THE EFFECT OF HEMIACETAL OF CHLORAL AND STARCH ON THE EFFICIENCY OF ENERGY UTILIZATION AND PERFORMANCE OF BEEF STEERS

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CHAPTER I

INTRODUCTION

There is a need for continual improvement in performance and efficiency of feed utilization by beef cattle. Currently the possibility of a major breakthrough in improvement of beef cattle performance appears slim. Recent bans on the use of diethylstilbesterol in beef cattle fattening rations have further forced the animal scientist to search for the small improvements in performance that can improve profits. Recent research has looked at the possibility of improving the economic efficiency of the ruminant by reducing a seemingly minor loss of energy, ruminal methanogenesis. This may be a method of maximizing the energetic efficiency of the rumen microbial population and in turn improving the energetic efficiency of the animal.

The use of respiration calorimetry and digestion trials is a means for fractionating the gross energy of a feed into its various energy components (DE, ME, NE, heat) and thus determining the actual usefulness of a ration for a specific purpose. Using these techniques the effect of inhibition of methane production on energetic efficiency can be studied.

The purpose of this study was to investigate the effects of inhibition of methane production on performance and energetic efficiency of beef steers as determined by respiration calorimetry and carbon-nitrogen balance.

CHAPTER II

REVIEW OF LITERATURE

Introduction

The ruminant is limited in its utilization of high concentrate rations due to microbial fermentation in the rumen. Although ruminal digestion accounts for 70-85% of the total digestion of dry matter in the digestive tract (Annison, 1956), it is a slow process which rarely goes to completion and much of the energy liberated in this process is utilized by the microorganisms or lost as heat of fermentation or as methane. Volatile fatty acids, the end products of microbial fermentation, are also utilized less efficiently than glucose, the end product of enzymatic digestion in the small intestine (Blaxter, 1962). The major losses of energy by the ruminant are heat, fecal energy and methane. Heat production is a necessity to the animal and researchers are limited in the ability to alter this energy loss. Fecal energy losses have been a source of investigation for a number of years, but until recently little work has been done to decrease energy losses due to methane production in the rumen.

The primary pathway of ruminal methane production is the reduction of carbon dioxide produced by microbial digestion (Carroll and Hungate, 1955) and appears to follow the reaction:

$$4\ H_2 + CO_2 \qquad\qquad CH_4 + 2\ H_2O.$$

The major methanogenic bacteria in the rumen, which carries out this reaction is Methanobacterium ruminatium (Smith and Hungate, 1958).

Bratzler and Forbes (1940) noted a high correlation between methane production and carbohydrate digested. The relationship between methane production and carbohydrate digested in cattle was expressed by the equation:

$$CH_4 \text{ (grams)} = (4.012 \times 100 \text{ g CHO digested}) + 17.68.$$

With sheep fed varying levels of corn oil, Swift et al. (1948) obtained the formula:

$$CH_4 \text{ (grams)} = (2.41 \times 100 \text{ g of CHO digested}) + 9.80.$$

Pilgram (1947) noted that methane production in sheep was greatest during the first four hours after feeding and declined during the remainder of the day. When animals were fasted for four days, methane production ceased, but after resumption of feeding, methane production reached previous levels within four days. Although hydrogen did appear in the rumen under some conditions, none was detected under normal feeding conditions. Graham (1967) noted no apparent difference in total methane production of sheep fed one or eight times each day, but noted markedly lower methane production in sheep fed every fourth day.

Most of the methane produced in the rumen is eructated, although it has been noted (Dougherty et al., 1964) that a small part was absorbed from the lungs and digestive tract into the blood. Later studies (Dougherty et al., 1967) indicated that some methane absorbed into the blood was oxidized, but to such a small extent as to not be an important factor in energy metabolism.

Swift et al. (1948) noted that 7.5% of the total energy intake of sheep was lost as methane. This agrees well with the values of 6.2 to

10.8% obtained by Blaxter and Clapperton (1965) in several years of study at the Hanna Dairy Research Institute. A number of other workers have noted similar values (Brody, 1964; Hershberger and Hartsook, 1968). Czerkawski (1969) estimated the daily production of methane from cattle and sheep to be 250 liters and 40 liters, respectively. With a caloric value of 9.45 kcal. per liter (Brouwer, 1965), this represents a loss of approximately 2360 kcal. daily for mature cattle and 280 kcal. daily for sheep. Studies indicate only small day to day variations in methane production by a single animal but marked differences between animals on the same ration (Blaxter and Clapperton, 1965).

Ruminal methane production is a substantial loss of energy to the ruminant and causes a decrease in the efficiency of energy utilization. In order to improve the utilization of dietary energy by ruminants, a number of workers have attempted to define the factors which influence methane production in the rumen. Coppock et al. (1965) noted that as concentrate replaced forage in the ration, energy lost as methane (expressed as a per cent of GE) increased significantly, but when expressed as a per cent of DE, methane production tended to decline. Similarly, at the maintenance level of feeding, methane production (as a per cent of GE) increased as the apparent digestibility of the ration increased (Blaxter and Clapperton, 1965). As the level of intake increased, methane production (as a per cent of GE) decreased when sheep were fed high quality feeds. When sheep were fed low quality feeds, however, level of intake had no effect on methane production.

Feeding rations of low digestibility and for low intake is not economically feasible when cattle are being grown for slaughter. Unfortunately, however, these are the conditions which seem to favor

lower methane production (Coppock, et al., 1965; Blaxter and Clapperton, 1965). A more suitable approach appears to be the addition of compounds to the ration that will reduce methane production without adversely effecting other digestive and metabolic processes of the animal. A variety of compounds have been found to have inhibitory effects on methane production.

Saturated Fatty Acids and Sulfur Containing Methane Inhibitors

Czerkawski, Blaxter and Wainman (1966c) tested the effects of saturated fatty acids on methane production and digestion in sheep. When 54 grams (4.5% of the ration) of stearic acid were fed to sheep daily, the digestion of cellulose and protein was decreased. Heat production was not affected, but a 29% decrease in methane production was noted. Energy retention was increased by 368 kcal. or 66% of the additional energy supplied by the stearic acid. Lauric acid infusions into the rumen caused a decrease in methane production, but there was an accompaning increase in heat production and a decrease in feed intake and energy retention. Similar results were obtained with ruminal infusions of sulfated long chain alcohols.

The continuous infusion of tertiary branched chain carboxylic acids into the rumen of sheep caused a significant decrease in methane production of 29% (Clapperton and Czerkawski, 1971). There were also significant decreases in the digestion of dry matter, organic matter and energy, and a 1.5% decrease in ME. When the acids were added to the ration, methane production was significantly decreased, but there were no significant effects on digestibility or ME.

Sodium sulfite was shown to inhibit methane production both *in vitro* and *in vivo* by Van Nevel *et al.* (1970). *In vitro*, additions of sodium sulfite resulted in the accumulation of hydrogen and a decrease in total VFA concentration. Infusion of 5 grams of sodium sulfite into the rumen of sheep resulted in a marked inhibition of methanogenesis for up to 5 hours. Rumen concentrations of propionate and butyrate increased while acetate concentrations and total VFA concentrations decreased. Overall, digestibilities tended to decrease, but this decrease was significant only for the NFE. Nitrogen retention was significantly ($P<.01$) improved. Krabill, Alhassan and Satter (1969) fed sodium sulfite at three levels to steers and found no effect on the apparent digestibility of dry matter, protein and energy or on nitrogen retention, although considerable variability in feed intake was noted. With *in vitro* studies, ingesta from rations which contained sodium sulfite produced less acetate, and more propionate, butyrate and isovalerate without affecting total VFA production. *In vitro* production of methane and carbon dioxide was significantly decreased.

Halogenated Methane Inhibitors

Effects on Gas Production

Bauchop (1967) found that a number of methane analogues had an inhibitory effect on *in vitro* methane production by rumen microorganism. Chloroform, carbon tetrachloride and methylene chloride caused marked reductions in methane production with an accompaning increase in hydrogen concentration. Rufner and Wolin (1968) noted similar results in continuous *in vitro* cultures with carbon tetrachloride additions except that higher concentrations were required to inhibit methanogenesis.

Methane production ceased within 29 hours after the addition of carbon tetrachloride, and several days were required for methane production to return to pretreatment levels. An increase in hydrogen concentration accompanied the decreased methanogenesis. Higher concentrations of carbon tetrachloride were required to inhibit methanogenesis *in vivo* than *in vitro*, but methane production ceased almost instantly and remained very low for three days.

Low concentrations of halogenated methane analogues inhibited methane production in extracts of *Methanobacillus omelianskii* by competitively inhibiting the factor III enzyme involved in cobamide-dependent methyl-transfer reactions (Wood, Kennedy and Wolfe, 1968).

Bromochloro methane (BCM) was shown to cause nearly complete inhibition of methanogenesis *in vitro* at concentrations of 3 parts per million (Trei and Olson, 1969). When Johnson *et al.* (1971) fed 5.5 grams of BCM per day to sheep, ruminal methane concentrations were reduced from 21.8% for controls to 2.9% for treated animals. Maximal inhibition occured within 6 hours post-feeding. Sawyer, Hoover and Sniffen (1971) reported greater than 80% reduction in rumen methane production in respiration experiments when BCM was fed at levels as low as 1.5 mg. per kg. body weight.

Singh, Trei and Scott (1971) noted a dose correlated reduction in ruminal methane concentrations of steers fed a 50% concentrate when a hemiacetal of chloral and starch (HCS) was added to the ration. A reduction in the inhibitory effect of the inhibitor was noted after 115 days on treatment when HCS was fed at a level of 1.5 grams per kg. of ration. Feeding HCS (2 grams per kg. of ration) to lambs on 50% and 80% concentrate rations resulted in greater than 80% reductions in

ruminal methane concentrations.

Johnson (1971) fed a pelleted 30% concentrate ration which contained 2 grams of HCS per kg. of ration to young rams at levels of 1.04 and 1.8 times maintenance. HCS reduced methane production ($P<.01$) by 86% at the low energy level and 56% at the high energy level. Hydrogen gas losses accounted for 2.08% of the gross energy intake or 47% of the decreased methane energy losses. In a later study (Johnson, 1972a), methane production was decreased 50% at the maintenance level of intake, and 82% at 2.1 x maintenance when HCS was fed at a level of 2.2 grams per head per day to sheep on a 60% concentrate ration. The increased hydrogen gas losses when sheep were fed HCS accounted for approximately 1.65% of the gross energy intake or about 40% of the energy saved in decreased methane production. Total gaseous energy losses were significantly ($P<.01$) reduced by feeding HCS.

Effect on Rumen and Blood Metabolites

Numerous workers have noted similar effects of halogenated methane inhibitors on VFA concentrations *in vivo* and *in vitro*. Decreased proportions of acetate accompanied by increased proportions of butyrate and propionate have been noted with carbon tetrachloride (Rufner and Wolin, 1968), chloral hydrate (Van Nevel *et al.*, 1968), BCM (Trei and Olson, 1969; Johnson, 1971) and HCS (Trei and Scott, 1971; Trei *et al.*, 1972). These shifts in VFA proportions were accompanied with no effect on total VFA concentrations except at high levels of the inhibitor (Trei *et al.*, 1972). *In vitro* lactic acid levels were increased with chloral hydrate (Prins and Seekles, 1968) and BCM (Trei and Olson, 1969) but in separate studies neither compound had an effect on rumen pH (Van Nevel *et al.*, 1968; Johnson, 1971).

Reduced rumen ammonia and plasma urea nitrogen levels were noted when HCS was fed to lambs (Trei and Scott, 1971; Trei et al., 1972). In vitro microbial protein synthesis was markedly increased by chloral hydrate (Van Nevel et al., 1968) suggesting an improved nitrogen utilization by the rumen microorganisms when a chlorated methane inhibitor is fed.

Effect on Digestion and Energy Utilization

Johnson (1971) noted no significant differences in digestion of dry matter, protein or energy by sheep due to HCS treatment. Nitrogen retention was not affected, but ME as a percent of GE was increased significantly ($P<.05$) by HCS treatment. Heat production and energy retention were not significantly effected although the HCS treatment group tended to have a slight advantage in energy retention when adjusted to equal GE intake per kg. metabolic size.

Sheep fed a 50% concentrate ration which contained 2 grams of HCS per kg. of ration had slightly higher digestibilities of dry matter, protein, fat and NFE (Singh and Trei, 1972). HCS treatment also tended to increase nitrogen retentions with a significant ($P<.01$) 21% increase over controls in one trial. A second study (Johnson, 1972a) indicated that HCS treatment improved DE at low intake levels (Maintenance), but depressed DE at high intake levels (2.1 x maintenance). Due to the decrease in gaseous energy losses with HCS treatment, ME was increased at the low level of feeding. Overall, HCS ($P<.05$) increased ME as a per cent of GE by an average of 2.7%. HCS had no significant effect on nitrogen digestion, nitrogen retention or on the efficiency of utilization of ME.

Effects on Performance

Trei and Scott (1971) fed feeder lambs a pelleted 60% concentrate corn based ration with HCS added at levels of 0.0, 0.5, 1.0 and 2.0 grams per kg of ration. Although there was a trend toward a dose correlated reduction in feed intake with the inhibitor, feed conversions were significantly ($P<.05$) improved at the two higher levels of HCS. Animals appeared to adapt to the inhibitor with time. Weight gains, feed conversions and feed intakes improved after the first 30 days of HCS feeding for the lambs at the two highest levels of HCS. The greatest response the first 30 days was with the 0.5 gram level, but during the last 60 days on trial the 2.0 gram level group had the best performance. In a second study (Trei et al., 1972), rates of gain were significantly improved when animals were fed HCS at levels of 1.0 and 2.0 grams per kg. of ration.

Unsaturated Fatty Acids as Methane Inhibitors

Effects on Gas Production

Czerkawski, Blaxter and Wainman (1966a) hypothesized that adding hydrogen acceptors other than carbon dioxide to the rumen might reduce methane production. Six wether sheep were fed 900 or 1000 grams of high quality dried grass daily in two meals. Impure emulsions of oleic (18:1) linoleic (18:2), linolenic (18:3) or palmitic (16:0) acid were infused directly into the rumen at a constant rate for eight to twenty-four days. There were marked decreases in methane production with all the acids tested. A three to eight day period elapsed before methane production reached a stable low value and, after infusions were stopped,

about 12 days passed before production of methane returned to pre-treatment levels. With all acids tested, the depression in methane production was broadly proportional to the amount of acid infused with the highest rate of infusion resulting in the greatest depression in methane production. The depression of methanogenesis with infusions of palmitic acid indicated that decreased methane production was not completely dependent upon the unsaturation of the fatty acid. Larger reductions in methane production, however, were noted with increase unsaturation. Linolenic acid infusions resulting in the greatest depression in methane production. The average decrease in methane production, expressed as kcal. of methane per 100 kcal. of fatty acid infused, were 13.8 kcal. for oleic, 14.2 kcal. for linoleic, and 16.4 kcal. for linolenic. With all the unsaturated fatty acids used, infusions of over 500 kcal. per day were required to cause marked reductions in methane production.

In a similar study (Czerkawski, Blaxter and Wainman, 1966b), linseed oil glycerides and linseed oil fatty acids were incorporated into a pelleted high concentrate ration for sheep. The fatty acid content of the glycerides and fatty acid mixtures were similar, being mostly composed of oleic, linoleic, and linolenic acid. The control and fat treated rations were also similar in fatty acid content except in the case of linolenic acid which was markedly higher in the high fat rations. The lipids were added to the diet at levels of 30 or 60 grams per day. Methane production of the sheep on the fat treated rations was significantly ($P<.001$) lower than the control group. Methane production was depressed 25 to 29 kcal. for each 100 kcal. of fat added to the ration. These depressions in methane production were greater than when

fatty acids were constantly infused into the rumen (Czerkawski et al., 1966a). In an attempt to explain this difference, fatty acids were rapidly infused into the rumen of a sheep at feeding time. The resulting depression in methane production was 28 kcal. per 100 kcal. of fatty acid infused. This suggested that the depression in methanogesesis was more dependent on the concentration of fatty acids in the rumen at any one time rather than the amount present over a 24 hour period.

The effects of a gradual increase in the fatty acid content of the ration on methane production were studied by Czerkawski (1966). Sheep were fed a mixed pelleted ration in which linseed oil fatty acids were added at an increasing level over an eight-week period. There was a 30% decrease in methane production during the eight-week period which was equivalent to a decrease of 17 kcal. of methane per 100 kcal. of fatty acid ingested. These results appeared to indicate an adaptation by the rumen microbial population to the fatty acids fed.

When linolenic acid was added to in vitro substrates of pyruvate, formate or glucose, Demeyer and Henderickx (1967) noted marked reductions in methane production. A number of other C-18 unsaturated fatty acids were also tested, but none was as effective in inhibiting methane production as linolenic acid. With formate as the substrate, the decrease in methane production was accompanied by an accumulation of hydrogen. This increased hydrogen concentration had not been noted in previous in vivo studies with unsaturated fatty acids. In in vitro studies with substrates of sugar beet pulp and sucrose, Czerkowski and Breckenridge (1969) also noted hydrogen accumulation with decreased methanogenesis when linseed oil fatty acids were added to the closed system.

Effects on Rumen Metabolites

A number of workers have noted reductions in proportions of acetate with accompaning increases in propionate proportions when fatty acids were fed or infused into the rumen of sheep (Shaw and Ensor, 1959; Robertson and Hawke, 1964; Demeyer *et al.*, 1969). Similar results have been obtained *in vitro* with pyruvate as the substrate (Demeyer and Henderickx, 1966) and with sugar beet pulp as the substrate (Czerkawski and Breckenridge, 1969). The effects on butyrate and total VFAs in these studies were usually small and inconclusive. Armstrong *et al.* (1958) suggested that a lowered acetate to propionate ration improved the utilization of ME for gain. Demeyer and Henderickx (1967) therefore hypothesized that the decreased acetate to propionate ratio noted with methane inhibition could be an added advantage of inhibition of methane production.

Effect on Energy Utilization and Digestion

No consistent effect on digestion of dry matter, energy, protein of crude fiber by sheep was noted by Swift *et al.* (1948) when ether extract levels in the ration were increased, but digestion of NFE decreased and digestion of ration lipids increased. Czerkawski *et al.* (1966b) and Czerkawski (1966) also noted increased digestion of lipids and decreased carbohydrate digestion with fat added to the ration. Cellulose digestion was also decreased, but the decrease was not enough to account for all the reduction in methane production. The adverse effect of fat on cellulose digestion appears to be greatest with rations of low quality roughages. Addition of alfalfa ash or calcium can partially reverse this depression (White *et al.*, 1958; Davidson and Woods, 1961, 1963;

Ward et al., 1957).

Swift et al. (1948) noted no marked effect of ration ether extract level on the ME of the ration. The ME of the fatty acids infused into the rumen of sheep in studies by Czerkawski et al. (1966a) was 104% of GE. The efficiency of utilization of the ME of the fatty acids for gain tended to increase with increased unsaturation with an average of 80% for all the fatty acids tested. Nitrogen retention was not affected indicating that the fatty acids were not degraded to any appreciable extent, but were absorbed and incorporated directly into tissue lipids. The addition of linseed oil fatty acids and glycerides to rations resulted in increases in percent ME and increases in the net efficiency of utilization of ME for maintenance and for gain (Czerkawski et al., 1966b).

Effects on Performance

Shaw and Ensor (1959) noted that addition of 300 ml. per day of cod liver oil, oleic acid or linoleic acid to normal rations of lactating cows resulted in a marked reduction in milk fat percentage. Clapperton (1969) noted slight increases in voluntary feed intake when linseed oil fatty acids were added to sheep rations at levels of 2 and 4%.

Summary

Research has shown that ruminal methane production can be reduced by compounds that are either selectively toxic to methanogenic bacteria or that act as alternate hydrogen acceptors in the rumen. In vitro studies (Singh and Trei, 1972b) suggest from calculations based on the theoretical reaction scheme of carbohydrate metabolism in the rumen, that

40-60% of the carbon and hydrogen conserved in reduced methane production can be converted to useful metabolites. At the current time HCS appears to be one of the more favorable methane inhibitors since it is a potent methane inhibitor that can be readily added to rations and easily stored for long periods of time (Trei et al., 1972).

Most of the current research with HCS has been done with sheep fed rations containing less than 65% concentrate. There is also a limited amount of work on the overall energetic efficiency of animals fed HCS. Studies indicate that high levels of HCS inhibit feed intake, but also provide the greatest improvement in performance. With these ideas in mind, the following study was undertaken to determine the effects of HCS on the energetic efficiency of beef steers fed a high concentrate ration and to determine the effects of a gradual increase in ration HCS levels on the performance of beef steers.

CHAPTER III

MATERIALS AND METHODS

Energy Balance Trials

Twelve steers of Hereford and Angus breeding averaging 262 kg. (range: 225 to 315 kg.) were paired according to shrunk weight and randomly alloted to two treatments. All steers were fed an 80% concentrate ration twice daily in individual stalls for four weeks prior to being started on treatment. Treatment group I was fed the basal ration alone while treatment group II (HCS steers) received the same basal ration plus 3 grams of the methane inhibitor HCS per kg. of ration. After the first week in the digestion stalls all steers were fed at 90% of their maximum intake obtained in the stalls in order to keep intakes constant. A 14 day adjustment period was followed by a 7 day fecal and urine collection period. Urine and feces were collected and weighed daily and 10% aliquots were stored at $4^{o}C$ until completion of the collection period. The daily samples were then mixed, subsampled and stored in plastic bottles at $0^{o}C$ for future analysis. Urine was acidified with HCl. An additional sample of feces were dried at $60^{o}C$ in a forced air oven, ground through a 1 mm screen in a Wiley mill and stored at $4^{o}C$.

Following the excreta collection period, steers were placed in one of two open circuit respiration chambers similar to those described by Flatt et al. (1958) for three days, the last two of which included

two consecutive 24 hour gas collection periods. The chambers were sealed at least 16 hours prior to the start of gas collection. Outdoor air was pulled into the chambers at a rate of 300 liters per minute. The chamber temperature was maintained at approximately 19°C and air was circulated by a fan. Exhaust air volume was measured by dry gas meters. Two spirometers constantly sampled the air passing through each chamber. Beckman IR-315 infrared analyzers were used to measure CO_2 and CH_4 and oxygen was measured by a Beckman para magnetic analyzer.

The gas meters were read and residual chamber air was analyzed at the start and end of each 24 hour period. Barometric pressure, room temperature, chamber temperature and humidity and exhaust air wet bulb and dry bulb temperatures were recorded each time for correction of gas volumes to standard temperature and pressure.

Upon completion of gas collection the steers were returned to feeding pens where they were fed in individual stalls twice daily at the same levels as previously described. Approximately three days following completion of gas collection rumen samples were collected at 4 hours postfeeding via stomach tube. The pH was taken of the whole rumen contents. Rumen contents were then filtered through 4 layers of cheesecloth and frozen for future analysis. One ml. of saturated mercuric chloride was added per 50 ml. of strained rumen fluid to stop bacterial fermentation.

On approximately day 100 on treatment, steers were returned to the metabolism stalls for a second energy balance trial conducted as previously described. Steers were placed in the respiration chambers on approximately day 120 on treatment. Following completion of gas collection, rumen samples were obtained as described previously.

During the energy balance trials, feed samples were collected daily, composited, subsampled and ground through a 1 mm screen in a Wiley mill. The ground samples were then stored in plastic bags at 4°C for future analysis.

Performance Trial

Twenty-seven steers of Hereford and Angus breeding averaging 316 kg. (range: 290 to 335 kg.) were randomly alloted to one of 9 pens with 3 pens per treatment. Treatments were then randomly alloted to pens with 3 pens per treatment. Treatments consisted of : 1) basal ration only (control), 2) basal ration + 0.2% HCS (CHCS) and 3) basal ration + an increasing level of HCS (IHCS). HCS levels for the IHCS treatment group were 0.1% for the first 30 days, 0.2% for the second 30 days and 0.3% for the last 30 days on trial. Steers were kept on concrete slatted floors and were allowed to eat ad libitum from bulk feeders. Weights were taken at 30 day intervals. Initial and final weights were taken after a 14 hour shrink and intermediate weights were pencil shrunk 4%. Feed samples were taken weekly at feeding and composited for each 30 day period. Subsamples were ground through a 1 mm screen in a Wiley mill and stored in plastic bags at 4°C for future analysis.

Laboratory Analysis

Wet fecal samples and feed were analyzed for dry matter and nitrogen by the methods of the A.O.A.C. (1960). Feed, fecal and urine energy were determined by combustion in a Parr adiabatic oxygen bomb calorimeter and carbon was determined in a Leco carbon analyzer as described by Smith et al. (A.O.A.C., 1965). Urine samples were filtered prior to

being analyzed for nitrogen, carbon and energy content. Urine was dried on cellulose at 20°C in a vacuum oven prior to determination of gross energy and dried in aluminium cups prior to carbon analysis.

Volatile fatty acids were determined by the method of Erwin, Marci and Emery (1961) using a Bendix 2500 gas chromatograph[1] equipped with a hydrogen flame ionization detector. A glass U shaped column (183 cm. long and inside diameter of 2 mm) was packed with 10% SP 1200[2] on Chromsorb W, acid washed with 1% H_3PO_4, 80/100 mesh. Flow rates of nitrogen (carrier gas), hydrogen and air were maintained at 60, 40 and 1.6 cc/min., respectively. Temperature of the column and detector were maintained at 115°C and 250°C, respectively. Peak areas were measured by an Autolab 6300 digital integerator[3]. Rumen ammonia nitrogen was measured by the procedure of Conway (1953).

Statistical Analysis

Digestibility, nitrogen retention, VFA and rumen ammonia-nitrogen data were analyzed by analysis of variance as a split plot design with treatments as the main plots and days on feed being sub-plots. Total energy balance data were analyzed by analysis of variance as a split-split plot with main plots being three 2 x 2 latin squares with rows being pairs and columns being chambers. Simple effects were tested by least significant difference (Steel and Torrie, 1960).

Data for the performance trial were analyzed by analysis of variance and least significant difference as a completely randomized design with

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[1]The Bendix Corporation, Ronceverte, W. Va.

[2]Supelco, Inc., Bellefonte, Pa.

[3]Vidar autolab, Mountain View, Calif.

pens as the experimental unit.

Calculations

Heat production was calculated from oxygen consumption, carbon dioxide and methane production and urinary nitrogen excretion by the formula of Brouwer (1965). The equation used was:

$$(HP = 3.866 \times CO_2) + (1.2 \times CO_2) - (0.518 \times CH_4) - (1.413 \times UN)$$

where HP is total heat production in kcal. per 24 hours, O_2 is liters of oxygen consumed, CO_2 is liters of carbon dioxide produced, CH_4 is liters of methane produced and UN is grams of urinary nitrogen excreted per day. Total energy retention was then determined by the formula

$$\text{Energy Retention (kcal/24 hours)} = ME - HP.$$

Energy retention was also determined by carbon-nitrogen balance using the equation and factors of Blaxter and Rook (1953). Their equation was:

$$\underset{\text{(kcal.)}}{\text{Energy Retention}} = (12.55 \times \underset{\text{(grams)}}{\text{C retained}}) - (6.9 \times \underset{\text{(grams)}}{\text{N retained}})$$

where all measurements are on the basis of 24 hours. Heat production could then be calculated using the equation

$$HP = ME - \text{Energy Retention}.$$

Level of feeding was calculated using the equation of Blaxter (1962) to calculate the ME maintenance requirement (M).

$$M = 1356 + 16.6 \times (\text{weight in kg.}).$$

The calculated M value was then divided by ME intake to give an estimated level of feeding as a multiple of maintenance. Energy retained as protein was calculated using the constants of Brouwer (1965). The equation used was:

$$\text{Protein gain} = \text{N retention} \times 6.25 \times 5.7$$

where 6.25 is the factor for conversion of nitrogen to protein, 5.7 is the caloric value of 1 gram of protein, N retention is in grams per day and protein gain is in kcal. per 24 hours. Fat gained was then calculated as total energy retention minus protein energy retention.

CHAPTER IV

RESULTS AND DISCUSSION

Energy Balance Trials

Energy Balance Trial I

The ingredient and chemical compositions of the basal ration are shown in Tables I and II, respectively. The basal ration was markedly higher in crude protein than the HCS ration. This may have been due to errors in the mixing of the ration or differences in the protein content of the ingredients used. Gross energy composition of both rations, however, was similar.

Energy balance trial I was conducted after the steers fed the basal + HCS ration (HCS group) had been on feed approximately 30 days to compare the short term effects of HCS on energy utilization by beef steers. Average steer weights and feed intakes are shown in Table III. Weights were taken 14 hours after the evening feeding 2 days before the start of the digestion phase, and the day of completion of gas collection. Steers were placed in the respiration chambers by pairs according to a schedule which insured an equal representation of each treatment in each chamber. Animals were maintained on the same level of intake throughout the total energy balance trial. Intakes of total dry matter and gross energy (GE) were similar for both treatment groups, but the control group had a significantly ($P<.05$) higher protein intake due to

TABLE I

INGREDIENT COMPOSITION OF THE BASAL RATION ENERGY BALANCE TRIALS[1]

Ingredient	Per cent
Rolled grain sorghum	62.97
Dehydrated alfalfa pellets	8.00
Cottonseed hulls	12.00
Soybean meal	11.00
Dried cane molasses	5.00
Trace mineralized salt	0.50
Calcium chloride	0.50
Aurofac-50	0.03

[1] on an as-fed basis

TABLE II

CHEMICAL ANALYSIS OF RATIONS[1]

Item	Basal		Basal + HCS	
	Trial I	Trial II	Trial I	Trial II
Dry matter (%)	88.23	89.01	88.51	88.13
Crude protein[2](%)	15.97	15.38	12.85	14.70
Carbon (%)	43.76	42.75	41.38	42.43
Ash (%)	4.43	5.93	4.74	4.52
ADF (%)[3]	16.64	18.69	16.30	16.48
GE (Mcal/kg)	4.55	4.48	4.47	4.58

[1]all figures except dry matter are on a 100% dry matter basis

[2]nitrogen x 6.25

[3]acid detergent fiber

TABLE III

STEER WEIGHTS AND DAILY FEED INTAKES:
ENERGY BALANCE TRIAL I

Item	Control	HCS
Average weight (kg)	293.93 ± 10.80[1]	300.13 ± 13.71
DM intake (kg/day)	4.53 ± 0.14	4.55 ± 0.14
GE intake (mcal/day)	20.61 ± 0.62	20.31 ± 0.62
GE intake (kcal/$W_{kg}^{0.75}$)	290.42 ± 8.80	282.29 ± 8.53
Protein intake (g/day)	723.80 ± 21.94	584.35* ± 17.70
N intake (g/day)	115.80 ± 3.51	93.49* ± 2.83
Level of feeding[2]	1.58 ± 0.07	1.46 ± 0.03

[1]values for this and subsequent tables are given as the mean ± the standard error of the mean

[2]as a multiple of maintenance

*significantly different from controls ($P<.05$)

the higher protein content of the control ration. There was some difficulty in getting the steers in both groups to consume feed at a desired level (2 x maintenance) in the digestion stalls even after an apparently adequate adaptation period. Control steers were on a slightly higher level of feeding (1.58 vs 1.46 x maintenance) than HCS steers, but this was not statistically significant.

Ration digestibilities are shown in Table IV. Control steers had significantly ($P<.05$) higher digestibilities of GE and crude protein, and significantly ($P<.05$) higher levels of nitrogen absorbed. Several studied (Johnson, 1971; Singh and Trei, 1972) have noted no significant effect of HCS feeding on digestion of energy, protein or dry matter. Johnson (1972) noted higher digestibilities of energy when HCS was fed to sheep on a maintenance ration, but noted lower energy digestibilities at a level of 2 x maintenance for HCS fed rams. The marked depressions in digestibilities in this trial suggests that the level of HCS used (0.3%) may have been too high and adversely affected the overall rumen fermentation to such an extent that digestion in the lower gut could not compensate for this decrease.

Table V shows the individual energy losses of each treatment group. Fecal energy losses (FE) were significantly ($P<.05$) higher for the HCS steers because of the lower digestibilities with this ration. Urine losses (UE) were similar, although the control group tended to have slightly higher losses probably due to their higher nitrogen intakes. HCS steers had significantly ($P<.05$) lower methane losses with a 42% decrease over the controls. Other studies have shown greater inhibition of methanogenesis than noted in this trial. The higher level of concentrates used in this study as compared to other studies may be the reason

TABLE IV

DIGESTIBILITY OF RATIONS:
ENERGY BALANCE TRIAL I

Item	Control	HCS
Energy (% of GE)	73.70 ± 1.77	69.60* ± 0.66
Energy (mcal/kg DM)	3.35 ± 0.80	3.11* ± 0.30
Dry matter (%)	75.10 ± 1.79	72.00 ± 0.66
Protein (%)	67.00 ± 2.15	58.00* ± 1.03
N absorbed (g)[1]	77.58 ± 3.23	54.24* ± 1.84

[1]N intake - fecal N

*significantly different from control ($P<.05$)

TABLE V

ENERGY LOSSES: ENERGY BALANCE TRIAL I

Item	Control	HCS
GE (mcal/day)	20.61 ± 0.62	20.31 ± 0.62
FE (mcal/day)	5.44 ± 0.44	6.18* ± 0.31
FE (% GE)	26.30 ± 1.77	30.37* ± 0.66
UE (mcal/day)	0.74 ± 0.05	0.68 ± 0.02
UE (% GE)	3.59 ± 0.16	3.39 ± 0.12
CH_4 (mcal/day)	1.11 ± 0.06	0.64* ± 0.06
CH_4 (% GE)	5.43 ± 0.34	3.18* ± 0.29
H_2 (est. mcal/day)[1]	0.00	0.21
H_2 (est. % GE)[1]	0.00	1.01
HP (mcal/day)	10.83 ± 0.37	11.41 ± 0.29
HP (% GE)	52.51 ± 1.20	56.14 ± 0.51

[1] estimated from the factors of Johnson (1971, 1972a)

* significantly different from control ($P<.05$)

for the lower inhibition. Johnson (1971) noted that hydrogen energy losses compensated for about 45% of the decreased methane losses when HCS was fed. In this study, hydrogen losses would represent about 200 kcal or 1% of the GE intake.

Respiratory quotients in this trial tended to be relatively high (>1.2) and appeared to indicate either a low recovery of oxygen or an over estimation of carbon dioxide production or both. Also, the validity of the equation of Brouwer (1965) under the circumstances of this study (i.e. lower methane production and markedly different protein levels) may be questioned since these parameters may have a marked effect on calculated heat productions, but only a small effect on actual heat production. For these reasons, heat production obtained by the respiratory exchange and those obtained by carbon-nitrogen balance were pooled and their mean value used as the calculated heat production in an attempt to get a more accurate estimate of actual heat production. Heat productions (HP) were not significantly different between treatment groups, although the HCS group tended to have higher values (Table V).

Energy retentions are shown in Table VI. Total energy retention (ER) appeared to be higher for the control group, but this was not statistically significant. Much of this marked difference could be accounted for in the higher level of feeding of the control group. A greater proportion of the GE and ME intakes of the control group appeared as body energy gain. As would be expected from their higher protein intakes and digestibilities, control steers had significantly ($P<.05$) higher retentions of protein (PR) and nitrogen (NR). When nitrogen retention was corrected to equal intakes and digestibilities

TABLE VI

ENERGY RETENTION: ENERGY BALANCE TRIAL I

Item	Control	HCS
ER (mcal/day)	2.48 ± 0.35	1,38 ± 0.16
ER (kcal/$W_{kg}^{0.75}$)	35.36 ± 5.11	19.36 ± 2.35
ER (% GE)	12.17 ± 1.77	6.91 ± 0.88
ER(% ME)	18.49 ± 2.50	10.88 ± 1.32
PR (g)	154.33 ± 24.26	80.02* ± 47.59
PR (mcal/day)	0.88 ± 0.14	0.46* ± 0.05
PR (% ER)	37.96 ± 2.50	37.33 ± 4.30
NR (g)	24.69 ± 3.89	12.80* ± 1.34
NR(% of N intake)	21.56 ± 3.60	13.80* ± 1.56
NR (% of N absorbed)	31.75 ± 5.00	23.70* ± 2.60
Fat gain (mcal/day)	1.60 ± 0.27	0.92 ± 0.16
Fat gain (% ER)	62.04 ± 2.50	62.66 ± 4.30

*significantly different from control ($P<.05$)

(NR/absorbed N), control steers still showed a significant ($P<.05$) advantage. Other studies (Johnson, 1972; Singh and Trei, 1972) have noted no effect of HCS on nitrogen retention when all animals were on similar nitrogen intakes. HCS feeding did not appreciably alter the composition of body energy gain with protein energy accounting for approximately 37% of total energy retention in both treatment groups. Similar values were noted by Johnson (1972).

The gross energy intake is partitioned into its various components in Table VII. Total ME intake was significantly ($P<.05$) higher for the control group (13317 vs 12793 kcal/day), but when ME was corrected to equal dry matter intake and weight (kcal/kg DM/$W_{kg}^{.75}$) the differences were not significant. Control steers had slightly higher values for ME as a percentage of GE (ME/GE) mostly due to their significantly ($P<.05$) higher DE values. ME as a percentage of DE (ME/DE) was significantly ($P<.05$) higher for the HCS steers, but if estimated hydrogen energy losses are included, the values are not significantly different ($P<.10$). Johnson (1972) noted a 2.7% increase in ME as a percentage of GE with HCS feeding (hydrogen losses included). In this trial a decrease in ME as a percent of GE of 1.6% was obtained. When estimated hydrogen losses were included, this decrease approached 2.7%. Values for ME/DE were slightly higher than the normally accepted 82% value (N.R.C., 1971), but values of this size have been reported (Graham and Searle, 1972; Webster et al., 1972; Brown et al., 1968).

Although fasting heat production was not measured in this study, NEm+g was calculated using the equation

$$NEm+g = 77\ W_{kg.}^{0.75} + ER$$

where NEm+g is total net energy for maintenance + gain in kcals., ER is energy retention and 77 is the assumed fasting heat production per

TABLE VII

ENERGY UTILIZATION: ENERGY BALANCE TRIAL I

Energy Fraction	Control	HCS
GE[1]	64.32 ± 1.66	62.28 ± 1.80
DE[1]	47.43 ± 1.77	43.41* ± 1.58
DE (% GE)	73.70 ± 1.77	69.63* ± 0.66
ME[1]	41.61 ± 1.04	39.28 ± 0.85
ME (mcal/kg DM)	2.94 ± 0.05	2.82* ± 0.02
ME (% GE)	64.68 ± 1.10	63.06 ± 0.44
ME (% DE)	87.75 ± 0.40	90.58* ± 0.45
NEm+g (mcal/kg DM)	1.76 ± 0.08	1.53 ± 0.05
NEm+g (% GE)	37.97 ± 1.85	32.43 ± 1.13
NEm+g (% ME)	58.77 ± 2.77	51.42 ± 1.54

[1] on the basis of kcal/kg DM consumed/$W_{kg}^{0.75}$

* significantly different from control ($P<.05$)

kg. metabolic size. The value of 77 was selected from studies conducted earlier in this laboratory (Kiesling, 1972). Total NEm+g was not significantly different (7911 kcal for controls vs 6901 kcal. for HCS) although the control group tended to be higher. The higher values for NEm+g as a percent of ME would suggest a more efficient utilization of ME by control steers. Johnson (1972) indicated that inhibition of methane by HCS had no effect on the net efficiency of utilization of ME. The values for NEm+g per kg. of dry matter consumed are slightly lower than those obtained in previous studies in this laboratory (Kiesling, 1972), but similar values have been reported (Lofgreen, Bath, and Strong, 1963).

Energy Balance Trial II

Ration ingredient composition was the same as in trial I (Table I). Table II shows the chemical composition of the rations in trial II. Trial II was conducted after the steers on the HCS ration had been on treatment for approximately 120 days to test the long term effects of HCS on energy utilization by beef steers. One control steer died of bloat while in the holding pens leaving only 5 steers for that treatment group in trial II. The steer was a chronic bloater and his death was not attributed to the treatment.

The average steer weights and feed intakes for trial II are shown in Table VIII. Although all steers were heavier than in trial I, feed intakes did not increase probably due to the increased discomfort in the digestion stalls. This resulted in both groups being on slightly lower levels of feeding in this trial. Control steers had significantly ($P<.05$) higher protein intakes but this difference was not as great as

TABLE VIII

STEER WEIGHTS AND DAILY FEED INTAKES:
ENERGY BALANCE TRIAL II

Item	Control	HCS
Average weight (kg)	334.85 ± 15.94	328.18 ± 12.90
DM intake (kg/day)	4.54 ± 0.15	4.45 ± 0.15
GE intake (mcal/day)	20.24 ± 0.67	20.40 ± 0.68
GE intake (kcal/$W_{kg}^{0.75}$)	264.24 ± 8.59	264.99 ± 8.85
Protein intake (g/day)	697.51 ± 19.64	654.19* ± 21.80
N intake (g/day)	111.60 ± 3.14	104.67* ± 3.49
Level of feeding[1]	1.37 ± 0.12	1.44 ± 0.03

[1] as a multiple of maintenance

* significantly different from control ($P<.05$)

in trial I.

The digestibility of the ration components are shown in Table IX. There were no marked effects on digestion of dry matter or energy by HCS treatment, but HCS steers had significantly ($P<.05$) lower digestion coefficients for crude protein. These results have been discussed in earlier sections of this report.

No significant treatment differences were noted in energy losses in feces, urine or as heat (Table X). HCS feeding resulted in a 25% decrease in methane losses, but this was not significant ($P<.10$). Estimated hydrogen gaseous losses accounted for less than 1% of the total gross energy intake.

Energy retentions are shown in Table XI. Both treatment groups had similar values for total energy retention (ER), energy retention per kg. metabolic size, energy retention as a percent of GE and energy retention as a percent of ME. Total protein (PR) and nitrogen (NR) retentions were almost equal for both groups, although HCS steers showed a marked advantage in nitrogen retention as a percent of N intake and in nitrogen retention as a percent of absorbed nitrogen (41 vs 46%). There was no apparent effect on composition of the energy gain due to HCS with protein accounting for an average of 25.2% of total energy retention. The effects of HCS on energy utilization are shown in Table XII. HCS steers had significantly higher ($P<.05$) total ME intakes and tended to have higher values for ME as a percent of GE. This resulted in significantly ($P<.05$) higher values for ME per kg. of dry matter consumed and ME as a percent of DE for the HCS steers. If estimated hydrogen losses are included, HCS steers still had a significant ($P<.05$) advantage in values for ME per kg. of dry matter, but the advantage in ME as a per cent of DE only approached significance ($P<.10$).

TABLE IX

DIGESTIBILITY OF RATIONS:
ENERGY BALANCE TRIAL II

Item	Control	HCS
Energy (% GE)	70.85 ± 2.31	71.20 ± 0.87
Energy (mcal/kg DM)	3.18 ± 0.11	3.26 ± 0.04
DM (%)	72.50 ± 2.38	72.15 ± 1.13
Protein (%)	65.03 ± 2.16	61.88* ± 1.00
N absorbed (g)	72.66 ± 3.54	64.77* ± 2.42

*significantly different from control ($P<.05$)

TABLE X

ENERGY LOSSES: ENERGY
BALANCE TRIAL II

Item	Control	HCS
GE (mcal/day)	20.24 ± 0.67	20.40 ± 0.68
FE (mcal/day)	5.89 ± 0.46	5.88 ± 0.32
FE (% GE)	29.14 ± 2.31	28.79 ± 0.87
UE (mcal/day)	0.66 ± 0.04	0.61 ± 0.03
UE (% GE)	3.28 ± 0.14	2.99 ± 0.12
CH_4 (mcal/day)	0.95 ± 0.06	0.72 ± 0.05
CH_4 (% GE)	4.76 ± 0.32	3.52 ± 0.28
H_2 (est. mcal/day)[1]	0.00	0.10
H_2 (est % GE)[1]	0.00	0.50
HP (mcal/day)	8.41 ± 0.22	8.90 ± 0.32
HP (% GE)	41.64 ± 1.14	43.63 ± 1.28

[1]estimated from the factors of Johnson (1971, 1972a)

TABLE XI

ENERGY RETENTION: ENERGY BALANCE TRIAL II

Item	Control	HCS
ER (mcal/day)	4.33 ± 0.39	4.29 ± 0.23
Er (kcal/$W_{kg}^{0.75}$)	56.07 ± 4.15	55.87 ± 3.21
ER (% GE)	21.18 ± 1.51	21.07 ± 1.14
ER (% ME)	33.44 ± 1.81	32.61 ± 1.82
PR (g)	187.03 ± 19.75	185.08 ± 6.88
PR (kcal/day)	1066.07 ± 112.61	1054.96 ± 39.21
PR (% ER)	25.16 ± 1.32	25.24 ± 1.19
NR (g)	29.92 ± 3.16	29.62 ± 1.10
NR (% N intake)	26.77 ± 2.68	28.47 ± 1.54
NR (% N absorbed)	40.92 ± 3.49	46.05 ± 2.48
Fat Gain (mcal/day)	3.26 ± 0.34	3.23 ± 0.21
Fat Gain (% ER)	74.84 ± 1.32	74.76 ± 1.19

TABLE XII

ENERGY UTILIZATION: ENERGY BALANCE TRIAL II

Energy Fraction	Control	HCS
GE[1]	58.88 ± 2.08	59.71 ± 1.70
DE[1]	47174 ± 2.17	42.53 ± 1.35
DE (% GE)	70.85 ± 2.31	71.21 ± 0.87
ME[1]	36.98 ± 1.34	38.64 ± 0.80
ME (mcal/kg DM)	2.82 ± 0.08	2.96* ± 0.02
ME (% GE)	62.82 ± 1.68	64.70 ± 0.33
ME (% DE)	88.57 ± 0.56	90.88* ± 0.38
NEm+g (mcal/kg DM)	2.08 ± 0.07	2.10 ± 0.05
NEm+g (% GE)	45.92 ± 1.25	45.22 ± 2/97
NEm+g (% ME)	73.38 ± 1.94	70.02 ± 4.62

[1] on the basis of kcal/kg DM consumed/$W_{kg}^{0.75}$

* significantly different from controls ($P < .05$)

A number of studies have indicated that heat production on fast tends to decline with age and increased weight (Ritzman and Colovos, 1943; Graham and Searle, 1972) and with improved adaptation to respiration chambers (Graham, 1962). Therefore, a value of 66 kcal/$W_{kg}^{0.75}$/day (Kiesling, 1972) was used in trial II for the calculation of NEm+g. Calculated values for both treatment groups were similar and tended to agree with values previously obtained at this laboratory (Kiesling, 1972).

Comparison of Treatments

By combining the results of both energy balance trials, an estimate of the effect of HCS on energetic efficiency of steers over a long feeding period can be obtained. Table XIII gives average steer weights and feed intakes for the 120 day period. There was a significant trial x treatment interaction ($P<.05$) for protein intake and feeding level, and therefore they are omitted. It is known that a protein deficiency in a ration has a marked effect on an animal's energetic efficiency (Reid, 1970). Although the control group had higher intakes of protein in both trials, the level of protein in the HCS ration was adequate (NRC, 1972). The efficiency of utilization of protein for maintenance appears to be greater than for carbohydrates, but the efficiency of utilization of protein for gain appears to be less than for carbohydrates (Martin and Blaxter, 1960, 1961). The possibility of the higher levels of ration protein in the control group having an effect on the overall energetic efficiency is, therefore, questionable. The possibility of synergestic effects further prohibits the making of a definite conclusion.

Significant trial x treatment interactions were noted for digestion of protein ($P<.001$), energy ($P<.005$) and dry matter ($P<.05$) suggesting

TABLE XIII

STEER WEIGHTS AND DAILY FEED INTAKES
COMBINED ENERGY BALANCE TRIALS

Item	Control	HCS
Average Weight (kg)	314.08 ± 11.20	314.16 ± 9.93
DM Intake (kg/day)	4.54 ± 0.10	4.50 ± 0.07
GE Intake (mcal/day)	20.42 ± 0.41	20.36 ± 0.44
GE Intake (kcal/$W_{kg}^{0.75}$)	277.30 ± 5.52	273.60 ± 5.86

an adaptation to the HCS by the steers or their microbial population. It also suggests that an adaptation period is required to obtain the maximum benefit from inhibition of methane production with HCS.

Average energy losses and energy retentions for both trials are shown in Tables XIV and XV. The decreased methane losses resulting from HCS feeding were compensated for by significantly ($P<.05$) higher heat losses resulting in the control group having significantly higher energy retentions ($P<.05$) and NEm+g ($P<.01$). It must be remembered, however, that the calculated heat productions are not independent variables, but that methane production and urinary nitrogen excretion are used in the calculations. Methane production and urinary nitrogen excretion were both lower in the HCS group due to HCS treatment and lower nitrogen intakes, respectively. This would result in higher calculated heat productions for the HCS steers with all other factors being equal. Since oxygen consumption and carbon dioxide production were almost the same for both groups the major difference in the calculated heat productions was due to methane and urinary nitrogen. Heat production calculated from the oxygen consumption and carbon dioxide production (caloric value of 5.047 kcal/liter) results in very similar values (9.12 mcal for controls vs. 9.19 mcal/day for HCS steers). This also results in similar energy retentions for both groups (3.92 and 3.66 mcal/day for controls and HCS steers respectively).

Both treatment groups were more efficient in trial II than in trial I suggesting that the adaptation period for trial I was too short for the animals to become completely adapted to the digestion stalls and respiration chambers. Data from trial I, however, tends to agree with results of comparative slaughter trials where animals are fed under practical

TABLE XIV

TOTAL ENERGY LOSSES AND ENERGY RETENTIONS:
COMBINED ENERGY BALANCE TRIALS

Energy Fraction	Control	HCS
GE (mcal/day)	20.42 ± 0.41	20.36 ± 0.44
Feces (mcal/day)[1]	5.66 ± 0.31	6.03 ± 0.22
UE (mcal/day)	0.70 ± 0.03	0.65 ± 0.02
CH_4 (mcal/day)	1.02 ± 0.06	0.67* ± 0.06
H_2 (mcal/day)[2]	0.00	0.16
HP (mcal/day)	9.62 ± 0.33	10.16* ± 0.34
ER (mcal/day)	3.41 ± 0.32	2.83* ± 0.33
NEm+g (mcal/day)	8.67 ± 0.32	8.15* ± 0.29

[1] significant trial x treatment interaction ($P<.05$)

[2] estimated from the factors of Johnson (1971, 1972a)

* significantly different from control ($P<.05$)

TABLE XV

ENERGY LOSSES AND ENERGY RETENTIONS:
COMBINED ENERGY BALANCE TRIALS

Energy Fraction	Control	HCS
Feces (% GE)[1]	27.71 ± 1.46	29.61 ± 0.53
UE (% GE)	3.42 ± 0.11	3.19 ± 0.10
CH_4 (% GE)	4.99 ± 0.24	3.29* ± 0.20
H_2 (est % GE)[2]	0.00	0.78
HP (% GE)	47.07 ± 1.40	49.88* ± 1.47
ME (% DE)	87.90 ± 0.35	90.70* ± 0.29
ER (% GE)	16.67 ± 1.48	13.99 ± 1.64
NEm+g (% GE)	41.94 ± 1.37	38.82 ± 1.81
NEm+g (mcal/kg DM)	1.91 ± 0.06	1.82* ± 0.07

[1] significant trial x treatment interaction

[2] estimated from the factors of Johnson (1971, 1972a)

* significantly different from control ($P<.05$)

conditions. This suggests that animals poorly trained to respiration chambers may have similar efficiencies of energy utilization to those of steers fed under practical conditions.

There was a significant ($P<.05$) trial by treatment interaction for all comparisons of ME except ME as a per cent of DE which was significantly ($P<.01$) higher for the HCS steers. If estimated hydrogen energy losses are included, the HCS group still had a significant ($P<.05$) advantage. Total ME intake and ME/kg DM declined significantly ($P<.05$) between trials in the control group, but increased significantly ($P<.05$) between trials for the HCS steers. ME as a per cent of GE was fairly constant across trials for both groups.

A plot of ME intake against energy gain, expressed as kcal/$W_{kg}^{0.75}$/day was used to estimate the net efficiency of utilization of ME for energy gain (Figure 1). The point representing energy gain at maximum intake is connected with a straight line to the point representing energy loss at zero feed intake. The point where the line crosses zero energy gain estimates the ME maintenance requirement. The slope of the line represents the net efficiency of utilization for body gain of ME ingested above maintenance. The plot representing the averages for each group in both trials are shown in Figure 1. The average energy equilibrium for the control and HCS group in trial I were 129 and 143 kcal/$W_{kg}^{0.75}$/day, respectively. In trial II the values for the treatment groups were 90 and 93 kcal/$W_{kg}^{0.75}$/day, respectively. The marked difference between trials for both treatments represents the decreased energy expenditure after the better adaptation to the respiration chambers. The net efficiencies of utilization of ME are shown in Table XVI. Values for the second trial are higher than the normally accepted limit of the

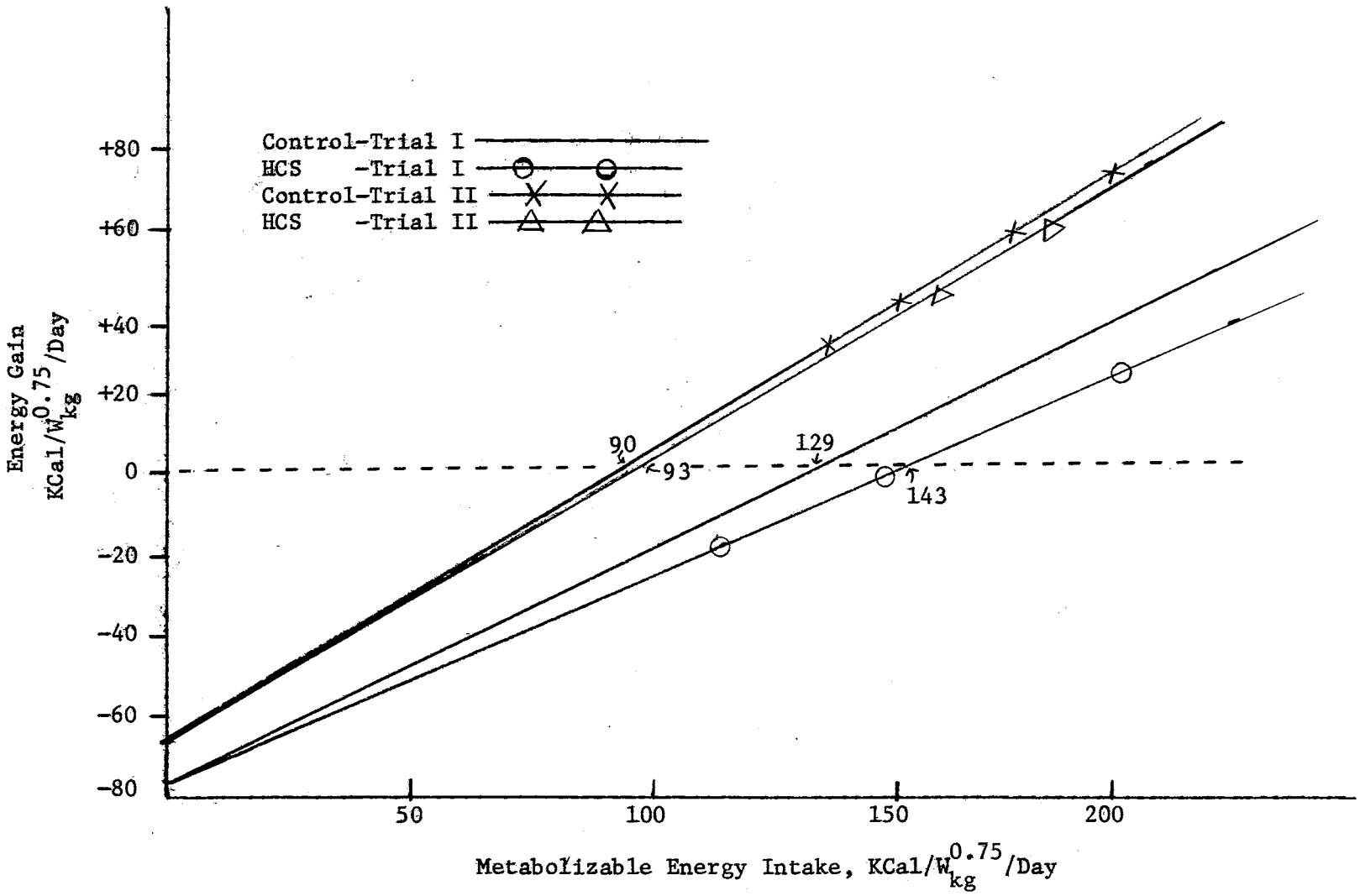

Figure 1. Relationship Between Energy Gain and Metabolizable Energy Intake

TABLE XVI

NET EFFICIENCY OF UTILIZATION OF ME (%)

Item	Trial I		Trial II	
	Control	HCS	Control	HCS
For Maintenance[1]	74.01	73.44	73.56	74.01
For Gain[1]	55,41	53.87	54.11	55.41
For Gain[2]	59.78	54.11	73.56	71.22

[1]determined by the equation of Blaxter (1961)

[2]determined by the plot of ME vs EG

ruminant animal of 70% (Reid, 1970). It should be remembered, however, that the fasting heat productions used in these calculations are assumed values and that in reality these lines are not linear between a level below and above maintenance. A decrease of only 5 kcal ME/$W_{kg}^{0.75}$/day would result in a decrease of almost 5 percentage units in the net efficiency of utilization of ME for gain. The values obtained using the assumed fasting heat productions, however, do tend to indicate that HCS had no effect on the net utilization of ME. The values for trial I tend to agree with the results of Johnson (1972a, 1972b) who also noted no effect of HCS on the net efficiency of utilization of ME for gain.

Values for the net utilization of ME for maintenance and for gain were also calculated using the equations of Blaxter (1961). These values are presented in Table XVI. Values calculated by both methods tended to agree in trial I but were markedly different in trial II. The values calculated by the equations of Blaxter, however, also tend to indicate that HCS feeding had little or no effect on the efficiency of utilization of ME.

Rumen VFA and Ammonia-N

Trial I

Rumen volatile fatty acid and ammonia-nitrogen levels are presented in Table XVII. Concentrations of total VFAs, acetate and propionate tended to be higher in the control group. The lower total VFA concentrations in the HCS group suggest a lowered rumen fermentation rate due to the HCS. HCS steers tended to have higher concentrations of butyrate, valerate and isovalerate, but these were significant ($P<.05$) only for valerate.

TABLE XVII

CONCENTRATIONS OF VOLATILE FATTY ACIDS AND RUMEN AMMONIA-N

Item[1,2]	Trial I		Trial II	
	Control	HCS	Control	HCS
Acetic[3]	47.49	38.01	72.48	44.14*
Propionic	41.72	29.23	32.77	30.56
Butyric	19.26	22.25	25.98	22.41
Valeric	3.86	6.72*	3.70	6.60*
Isovaleric	4.72	8.00	7.20	8.05
Total VFA	117.05	104.22	142.14	111.76
NH_3-N[4]	95.93	121.98	102.04	124.72
pH	5.90	5.90	5.70	5.90

[1] at four hours postfeeding

[2] average of 6 steers except Control-Trial II which is average of 5 steers

[3] VFA concentrations in umoles/ml

[4] NH_3-N concentrations in ugrams/ml

* significantly different from value for control in the same trial ($P<.05$)

The molar proportions of volatile fatty acids are given in Table XVIII. Control steers tended to have higher prportions of acetate and had significantly ($P<.05$) higher proportions of propionate. The lower proportions of propionate noted in this study are contrary to results noted with HCS feeding in other studies. The time and method of rumen sampling may be the reason for these results. HCS steers, however, did have significantly ($P<.05$) higher proportions of butyrate, valerate and isovalerate.

The HCS group tended to have higher rumen ammonia-N levels but these were not significant. Previous studies have noted lower rumen ammonia-N levels with HCS feeding (Singh et al., 1971). Time and method of sampling must again be considered in interpreting these results.

Trial II

Concentrations of volatile fatty acids in trial II are presented in Table XVII. Control steers had significantly ($P<.05$) higher concentrations of acetate and tended to have higher concentrations of total VFA. Concentrations of propionate and butyrate were similar for both groups. HCS steers had significantly ($P<.05$) higher concentrations of valerate.

Control steers had significantly ($P<.05$) higher molar proportions of acetate while HCS steers had slightly higher proportions of propionate, butyrate and isovalerate and significantly ($P<.05$) higher proportions of valerate.

No significant differences were noted in rumen ammonia-N concentrations or in rumen pH.

The occurance of a number of trial x treatment interactions

TABLE XVIII

MOLAR PROPORTIONS OF VOLATILE FATTY ACIDS

Item[1,2]	Trial I		Trial II	
	Control	HCS	Control	HCS
Acetic	39.95	37.17	50.58	39.84*
Propionic	37.03	27.56*	23.14	27.14
Butyric	16.02	20.93*	18.24	20.10
Valeric	3.37	6.46*	2.73	5.89*
Isovaleric	3.63	7.87*	5.31	7.02

[1]at four hours postfeeding

[2]all values are moles/100 moles total VFA

*values for a given acid in the same trial are significantly different from controls ($P<.05$)

prevented the pooling of VFA data from trial I and trial II.

Feedlot Performance

The ingredient composition of the basal ration and the chemical composition of the treatment rations are shown in Tablex XIX and XX, respectively. The basal ration was slightly higher in crude protein and acid detergent fiber content, but the gross energy content of the rations were almost identical. Average daily gain, feed efficiencies and average daily feed intakes are shown in Table XXI. The IHCS group tended to have lower average daily weight gains than the control and CHCS groups, but this was not statistically significant. Weight gains for the control and CHCS groups were similar. Previous studies (Trei and Scott, 1971; Trei et al., 1971) have shown a slight improvement in daily weight gain when animals were fed rations containing HCS. Rations in these studies contained 50 to 60% concentrate while the rations used in this study contained 80% concentrate. This suggests that the improvement in performance due to HCS feeding may decrease as the concentrate level of the ration increases.

The CHCS group had a slight advantage in feed efficiency over the control and IHCS groups (6% and 19%, respectively), but this difference was not significant. Dry matter intakes were similar for both HCS groups with the control group have slightly higher intakes. Previous studies have noted similar effects of HCS feeding at a constant level on feed efficiency and feed intake (Trei and Scott, 1971a, 1971b; Trei et al., 1971; Trei et al., 1972).

Trei et al. (1971) suggested that a constant increase in the level of HCS in the ration might improve animal performance without reducing

TABLE XIX

INGREDIENT COMPOSITION OF BASAL RATION:[1]
FEEDLOT PERFORMANCE TRIAL

Ingredient	Percent
Rolled grain sorghum	62.99
Dehydrated alfalfa pellets	7.50
Cottonseed hulls	11.50
Soybean meal	10.99
Dried cane molasses	4.99
Trace mineralized salt	0.50
Dicalcium phosphate	0.50
Calcium carbonate	0.50
Ammonium chloride	0.50
Aurfac-50	0.03

[1]on an as-is basis

TABLE XX

AVERAGE CHEMICAL COMPOSITION OF RATIONS:
FEEDLOT PERFORMANCE TRIAL

Ingredient[1]	Control	CHCS	IHCS
Dry matter (%)	88.05	87.66	87.48
Crude Protein (%)	16.46	15.33	15.62
Acid Detergent Fiber (%)	17.79	14.84	14.78
Ash (%)	6.27	5.49	5.46
Gross Energy (kcal/kg)	4514.37	4546.77	4558.77

[1]all figures except dry matter are on a 100% dry matter basis

TABLE XXI

ANIMAL PERFORMANCE IN THE FEEDLOT[1]

Item	Control	CHCS	IHCS
Initial Weight (kg)	311.86 ± 3.54[2]	316.94 ± 3.38	317.05 ± 2.51
Final Weight (kg)	431.98 ± 5.99	433.24 ± 8.74	412.07 ± 8.63
Total Gain (kg)	120.12 ± 6.66	116.30 ± 6.77	95.02 ± 6.48
Daily Gain (kg/day)	1.40 ± 0.08	1.35 ± 0.08	1.10 ± 0.08
Feed/Gain (kg)	8.84 ± 0.26	8.31 ± 0.10	9.86 ± 0.35
DM/Gain (kg)	7.78 ± 0.23	7.29 ± 0.09	8.62 ± 0.31
Feed Intake (kg/day)	12.33 ± 0.57	11.27 ± 0.32	10.91 ± 1.18
DM Intake (kg/day)	10.85 ± 0.51	9.86 ± 0.28	9.54 ± 1.03
GE Intake (mcal/day)	48.98 ± 2.29	44.83 ± 1.26	43.49 ± 4.71
GE/Gain (mcal/kg)	35.07 ± 1.03	33.15 ± 0.39	39.36 ± 1.41

[1]each figure is the average of 9 steers

[2]standard error of the mean

feed intake. Table XXII shows the average daily feed intakes for each treatment group during each period of the feeding trial. Periods 1, 2 and 3 represent successive 30 day periods on treatment. The CHCS group had slight increases in dry matter intake between each period while the IHCS group had slight decreases. This suggests that the steers in the IHCS group did not adapt to the increasing level of HCS in the ration.

Table XXI shows the average daily gross energy intakes and the gross energy intakes per kg. of body weight gain. Average daily GE intakes were not significantly different although the control group tended to have slightly higher intakes. The CHCS group had the greatest efficiency of conversion of gross energy to body weight gain although the difference was not statistically significant. This suggests that the 0.2% level of HCS used in the growth trial may be more satisfactory than the 0.3% level used in the previously reported energy balance trials.

TABLE XXII

AVERAGE DAILY FEED INTAKE PER HEAD BY PERIOD

Item	Control	CHCS	IHCS
Period 1			
Feed Intake (kg)	11.56 ± 0.89[1]	10.63 ± 0.44	11.24 ± 1.35
DM Intake (kg)	10.28 ± 0.79	9.34 ± 0.39	9.89 ± 1.17
Period 2			
Feed Intake (kg)	12.65 ± 0.49	11.45 ± 0.48	10.22 ± 0.95
DM Intake (kg)	11.18 ± 0.43	10.01 ± 0.46	9.80 ± 0.85
Period 3			
Feed Intake (kg)	12.84 ± 0.49	11.70 ± 0.48	10.25 ± 1.06
DM Intake (kg)	11.15 ± 0.44	10.26 ± 0.35	8.91 ± 1.10

[1]standard error of the mean

CHAPTER V

SUMMARY AND CONCLUSIONS

Twelve steers were used to invetigate the effect of inhibition of methane production on the energetic efficiency of beef steers fed a high concentrate ration. One group of 6 steers was fed a basal ration containing 80% concentrate and a second group was fed the same ration containing 0.3% of a methane inhibitor, HCS. Animals were individually fed twice daily.

Total energy balance trials were conducted at 30 and 120 days on feed. Feces and urine were collected over a seven-day period and gaseous exchange was measured for two consecutive 24-hour periods in each trial. Rumen fluid samples were taken in each trial for determination of VFA and ammonia-N.

HCS additions had a significant detrimental effect on digestion of protein and energy ($P<.05$) at 30 days on feed. At 120 days on feed, only protein digestibilities were significantly lower for the HCS group. Methane production was significantly lower ($P<.05$) for HCS steers in trial I, but not in trial II.

Control steers tended to have higher energy retentions and had significantly ($P<.05$) higher ME intakes in trial I while HCS steers had significantly ($P<.05$) higher ME intakes in trial II. ME/GE values were similar for both treatment groups in both trials, but HCS steers had significantly ($P<.05$) higher values for ME/DE in both trials. HCS had

no marked effect on the net utilization of ME for gain.

HCS steers tended to have higher levels of rumen ammonia-N, but lower concentrations of total VFAs. No consistent effect of HCS on the major volatile fatty acids was noted.

Twenty-seven steers were randomly alloted to one of three treatments in a growth trial. Treatments consisted of 1) basal ration, 2) basal + 0.2% HCS (CHCS) and 3) basal + an increasing level of HCS (IHCS).

There were no significant differences in average daily gain, feed efficiencies, or feed intake, although the IHCS group tended to have poorer performance than the other two treatment groups. CHCS steers had a slight advantage over the control and IHCS groups in feed efficiency (6 and 19% respectively). Steers on the increasing level of HCS apparently were unable to adapt to the inhibitor and had reduced feed intakes each month of the trial.

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