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A STUDY ON THE EFFECT OF THE COMBINATION OF MONENSIN AND ISOACIDS ON RUMEN FERMENTATION *IN VITRO*.

BY

Patrice Kone

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ABSTRACT

A STUDY ON THE EFFECT OF THE COMBINATION OF MONENSIN AND ISOACIDS ON RUMEN FERMENTATION IN VITRO.

By

Patrice Kone

A semi-continuous technique was adapted to investigate the interaction of isoacids and Monensin on rumen fermentation. The culture was established using inoculum from a cow fed timothy hay. The media contained timothy hay, urea and ammonium sulfate (1.0, 0.02 and 0.003 g/100 ml, respectively), minerals and vitamins. The culture was for 12 days and was allowed to stabilize 4 days before adding treatment level of isoacids and Monensin. Comparisons were made for the last 2 days of each trial. Isoacids (equal proportions of isobutyric, 2-m-butyric, isovaleric and valeric acids) at 15 mg/100ml increased acetate (6.11 vs 5.60 meq/100ml) and total volatile fatty acid production (8.97 vs. 8.13 meq/100ml); Monensin at 150 ug/100ml reduced acetate (3.99 vs. 6.08 meq/100ml) and VFA (6.84 vs. 8.54 meq/100ml) but increased propionate production (2.28 vs. 1.73 meq/100ml). The combination of isoacids and Monensin increased acetate in relation to Monensin (5.24 vs. 4.00) but did not eliminate the effect of the ionophore on propionate. Total gas production, hydrogen and ammonia-nitrogen levels were not influenced by isoacids and Monensin.

DEDICATION

To my parents, who instilled in me the belief that education was the greatest gift they could bestow in me, I thank them for both moral and financial support; to my wife Rachel who has been a source of constant strength during the course of this study.

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LIST OF ABBREVIATIONS

C2	Acetate
C3	Propionate
C2C3	Ratio of C2 to C3
C4	Butyrate
C4-C5	Four and five-carbon branched-chain fatty acids
CS	Corn silage
CH4	Methane
C02	Carbon Dioxide
GH	Grass hay
GP	Gas Production
H2	Hydrogen
IVA	Isovalerate
ISO	Equimolar weight mixture of isobutyric acid, isovaleric acid, 2-methyl butyric acid and valeric acid.
MON	Monensin
2MB	2-Methyl Butyrate
N-NH3	Ammonia nitrogen
VAL	Valerate

INTRODUCTION

The efficiency of nutrient utilization by ruminants is influenced by the balance of rumen fermentation end-products, which is dependent upon the types of microorganisms present in the rumen. Consequently, effective manipulation of microbial species in the rumen and their activities can result in improved animal performance.

Monensin has been used extensively in diets for feedlot cattle since 1975. Recently, isoacids have been commercialized for dairy cattle and may improve growth of feedlot cattle. However, little is known about the effects of the combination of the two compounds in the rumen.

In vitro rumen fermentations have shown that Monensin decreases the acetate:propionate ratio (Smith, 1971; Richardson et al., 1976; Chalupa, 1977). These results have been attributed to a toxic effect of the antibiotic upon Ruminococci (Brulla and Bryant, 1980). Isoacids (isobutyric, isovaleric, 2-methylbutyric, and valeric acids), however when added to in vitro systems, have increased acetate production due to an enhanced growth of Ruminococci (Allison and Bryant, 1958; Gorosito et al., 1985).

The mode of action of the ionophore is believed to be through an interruption in Na+/K+ transport at the membrane level (Romatowski, 1979). Isoacids have their effect at the membrane level too. They are carbon skeletons for the synthesis of branched chain aminoacids that are incorporated into membrane proteins (Allison and Bryant, 1958).

The objective of this work was to study the effects of adding Isoacids to an *in vitro* rumen fermentation system containing Monensin.

LITTERATURE REVIEW

MONENSIN.

Monensin, a carboxylic polyether ionophore, is a biologically active compound produced by Streptomyces Cinnamonensis (Haney and Hoehn, 1967). Initial use of Monensin as an anticoccidial feed additives was launched by the poultry industry. However during the past 15 years chemical agents including Monensin have been identified as having the potential to affect rumen metabolism. Thus, because of its potent effect on ruminal ecology and animal production, Monensin has been approved since 1975 by the Food and Drug Administration as a feed additive for cattle.

Animal response to monensin

Numerous studies conducted on animal response to monensin, have shown that when it is incorporated at a recommended level into high concentrate diets for finishing cattle, body weight gain is unchanged, even though feed intake is generally depressed. Therefore, the efficiency of feed conversion is improved (Owen, 1980; Bergen, 1984). The influence of Monensin on feed intake is well documented. Furthermore, studies have shown that in high grain diets, supplementation with Monensin depressed feed intake by 5 to 6% (Anonymous, 1975; Owens, 1980).

When animals were fed roughage, Monensin did not depress intake and body weight gain improved (Potter and Richardson 1975). Therefore, efficiency of feed conversion is improved.

Cattle receiving Monensin under pasture conditions have responded with a 17% increase in rate of gain (Anonymous 1975).

Monensin has been shown to increase the efficiency of feed utilization (kg of feed per kg of live weight gain) in both sheep and cattle fed a variety of rations (Potter et al 1976). Carcass data analysis indicated that the main effect of the Monensin is to increase the efficiency of dietary energy retention in the carcass.

Effect on Rumen Fermentation.

The effect of Monensin on rumen fermentation is well known. Monensin has consistently increased the molar proportion of propionate with a concomitant decline in the molar proportion of acetate and butyrate (Chalupa, 1980; Bergen and Bate, 1984). Van Maanen et al., (1978) found using isotope dilution techniques that higher production of propionate occurs at the expense of acetate. The shift in VFA is often associated with a decrease in methane production without accumulation of gaseous hydrogen (Van Nevel and Demeyer, 1979; Chalupa, 1980).

The concept that propionate is more efficiently utilized than acetate is based on two hypotheses. Hungate (1966) estimated the efficiency of conversion of hexose to propionate to be higher than the efficiency of conversion of hexose to acetate or butyrate. Therefore, it was proposed that propionate is more efficiently utilized than acetate and butyrate. The second theory proposed by Smith (1971) was more controversial. Smith proposed that propionate is more

efficiently used than acetate by the tissue.

Allen and Harrison (1979) reported a decrease in branched chain fatty acids when Monensin was added at 22 ppm to the diet of sheep fed dried grass/maize.

In vitro studies have indicated a decrease in microbial methane production with monensin (Barley et al., 1979; Chalupa et al., 1980). Similar responses have been obtained in vivo. The inhibition of methane production by methane producers is only partial. Studies have shown a decrease of 4% to 31%. Van Nevel and Demeyer (1977) reported a decrease in the metabolism of formate to carbon dioxide and hydrogen when Monensin was fed and proposed that this effect of Monensin could account for the decrease in methane production. Monensin has no effect on carbon dioxide production at low level, but high levels of the chemical significantly depresses carbon dioxide (Bartley et al., 1979; Chalupa et al., 1980). Chen and Wolin (1979) reported a selection against hydrogen-producing rumen bacteria and a selection in favor of succinate-forming bacteria. They proposed that the decrease in hydrogen and carbon dioxide production could also account for the decrease in methane production when Monensin is fed.

Other ruminal effects due to Monensin were: a lower ruminal lactate production in stressed animals (Denis et al., 1980), an increase in ruminal forage fill (Ellis and Delaney, 1982), a decrease in ruminal rate of passage (Lemenger et al 1978), and an increase in dry matter digestibility and ammonia nitrogen retention (Rust et al., 1978). Schelling (1983) has

defined seven probable system modes of action of Monensin: modification of acid production, modification of feed intake, change in gas production, modification in digestibility, changes in protein utilization, modification in rumen fill and rate of passage, and perhaps other ruminal modes of action.

The effect of Monensin on volatile fatty acids is accepted as one of the main modes of action. However this probably does not account for all the effects of Monensin.

Reilly and Ford (1971) suggested that Monensin spares amino acids normally used for gluconeogenesis. Eskeland et al. (1974) proposed a possible stimulation of protein synthesis when Monensin is fed and Smith (1979) suggested that the increase in propionate may also lower heat increment.

Effect on rumen microbes

Numerous in vivo and in vitro studies were conducted to determined how Monensin controlled microbial activity. Chen and Wolin (1979) and Denis et al. (1981) found that Ruminoccoccus albus, Ruminoccocus flavefaciens and Butyrivibrio fibrisolvens are very sensitive to Monensin. These species are very important in the production of acetate, butyrate, carbon dioxide and hydrogen. Selenomonas ruminantium which decarboxylate succinate to propionate were resistant to Monensin. Bacteriodes, Selenomonads and Succinivibrio, all succinate producers, were not inhibited by Monensin. Lactate producing species such as Lactobacillus vitulinus and Lactobacillus ruminus were inhibited by Monensin, whereas at

the same level, lactate fermentors (Megasphaera elsdenii and Selenomonas ruminantium) were not sensitive to Monensin. Therefore it was proposed that Monensin could be effective in preventing lactic acidosis. These findings have been confirmed by Nagaraja et al., (1981).

Attempts have been made to explain the changes brought about by Monensin on the predominant rumen microbes (Chen and Wolin, 1979; Anderson et al., 1981). The sensitivity pattern suggested that the antibiotic acted by selecting for the rumen microbes that produce proportionally more propionate. Consequently, species such as Selonomonas ruminantium and Bacteriodes ruminicola were selected. Ruminoccocci and Butyrivibrio that are major producers of acetate, butyrate, hydrogen and carbon dioxide were inhibited.

ISOACIDS

Branched-chain carbon skeletons referred to as isobutyric acid, 2-methylbutyric acid and isovaleric acid are required for growth by a wide variety of cellulolytic anaerobic microorganisms (Hungate, 1966; Cook, 1985). Valeric acid has been shown to improve cellulose degradation in vitro (Cummins and Papas, 1984; Amos and Little, 1971). The ruminal source of branched-chain fatty acids are degraded feed protein and endogenous branched-chain aminoacids (Pittman and Bryant 1964).

Addition of isoacids to in vitro fermentation systems has increased digestibility of soybean stover (Soofi et al., 1982; Cummins and Papas, 1984).

In the rumen, bacteria use branched chain fatty acids to synthesize aminoacids, but they also use isoacids for the biosynthesis of long chain fatty acids (Allison et al., 1961 Dehority et al, 1967).

In vitro studies by Felix (1976) and by Cummins and Papas (1984), have shown that the addition of isoacids increased microbial growth and dry matter digestion. By the same token, Gorosito and Russel (1984) have reported that the addition of these acids not only increases cell wall digestion of intact forage but also increases ammonia nitrogen utilization by the rumen microbes.

In vivo studies reported that isoacids have constantly increased nitrogen retention and have lowered urinary nitrogen loss when steers were fed isolated soy protein (Oltjen et al., 1970). Felix and Cook, (1980) and Cook, (1985) pointed out that isoacids plus urea improved milk production. These authors reported that isoacids increased growth rate in young animals but not in older animals and that the supplementation of Isoacids and urea to high producing cows fed corn silage as the sole roughage had a positive effect on milk production, persistancy of lactation, body weight, feed intake and nitrogen balance.

The requirement for branched chain fatty acids by the rumen microbes is well known. Allison and Bryant (1963) and Yokoyama and Johnson (1984) reported a requirement of branched chain fatty acids for growth of several rumen cellulolytic bacterial species including Ruminococcus albus and Bacteriodes

succinogenes. Isoacids are also required for growth of methanogenic bacteria, Treponema and Megasphaera elsdenii.

Towns and Cook (1984) reported an alteration of growth hormone and insulin and an increase in milk production in lactating cows fed high concentrate diets. Fieo et al. (1984) also found a higher growth hormone concentration.

Towns and Cook (1984) found that during an eight-hour sampling period, isoacid treated cows had higher growth hormone but lower blood glucose than control cows.

Bines and Hart (1984) and Istasse and Orskov (1984) reported that the decrease in propionate production in the rumen resulted in lower stimulus for insulin secretion.

Brondani (1986) found that the decrease in insulin found for the first time by Towns and Cook (1984) when cows were fed high concentrate diet plus isoacids was due to a decreased propionate production in the rumen.

MONENSIN AND ISOACIDS

Monensin, when used in lactating cow diets, has caused a decrease in milk production and milk fat percentage. This response may be due to a high level of propionate in relation to acetate and butyrate, which stimulates the release of insulin. Insulin is responsible for partitioning nutrients away from milk production to fat deposition. The addition of isoacids in the diets of cows receiving Monensin could increase the acetate to propionate ratio, because isoacids would increase acetate production. The problem of combining the two chemicals is that isoacids is a growth factor for cellulolytic

bacteria and Monensin is toxic to this class of microorganisms. Thus, our intent was to investigate the effect of the two compounds on the rumen microorganism in vitro using a long-term culture technique.

LONG TERM CULTURE EXPERIMENTS

Numerous in vitro continuous culture experiments have attempted to use substrate concentrations characteristic of the rumen and to substitute artificially for the supply and removal functions of the rumen (Hungate et al., 1942; Gray et al, 1962; Short, 1978). Survival of protozoa in number and kinds comparable to the rumen is the easiest means to ascertain whether the rumen population is maintained. Rates of production of fermentation acids and gases are criteria for comparing in vitro activity with that in the rumen (Hungate, 1966).

The objectives of the present study were first to duplicate the rumen conditions sufficiently well to support the fermentation rate characteristic of the rumen, secondly to investigate through a quantitative measurement of the production of VFA and gasses under completely controlled conditions, and finally to determine the effects of the combination of Isoacids and Monensin on rumen microbes.

MATERIALS AND METHODS

A. MATERIALS

A series of *in vitro* experiments were conducted to investigate the effect of Monensin and isoacids upon a mixed population of rumen microorganisms. The experimental technique consisted of replacing one half the volume (50 ml) of a 24-hour culture by a new medium of equal volume. There were ten trials lasting from eight to twelve days, with some variations in the cow diet, the media composition and the concentration of chemicals.

A mature non-pregnant and non-lactating rumen-canulated Holstein cow of approximately 550 kg body weight served as a donor. Throughout the entire experimental period, the cowwas fed four different diets, one of each separated by a ten-day period of adaptation. The cow was fed three hours prior to taking the rumen samples. Samples from different parts of the rumen were strained through two layers of surgical gauze into 1-liter glass bottles kept at 40 degrees Celsius and rapidly brought to the laboratory. A total of 1 liter of rumen fluid was added to the same amount of medium. The medium composition is shown in Tables 1, 2 and 3.

Table 1. COMPOSITION OF 1 LITER OF MEDIUM

Dry nutrients:	
Grass hay	20.00 gr
Urea	.40 gr
Ammonium Sulfate	.06 gr
Mineral solution 1	80 ml
Mineral solution 2	80 ml
Micromineral solution	20 ml
Sodium Bicarbonate solution 6.32%	110 ml
Sodium Sulfide solution	5 ml
Vitamins	1 ml
Distilled water	685 ml

Adapted from Phillips, D.S., and J.M Tadman (1980) (unpublished data)

Table 2. COMPOSITION OF MEDIUM STOCK SOLUTIONS

Mineral solution 1	g/liter
K2HPO4.3(H2O)	12.5
Mineral solution 2	
KH2PO4	12.5
MgSO4.7(H2O)	3.0
NaCl	12.0
CaCl2.2(H2O)	1.6
Micromineral solution	
Disodium Dihydroxide. E.D.T.A	5.000
FeSO4.7(H2O)	2.000
H3Bo3	0.030
CoCL2.6(H2O)	0.020
ZnSO4.7(H2O)	0.010
MnCl2.4(H2O)	0.003
Na2MoO4.2(H2O)	0.003
NiCl2.6(H2O)	0.002
CuCl2.2(H2O)	0.001
Sodium Bicarbonate 6.33%	
NaHC03	63.300
Sodium Sulfide 2.5%	
Na2S.9H20	25.000

Adapted from Phillips and Tadman 1980 -(unpublished data)

Table 3. COMPOSITION OF VITAMIN SOLUTION

Composition	g/liter
Pyridoxamine Hydrochloride	2.0
Riboflavin	2.0
Thiamine Hydrochloride	2.0
Nicotinamide	2.0
Calcium Panthotenate	2.0
Lipoic Acid	1.0
Para-Aminobenzoic Acid	.1
Folic Acid	.05
Biotin	.05
Coenzyme B12	.05

DESCRIPTION OF THE INCUBATION VESSEL

A 250 ml erlenmeyer flask, with a liquid port on one side for culture sampling and a gas sampling port on the other side, was fitted with a graduate cylinder for gas measurements (Figure 1). A 12 cm flexible tube was placed at the extremity of the liquid port in order to allow for easy transfer. A pinch clamp was placed at the extremity of each tube to avoid any oxygen entry into the flask (Figure 1).

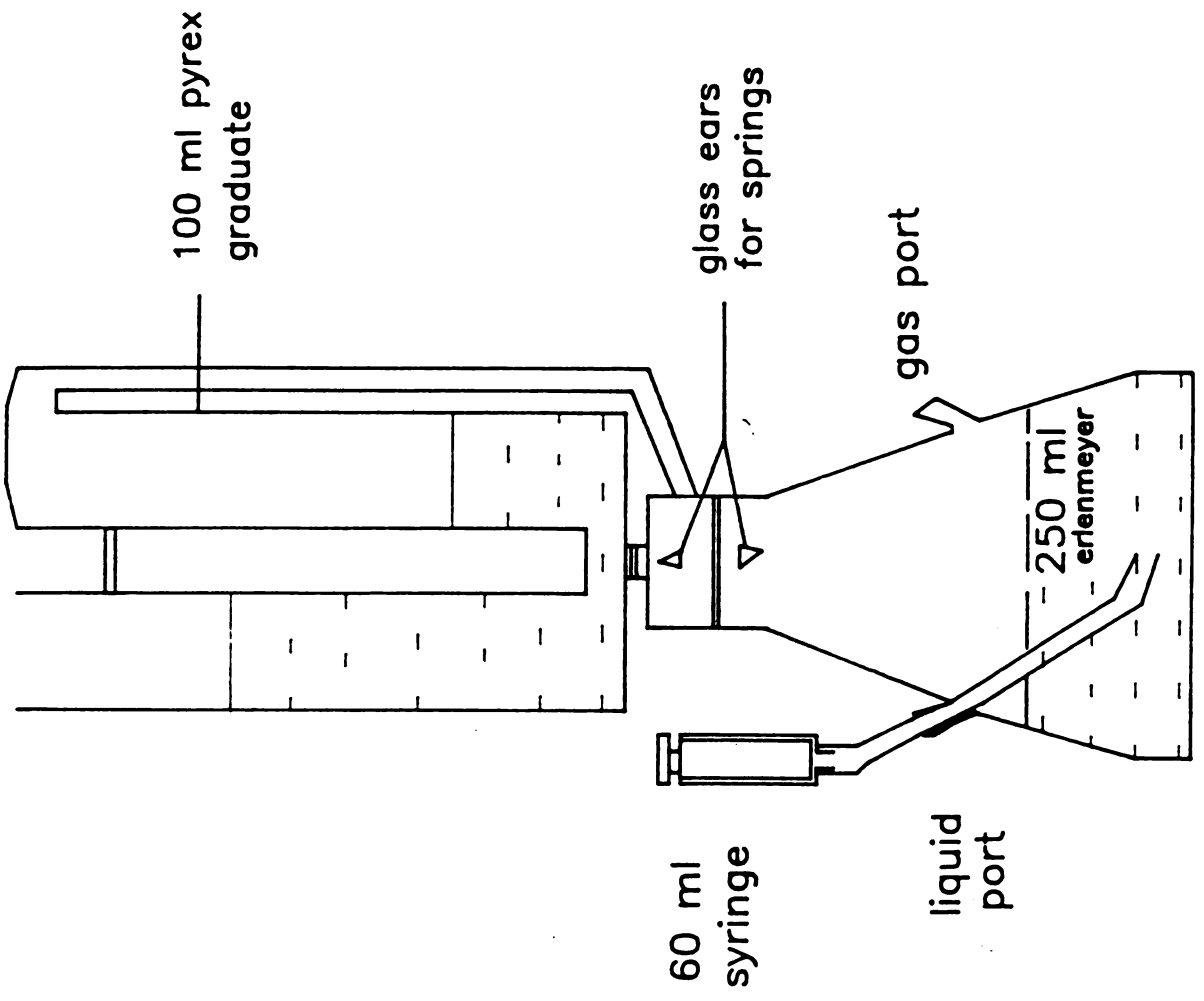

Figure 1. Anaerobic Digestor and Gas measuring Apparatus.

ESTABLISHMENT OF CULTURE

On the first day of culture establishment, 1 liter of medium was prepared and the same amount of rumen fluid was collected from a cow 3 hours after feeding. Inoculum and medium were mixed under C02. A 100 ml aliquot of this mixture was then dispensed anaerobically into the incubation flasks flushed with ammonia nitrogen. The fermentation flasks were then placed in a Grant water bath shaking at a rate of 45/min and the temperature of the water was maintained at (39°C)[1]

TRANSFER OF CULTURE

The procedure was adapted from Short (1980) and modified as follows: after 24 hrs of incubation, one half (50 ml) of the incubated culture was replaced by 50 ml of a new medium in the following way. The digestors were individually removed from the water bath. Fifty ml of the old medium was withdrawn while the fermentor was being flushed with nitrogen. Then, 50 ml from a fresh medium were collected from a round flask gassed with 100% C02 and transferred via a 60 ml syringe into the flask containing the remaining 50 ml of the old culture.

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[1]Bench Scale Equipment Co., Dayton, Ohio.

PREPARATION OF ISOACIDS AND MONENSIN SOLUTIONS

Isoacids (isobutyric, 2-methylbutyric, isovaleric, and valeric acids) were obtained from Eastman Chem. Co. The four isoacids were mixed at equimolar concentration, then neutralized with sodium hydroxide and diluted in double-distilled water to final concentrations of 10, 15, 20, and 40 mg/dl in the fermentors.

Monensin, obtained from Sigma Company, was primarily dissolved in 10 ml of methanol and then diluted with double-distilled water. The final medium concentrations were 100, 150, 200, and 400 ug/dl.

SAMPLING THE CULTURE MEDIA

Fifty milliliters of a 24-hour culture were collected daily from each digestor and divided as follows: 25 ml was used to measure the pH. The remaining 25 ml was centrifuged at 1800 x g. Two ml of the supernatant was treated with sulfuric acid (1N) and sodium tungstate (10%), and was then saved for ammonia nitrogen determination. One ml was treated with 200 ul of formic acid (88%) and immediately analyzed for volatile fatty acids.

MEASUREMENT OF TOTAL GAS PRODUCTION

The system used to measure the rate of gas production was devised by Phillips and Tadman (1973), and is based on the *in vitro* technique of using gas production rates for obtaining an estimate of microbial net growth (El-Shalzy and Hungate, 1965). The gas produced in the fermentation vessel caused the barrier solution to move down in the manometer, and is monitored as a function of time. The barrier solution was double distilled water containing 20% NaCl. A vacuum pump was used to bring the solution to the 90 ml mark. Readings were taken during the next three hours.

B - ANALYTICAL PROCEDURE

Gas composition

After 24 hrs, 0.5 ml gas samples were collected and allowed to remain in the Pressure-Lok A2 gas syringes[3] until injection into the gas chromatograph. A Hewlett-Packard 5750 Gas Chromatograph[4] equipped with a thermal conductivity detector was used for the analysis. Analysis was performed at the following operating conditions:

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3 Precision Sampling Corp., Baton Rouge Louisiana.
4 Hewlett-Packard. Route 41, Avondale, Pensylvania.
5 Supelco, Inc. Bellefonte, Pensylvania.

1. Column: Pyrex, 3m long x 2 mm i.d. packed with carbosieve, 100/120 mesh.[5]
2. Detector temperature : 250°C
3. Injection port temperature : 150°C
4. Column temperature : 125°C
5. Carrier gas (Argon) flow rate : 20 ml/min
6. Bridge current : 100 milliamperes

A model 7227A Strip Chart Recorder and a Model 3370B Integrator were used for recording.

Ratio between gases were calculated, based upon peak length of individual gases detected by the gas chromatograph and recorded on the Strip Chart Recorder.

AMMONIA NITROGEN

Ammonia nitrogen from rumen and fermentor samples were analyzed using the indophenol reaction (Chaney and Menbach, 1961) adapted from the determination of urea after hydrolysis with urease. Samples were prepared according to the method of Kulasek (1959) and Okuda *et al.* (1963).

VOLATILE FATTY ACIDS

Prior to the injection, 1 ml of incubation medium was acidified using formic acid. Injection volume was 1 microliter. The standard was obtained by a 1/10 dilution of the stock solution (Table 4).

TABLE 4. STANDARD STOCK SOLUTION

VFA	(umoles/ml)
ACETATE	57.430
PROPIONATE	19.490
ISOBUTYRATE	0.518
BUTYRATE	14.470
2-METHYLBUTYRATE	1.420
ISOVALERATE	1.864
VALERATE	2.924

One ml of the supernatant from the fermented sample was mixed with 200 microliter of 88% formic acid inside an automatic sampler vials (Hewlett-Packard Co.). The samples were then loaded onto an automatic injector.

Volatile fatty acids (VFA) were determined on a Hewlett Packard Gas-Liquid Chromatograph equipped with flame ionization detector. Analysis was performed at the following operating conditions:

1. Column: Pyrex, 6 ft long x 2 mm i.d. packed with 3% carbowax. 20M + 5% HC00H on 60/80 carbopack B 1-1825 (Supelco. Inc)
2. Detector temperature : 200^{o}C
3. Injection port temperature : 200^{o}C
4. Column (Oven) temperature: program 116^{o}C to 180^{o}C 8^{o}C/min
5. Carrier gas (Nitrogen) flow rate : 20 ml/min
6. Hydrogen pressure: 30 psi

7. Air pressure: 40 psi

An integrator-recorder, Model 3380A, was used for recording.

C. EXPERIMENTAL DESIGN.

A series of ten experiments was conducted, first to validate the *in vitro* system and then to investigate the interaction of isoacids and monensin on rumen microbes. See summary Table 5.

TABLE 5 Number of experiments to validate the system and test the effect of isoacids and Monensin.

	[1]Inoc.	[2]Subs.	[3]Prot.	[4]Mon	[5]Iso	[6]M+I
VALIDATION	2	3	2	-	-	-
INTERACTION	-	-	-	1	1	1

[1]inoculum [2]substrate, [3]protein level.
[4]monensin, [5]isoacids, [6]monensin + isoacids.

Validation of the System.

To reach the first objective, seven experiments were conducted over a period of eight days each, with a variation in the Inoculum, the Substrate and the Urea level. In addition, the effect of methanol and time were also evaluated.

In order to evaluate the Inoculum effect, two experiments were conducted: diets were changed after each experiment and a ten-day adaptation period was observed. The basal diets were corn silage, and grass hay. The proximal analysis of the ration is shown in table 6. The difference between rations could not be tested with complete validity because cows were not replicated within ration.

Table 6. Nutrient Content of the Source of Inoculum.

	DM	CP	NDF	NEM	NEG	NEL
grass hay	100	6.8	66.8	1.23	1.15	.50
corn silage	100	8.7	42.8	1.60	1.58	.99

In the second phase, three experiments were conducted. The objectives were to investigate the effects of the substrate on the fermentation pattern. Straw, grass hay, and corn silage were used (Table 7). Data were analyzed by AOV, using a completely randomized design. Differences between treatment means were tested using orthogonal contrasts.

TABLE 7. Nutrient Content of the Substrate.

	DM	CP	NDF	NEM	NEG	NEL
grass hay	100	6.8	66.8	1.23	1.15	.50
corn silage	100	8.7	42.8	1.60	1.58	.99
straw	100	4.3	73.2	0.90	0.20	1.02

In the third phase, two experiments were conducted where the Inoculum and the substrate used were the same in both experiments. To assess the effects of low and high concentrations of nitrogen, 0.2 g of urea were added to a liter of medium for the first experiment vs. 0.6 g of urea for the second experiment. These two levels were chosen based on previous dose response study on the effect of different levels of urea on ammonia concentration in the culture media. Because Monensin would not dissolve in aqueous solution, it was therefore necessary to find an adequate solvent that would not affect the fermentation pattern. Thus, for this purpose, ethanol and methanol were tested at 10% and 1% of the medium volume. During these experiments, the effect of time was investigated as well. Consequently, daily measurements of VFA, gas composition, and ammonia nitrogen content were obtained throughout the 8-day period and comparisons of all the data were made. Protein level data were analyzed using a 2x2 factorial.

Effect of Chemicals.

To reach the second objective, three trials were conducted in order to observe the artificial rumen fermentation as affected by isoacids and Monensin. The first two experiments had either isoacids or Monensin as treatments whereas the third received a combination of the two chemicals. The incubation time was twelve days using triplicate fermentors. Isoacids and Monensin were added to the flasks after the fourth day of incubation. The final concentrations of isoacids in the fermentors were 10, 15, and 20 mg/dl of medium in trial one. In trial two, Monensin concentrations were 100, 150, and 200 ug/dl whereas in trial three, levels were 150 ug/dl for Monensin and 10 and 15 mg/dl for isoacids.

All statistical analysis were carried out using the Statistical Analysis System (SAS) (SAS-MSU,1982). Data were analyzed by analysis of variance using split-plot for repeated measurements. Differences between treatment means were tested by Tukey's test for all experiments.

RESULTS AND DISCUSSION

VALIDATION OF THE SYSTEM

In order to set up a semi-continuous culture technique that will approach the rumen conditions, a series of seven experiments were carried out. Source of inoculum, substrate, protein level and other factors affecting microbial activity in the rumen were investigated.

Source of inoculum

Two experiments were conducted to investigate the effect of the type of ration on the long term culture technique over a period of eight days. Ground hay was used as substrate in both trials. The results are summarized in Table 8.

Table 8. Effect of the inoculum on Rumen VFA concentration and total gas production in vitro using grass hay as substrate.

	day 1		day 4		DAY 8	
Variable	CS[1]	GH[2]	CS	GH	CS	GH
	mmoles /dl					
C2	8.03	6.50	6.63	6.65	6.64	6.50
C3	2.77	1.64	1.88	1.86	1.87	1.84
C4	1.59	1.23	0.79	0.78	0.78	0.79
ISO	0.26	0.21	0.15	0.13	0.14	0.13
TOT	12.60	9.62	9.45	9.42	9.43	9.26
	ml/hr					
GAS	28	20	18	18	17	17

[1]rumen fluid from a cow fed corn silage.
[2]rumen fluid from a cow fed grass hay.

VFA are expressed in mmoles/dl of the culture volume, and gas production in ml/hr. On the first day of incubation acetate, propionate, butyrate and total gas production were affected by the source of inoculum, however on the fourth day of incubation and thereafter there was no difference on rate of gas production nor on VFA production between the two types of ration.

One of the main factors influencing the rumen fermentation is the variability in the components of the feed (Hungate 1955). The rumen microbial population depends on the continuous supply of the digestible feeds included in the ration. In this study, since the substrate used was the same in both experiments, an adaptation of the microbial population to thesubstrate may have occurred over time.

Substrate

After determining that source of inoculum had no effect on the system the effect of substrate on the fermentation was studied. The results are presented in Table 9.

VFA and gas productions were higher when corn silage was fed compared to grass hay, and lower than for grass hay when straw was used as substrate.

Of the digestible feeds, carbohydrates are the most important quantitatively because of their superiority as a source of energy under anaerobic conditions (Hungate,1955). Soluble carbohydrates, starch, and insoluble carbohydrates are the common types present in forage plants. Protein, in

addition to nitrogen and carbohydrates, is also required for fermentation and growth. Protein may influence rumen fermentation, not only directly as a source of nitrogen for assimilation, but also as a source of isoacids.

Table 9. Effect of substrate on rumen VFA concentration and total gas production *in vitro* using inoculum from a cow fed grass hay.

	Substrate			
Variable	Corn silage	Grass hay	Straw	SEM
		mmoles /dl		
VFA	11.22a	9.98b	7.75c	.51
Acetate	6.37a	5.93b	5.45c	.13
Propionate	2.86a	1.76b	1.77b	.18
Butyrate	.87a	.87a	.97a	.02
		molar %		
Isoacids	1.65a	1.47b	.83c	.12
		ml/hr		
Gas	41.44a	24.77b	16.77c	3.63

a,b,c Means in a row with different superscript differ p(<.05).

In the present study the difference in VFA production was due to a difference in energy and/or protein content of the different substrates used. When straw was used as substrate, isoacid concentrations remained very low in the culture. Therefore, they may have been a limiting factor for growth of cellulolytic bacteria.

Protein Level

Two experiments were conducted to investigate the effect of protein level on the semi-continuous culture system and to adjust the ammonia nitrogen level in the medium. The effect of two components of protein degradation ammonia nitrogen and isoacids on rumen fermentation, was studied (Table 10). In order to keep energy constant, the same substrate (Grass hay) was used in both trials.

Table 10. Effect of ammonia nitrogen level and Isoacid concentration on rumen VFA and Total gas production in vitro using inoculum from a cow fed grass hay.

	Ammonia Nitrogen Level (mg/dl)				
	5.57		0.24		
Variable	[1]ISO-	[2]ISO+	ISO-	ISO+	SEM
	mmoles/dl				
VFA	8.114a	8.918b	7.955a	8.107a	.10
Acetate	5.607a	6.118b	5.510a	5.408a	.02
Propionate	1.637a	1.679a	1.630b	1.635b	.02
Butyrate	0.609a	0.667b	0.602a	0.604a	.03
Isoacids	0.261a	0.454b	0.213a	0.460b	.1
	ml/hr				
Total gas	27.72a	28.49a	26.33a	27.40a	2.5

[1]isoacids not added.
[2]isoacids at 10 mg/100ml of final incubation media.
a,b,c Means in a row with different superscript differ ($p<.05$)

There were no differences in total gas production and VFA production when ammonia concentrations in the media were low (0.24 mg/dl) or high (5.57 mg/dl) and when isoacids were not added to the media. The addition of isoacids to the culture containing low ammonia nitrogen resulted in no change in VFA and gas production. However, when urea was added in larger amount to the medium, the addition of isoacids significantly increased acetate and total VFA concentrations.

Several studies related to protein metabolism have been conducted. Bergen (1979) reported that the ruminal fermentation is a coupled process between carbohydrate degradation and microbial cell synthesis. Ammonia-N, isoacids and other factors such as carbon skeletons and sulfur are required for this process (Brondani, 1986). A major concern in ruminant nutrition is to define the nutrients required by rumen microorganisms for maximum fermentation of feedstuffs, particularly for low protein, high fiber plant material Cook (1985). Cook and Felix (1976) found that isoacids were limiting factors for growth of rumen microorganisms when animals were fed high levels of urea as source of supplemental nitrogen. Urea is hydrolyzed in the rumen to ammonia and carbon dioxide (Hungate and Gall, 1955). Many types of bacteria contribute to this process (Muhrer and Caroll, 1964). Since little energy is released, the splitting of urea to ammonia would be of value to the microorganisms mainly for growth.

In our study no difference was observed between low and high ammonia concentration (Table 10), although VFA

production was slightly lower when ammonia concentration was low. The low concentration of isoacids may have limited the microbial growth in the culture containing sufficient amount of nitrogen. The addition of isoacids to the culture containing 5.57 mg/dl N-NH3 increased total VFA and gas production whereas isoacids had no effect when added to the culture containing 0.24 mg/dl of N-NH3. Ammonia nitrogen may have been a limiting factor for growth in this case. Previous experiments conducted using grain as substrate failed to show a significant increase in VFA production when isoacids were added to the system. The probable reason for the no effect of isoacids on VFA may be that the high level of protein present in the Substrate provide sufficient branched chain fatty acids for the bacteria. Therefore isoacids were not limiting in the fermentation process.

Ethanol and Methanol

In order to find an adequate solvent that would dissolve monensin, the effect of ethanol and methanol was assessed.

In vitro and in vivo studies related to ethanol metabolism in the rumen have shown that Ethanol is formed in pure cultures of a number of species of rumen bacteria, but does not occur in the normal rumen at a significant concentration. Emery et al (1959) reported that Ethanol added to the rumen exerts little effect on oxygen consumption and methane production. Ethanol was not rapidly metabolized in the rumen, nor was it rapidly attacked in vitro (Leroy, 1958; Emery 1959). Ethanol slowly disappeared, probably by absorption and passage to the omasum.

In our study Ethanol at 1% caused a drastic decline in all parameters after 48 hrs of incubation. The explanation of the decrease in VFA and GAS production when Ethanol was added to the system is uncertain. Ethanol may have been toxic for certain rumen microbes. Therefore, we did not use ethanol as a solvent for Monensin even though it is widely used in pure culture as a solvent.

Czerkawski and Breckenridge (1972) investigated the metabolism of the primary alcohols, methanol through butanol, by rumen microorganisms in vitro. They reported that methanol is initially oxidized to formic acid with the resulting hydrogen used by other microorganisms for methane production. The rate of oxidation of these alcohols are relatively slow in comparison to the rate of hexose fermentation. When methanol was added to the fermentation vessel Czerkawski and Breckenridge (1972) found no change in the amount of acetic, propionic or butyric acids produced, but there was an increase in methane production.

In our study, addition of methanol at 1% of incubation medium decreased VFA, C02 and Total gas production and increased methane production. However, at 0.1% there was no change in total VFA nor C02 concentration and only a slight increase in methane production was observed. In order to eliminate any possible error due to addition of methanol, during the treatment period the control flasks received 0.1% of methanol.

The length of the long term culture technique was then extended over a period of twelve days in order to observe the effect of time on the system. There was a decrease of the variables measured, from day 1 to day 3, followed by a relatively a stable condition from day four to day twelve. a summary of these results is presented in Figures 2 and 3.

Based on these results, the fourth day of incubation was chosen as the first day of the treatment. The treatment injected through the gas port of the incubations flasks.

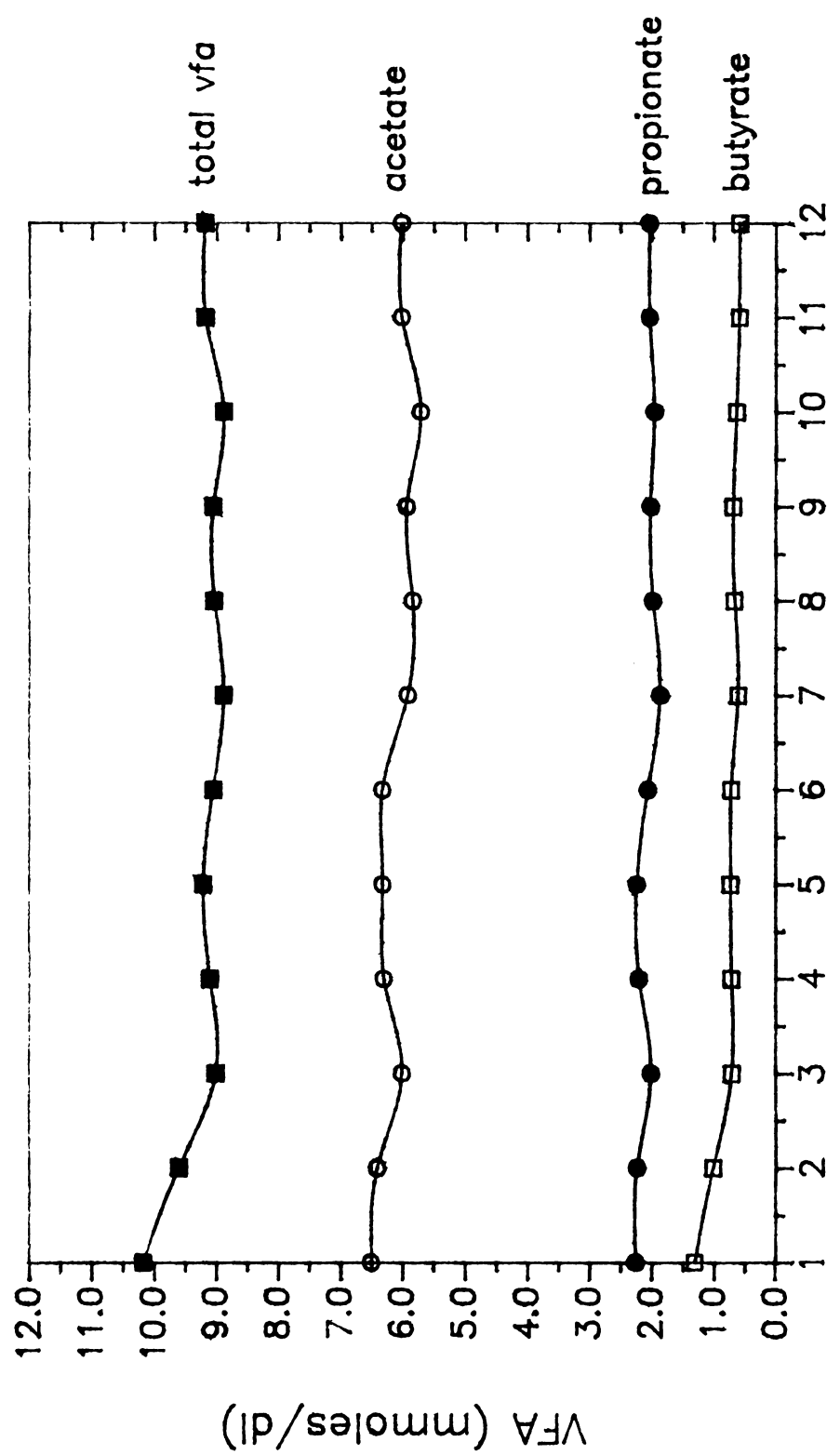


Figure 2. Daily rumen VFA concentration in vitro using grass hay as substrate.

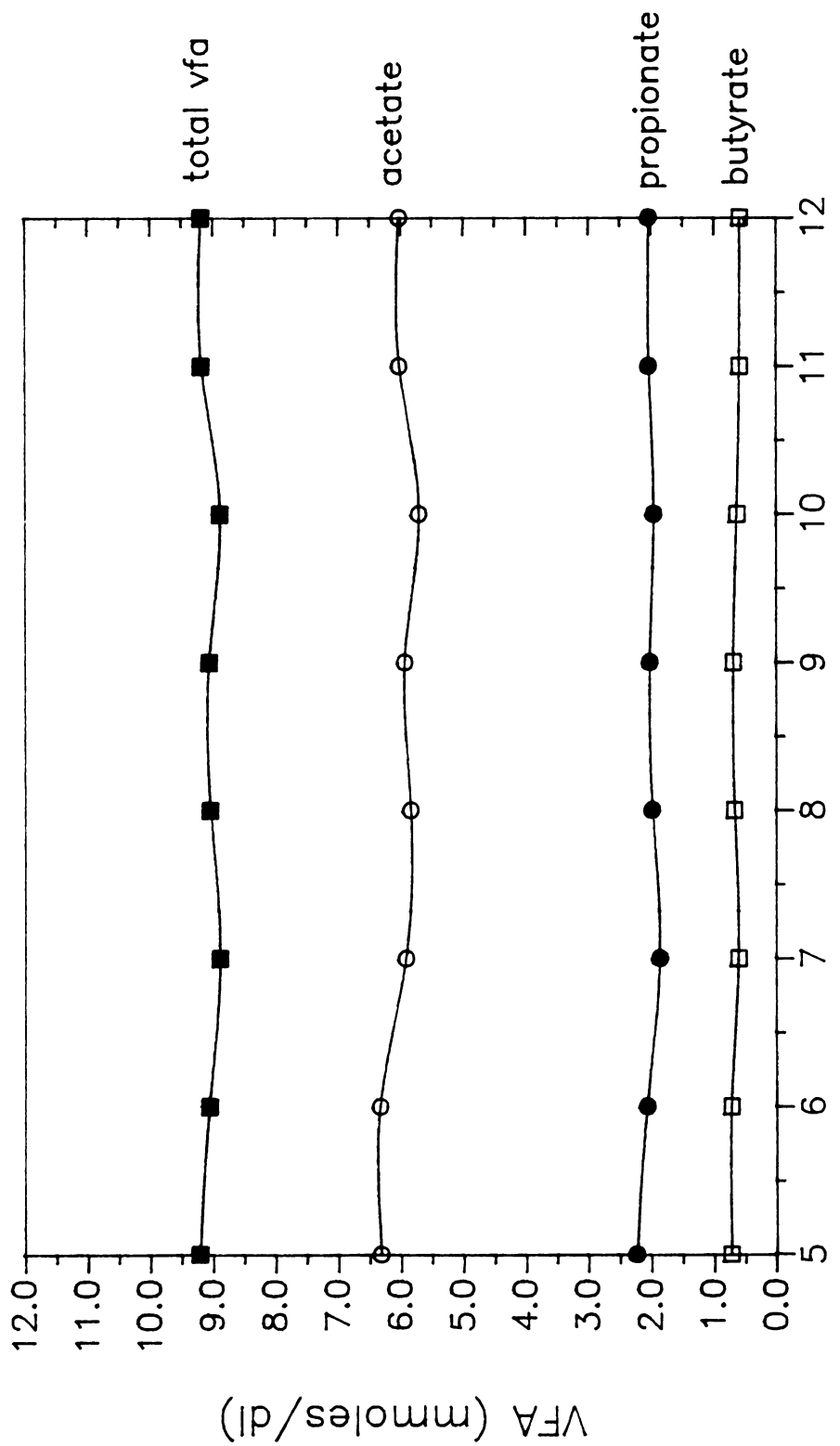


Figure 3. Daily rumen VFA concentration in vitro using grass hay as substrate.

INFLUENCE OF CHEMICALS ON RUMEN FERMENTATION.

The effects of different concentrations of Isoacids are summarized in Tables 11 and 12.

TABLE 11. Effect of isoacids on rumen VFA concentration *in vitro* using inoculum from a cow fed high roughage diet.

	Isoacid concentration (mg/dl)				
Variable	CTRL	10	15	20	SEM
	mmoles/dl				
VFA	7.878c	8.728a	8.939a	8.413b	.08
Acetate	5.480b	6.072a	6.170a	5.613b	.07
Propionate	1.552b	1.698a	1.638a	1.520b	.02
Butyrate	.609a	.586a	.667a	.659a	.03
Isoacids	.284d	.406c	.498b	.597a	.01
Isobutyrate	.034d	.060c	.076b	.089a	.001
2-Methylbutyrate	.066d	.108c	.134b	.150a	.003
isovalerate	.049d	.103c	.129b	.133a	.003
Valerate	.127c	.143c	.169b	.220a	.006

a,b,c Means in a row with different superscript differ ($p<.05$)

Isoacids at 10 and 15 mg/dl increased acetate concentration but had no effect on acetate concentration at 20 mg/dl.

Isoacids at 10 and 15 mg/dl of the culture media, increased total VFA, but there was a trend toward a decrease at 20 mg/dl compare to the previous levels. As expected, there

was an increase in branched-chain fatty acid concentrations in respect to the amounts added in the media.

Isoacids at 10 mg/dl and 15 mg/dl increased propionate concentration. However, a trend toward a decline of propionate production back to the control level was observed at 20 mg/dl. Figure 4.

Previous studies conducted by Felix and Cook (1980), reported that isoacids increased growth rate in young animals but not in older animals and that the supplementation of Isoacids and urea to high producing cows fed corn silage as the sole roughage had a positive effect on milk production, persistancy of lactation, body weight, feed intake and nitrogen balance. Towns and Cook, (1984) found that during an eight-hour sampling period, isoacid-treated cows had higher growth hormone but lower glucose and lower insulin. Brondani (1986) found that during an eight hour sampling period, a low concentration of insulin observed in isoacid-treated cows was associated with a decrease in propionate production. Brondani therefore pointed out that the decrease in insulin found for the first time by Towns and Cook, (1984) when cows where fed a high concentrate diet plus isoacids was due to a decreased propionate production in the rumen.

The present dose response study of the effect of isoacids on mixed rumen microbes was the first to demonstrated that isoacids have different effects at different concentrations. At low concentrations of branched chain fatty acids, the supplementation of isoacids increase all VFA. Perhaps by serving as a growth factor for cellulolytic bacteria.

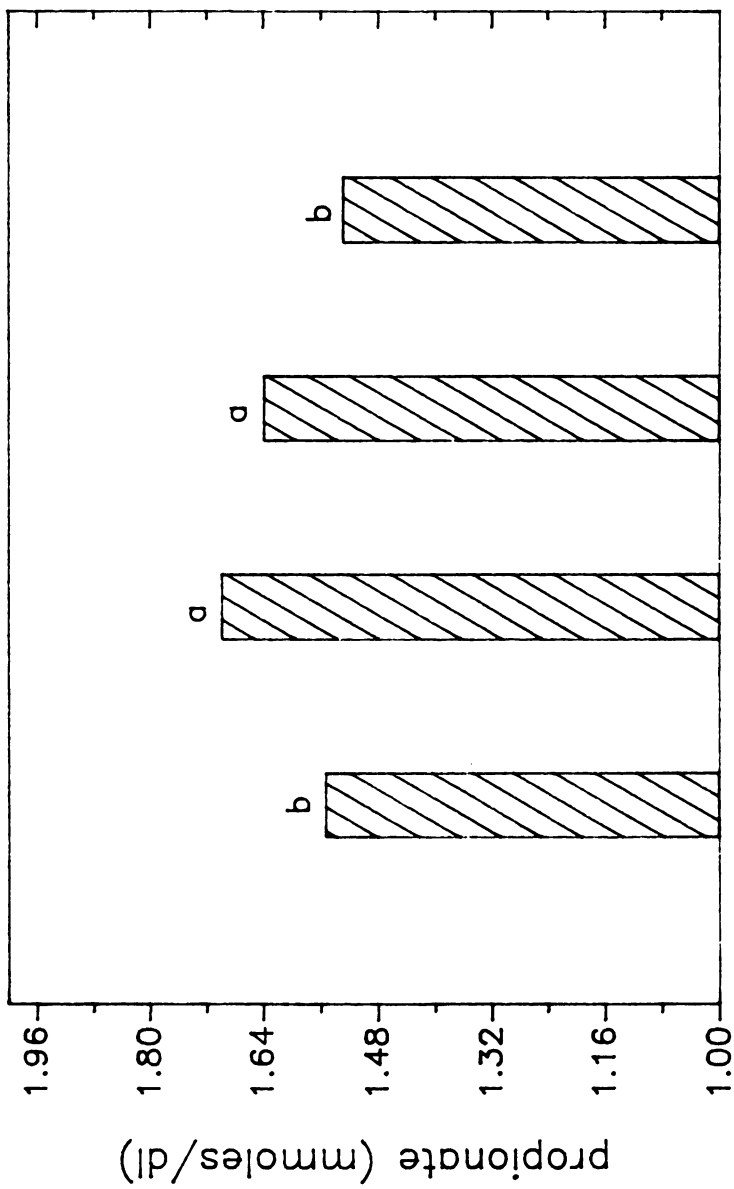


Figure 4. Effect of Isoacids on average daily rumen propionate concentration in vitro using grass hay as substrate.

However, when the concentration is optimal, further supplementation of isoacids tend to shift the carbon flux toward more acetate, less propionate, more microbial growth and a trend toward more butyrate (Table 11). These results clearly indicate that it is likely that the decrease in propionate found by Brondani, (1986) was due to the high ruminal concentration of isoacids brought about by the diet and the supplementation of isoacids.

The mechanism by which isoacids induces these changes in bacteria is unknown. The fact that propionate concentration decreased suggests that propionate producers are the primary targets and that metabolic adaptations occur in these species. The fact that growth yield increased suggests that a shift in bacterial electron flow occurs such that the production of end products is couple with optimal response in net microbial growth.

In the present study it was also noted that high concentrations of isoacids tend to decrease VFA and gas production. This suggests that supplementation of isoacids far above normal concentrations may affect the efficiency of fermentation.

TABLE 12. Effect of Isoacids on rumen gas production and ammonia nitrogen concentration in vitro using inoculum from a cow fed high roughage diet.

	Isoacid concentration (mg/dl)				
Variable	Control	10	15	20	SEM
			ml/hr		
Total gas	27.72a	27.71a	28.49a	27.55a	0.4
			percent		
CH4	35.15a	36.15a	34.38a	33.08a	1.99
CO2	49.02a	54.94a	49.03a	47.37a	3.15
H2	3.83a	4.00a	3.67a	3.83a	0.2
			mg/dl		
NNH3	5.57b	6.10ab	6.34a	6.11ab	0.05

a,b Means in a row with different superscript differ p(<.05).

In contrast to VFA production, total gas production as well as CH4, CO2 and H2 were not affected by isoacids. An in vitro study conducted by K.A Cummins (1984) using ammonium salt of isoacids at 1% of dry matter in a diet composed of cottonseed meal or alfalfa haylage and soybean meal (16% CP), showed an increased microbial nitrogen incorporation, increased dry matter digestion and microbial growth. But, when isoacids were added above 1% of the dry matter, digestion was decreased.

In our study, we did not observe any significant change in ammonia nitrogen concentration in the medium. However, in the presence of very low concentrations of ammonia nitrogen, isoacids did not increase acetate nor total VFA. This suggests that the increase in VFA production was associated

with an increase in ammonia nitrogen.

The effect of different concentrations of Monensin are summarized in Tables 13 and 14.

Table 13 Effect of monensin on rumen VFA concentration *in vitro* using inoculum from a cow fed high roughage diet.

	Monensin Level (ug /100ml				
Variable	Ctrl.	100	150	200	SEM
	mmoles / dl				
VFA	8.543a	7.221b	6.847b	6.887b	0.14
Acetate	6.026c	4.286b	3.743a	3.601a	0.11
Propionate	1.736c	2.272b	2.281b	2.420a	0.03
Butyrate	0.523a	0.467b	0.429ab	0.359a	0.02
Isoacids	0.184	0.162	0.162	0.195	0.01
Isobutyrate	0.027a	0.028a	0.027a	0.027a	0.01
2M-butyrate	0.042a	0.038a	0.036a	0.054a	0.01
Isovalerate	0.035a	0.028a	0.030a	0.047a	0.01
Valerate	0.080a	0.072a	0.069a	0.067a	0.01
AC/PR	3.509a	1.907b	1.755bc	1.614c	0.07

a,b,c Means in row with different superscript differ p<.05)

In contrast to isoacids, Monensin drastically decreased acetate concentration at all levels tested. Propionate concentration was significantly increased at all levels tested whereas butyrate concentration dramatically decreased at all levels tested. A summary of these results is presented in Figures 5 and 6.

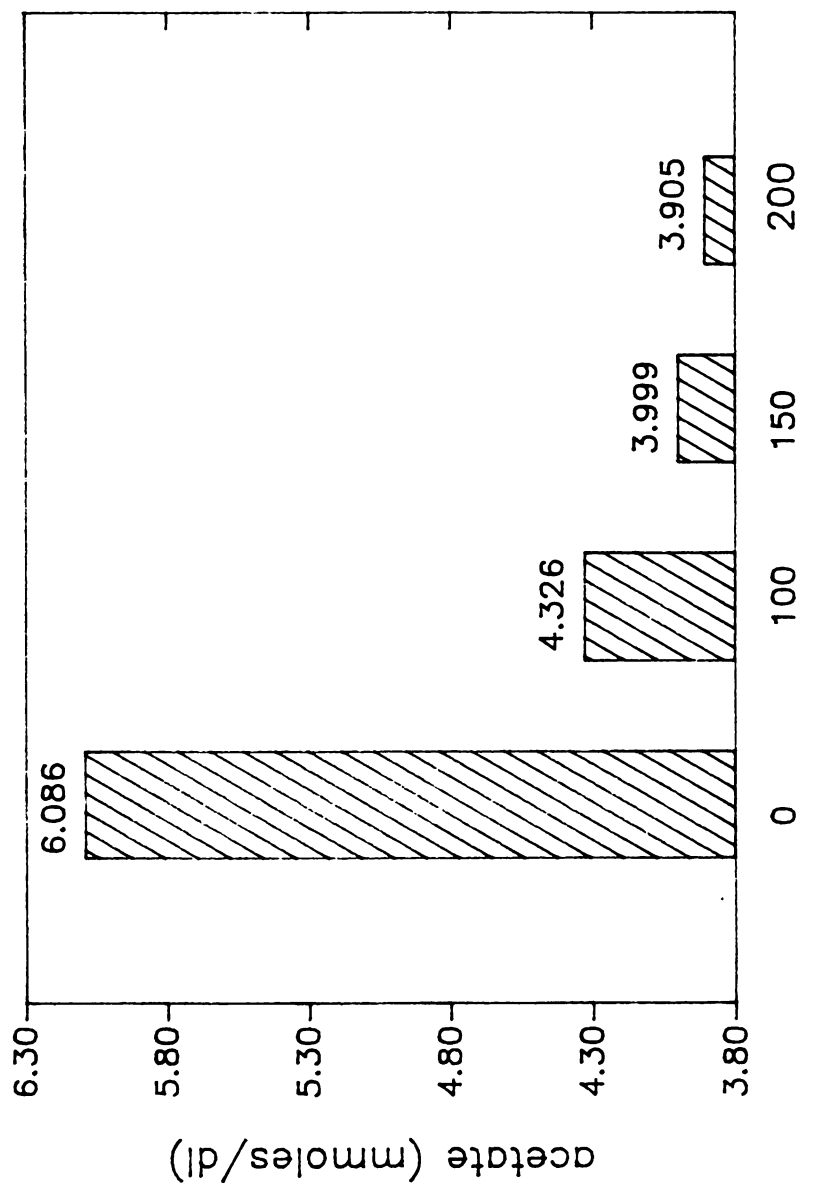


Figure 5. Effect of Monensin on average daily acetate concentration in vitro using grass hay as substrate

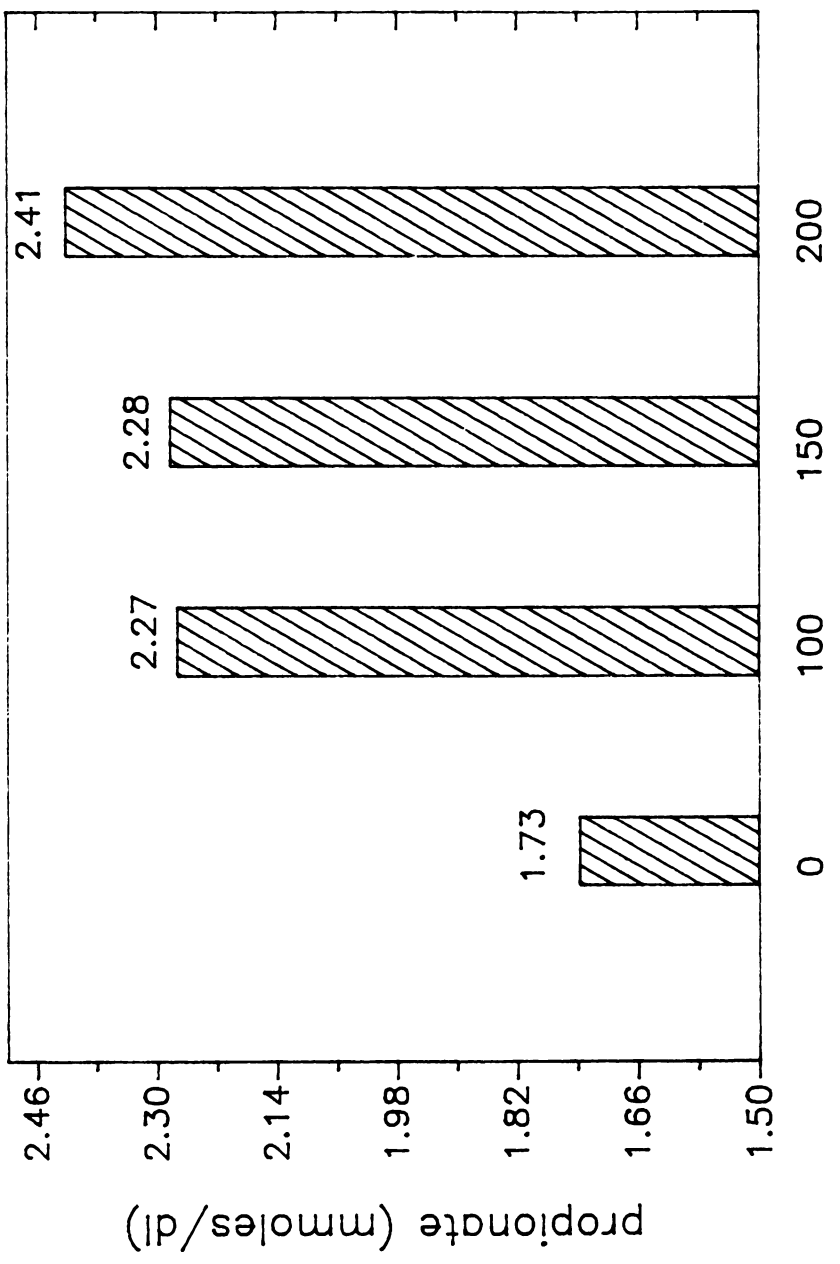


Figure 6. Effect of Monensin on average daily rumen propionate concentration <u>in vitro</u> using grass hay as substrate.

Table 14. Effect of Monensin on rumen gas production and ammonia nitrogen concentration in vitro using inoculum from a cow fed high roughage diet.

	Monensin level (ug/100ml)				
Variable	Control	100	150	200	SEM
	ml/hr				
Total gas	25.88a	22.93b	20.72c	20.16c	0.68
	%				
CH4	36.33a	27.07b	26.77b	26.00b	0.91
C02	49.83a	48.33a	42.50a	47.50a	3.2
H2	3.5b	3.5b	3.5b	4.0a	0
	mg/dl				
N-NH3	3.37a	4.25a	4.27a	4.37a	0.76
PH	6.80c	6.82c	6.85b	6.89a	0.07

a,b,c Means in a row with different superscript differ p(<.05).

The addition of Monensin decreased total gas production at all levels, and a drastic decrease in methane production. Neither C02 nor H2 were significantly affected. The results from the Monensin experiment agreed with similar studies conducted by Short (1976) and Chalupa (1977). Monensin constantly decreased acetate and butyrate production, and increased propionate production. This shift in rumen fermentation by Monensin is due to a selective action of the antibiotic on the rumen microbes by interfering with the passage of ions across cell membranes (Bergen, 1984).

Isoacid concentrations did decrease in the presence of Monensin, but not at a significant level. Matsumoto et al., (1984) reported a decrease in NH3 and total amino acids, a decrease in protozoa number and a decrease in isoacid concentration whereas leucine, isoleucine and valine concentrations were higher when 40ppm monensin was added to the diet of steers fed concentrate and rye grass silage. Monensin, by increasing ammonia nitrogen utilization, also increased isoacid utilization.

The effect of the combination of isoacids and monensin are presented in tables 15 and 16.

Table 15. Effect of Isoacids and Monensin on rumen VFA concentration and gas production in vitro using inoculum from a cow fed a high roughage diet.

	Treatment					
Variable	Ctrl.	[1]ISO-15	[2]MON-15	[3]M+I-10	[4]M+I-15	SEM
	mmoles/dl					
VFA	8.835ab	9.268a	6.850c	8.530ab	8.350b	.25
C2	6.047a	6.335a	3.743c	5.245b	4.986b	.11
C3	2.033ab	1.821b	2.282a	2.267a	2.320a	.11
C4	.579a	.594a	.405a	.620a	.589a	.01
ISO	.252b	.647a	.162b	.398ab	.435ab	.09
IB	.034b	.143a	.027c	.065b	.076b	.003
2MB	.050b	.128a	.036c	.092b	.107ab	.01
IV	.052b	.153a	.030c	.094b	.115b	.01
VA	.127b	.263a	.069c	.149b	.161b	.01
	ml/hr					
Gas	25.55ab	26.50a	20.72c	24.50ab	23.72b	.79

[1]Isoacids at 15mg/100ml of final incubation media.
[2]Monensin at 150ug/100ml of final incubation media.
[3]Monensin at 150ug/100ml and isoacids at 10mg/100ml.
[4]Monensin at 150ug/100ml and isoacids at 15mg/100ml.
a,b,c,d Means in row with different superscript differ ($p<.05$)

Table 16. Effect of isoacids and monensin on rumen gas production, ammonia nitrogen concentration and pH *in vitro* using inoculum from a cow a fed high roughage diet.

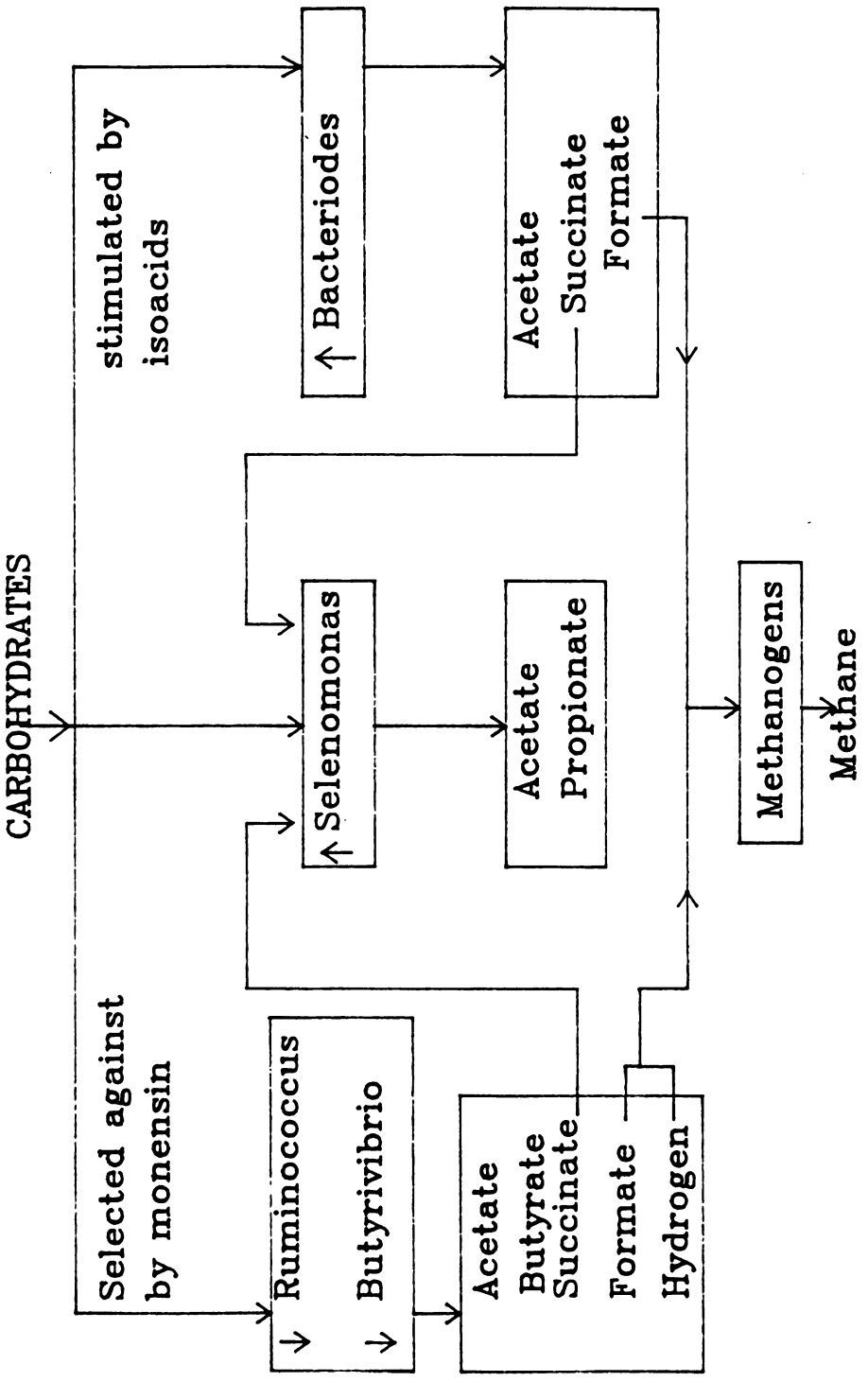
	Treatment					
Variable	Ctrl.	[1]ISO-15	[2]MON-15	[3]M+I-10	[4]M+I-15	SEM
			ml/hr			
gas	25.55ab	26.50a	20.72c	24.50ab	23.72b	.79
			%			
CH4	35.15a	34.38a	26.90b	29.40ab	28.35b	1.06
C02	49.37a	49.03a	32.65b	48.03a	49.20a	2.01
			mg/dl			
N-NH3	4.90a	4.40a	4.37a	4.68a	5.13a	.19
ph	6.79a	6.78a	6.87b	6.79a	6.79a	.01

[1]Isoacids at 15mg/100ml of incubation media.
[2]Monensin at 150ug/100ml of incubation media.
[3]Monensin at 150ug/100ml and isoacids at 10mg/100ml.
[4]Monensin at 150ug/100ml and isoacids at 15mg/100ml.
a,b,c, Means in row with different superscript differ ($p<.05$)

The addition of Monensin caused a decrease in acetate and propionate after 24 hrs of incubation, but after 48 hrs, propionate was higher than control values whereas acetate remained lower than the controls. (Figures 8 and 9). The addition of isoacids at 10 mg/100ml to flasks containing monensin increased acetate and total VFA concentration compared to monensin alone, and increased butyrate concentration above the control. However, the addition of isoacids to flasks containing Monensin did not alter propionate methane and total gas production.

The possible mode of action of the combination of isoacids and Monensin is shown in figure 7. Bryant (1964), reported the distribution of predominent rumen bacteria cultured from rumen contents of cattle fed differents diets. Bacteriodes succinogenes, Butyrivibrio fibrisolvens, Bacteriodes ruminicola and Ruminococcus albus were the most predominant bacteria when cows were fed wheat straw. They represented respectively 20, 19, 12 and 8% of total isolates. According to Wolin and Miller (1983), Monensin affects the rumen fermentation by selecting for organisms that participate in the production of relatively more propionate and against those that contribute to the production of relatively more acetate, butyrate and precursors of methane. Ruminococci and Butyrivibrio are inhibited by very low concentrations of monensin. These species are important producers of acetate, butyrate and the substrate for methanogens, H2 and C02. Selenomonads are very insensitive, whereas Bacteroides, although sensitive, rapidly become resistant to the antibiotic. Both organisms are important in the production of propionate. The addition of isoacids to the culture containing Monensin would cause an outgrowth of . Bacteroides resulting in more acetate, succinate, and formate. Succinate would be decarboxylated to propionate by selenomonads and formate would be used as an energy source by methanogens. The end result would be an increase in acetate, propionate and probably more substrate degradation as indicated by higher VFA and total gas production observed in the last experiment (Table 15).

Figure 7. Proposed mechanism of action for the effect of the combination of isoacids and Monensin on rumen fermentation


CARBOHYDRATES
stimulated by isoacids
↑ Bacteriodes
Acetate
Succinate
Formate
↑ Selenomonas
Acetate
Propionate
Methanogens
Methane
Selected against by monensin
↓ Ruminococcus
↓ Butyrivibrio
Acetate
Butyrate
Succinate
Formate
Hydrogen

The addition of isoacids to a culture containing Monensin will serve as a factor for growth not only for Bacteriodes succinogenes but also for Ruminoccoccus flavefaciens and methanogenic bacteria, which require also isoacids. Bacteriodes succinogenes in concert with Ruminoccoccus flavefaciens and Methanogenic bacteria will produce more acetate and methane. This was confirmed in our experiment by an increase in acetate and a trend toward an increase in methane production initially depressed by Monensin Figures 8 & 10. Based on these results we proposed that the combination of isoacids and Monensin may be of practical application in cattle rations. The addition of Monensin alone to the diet of growing steers increase the efficiency of growth by increasing propionate production. Because of the importance of propionate in the metabolism of glucose and the regulation of insulin secretion in ruminants, the increase in ruminal propionate production will promote growth. Isoacids increase acetate, total VFA and microbial synthesis. Since the addition of isoacids to cultures containing Monensin does not alter the effect of Monensin on propionate production the excess of acetate and microbial protein may further improve the efficiency of growth in these animals.

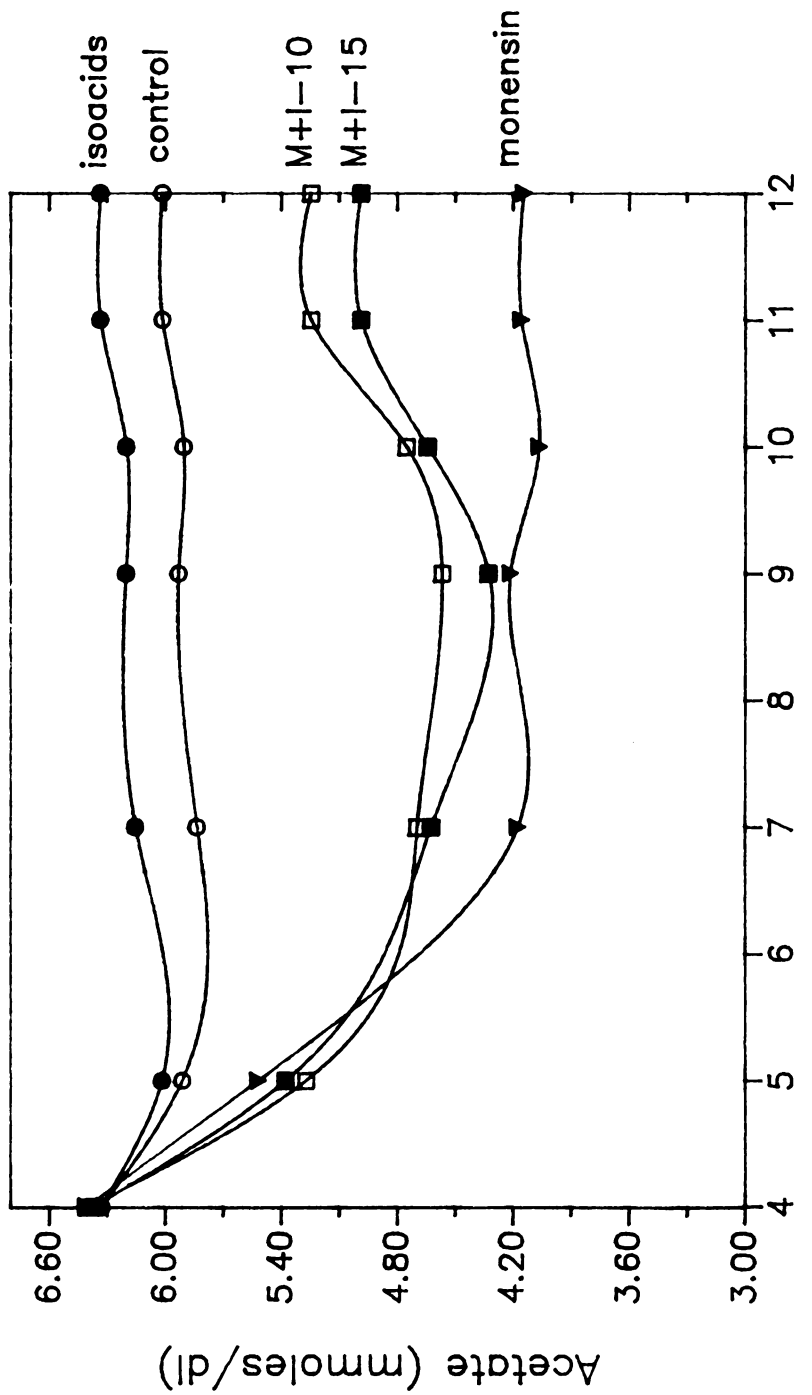


Figure 8. Effect of the combination Isoacids and Monensin on Daily rumen Acetate concentration in vitro using grass as substrate.

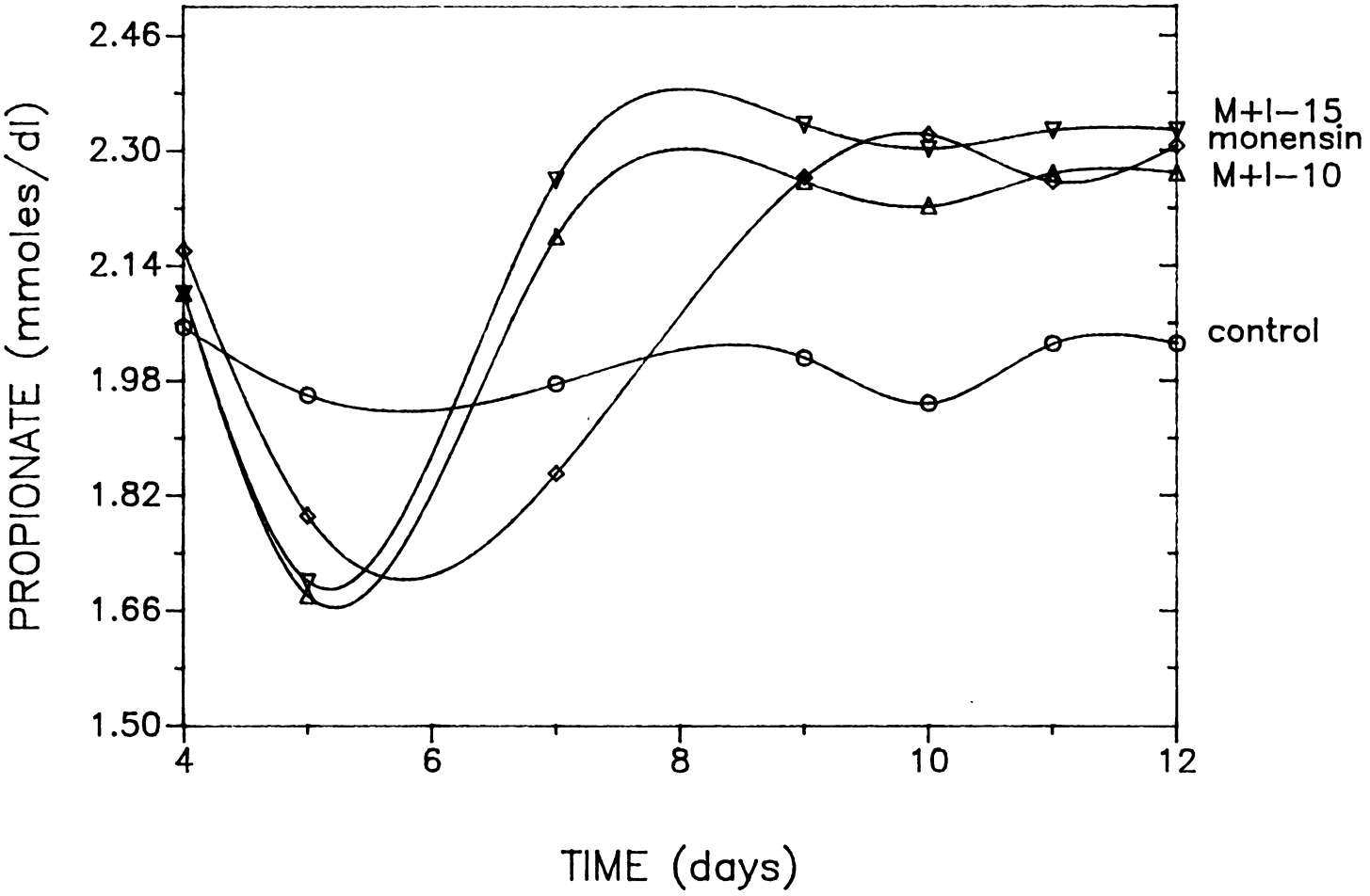


Figure 9. Effect of the Combination isoacids&Monensin on daily propionate concentration in vitro using grass hay as substrate

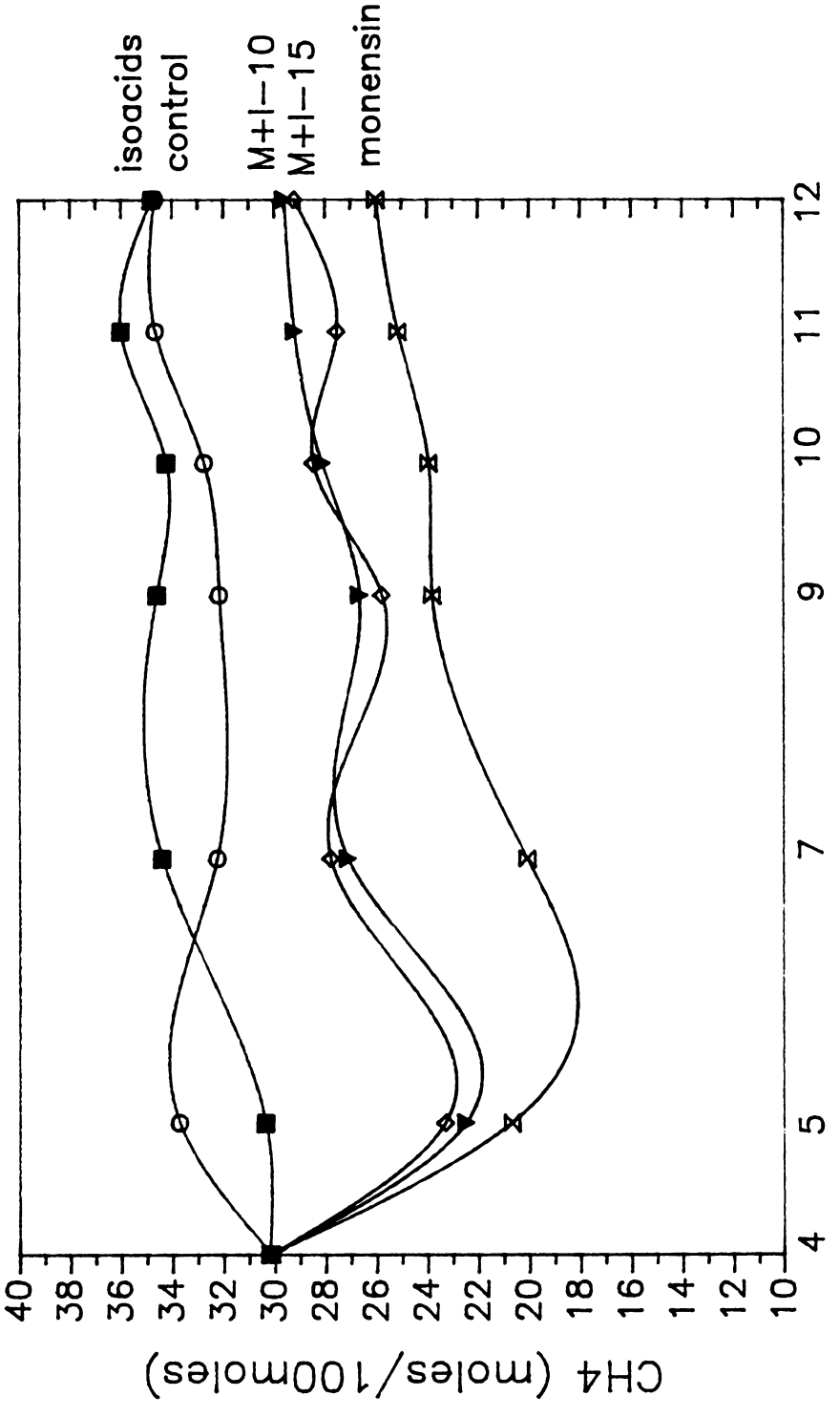


Figure 10. Effect of the combination Isoacids and Monensin on Daily rumen Methane concentration in vitro using grass hay as substrate

SUMMARY AND CONCLUSIONS.

A semi-continuous culture technique was set up to investigate the interaction of isoacids and Monensin on rumen fermentation *in vitro*. Initial studies validated the system. Inoculum, Substrate and protein level were evaluated. Three experiments were then conducted. The first two tested different levels of Isoacids and Monensin seperately. The third investigated the interaction of the two chemicals. The dose level study and the interaction of the two chemicals led to the following conclusions:

-Isoacids at 1% of DM increased acetate and propionate production and consequently total VFA production. At 1.5% of DM isoacids increased acetate and total VFA production and did not change propionate production. At 2% Isoacids decreased propionate production, but total VFA was not significantly affected.

-In contrast to isoacids the effect/response of Monensin drastically decreased acetate, butyrate, methane and total VFA production at all levels tested, and dramatically increased propionate production at all levels tested.

-Isoacids when added to a system containing Monensin restored acetate, methane and total VFA production to the control level and increased propionate production above the control.

-Isoacids when added to a culture containing Monensin may favor an outgrowth of *Bacteriodes succinogenes* and perhaps *Ruminoccoccus flavefaciens* and methanogens that require

isoacids as growth factor. The addition of isoacids and Monensin may have practical application in cattle rations.

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