

samples are contaminated with saliva and produce lower values (Lane et al., 1968). Bloomfield et al. (1963) reported VFA are absorbed at the rate of .4 to 1.8 mM. %/hr. between pH 6.2 and 7.6 and samplings from the slaughtered animal would cause some increase due to inhibited absorption from the rumen. The high level of protein in these rations may also be a factor, as several reports (Shaw et al., 1959; Davis et al., 1957) indicate an increase in VFA as protein levels increase.

Uesaha et al. (1968) reported increased propionic acid concentrations, narrower acetate/propionate ratios and increased total VFA concentrations with urea supplements to high concentrate rations. Oltjen and Davis (1965), Satapathy and Leffel (1962) and Satapathy et al. (1964) showed increased total VFA levels and increased propionate production with fattening rations. Stewart and Schultz (1958) found that urea consistently increased VFA production in vitro regardless of substrates, but the increases were not as large in vivo. Some reports show that urea has no effect, or decreases total VFA concentration (Raun, 1962; Davis and Stallcup, 1967; and Clifford and Tillman, 1968). These results may be attributed to inefficient use of ammonia due to a deficiency of RAC, or rations containing enough natural protein to support growth without the addition of urea. The type of carbohydrate used to replace the energy of the protein also affects the VFA concentrations (Briggs, 1967).

Both cellulolytic and amyolytic bacteria have a requirement for ammonia nitrogen (Hungate, 1966). Bruggemann et al. (1962), using in vitro system, reported that cellulolytic and amylolytic activity of mixed rumen microorganism increased when urea replaced soybean meal as a supplement for cattle. Belasco (1954), Chalupa et al. (1963), and Oltjen and Putnam (1966) reported that improvement in starch and cellulose digestion resulted in increased acetate and propionate levels. Bennett and Elliott (1959) demonstrated that urea improved the fermentation of all carbohydrates, resulting in increased total VFA as well as acetic, propionic and butyric concentrations over the control.

The literature indicates that molasses decreases acetate production and increases propionate and butyrate production (Steward and Schultz, 1958; Meriono et al., 1965; Parks et al., 1964). The increase in acetate production noted in the experiments reported herein was due to the interaction between urea and molasses. This resulted because the low-urea, low-molasses ration was not utilized as efficiently by rumen organisms. Increasing either urea or molasses improved fermentation to a level that was not improved upon by a combination of the two. This same type of interaction also appeared with butyric and valeric acids. Beames (1959) and Coombe and Tribe (1962) also observed interactions between urea and molasses. There are many reports of molasses and urea

combinations being used efficiently at high levels (Putnam et al., 1964; Thompson, 1961; Bradly et al., 1962; and others).

Isovaleric acid comes from the degradation of protein by microbial action (el-Shazley, 1952; Annison, 1954). The rations containing 0.5% urea had more native protein than the rations containing 1.5% urea and produced more isovaleric acid. The ration containing 1.5% urea and 7.5% molasses had no detectable isovaleric acid present in the rumen contents, indicating that it was being utilized in protein synthesis or for energy by bacteria. The utilization of isovaleric acid closely parallels that of ammonia in the rumen, indicating its importance in the synthesis of bacterial protein. Allison et al. (1966) showed that isovaleric acid was incorporated into leucine. Cline et al. (1966) showed that branched-chain VFA increased nitrogen retention. Oltjen and Putnam (1966) found that substituting urea for soybean meal in purified diets resulted in lower plasma levels of valine, isoleucine, and leucine and attributed this to insignificant amounts of branched-chain VFA in the rumen. Branched-chain VFA are also required for growth of certain bacteria (Bryant and Doetsch, 1954, 1955; Allison et al., 1958).

These results indicate that, when higher levels of urea (30% of the protein) were fed, increasing the RAC in the ration increases microbial fermentation of the ration and ammonia nitrogen utilization. However, if the level of protein is nearly sufficient to meet

the animals' requirements, the addition of 0.5% urea is inefficiently used, producing higher rumen ammonia levels and lower VFA concentration in the rumen.

Carcass Characteristics

The mean values of various carcass characteristics for each treatment group are presented in Table 5. The relationships between the concentration of VFA in the rumen or their molar percentage and the carcass characteristics studied were non-significant ($p \geq .6$). The analysis of variance for the urea and molasses treatments is presented in Appendix Table 2. Correlation coefficients between VFA concentrations and carcass characteristics were generally very low and non-significant ($p > .2$). These are presented in Appendix Table 3. No significant difference ($p > .05$) was found to exist between urea or molasses treatments and carcass characteristics.

Many researchers have observed that urea could replace protein in the ration without affecting performance if sufficient RAC were supplied (Mills et al., 1942; Johnson et al., 1942; Ellis and Pfander, 1958; Blaylock et al., 1965; and others). Oltjen et al. (1965), Kercher and Paules (1967), Clifford and Tillman (1967), Putnam et al. (1968) and Richardson et al. (1966) concluded that replacing protein with urea in fattening rations did not affect carcass

Table 5. Mean values of carcass characteristics and average daily gain for steers in the feed-lot trial.

Item	Ration Number			
	1	2	3	4
Average daily gain (kg.)	1.27	1.36	1.35	1.41
Marbling score *	12.0	11.8	12.6	13.2
Kidney fat of carcass (%)	2.27	1.88	2.0	2.05
Backfat thickness (cm.)	1.15	1.27	1.54	1.39
Ribeye area cm./100 kg. carcass	3.83	4.31	4.46	4.40
Yield (%)	60.0	59.1	59.8	60.6
Grade **	5.0	5.1	5.3	5.2

*Slight, 11 to Moderate, 14.

**Low choice, 4; Choice, 5; High choice, 6.

Note: All differences between ration non-significant ($p = .2$).

variability.

In summary, the results of this work support numerous other workers by finding that urea can replace up to 30% of the natural protein in fattening rations without affecting gain or carcass characteristics. The results reported here also agree with previous workers that VFA concentrations and molar % are poor indicators of the performance or carcass characteristics due to the high variability between animals consuming the same ration.

In contrast to much of the literature these findings indicate that a combination of soluble sugars and starch produce lower rumen ammonia levels than starch alone in animals adapted to urea supplemented rations. A significant interaction also exists between urea and molasses and total volatile fatty concentrations, and all individual acids measured except propionic acid. Increases in either urea or molasses increase the concentration of acetic, propionic and total VFA levels.

Metabolism Trial

The coefficient of apparent digestibility of the rations appear in Table 6 along with the percent of nitrogen absorbed that was retained. There was no significant difference ($p > .05$) between the urea-containing diets and the all-protein diets. The approximate significance level is ($p = .6$) when the rations are adjusted to equal

Table 6. Means of digestion coefficients and nitrogen retention for metabolism trials I and II.

Ration number	% RAC	Percent digestibility, dry basis						% absorbed-N retained	Net protein retained
		Dry matter	Crude protein	acid detergent fiber	Ether extract	Ash	Energy		
Ration 1	42.0	73.1	70.3	38.4	81.4	33.7	53.6	55.5	39.1
Ration 2	44.3	76.7	77.3	48.5	85.0	36.6	56.3	63.8	49.4
Ration 3	53.1	75.8	70.6	42.7	73.2	39.0	55.1	62.1	43.8
Ration 4	50.2	78.5	74.5	54.1	83.8	41.0	61.8	70.8	52.7
Ration 5	45.3	79.1	76.3	47.7	85.5	35.9	58.2	60.5	46.0
Ration 6	47.0	73.1	69.5	43.8	73.4	37.5	54.7	68.6	47.6

*Net protein retained = crude protein digestibility x % absorbed-N retained.

Note: differences between ration means are non-significant ($p = .2$) for digestibility of nutrients.

RAC levels of 42, 47, and 53% for rations 1 and 2, 5 and 6, 3 and 4, respectively. For the second trial, the digestibility was significantly higher ($p < .01$) than the first. The statistical analysis is presented in Appendix Table 3.

The trial difference may have been caused by an adaptation response. The animals may not have been fully adapted to the high concentrate diets due to the drastic change from grazing grass stubble to good quality alfalfa and then to a concentrated diet in only 28 days. Lloyd et al. (1956) indicate that, when diets are changed, large variations in digestion coefficients are noted and three or more weeks may be required before the digestibility of dry matter, protein and fiber is stabilized. Church (1969) states that the adaptation period for vastly different diets is variable and the shift from a forage diet to a concentrate diet may require as long as 4-6 weeks.

The apparent digestibility of dry matter and fiber appear to be related. With rations containing no urea (rations 1, 5, 3) the digestibility of fiber was 38.4%, 47.7%, 42.7% and dry matter was 73.1%, 79.1%, 75.8%, respectively, with increasing RAC levels as compared to 48.%%, 43.8%, 54.1%, and 76.7%, 78.5%, 73.1% respectively, for the urea-containing diets (rations 2, 6, 4). Although these rations contained approximately the same amount of fiber, the digestibility of the fiber was highly variable. Van Soest (1967) reports an inverse relationship between the digestibility of

the fiber and the lignin content, which increases with plant maturity. A possible explanation may be that optimum conditions were obtained in ration 5 to support maximum digestion when compared to the other protein diets. Ration 6 contains considerably more roughage than either ration 2 or 4 and has a higher lignin content which would account for a lower digestibility of the fiber. This lower fiber digestibility would also be reflected in the dry matter digestibility. Crampton (1959) concluded that the digestibility of fiber from concentrates is approximately twice that of poor quality roughages, and Morrison (1956) shows the digestibility of the fiber of wheat or barley to be considerably higher than that of the grass hay and alfalfa which was used as the primary replacement for the grain in ration.

The net protein retained (digestibility of crude protein x percent absorbed - N retained) is significantly higher ($P < .05$) for the urea containing diets than the non-urea containing diets. Ration 4 is significantly higher ($P < .01$) than ration 3 in net protein retained. The other urea containing rations tend to have a higher net protein value than the natural protein rations containing similar RAC levels, but these differences were non significant ($P > .1$).

Ration 4 was significantly higher in net protein value because nitrogen retention was greatest with the higher levels of RAC whereas with Ration 3 this was not true. With the natural protein rations optimum nitrogen absorption and retention were observed with

ration 5 (45% RAC); nitrogen retention then decreased with increasing RAC levels.

This work would indicate that the net protein retained is higher for urea-containing rations than for those rations that contain only natural protein. A possible explanation could be that the RAC supports a microbial population that can utilize the ammonia nitrogen more efficiently than amino nitrogen. This would support the findings of Bruggemann et al. (1962) who reported that an increase by 50% in the amylolytic activity of rumen contents when urea replaced soybean meal in the ration.

One of the primary objectives of this trial was to determine if increasing the RAC level would increase nitrogen absorption and retention. The percent nitrogen absorbed that is retained and the percent nitrogen retained were shown to be significantly ($p < .05$) increased with increasing RAC levels when subjected to a regression analysis. The nitrogen retained is linear, increasing 8.2% with an 11% increase in RAC. This may be expressed in the regression equation, $y = 46.82 + .7471 (x-47)$, and is shown in Figure 1.

The more important variable, percentage retention of absorbed nitrogen, may be expressed in a regression equation, $y = 64.983 + .8134 (x-47)$, and is shown in Figure 2. This represents a 0.09% increase in the percent of absorbed nitrogen retained for each 1% increase in RAC. It must be remembered that these

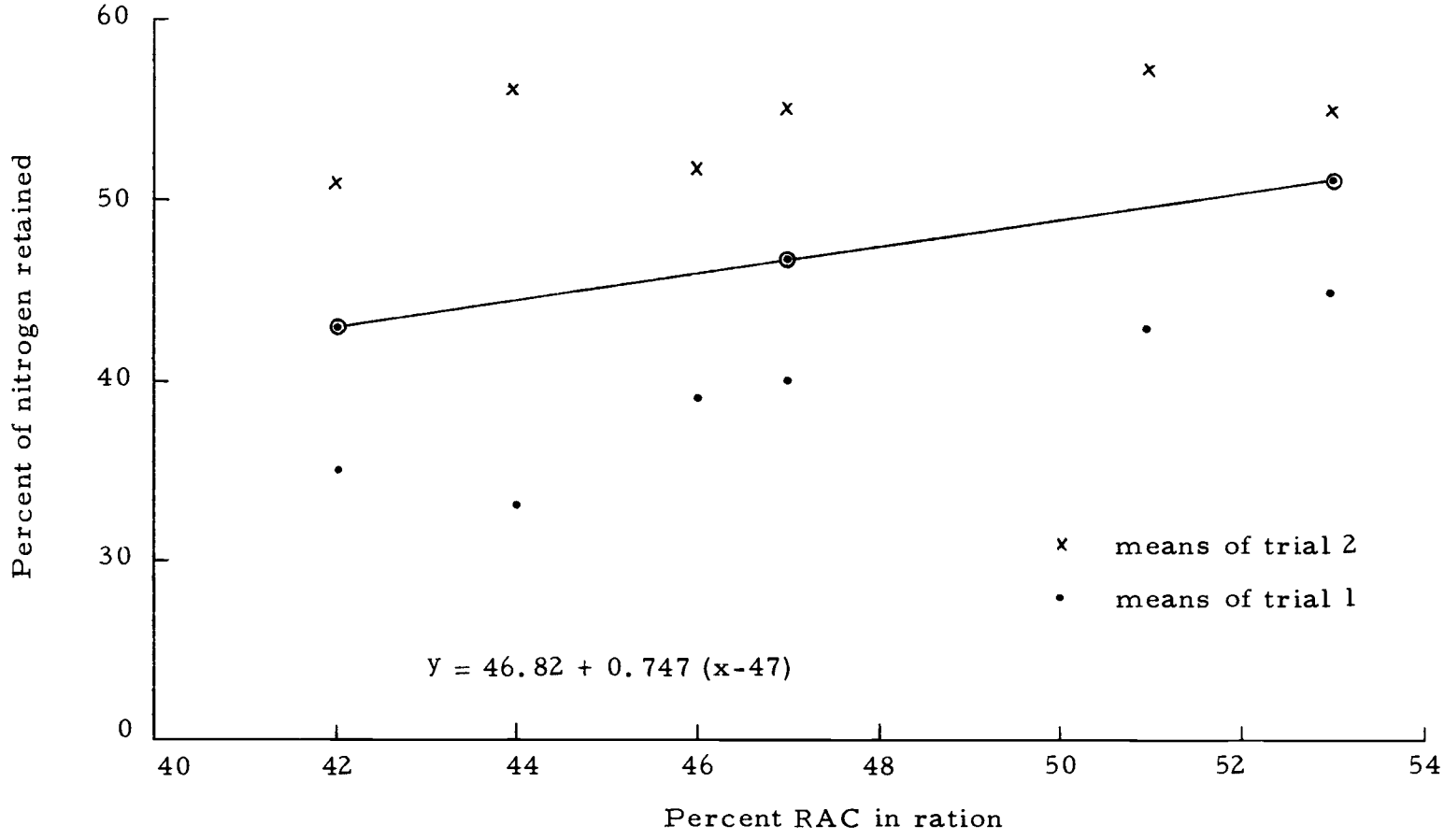

Figure 1. Percent of nitrogen retained at varying RAC levels.

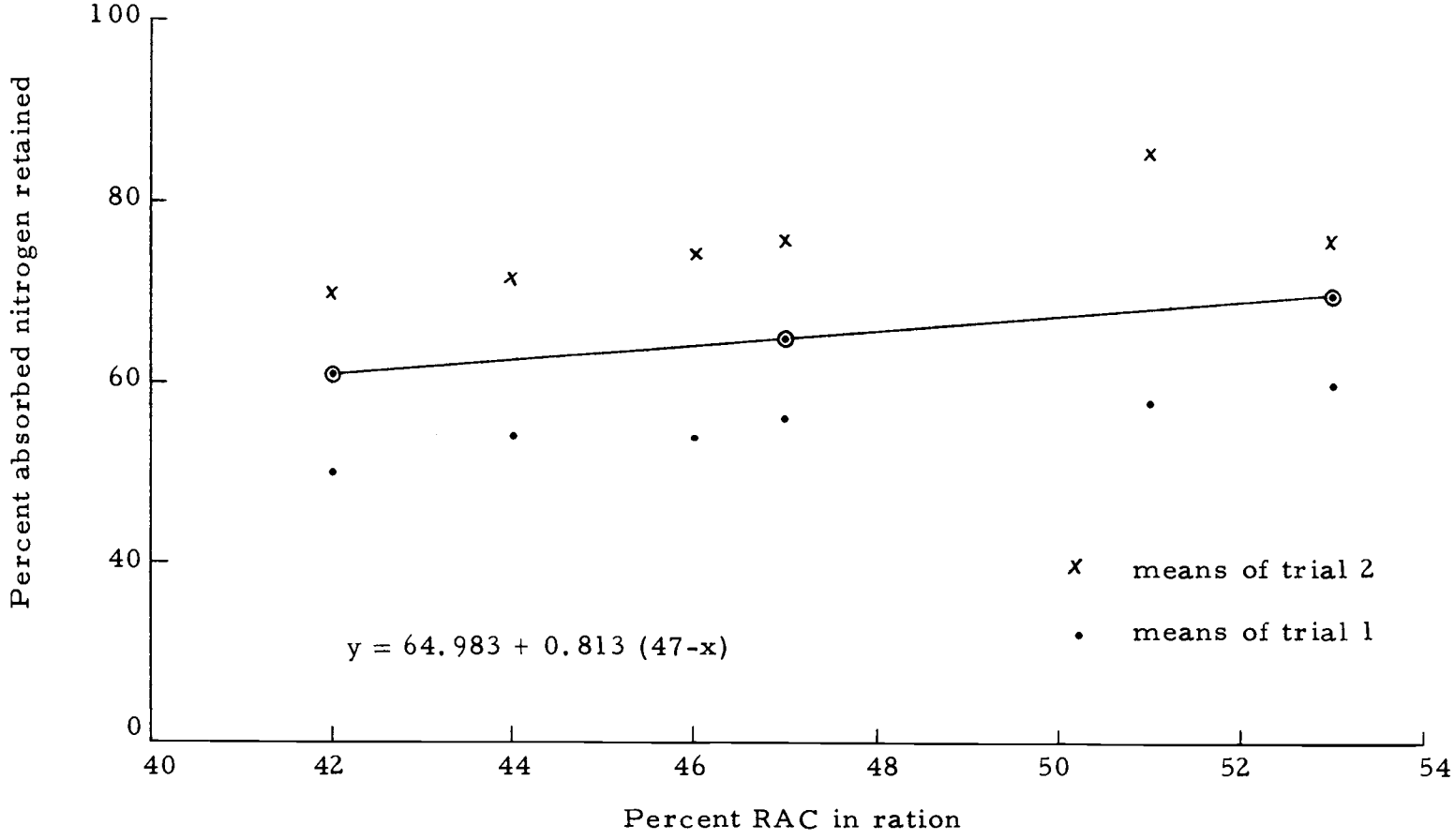

Figure 2. Percent of absorbed nitrogen retained at varying RAC levels.

regressions are valid only for the level of intake used in this trial.

By using the energy conversion figure of McLaren et al. (1965) for reducing sugar, 3.74 Kcal/gm., a range from 1,170 to 1,490 Kcal. is obtained for the rations used in this study. McLaren and co-workers and Barth (1962), using semi-purified diets and high levels of urea, obtained comparable results with a range from 564 Kcal. to 1,900 Kcal. supplied by RAC. Other workers have shown that the percent absorbed nitrogen retained increased with increasing RAC levels (Hamilton, 1942; Fontenot et al., 1955; Woods et al., 1956; and Ellis and Pfander, 1958).

This linear response in retention of absorbed nitrogen appears to remain relatively constant for diets ranging from 565 Kcal. to 1,900 Kcal. supplied by RAC. Barth states, "This relationship becomes curvilinear when maximum nitrogen values are obtained." This point has not been clearly defined but is in excess of 1,900 Kcal. of RAC in the diet. This area needs more research to determine what level of RAC best supports urea utilization. It would be most useful to know at what level this linear response becomes curvilinear.

In trying to estimate at what level of RAC this linear response becomes curvilinear it would be well to review the work of Ellis et al.

(1956), Gray et al. (1958), and Hungate (1966). These workers indicate that from 1.2-1.8 gm. of nitrogen are incorporated into microbial protein for each 100 gm. of carbohydrate fermented. Gray et al. (1958) reported that rumen microorganisms assimilate an amount of nitrogen not exceeding 1.2% of the dry matter of the feed consumed. On this basis the experimental rations used in the present research would supply 8 gm. of nitrogen for microbial protein. This would imply that a fermentable carbohydrate level of approximately 65% would support maximum nitrogen utilization by rumen microorganisms. There are many factors that would affect this utilization such as the digestibility of the dry matter and protein, the percent of protein, solubility of the carbohydrate, fiber content, and others.

This is an area that undoubtedly needs more research. Not only in regard to nitrogen utilization with relation to carbohydrate level in the rations, but the relationship to dry matter and fiber digestibility also need further investigation.

SUMMARY AND CONCLUSIONS

The object of this study was to investigate the effects of readily available carbohydrates (RAC) on the utilization of urea. The effect of urea on carcass characteristics and VFA levels was also determined. A feedlot and metabolism trial were used for this study.

In the feedlot trial urea and molasses combinations were investigated. Significant interactions ($p < .01$) resulted between the quantity of molasses and urea for rumen ammonia, acetic, valeric, and iso-valeric acid concentrations. The butyric acid concentration was significant ($p < .05$) for this interaction. This resulted because the low-urea, low-molasses ration had a low level of the VFA concentration, and increasing either the energy source or ammonia source supported rumen microbial populations that produced these acids at a rate comparable to a combination of the higher levels of molasses and urea. The interaction between rumen ammonia levels and iso-valeric concentration are similar, indicating their importance in microbial protein synthesis. Urea increased only acetate and propionate levels significantly and had no affect on the molar percentage of the VFA, while molasses decreased acetate and increased propionate molar percentage significantly.

The urea or molasses treatments had no affect on the carcass characteristics which affect quality or yield grade. This indicates

that urea can be supplemented for protein in fattening rations without affecting the value of the carcass. The correlations between rumen VFA concentration and carcass characteristics were low, accounting for less than 20% of the variability, thus indicating that VFA levels are a poor indicator of carcass composition as usually evaluated.

A linear response was obtained for the percent retention of absorbed nitrogen. A 2.8% increase in nitrogen retained occurred for each 100 Kcal of RAC supplied in the diet. With diets ranging from 41% to 53% RAC, urea can replace 30% of the crude protein without affecting digestibility of crude protein, fiber, ether extract, ash, energy, and dry matter significantly ($p > .1$). These results indicate that, at the level of intake and RAC investigated, urea utilization is improved as the percentage of RAC in the ration increases.

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Appendix Table 1. Summary analysis of variance for feed-lot trial.

Source	MS	Treatment t		
		Urea	Molasses	Urea x Molasses
Rumen ammonia	.002	.972	-5.698**	3.897**
VFA concentrations				
Acetic acid	1.502	7.951**	5.343**	5.482**
Propionic acid	3.330	2.967**	5.721**	1.301
Butyric acid	.808	1.296	1.021	2.115**
Valeric acid	.115	1.272	1.506	5.152**
Isovaleric acid	.012	.006	4.901**	3.221**
Total VFA	1.124	1.843	3.421**	2.885**
Molar %				
Acetic acid	37.162	.362	-2.083*	1.328
Propionic acid	48.792	1.044	4.333**	.006
Butyric acid	15.642	1.423	2.465*	.306
Valeric acid	2.401	.832	.814	1.745
Isovaleric acid	4.633	.365	1.544	.300
Carcass characteristics				
Average daily gain	.102	.578	1.437	.424
Marbling score	8.682	.962	.004	.041
Grade	.775	.728	.038	.422
% Kidney fat	.350	.299	.869	1.126
Backfat thickness	.043	1.471	.236	.723
Ribeye cm./cwt.	.071	1.512	.894	1.235
Yield	2.541	1.233	.106	1.687

df = 45

*t (.05) = 2.013

**t (.01) = 2.690

Appendix Table 2. Correlation coefficient between VFA concentrations and carcass characteristics.

Carcass Characteristic	Volatile fatty acid concentration			
	Acetic	Propionic	Butyric	Valeric
Average daily gain	-.15	.01	.13	.03
Backfat thickness (cm.)	-.04	.18	.15	.10
Loineye area (cm./cut)	.12	.12	-.04	.00
Marbling score	-.03	.06	-.20	.01
Kidney fat (%)	-.16	-.07	-.13	-.18
Yield	-.02	.12	.30	-.12
Grade	-.05	.07	-.26	.00

df = 45

*p < .05

**p < .01

Note: Significance level is p = .5 and larger.

Appendix Table 3. Summary of analysis of variance for the metabolism trial.

Source apparent digestibility	Treatment F			
	MS	Urea	Linear in RAC	Trial
Dry matter	11.98	1.750	1.819	7.739**
Crude protein	19.20	2.610	.106	13.020**
Acid-det. fiber	24.12	.819	3.558***	8.342**
Ether-extract	29.40	3.983***	.787	9.680**
Ash	15.13	.087	5.739*	8.431**
Energy	10.22	.568	2.571	9.561**
% N-retained	11.98	.507	5.654*	43.463**
% absorbed N retained	20.66	2.015	4.325*	82.951**

df = 24

*F (< .05)
**F (< .01)
***F (> .1)