COMPARATIVE RUMEN FERMENTATION ACTIVITY OF CATTLE WITH AND WITHOUT RUMEN CILIATE PROTOZOA

by

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B. S., Kansas State University, 1967
D.V.M., Kansas State University, 1969

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Dairy and Poultry Science

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1970

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INTRODUCTION

The protozoa found in the fore stomachs of ruminant animals have received much attention over the years. The earliest discovery of protozoa was probably by Leeuwenkoek of Delft, Holland who lived during the period 1632-1723. Leeuwenkoek is credited with the manufacture and use of microscopes of higher magnifying power then formerly used. He observed protozoa in an infusion of ground plant material and water after a few hours of incubation. The small single celled animals were observed to move readily throughout their environment by means of cilia. This class of protoza are referred to as Ciliata. According to Hungate (1966) ruminants consumed plants, water, and free-living protozoa. Over a period of years of evolution the protozoa became adapted to the rumen environment. Hungate (1966) stated that Gruby and Delafond in 1843 observed rumen protozoa ingesting plant cells and that the protozoa found in the ruminoreticulum were viable while those protozoa found in the omasum and abomasum were dead and disintegrating. Much of the early work with rumen protozoa involved studies pertaining to morphology and classification. Later work was directed toward studying nutritional requirements of various genera and their ecological distribution in order to help assess the contribution of rumen protozoa to their host.

Hungate (1950) has shown that the number of protozoa per gram of rumen fluid may exceed one million and that their mass may equal that of the rumen bacteria. Since large numbers of protozoa were observed in the normal ruminant, their presence was thought to make some essential contribution to their host. In vitro studies on holotrich protozoa by Heald and Oxford (1963), Gutierrez (1955), Howard (1959a,b) and Abou Akkada and Howard (1960) indicate that solu-

ble carbohydrates can be matabolized to VFA, gas, and storage polysaccharides. Hungate (1942,43) and later Abou Akkada et al. (1963) showed that many species of rumen protozoa contained active cellulase and could utilize cellulose. Williams et al. (1961) and Colemen (1963) showed that rumen protozoa can synthesize amino acids within their cells.

To determine the contribution that ciliate protozoa make to the host one needs to compare defaunated animals with those with protozoa. Isolating newborn animals from adult ruminants provides an effective and safe means of defaunation, but it is time consuming and not applicable to adult animals. Other methods of defaunation have been incomplete or have affected the health of the animals. Recently a simple chemical method for defaunation was developed at Kansas State University (Abou Akkada et al. 1968). The method employs dioctyl sodium sulfosuccinate as the defaunating agent.

The work reported here compares differences in rumen metabolism between faunated animals and those defaunated with dioctyl sodium sulfosuccinate.

REVIEW OF LITERATURE

Methods of Defaunation. The removal of ciliate protozoa from the rumen (defaunation) may be accomplished by several different methods. The purpose of defaunation is to provide a research tool to study the contribution of protozoa to their host (Abou Akkada and el-Shazly, 1964; and Klopfenstein et al. 1966). Eadie (1957) stated that defaunation, followed by a single species of protozoa, provides a method of growing and studying certain species of protozoa.

The early methods of defaunation were crude and often unsuccessful. Liebetanz (1910) defaunated goats by infusing acetic acid through a trocar inserted in the rumen. Becker et al. (1930) defaunated adult goats by using a modification of Liebetanz's technique. In addition to acetic acid, six eggs and one quart of milk were placed in the rumen to initiate a putrefactive process which destroyed ciliate protozoa but quite often killed the animal. Becker et al. (1930) also used 2% copper sulfate solution administered in a 50 cc dose per animal for two days. This method was effective for defaunation and less harmful than previous methods. However, Abou Akkada (1967) found certain species such as entodinia survived when copper sulfate was used for defaunation. Eadie (1957) developed a method to remove the holotrich ciliates from the rumen of adult sheep by removing the rumen contents. The emptied rumen was washed several times with saline and soft tap water. The rumen contents were heated to 50 C for 15 minutes before being returned to the rumen. A few oligotrichs would appear in 10-14 days when this method was employed. Abou Akkada et al. (1968) developed a method to remove all rumen ciliate protozoa by using dioctylsodium sulfosuccinate (Aerosol OT). Clark and Reid (1969)

reported on the use of dimetridazole (Emtryl) for defaunating cattle.

Most in vivo studies on rumen protozoa employed lambs or calves that were isolated from adult animals at birth and contained no rumen protozoa. Methods and techniques on isolation have been described by Abou Akkada and el-Shazly (1964), Bryant and Small (1960), and Eadie (1962).

General Performance of defaunated and faunated animals. Defaunation did not cause any ill effects on lambs or calves according to Pounden and Hibbs (1950) and Eadie (1962; 1966) except that the defaunated calves appeared to have rougher hair coats. A "pot belly" appearance was observed in both defaunated lambs and calves.

Becker and Everett (1930) showed that growing lambs without ciliate protozoa gained slightly less weight in a 3 month period than faunated controls. Similar results on growth rate and feed efficiency were observed by Abou Akkada and el-Shazly (1964), Christiansen et al. (1965) and Borhami et al. (1967). Eadie (1962, 1967) reported that there was no significant difference in weight gain, feed intake and general performance between defaunated lambs or calves and faunated controls. Under favorable conditions, the rumen ciliates may contribute up to 20 percent of the host's nutritional requirements according to Hungate (1955), Oxford (1955), and Gutierrez et al. (1960).

Comparison of rumen pH, ammonia, and VFA between defaunated and faunated animals. Abou Akkada and El-Shazly (1964) using lambs isolated at birth and faunated controls showed that total VFA concentration and rumen ammonia were higher in the faunated group. They found that the faunated group had a narrower acetic:propionic ratio than the defaunated group. Borhami et al. (1967) reported higher rumen ammonia and VFA concentrations in the faunated buffalo

calves than in defaunated calves. Christiansen et al. (1965), using wether lambs defaunated with cooper sulfate or isolated at birth showed higher rumen VFA and ammonia concentrations, a narrower acetic to propionic ratio and a lower rumen pH in the faunated group. Chalmers et al. (1968) and Klopfenstein et al. (1966) reported increased ammonia concentration in faunated lambs. Luther et al. (1966) employing in vitro techniques showed that the addition of protozoa to a bacterial fermentation increased VFA and ammonia production. They also reported that in an in vivo study using lambs, faunated lambs fed an 80 percent roughage ration had higher concentrations of ruminal VFA and ammonia than unfaunated lambs. Faunated lambs fed a ration containing 80 percent concentrate had higher concentrations of propionic acid but not higher total VFA concentration than did defaunated lambs. A narrower acetic to propionic acid ratio was observed in the faunated group during both ration treatments. Kurihara et al. (1968) observed higher VFA and ammonia production in faunated than in defaunated sheep. They believe that bacterial ammonia production may be enhanced by the presence of protozoa as suggested by Coleman (1964).

Comparison of cellulose digestion between defaunated and faunated animals. Conrad (1950) showed a greater cellulose and dry matter digestibility in faunated than in defaunated calves. Abou Akkada and el-Shazly (1965) and Klopfenstein et al. (1966) reported increased digestibility of dry matter in faunated than in defaunated lambs. Luther et al. (1966) was unable to show any difference in digestibility between defaunated and control groups of lambs. Yoder et al. (1964, 1966) studied in vitro cellulose digestion using washed-cell bacteria and protozoa suspensions. There was an increase in cellu-

lose digestion and VFA production when protozoa were added to in vitro bacterial fermentation systems. They conjectured as a result of these in vitro studies, that rumen protozoa might contain or produce unidentified factors that stimulate bacterial cellulose digestion.

EXPERIMENT I

INTRODUCTION

The object of this experiment was to study the diurnal variation of various rumen parameters that existed between defaunated and faunated adult cattle fed three different rations.

EXPERIMENTAL PROCEDURE

Animals. Four rumen fistulated Jersey cows were used. The experimental (defaunated) group contained two rumen fistulated Jersey cows 13 and 36. The control group contained two rumen fistulated Jersey cows 34 and 35. The two animals in the control group were kept in a drylot at the K.S.U. Dairy Research Center. The two animals to be defaunated (experimental group) were isolated from the other ruminants and kept in a metabolism room with controlled environment. This room is located in K.S.U.'s Call Hall. Animals in both groups were fed identical rations.

Rations. All animals were fed twice daily at 7:00 A.M. and 4:00 P.M. The experiment was divided into three periods and in each period a different ration was fed. The first ration fed in period A was roughage and grain fed in a 1:1 ration. The roughage was of good quality alfalfa hay and the grain mixture contained 27% cracked corn, 11% soybean meal, 24% dehyrated alfalfa pellets, 35% ground sorghum grain, 1% urea, 1% dicalcium phosphate, and 1% salt.

The second ration (fed in period B) was freshly cut immature alfalfa. Several small plots of alfalfa provided new fresh growth from previous cuttings.

The alfalfa was cut either with a small sickle-bar hand operated mower or a small tractor equipped with a 2 meter side-bar mower. The alfalfa was cut when it reached a height of about 30 cm. A quantity of alfalfa was cut each day with the mechanical mowers, raked by hand with a wooden hay rake, and hauled to the animals. One half of the freshly cut alfalfa was fed to the defaunated group of animals housed in the metabolism room and the remainder was fed to the control animals in the drylot. Animals kept in the metabolism room had their portion of the alfalfa removed first to reduce chances of contamination with ciliate protozoa.

The third ration (fed in period C) was a high grain low-roughage ration. The grain mixture was the same as the one used in period A. A small quantity (0.25 kg per head per day) of good quality alfalfa hay was fed.

Defaunation Procedure. The method used to remove ciliate protozoa was similar to that described by Abou Akkada et al. (1968). Dioctyl sodiumsulfosuccinate, also known as Aerosol O.T. or Sur-ten[1] was used to remove all species of rumen ciliate protozoa. Each animal to be defaunated received 30 g of Aerosol O.T. placed in a 32 g gelatin capsule and administered with a balling gun. The procedure was repeated on three consecutive days. Rumen samples were drawn daily during the dosing period to ascertain the amount of protozoal activity present. The rumen fluid samples were obtained from the fistulated Jerseys by aspirating with a syringe through the sampling tubes. The protozoal activity was determined by placing a drop of rumen fluid on a glass slide, covering the specimen with a cover slip, and observing under low power with a

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[1]Courtesy of Dr. J. Drain, American Cyanamid Co., Princeton, New Jersey

microscope. When no cililate protozoa could be seen after examining 4 to 5 specimens the animal was considered defaunated. After the initial dosing period, rumen samples from the defaunated animals were checked every 3 to 4 days to see if they remained ciliate free. When protozoal cells were observed by microscopic examination during check periods, the animals involved were retreated with Aerosol O.T. on an individual or group basis, depending on the degreee of contamination. The dosage level was increased for some animals to achieve a desired response. High dosage levels (30 to 45 g daily) would cause animals to show clinical signs such as either partial or complete anorexia, dehydration, rumen atony, and disappearance of ciliate protozoa. On discontinuing Aerosol O.T., the above signs would disappear in one or two days and feed intake would increase. In many instances where dosing intervals were frequent, the overall feed intake per day was lower in the defaunated group as compared with the control group.

Management Precautions. Precautions were taken to prevent contamination of the defaunated animals from outside sources. The metabolism room was kept locked to prevent unauthorized individuals and visitors from entering. The animals were cared for by one person who took special precautions not to expose himself to other ruminants. Clean coveralls and boots were kept in the metabolism room and were donned prior to working with the animals. Instruments such as stomach tubes, speculums, syringes, and balling guns were rinsed in 75 C water between animals to reduce chances of contaminating the defaunated animals.

Sampling Procedure. Samples of rumen fluid were drawn from the fistulated animals which were fitted with a plastic cannula and closed with a plastic cap.

A sampling tube, about 35 cm in length, was connected to a cylindrical strainer 16 x 3 cm which contained many perforations. The free end of the sampling tube was inserted through a hole in the center of the plastic cap. The strainer was positioned about 25 cm above the ventral floor of the rumen in order that representative rumen fluid samples could be removed by the use of a large 180 ml dosing syringe. The free end of the sampling tube that protruded to the exterior was closed with a pinch clamp.

During period A (hay and grain ration) the animals were sampled on two different days (A1, A2) and during period B (freshly cut alfalfa) and period C (high grain) samples were obtained on three different days (B1, B2, B3 and C1, C2, C3). On each sampling day rumen fluid was collected at 1.5, 3, 6, and 12 hours after feeding. Samples from each group were obtained individually, at the same time. The first syringe full of rumen fluid was discarded in order to prevent dilution or produce a sample not completely representative since fluid that remained in the sampling tube between sampling times or periods might have been static. The rumen fluid from each sampling time on a given sampling day was placed in 3 separate containers. One container consisted of a large-mouth 120 ml jar which held enough rumen fluid for pH and ammonia determinations. The second container was a 500 ml glass jar and was filled with rumen fluid for measurement of gas production. The third container was 100 ml plastic flask which was fitted with a secure cap and frozen for later VFA and lactic acid determinations. All samples were taken to the laboratory immediately after collection.

Analysis of pH and ammonia. The pH was determined with a Leeds and Northrup pH meter.

Rumen ammonia was determined by the modified microdiffusion method of Conway (1957). Each ammonia sample was run in duplicate and the results were averaged.

Gas production. The amount of microbial activity was estimated by gas production in vitro using the method described by el-Shazly and Hungate (1965). During each period the substrate was the same as the ration fed to the animals during that period. In all cases the total amount of substrate used was 10 g. A 50 ml quantity of rumen fluid was used as inoculum. The fermentation period was 60 min. The quantity of gas produced in 5 min. intervals was recorded. The results were expressed as milliliters of gas produced in 60 min. All samples were tested in duplicate and the results averaged.

Determination of VFA and lactate. The preparation of rumen fluid for volatile fatty acid determination utilized a technique described by Erwin et al. (1961). A 5 ml quantity of strained rumen fluid which was previously frozen was thawed and placed in a centrifuge tube and 1 ml of 25% metaphosphoric acid was added. After standing 30 min. the sample was centrifuged at 15,000 x g for 10 min. The supernatant was analyzed for VFA concentration by gas chromatoghaphy. An Aerograph Hi-Fi (oven Model 550 and electrometic model 500) and Leeds and Northrups Model H recorder with disc integrator were the instruments used. The column used was Teflon 122 cm x 3 mm O.D. packed with silanized polypack I (80-120 mesh). The carrier gas was helium at a rate of 20 ml per min. The rate of hydrogen of the flame ionization detector was 30 ml per min. The column temperature was 165 C and the injector temperature was 225 C.

Lactic acid was determined colormeterically by the method described by Barker and Summerson (1941).

RESULTS

Variations existed between animals in each group and between the control and defaunated groups in the parameters studied (pH, gas production, acetic: propionic acid ratio and the concentration of ammonia, lactic acid, total VFA, acetate, propionate, butyrate, isobutyrate, valerate, and isovalerate). In an attempt to interpret the variations that existed between individual animals and groups, a statistical 3-way analysis of variance was computed. Using the mean value of each parameter, statistical variance between animals, ration periods and sampling hours and their interactions were studied. The significant findings for each parameter studied are discussed individually. The significant correlations of various parameters in the control and defaunated group are tabulated.

pH. There was no significant difference in pH between the control and defaunated animals (Table 1). The mean pH values obtained during ration treatment periods B1, B2, and B3 were the highest followed by those obtained during A1 and A2. The lowest values were obtained during period C1, C2, and C3. The mean hourly pH of all animals in both groups for the 3 ration treatment periods exhibited a consistent trend (Fig. 1). The pH value was highest 12 hours after feeding. This value was significantly different ($p<.05$) from the 1.5 hour value (Table 1). The 3 and 6 hour samples had the lowest pH values. There was no significant difference between these samples. The trend was the same for each ration treatment, but the highest pH was on freshly cut alfalfa and lowest on the high-grain ration. The relationship between animals and ration treatments are shown in Figure 2. There appeared to be little

Table 1. Animal, ration, and sampling time means of various rumen parameters studied.

	PARAMETER					
	pH	Ammonia	Lactate	Total VFA	C2:C3 Ratio	Acetate
		(mg/100 ml)	(µg/ml)	(meq/liter)		(meq/liter)
Animals						
34*	6.50a	23.2ab	16.2a	100.5a	2.70b	60.4ab
35**	6.52a	25.9a	12.0a	101.3a	2.99a	63.8a
13**	6.51a	25.0ab	25.1a	79.7b	2.71b	62.2ab
36	6.56a	20.9c	18.1a	67.2c	2.38c	58.2b
Ration Periods						
A1	6.44cd	18.5d	20.7ab	103.8ab	2.22c	55.3cd
A2	6.60c	19.5d	50.8a	93.0bc	3.25b	65.2ab
B1	6.97a	18.3d	6.9b	66.6e	3.65a	68.6a
B2	6.91ab	30.3a	5.6b	80.5d	3.48a	67.8a
B3	6.79b	26.5b	7.9b	55.4e	3.44ab	69.3a
C1	6.13ef	24.5bc	12.1b	86.7cd	1.41d	49.1e
C2	6.04f	21.2cd	11.2b	103.2ab	1.67d	53.5de
C3	6.29de	31.0a	27.4ab	108.4a	2.44c	60.5bc
Sampling Time						
1.5 Hour	6.52a	31.1a	40.1a	93.4ab	2.82a	63.4a
3 Hour	6.34b	26.2b	12.5b	94.8a	2.72ab	61.3ab
6 Hour	6.35b	18.4c	8.9b	85.0b	2.65ab	62.4a
12 Hour	6.88c	19.2c	9.8b	75.6c	2.60b	57.6b

* = Control
** = Defaunated

a,b,c,d,e,f Values within each column sharing a common letter are not significantly different (P>.05)

Table 1 cont. Animal, ration, and sampling time means of various rumen parameters studied.

	PARAMETER					
	Proprionate	Isobutyrate	Butyrate	Isovalerate	Valerate	Gas production
Animals						
34*	24.8b	1.68b	8.86a	2.74a	1.43a	41.3b
35**	23.6b	1.23c	8.00b	2.19c	1.19bc	54.3a
13**	23.6b	1.46b	5.71c	2.50b	1.31ab	42.7b
36	26.9a	1.47ab	5.31c	2.25c	1.09c	23.3c
Ration periods						
A1	22.2d	0.77de	6.76b	1.08d	1.18b	25.6bc
A2	20.3e	1.65b	8.64a	2.85b	1.36b	26.4bc
B1	18.9e	2.21a	6.14bc	3.24a	0.85cd	16.6cd
B2	20.4e	2.26a	5.47cd	3.21a	0.92c	39.9b
B3	20.3e	2.08a	4.74d	2.90b	0.68d	17.9cd
C1	37.8a	0.67e	5.69e	1.72c	1.92a	69.0a
C2	32.7b	0.95cd	9.35a	1.72c	1.79a	48.0b
C3	25.5c	1.12c	8.96a	2.63b	1.34b	79.8a
Sampling time						
1.5 hour	24.6ab	1.47b	6.66b	2.44b	1.27ab	44.6a
3 hour	25.3a	1.30c	7.17ab	2.12c	1.34a	43.5a
6 hour	25.7a	1.31c	7.28a	2.06b	1.22ab	41.7ab
12 hour	23.4b	1.77a	6.76ab	3.04a	1.18b	31.7b

* = Control

** = Defaunated

Table 2. Analysis of variance (F-ratios) of various rumen parameters studied.

Source	pH	NH_3	Lactate	Total VFA	C2:C3 ratio	Acetate
Animal	0.4	5.9**	0.4	27.6**	12.3**	2.6
Ration treatment period	38.4**	16.7**	1.7	17.8**	74.0**	13.5**
Sample hour	38.9**	46.0**	3.2*	7.9**	1.7	2.9*
Animal X Ration treatment period	4.8**	6.9**	0.9	8.8**	3.2**	0.7
Animal X Hour	1.2	1.3	0.7	0.8	1.7	0.9
Ration treatment period X hour	1.7	4.0**	0.9	3.1**	1.3	1.9

* = $P < .05$

** = $P < .01$

Table 2 cont.

Source	Proprionate	Isobutyrate	Butyrate	Isovalerate	Valerate	Gas production
Animal	9.5**	13.3**	79.7**	11.3**	7.8**	12.5**
Ration treatment period	91.3**	85.2**	42.0**	58.9**	35.3**	21.4**
Sample hour	4.1**	18.7**	2.5	36.4**	1.8	2.7
Animal X Ration treatment period	4.8**	9.0**	12.3**	8.8**	7.7**	3.4**
Animal X Hour	1.0	0.9	1.3	0.9	0.4	0.5
Ration treatment period X hour	3.6	4.8**	4.1**	4.4**	2.3**	3.6**

* = $P < .05$

** = $P < .01$

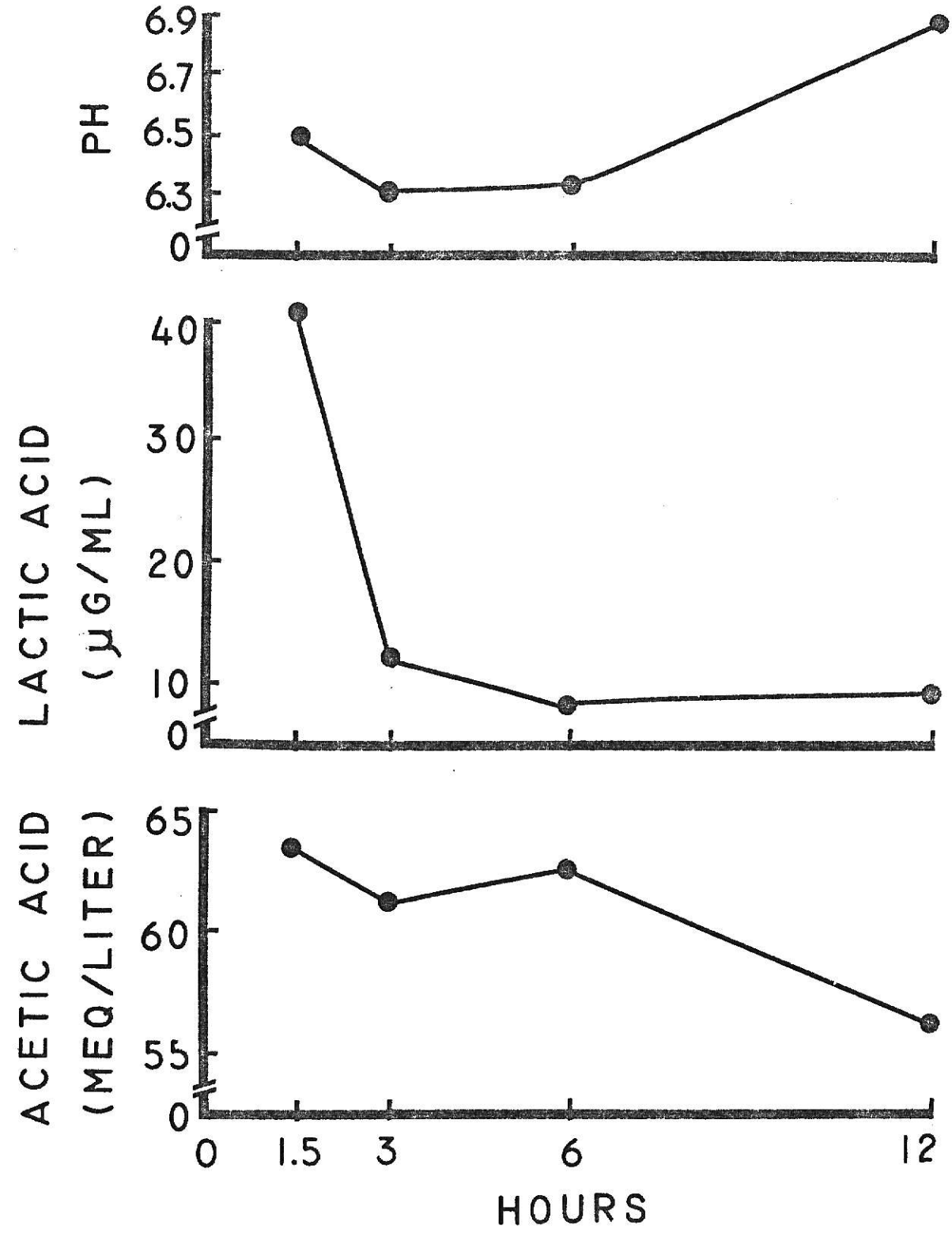

Figure 1. The mean pH, lactic acid and concentration of acetic acid at 1.5, 3, 6, and 12 hours. Each value is an average of all animals and ration treatments.

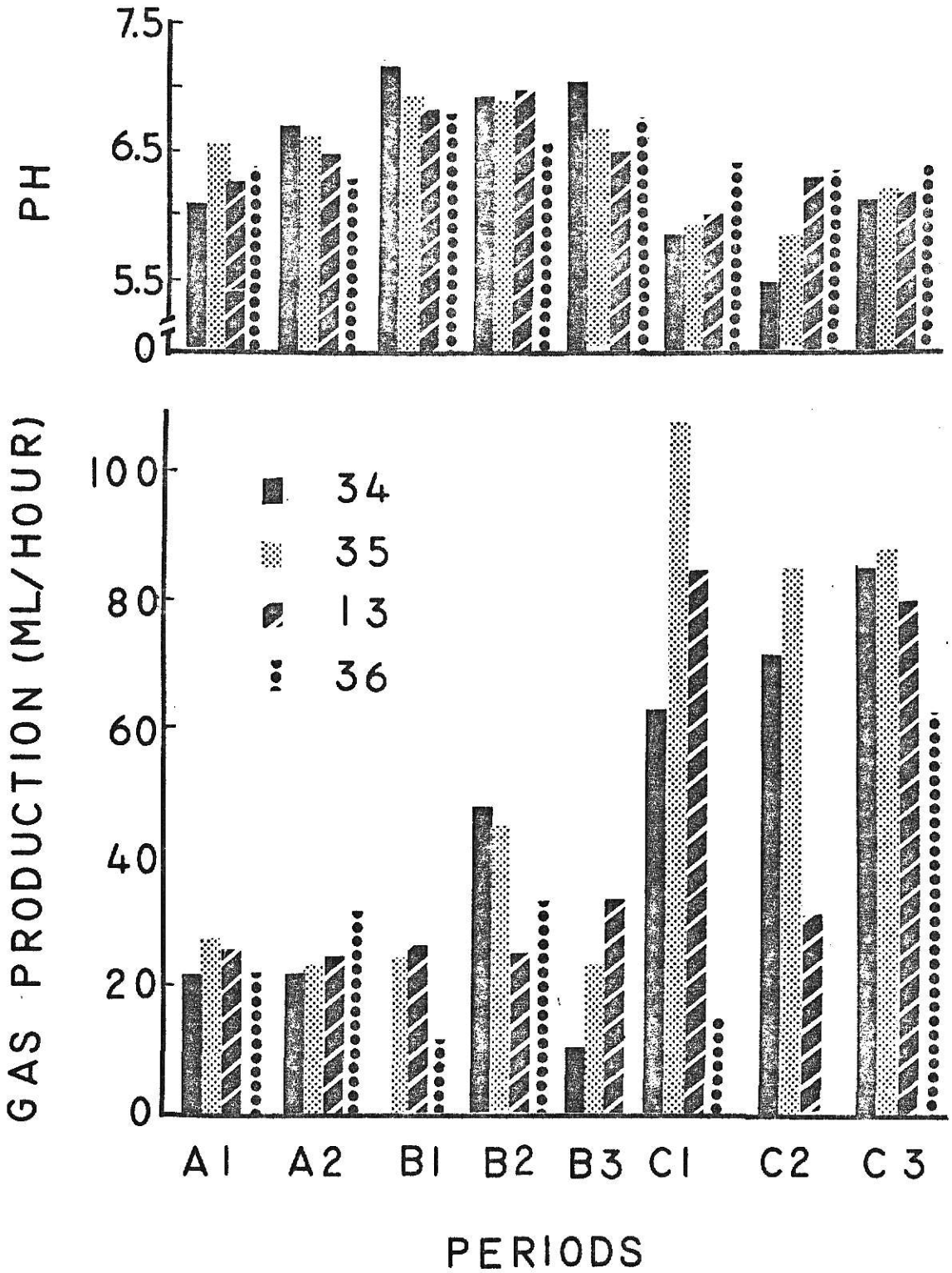

Figure 2. Effect of ration on mean daily pH and gas production. Rations were: hay and grain (A1, A2), fresh alfalfa (B1, B2, B3) high grain (C1, C2, C3). Defaunated cows were 13 and 16, faunated were 34 and 35.

difference between the control and the defaunated group. During periods C1 and C2 the control group had a lower pH which may have been due to increased rumen fermentation as gas production also increased (Fig. 2). There was a significant correlation between pH and acetate concentration during the hay and grain treatment periods A1 and A2 (Tables 3 and 4). Acetate and pH were negatively correlated in the defaunated animals but positively correlated in the controls.

Ammonia. The overall mean ammonia concentrations for all ration treatment periods showed a significant difference between animals in each group but not between the defaunated and control group (Table 1). The overall hour mean of all animals in both groups and all ration treatment periods showed the highest ammonia concentration in the 1.5 hour sample followed by the 3 hour sample with a significant difference between the two samples. The 12 hour and 6 hour samples had the lowest mean ammonia concentration, but the difference between them was not significant. There was a significant relationship between individual animals and ration treatments (Fig. 3). The defaunated group during sampling period C1 had a lower mean ammonia level than the control group, which might have been due to a decreased feed intake by the defaunated group on that particular sampling day (Fig. 3). The relationship between ration treatment and sampling hour shows that the mean ammonia level of all animals in both groups was highest in the 1.5 and 3 hour samples (Fig. 4). The correlations between ammonia and C2:C3 ratio showed that the data for all ration treatments were positive for the defaunated group and negative for the control group (Tables 3 and 4). There were negative and positive correlations between the two groups for ammonia, acetate, and propionate when the data of all ration treatments were pooled.

Table 3. Significant correlations of various parameters studied for the defaunated group.

Parameter	Parameter designation	Correlation coefficients					
pH	A	$-0.59^{a}D$	$-0.63^{b}D$	$-0.42^{d}D$	$0.52^{b}E$	$0.32^{d}E$	$-0.66^{a}F$
		$0.44^{b}F$	$-0.79^{a}G$	$-0.55^{b}G$	$-0.46^{d}G$	$0.87^{b}H$	$0.58^{d}H$
		$-0.65^{a}I$	$-0.43^{b}I$	$-0.36^{d}I$	$0.86^{b}J$	$0.50^{d}J$	$-0.53^{b}K$
		$-0.52^{d}K$	$-0.45^{d}L$				
Ammonia	B	$0.56^{c}C$	$0.28^{d}C$	$-0.45^{b}D$	$0.28^{d}E$	$0.28^{d}F$	$-0.27^{d}G$
		$0.59^{a}H$	$-0.53^{b}I$	$0.68^{a}J$	$0.33^{d}J$	$-0.51^{b}K$	$0.60^{c}L$
		$0.33^{d}L$					
Lactate	C	$0.56^{c}B$	$0.28^{d}B$	$0.55^{b}D$	$-0.51^{a}F$	$-0.51^{b}H$	$0.79^{b}I$
		$-0.52^{b}J$					
Total VFA	D	$-0.59^{a}A$	$-0.63^{b}A$	$-0.42^{d}A$	$-0.45^{b}B$	$0.55^{b}C$	$-0.64^{b}E$
		$-0.69^{b}F$	$0.67^{a}G$	$0.64^{b}G$	$-0.47^{c}G$	$-0.74^{b}H$	$-0.68^{c}H$
		$-0.44^{d}H$	$0.61^{a}I$	$0.68^{b}I$	$0.58^{c}I$	$0.58^{d}I$	$-0.81^{b}J$
		$0.65^{a}K$	$0.65^{b}K$	$0.48^{d}K$	$0.37^{d}L$		
C2:C3 ratio	E	$0.52^{b}A$	$0.32^{d}A$	$0.28^{d}B$	$-0.64^{b}D$	$0.70^{a}H$	$0.64^{b}H$
		$0.66^{d}H$	$0.65^{a}I$	$-0.54^{b}I$	$0.67^{c}I$	$0.62^{a}J$	$0.61^{b}J$
		$0.61^{d}J$	$-0.61^{b}K$	$-0.38^{d}K$			
Acetate	F	$-0.66^{a}A$	$0.44^{b}A$	$0.28^{d}B$	$-0.51^{a}C$	$-0.69^{b}O$	$0.53^{a}H$
		$0.60^{a}H$	$0.51^{d}H$	$0.73^{a}I$	$-0.71^{b}I$	$0.23^{c}I$	$0.60^{b}J$
		$0.52^{d}J$	$-0.69^{b}K$				

Table 3 cont.

Parameter	Parameter designation	Correlation coefficients					
Propionate	G	$-0.79^{a}A$	$-0.55^{b}A$	$-0.46^{d}A$	$-0.27^{d}B$	$0.67^{a}D$	$0.64^{b}D$
		$-0.47^{c}D$	$-0.67^{b}H$	$-0.38^{d}H$	$0.68^{a}I$	$0.46^{b}I$	$-0.77^{c}I$
		$-0.62^{b}J$	$-0.43^{c}J$	$-0.40^{d}J$	$0.54^{a}K$	$0.57^{b}J$	$0.44^{d}K$
Isobutyrate	H	$0.87^{b}A$	$0.58^{d}A$	$0.59^{a}B$	$-0.51^{b}C$	$-0.74^{b}D$	$-0.68^{c}D$
		$-0.44^{d}D$	$0.70^{a}E$	$0.64^{b}E$	$0.66^{d}E$	$0.53^{a}F$	$0.60^{b}F$
		$0.51^{d}F$	$-0.67^{b}G$	$-0.38^{d}G$	$-0.61^{b}I$	$0.93^{a}J$	$0.92^{b}J$
		$0.54^{c}J$	$0.79^{d}J$	$-0.64^{b}K$	$-0.38^{d}K$	$-0.34^{d}L$	
Butyrate	I	$-0.65^{a}A$	$-0.43^{b}A$	$-0.36^{d}A$	$-0.53^{b}B$	$0.79^{b}C$	$0.61^{a}D$
		$0.68^{b}D$	$0.58^{c}D$	$0.58^{d}D$	$0.65^{a}E$	$-0.54^{b}E$	$0.67^{c}E$
		$0.73^{a}F$	$-0.71^{b}F$	$0.25^{c}F$	$0.68^{a}G$	$0.46^{b}G$	$-0.77^{c}G$
		$-0.61^{b}H$	$-0.70^{b}J$	$0.61^{a}K$	$0.75^{b}K$	$0.57^{c}K$	$0.61^{d}K$
Isovalerate	J	$0.86^{b}A$	$0.50^{d}A$	$0.68^{a}B$	$0.33^{d}B$	$-0.52^{b}C$	$-0.81^{b}D$
		$0.62^{a}E$	$0.61^{b}E$	$0.61^{d}E$	$0.60^{b}F$	$0.52^{d}F$	$-0.62^{b}G$
		$-0.43^{c}G$	$-0.40^{d}G$	$0.93^{a}H$	$0.92^{b}H$	$0.54^{c}H$	$0.79^{d}H$
		$-0.70^{b}I$	$-0.68^{b}K$	$-0.29^{d}K$			

Table 3 cont.

Parameter	Parameter designation	Correlation coefficients					
Valerate	K	$-0.53^{b}A$	$-0.52^{d}A$	$-0.51^{b}B$	$0.65^{a}D$	$0.65^{b}D$	$0.48^{d}D$
		$-0.61^{b}E$	$-0.38^{d}E$	$-0.69^{b}F$	$0.54^{a}G$	$0.57^{b}G$	$0.44^{d}G$
		$-0.64^{b}H$	$-0.38^{d}H$	$0.61^{a}I$	$0.75^{b}I$	$0.57^{c}I$	$0.61^{d}I$
		$-0.68^{b}J$	$-0.29^{d}J$	$0.33^{d}L$			
Gas production	L	$-0.45^{d}A$	$0.60^{c}B$	$0.33^{d}B$	$0.37^{d}D$	$-0.34^{d}H$	$0.33^{d}K$

a Hay and grain ration
b Freshly cut alfalfa ration
c High grain ration
d Combination of all three rations

Table 4. Significant correlations of various parameters studied for control groups

Parameter	Parameter designation	Correlation coefficients					
pH	A	$0.57^{c}C$	$-0.75^{a}D$	$-0.74^{b}D$	$-0.60^{d}D$	$0.67^{a}E$	$0.71^{b}E$
		$0.41^{c}E$	$0.77^{d}E$	$0.71^{a}F$	$0.59^{b}F$	$0.73^{d}F$	$-0.68^{a}G$
		$-0.75^{b}G$	$-0.71^{d}G$	$0.88^{a}H$	$0.68^{b}H$	$0.75^{c}H$	$0.83^{d}H$
		$-0.45^{d}I$	$0.86^{a}J$	$0.67^{b}J$	$0.51^{c}J$	$0.64^{d}J$	$-0.61^{a}K$
		$-0.61^{b}K$	$-0.66^{d}K$	$-0.43^{c}L$	$-0.57^{d}L$		
Ammonia	B	$-0.50^{b}C$	$-0.29^{d}E$	$0.54^{b}F$	$-0.27^{d}F$	$-0.47^{b}G$	$0.32^{d}G$
		$0.43^{b}H$	$-0.57^{b}I$	$0.48^{b}J$	$-0.41^{b}K$	$0.35^{d}L$	
Lactate	C	$0.51^{b}D$	$0.33^{d}D$	$-0.59^{b}F$	$0.41^{b}G$	$0.67^{b}I$	$0.37^{d}I$
		$-0.25^{d}J$	$0.28^{d}K$	$0.57^{c}A$	$-0.50^{b}B$		
Total VFA	D	$-0.79^{a}E$	$-0.56^{b}E$	$-0.42^{d}E$	$-0.74^{a}F$	$-0.65^{b}F$	$-0.40^{d}F$
		$0.82^{a}G$	$0.68^{b}G$	$0.29^{d}G$	$-0.69^{a}H$	$-0.72^{b}H$	$-0.51^{c}H$
		$-0.68^{d}H$	$0.57^{b}I$	$0.55^{d}I$	-0.55^{a}	$-0.75^{b}J$	$-0.51^{c}J$
		$-0.60^{d}J$	$0.68^{b}K$	$0.55^{d}K$	$0.32^{d}L$	$-0.75^{a}A$	$-0.74^{b}A$
		$-0.60^{d}A$	$0.51^{b}C$	$0.33^{d}C$			
C2:C3 ratio	E	$0.67^{a}A$	$0.71^{b}A$	$0.41^{c}A$	$0.77^{d}A$	$-0.29^{d}B$	$-0.79^{a}D$
		$-0.56^{b}D$	$-0.42^{d}D$				
		$0.62^{a}H$	$0.43^{b}H$	$0.51^{c}H$	$0.66^{d}H$	$-0.26^{d}I$	$0.46^{b}J$
		$0.37^{d}J$	$-0.55^{b}K$	$-0.70^{d}K$	$0.42^{b}L$	$-0.46^{d}L$	

Table 4 cont.

Parameter	Parameter designation	Correlation coefficients					
Acetate	F	$0.71^{a}A$	$0.59^{b}A$	$0.73^{d}A$	$0.54^{b}B$	$-0.27^{d}B$	$-0.59^{b}C$
		$-0.74^{a}D$	$-0.65^{b}D$	$-0.40^{d}D$	$0.51^{a}H$	$-0.82^{c}H$	$0.59^{d}H$
		$-0.70^{b}I$	$-0.39^{d}I$	$0.27^{d}J$	$-0.68^{b}K$	$-0.75^{d}K$	$-0.48d_{L}$
Propionate	G	$-0.68^{a}A$	$-0.75^{b}A$	$-0.71^{d}A$	$0.32^{d}B$	$0.41^{b}C$	$0.82^{a}D$
		$0.68^{b}D$	$0.29^{d}D$	$-0.64^{a}H$	$-0.57^{b}H$	$-0.49^{c}H$	$-0.63^{d}H$
		$-0.42^{c}I$	$-0.60^{b}J$	$-0.32^{d}J$	$0.59^{b}K$	$0.63^{d}K$	$-0.41^{b}L$
		$0.48^{d}L$					
Isobutyrate	H	$0.88^{a}A$	$0.68^{b}A$	$0.75^{c}A$	$0.83^{d}A$	$0.43^{b}B$	$-0.69^{a}D$
		$-0.72^{b}D$	$-0.52^{c}D$	$-0.68^{d}D$	$0.62^{a}E$	$0.43^{b}E$	$0.51^{c}E$
		$0.66^{d}E$	$-0.41^{d}I$	$0.86^{a}J$	$0.96^{b}J$	$0.73^{c}J$	$0.83^{d}J$
		$-0.54^{b}K$	$-0.62^{d}K$	$-0.53^{d}L$			
Butyrate	I	$-0.45^{d}A$	$-0.57^{b}B$	$0.67^{b}C$	$0.37^{d}C$	$0.57^{b}D$	$0.55^{d}D$
		$-0.26^{d}E$	$-0.70^{b}F$	$-0.39^{d}F$	$-0.42^{c}G$	$-0.41^{d}H$	$-0.47^{b}J$
		$-0.40^{d}J$	$0.66^{b}K$	$0.48^{d}K$	$0.28^{d}L$		
Isovalerate	J	$0.86^{a}A$	$0.67^{b}A$	$0.51^{c}A$	$0.64^{d}A$	$0.48^{b}B$	$-0.25^{d}C$
		$-0.55^{a}D$	$-0.75^{b}D$	$-0.51^{c}D$	$-0.60^{d}D$	$0.46^{b}E$	$0.37^{d}E$
		$0.27^{d}F$	$-0.60^{b}G$	$-0.32^{d}G$	$0.86^{a}H$	$0.96^{b}H$	$0.73^{c}H$
		$0.83^{d}H$	$-0.47^{b}I$	$-0.49^{d}I$	$-0.53^{b}K$	$-0.45^{d}K$	$-0.32^{d}L$

Table 4 cont.

Parameter	Parameter designation	Correlation coefficients					
Valerate	K	$-0.61^{a}A$	$-0.61^{b}A$	$-0.66^{d}A$	$-0.41^{b}B$	$0.28^{d}C$	$0.68^{b}D$
		$0.55^{d}D$	$-0.55^{b}E$	$-0.70^{d}E$	$-0.68^{b}F$	$-0.75^{d}F$	$0.59^{b}G$
		$0.63^{d}G$	$-0.53^{b}H$	$-0.62^{d}H$	$0.66^{b}I$	$0.48^{d}I$	$-0.55^{b}J$
		$-0.45^{d}J$	$0.28^{d}L$				
Gas productions	L	$-0.43^{c}A$	$-0.57^{d}A$	$0.35^{d}B$	$0.32^{d}D$	$0.42^{b}E$	$-0.46^{d}E$
		$-0.48^{d}F$	$-0.41^{b}G$	$0.48^{d}G$	$-0.53^{d}H$	$0.28^{d}I$	$-0.32^{d}J$
		$0.28^{d}K$					

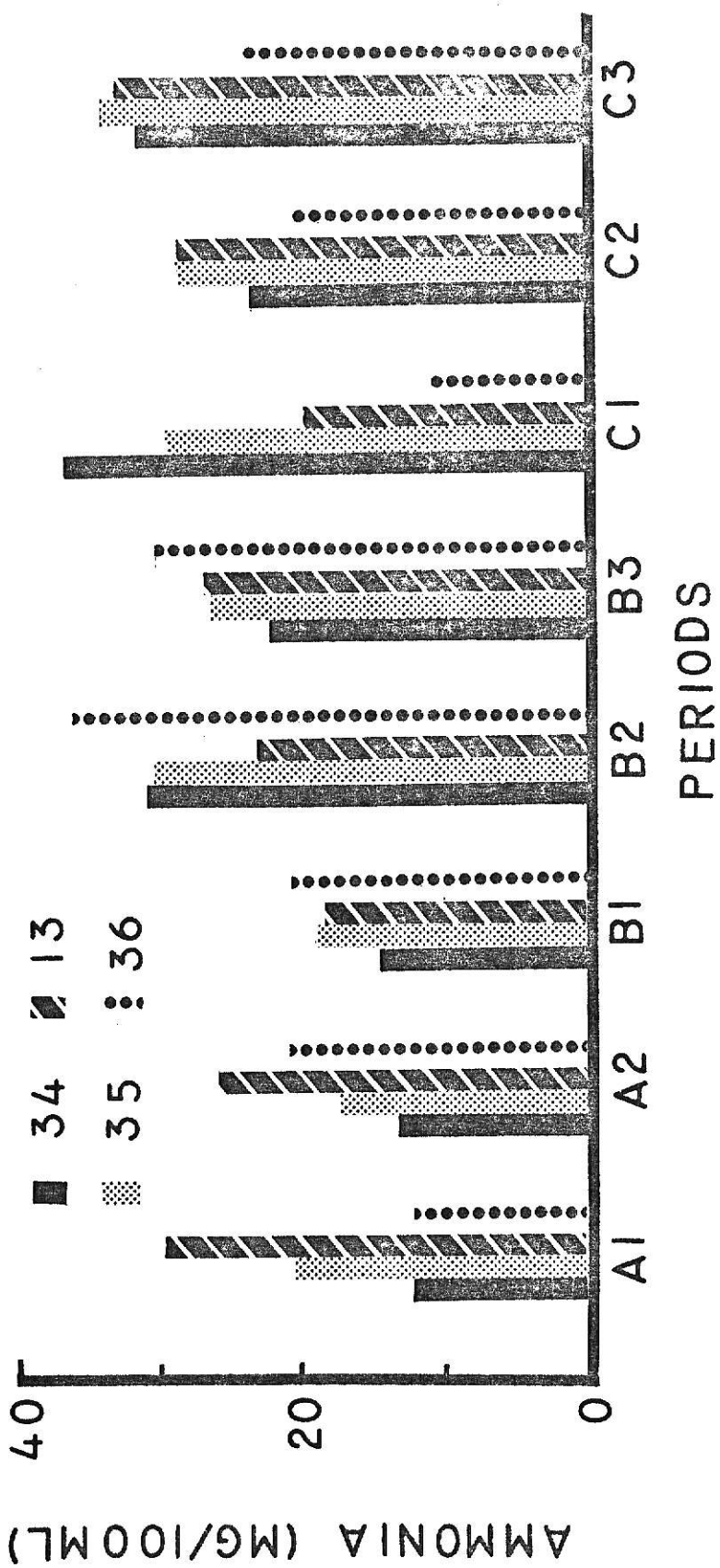

Figure 3. Effect of ration on mean daily concentration of rumen ammonia. Rations were: hay and grain (A1, A2), fresh alfalfa (B1, B2, B3), high grain (C1, C2, C3). Defaunated cows were 13 and 16, faunated were 34 and 35.

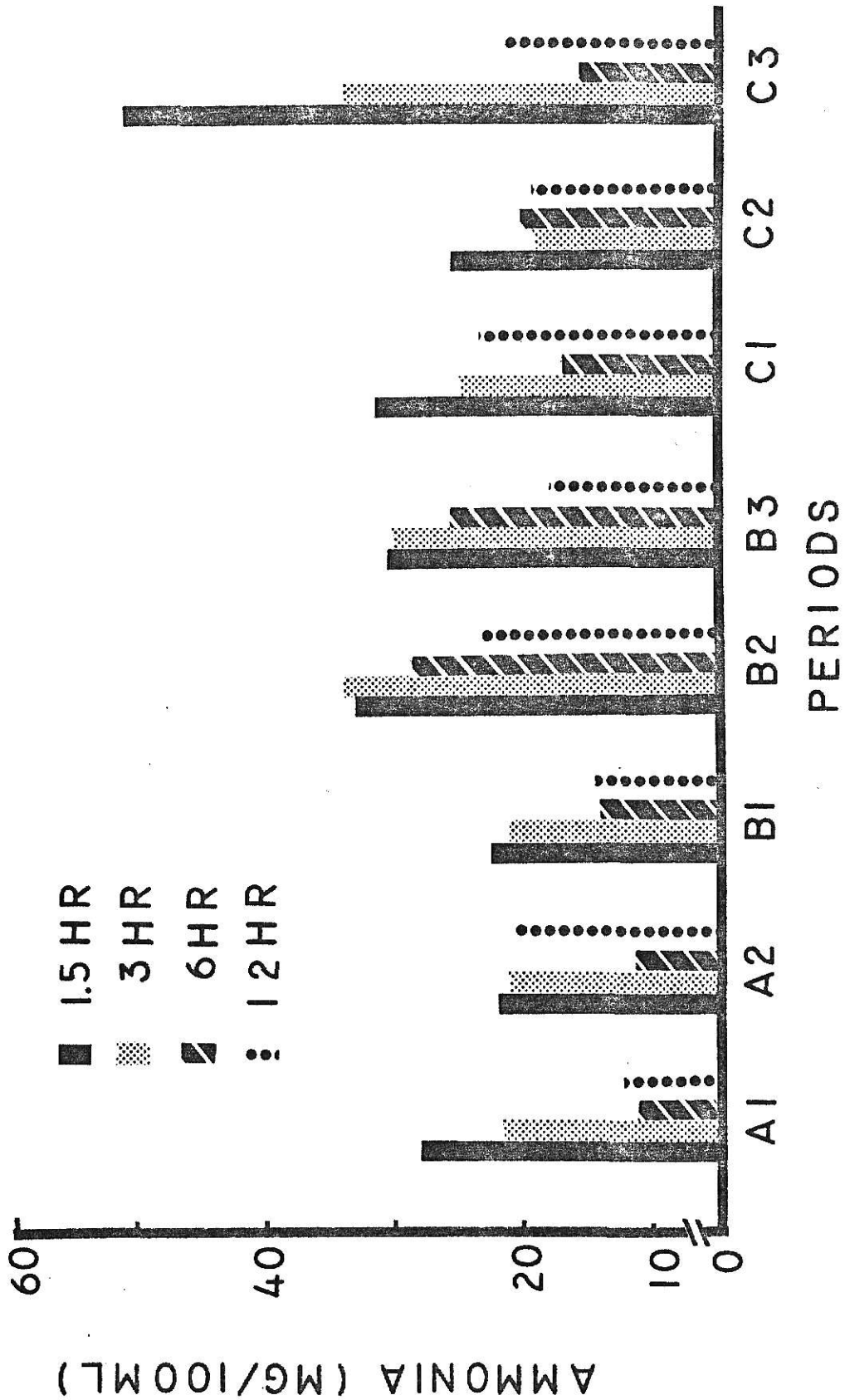

Figure 4. Effect of three ration treatments on rumen ammonia concentration at 1.5, 3, 6, and 12 hours. Rations were: hay and grain (A1, A2), fresh alfalfa (B1, B2, B3), high grain (C1, C2, C3). Each value is an average of two faunated and two defaunated cows.

Lactic acid. The mean lactic acid concentrations varied greatly between ration treatment periods (Table 1). There was no significant difference in the overall mean value between animals in both groups. The highest mean lactic acid value was obtained during period A2 (hay and grain) while the lowest mean values were obtained during the feeding of freshly cut alfalfa (periods B1, B2, and B3). When considering the hourly mean of all animals and ration treatment periods, the lactic acid concentration was greatest 1.5 hours after feeding and then declined (Fig. 1). There was a significant difference between the 1.5 and 3 hour values but differences between the 3, 6, and 12 hour sampling times were not significant (Table 1).

Total VFA. The overall mean total VFA concentration for all animals and ration treatments showed that the two animals in the control group (34 and 35) had the highest total VFA concentration with no significant difference between them (Table 1). The defaunated group (13 and 36) had the lowest mean concentration with a significant difference between the two animals. The treatments that produced the highest total VFA concentration were the high-grain and the hay and grain rations while the freshly cut alfalfa ration produced the lowest total VFA concentration. The highest mean VFA concentration (all animals and rations considered) occurred during the 1.5 and 3 hour samples. The 6 and 12 hour samples had lower concentrations. There was a significant difference between the 6 and 12 hour samples. There was a relationship between animals and ration treatment periods (Fig. 5). There appeared to be a greater difference between the two groups during ration treatment periods A1 and C2. The relationship between ration treatment periods and sampling time (Fig. 6), showed that the 12 hour concentration was lowest in periods A1, B3, and C3 while on other occasions it was as high as or higher

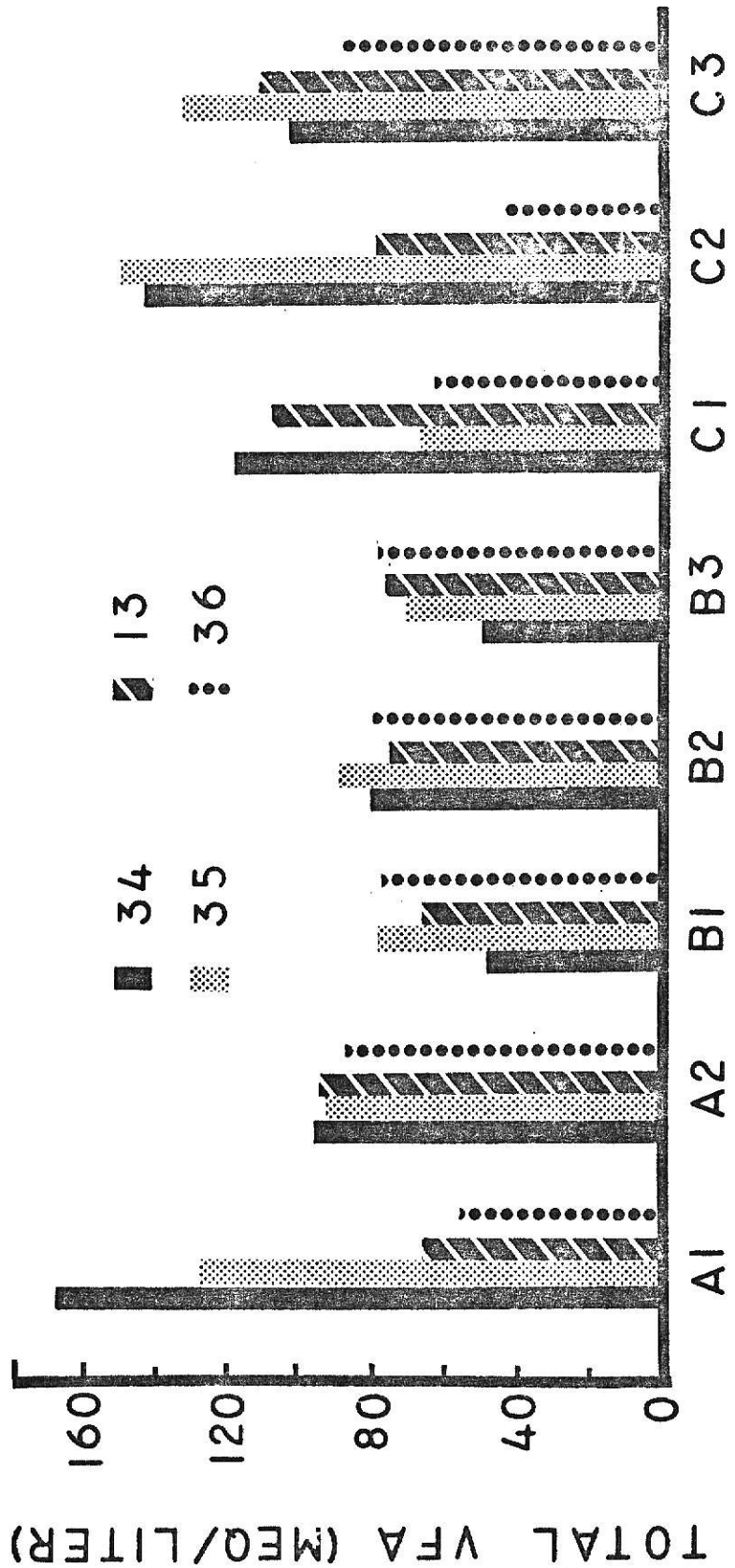

Figure 5. Effect of ration on mean daily concentration of total VFA production. Rations were: hay and grain (A1, A2), fresh alfalfa (B1, B2, B3), high grain (C1, C2, C3). Defaunated cows were 13 and 16, faunated were 34 and 35.

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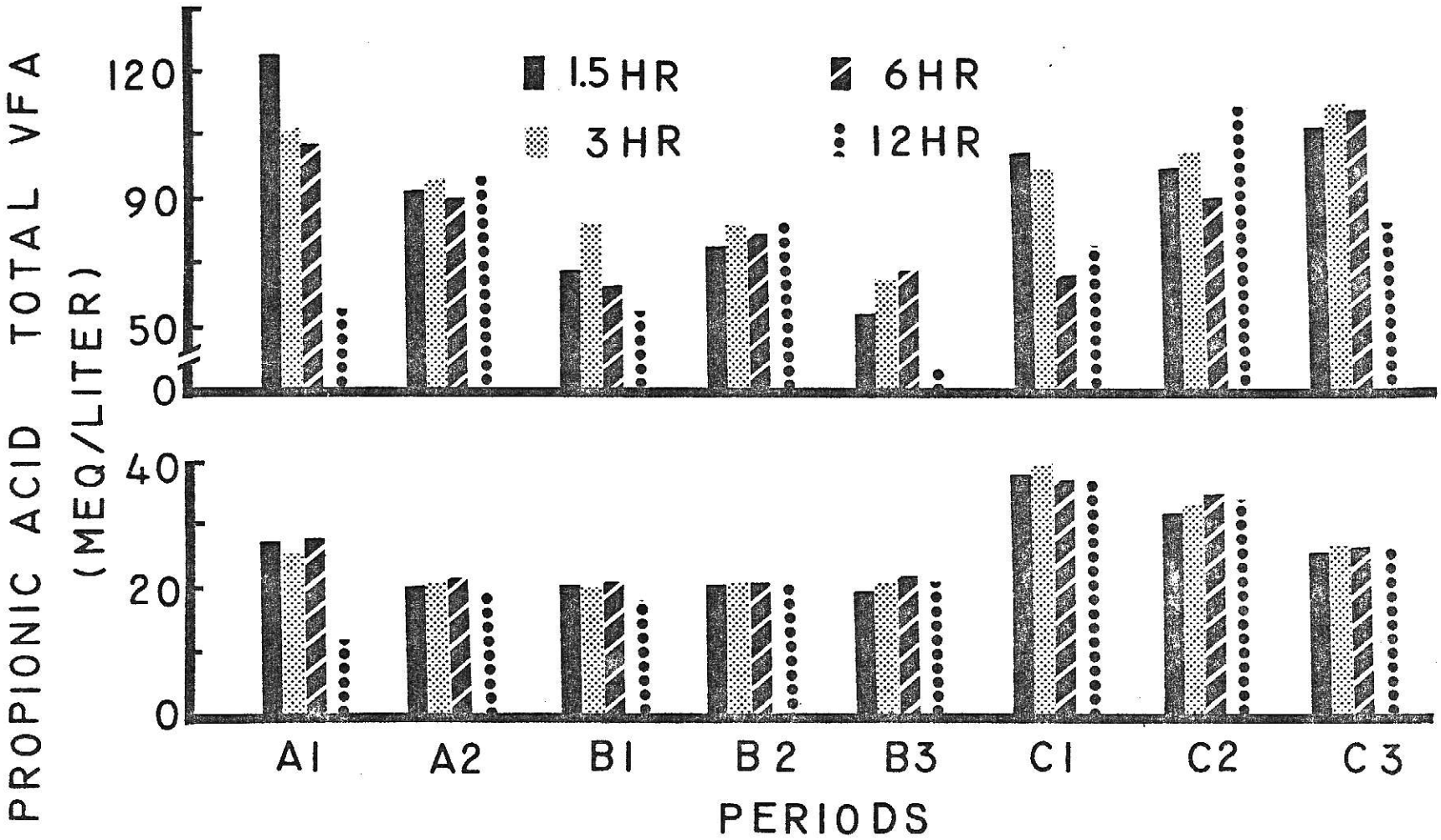

Figure 6. Effect of three ration treatments on the concentration of propionic acid and total VFA at 1.5, 3, 6, and 12 hours. Rations were: hay and grain (A1, A2), fresh alfalfa (B1, B2, B3), high grain (C1, C2, C3). Each value is an average of 2 faunated and 2 defaunated cows.

than that occurring during the other sampling hours.

C2:C3 Ratio. When all ration period treatments were considered, there was variation in the mean C2:C3 ratio between individual animals (Table 1). There was a significant difference between animals in both groups but no significant difference between cow 13 (defaunated) and cow 34 (control). There was a significant difference in the mean C2:C3 ratio between ration treatments. Freshly cut alfalfa produced the highest ratio. Hay and grain and high-grain rations produced lower mean ratios. The overall hourly mean C2:C3 ratio on all ration treatments and animals showed little variation. The 1.5 hour sample had the largest ratio followed by the 3, 6, and 12 hour samples. There was a significant difference only between the 1.5 and 12 hour samples.

There was a relationship between animals and ration treatment periods (Fig. 7). The highest mean ratio occurred during ration treatments A and B while ration treatments C1 and C2 were the lowest.

When all ration treatments were considered, there was a negative correlation between the C2:C3 ratio and ammonia concentration for the control group while the defaunated group showed a positive correlation between the same parameters (Tables 3 and 4).

Acetate. The overall mean acetate level of all ration treatments showed little difference between animals of both groups (Table 1). There was a significant difference in ration treatments (Fig. 8). The freshly cut alfalfa (B1, B2 and B3) produced the highest level of acetate while the lowest level was produced on the high-grain ration (C1 and C2). There was some variation in the overall hourly mean acetate values (Table 1). The 1.5 hour sample had the highest followed by the 6, 3, and 12 hour samples. There was a significant difference between the 1.5 and 12 hour samples but not between the others.

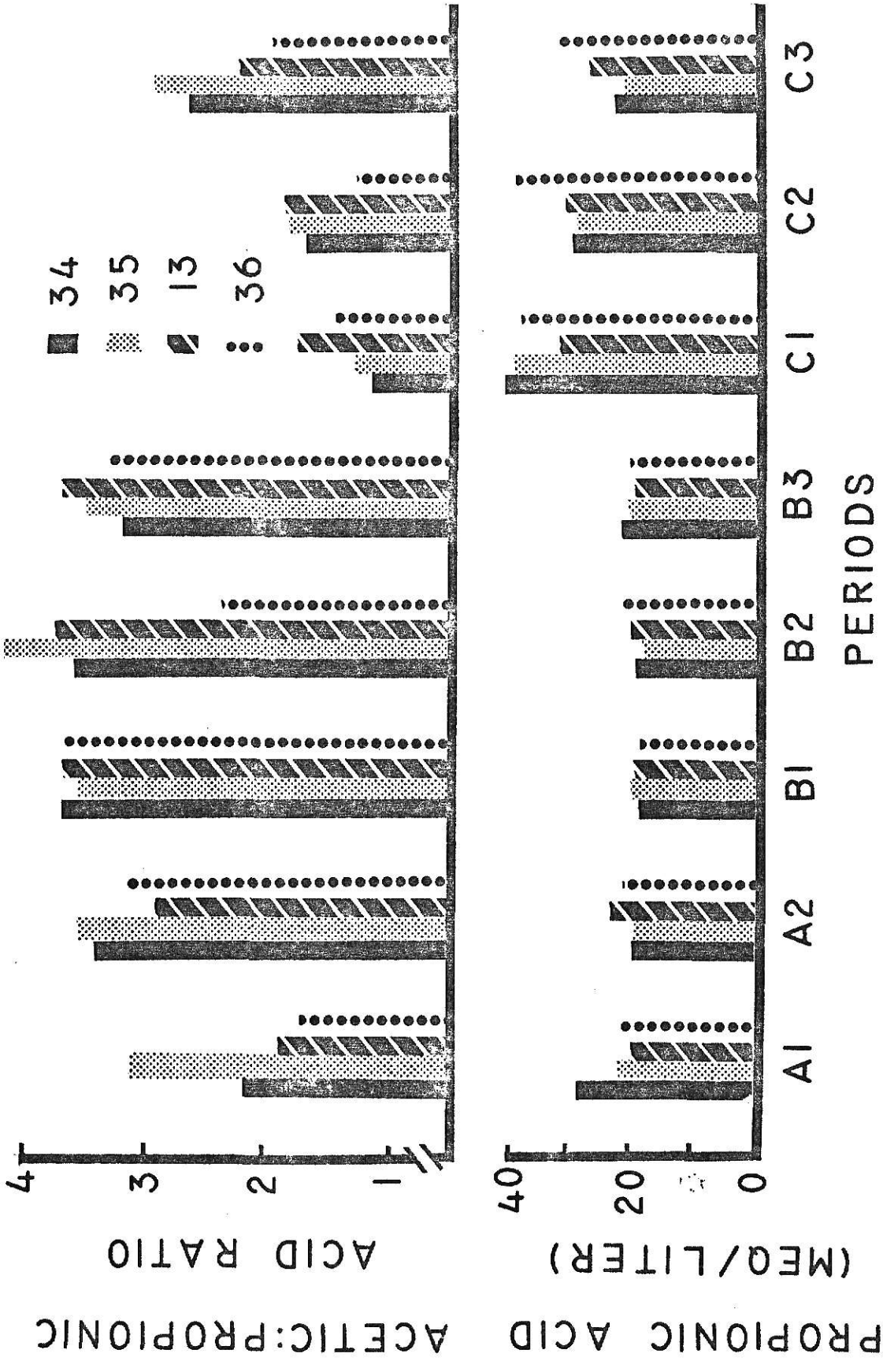

Figure 7. Effect of ration on mean daily concentration of propionic acid and acetic: propionic acid ratio. Rations were: hay and grain (A1, A2), fresh alfalfa (B1, B2, B3), high grain (C1, C2, C3). Defaunated cows were 13 and 16, faunated were 34 and 35.

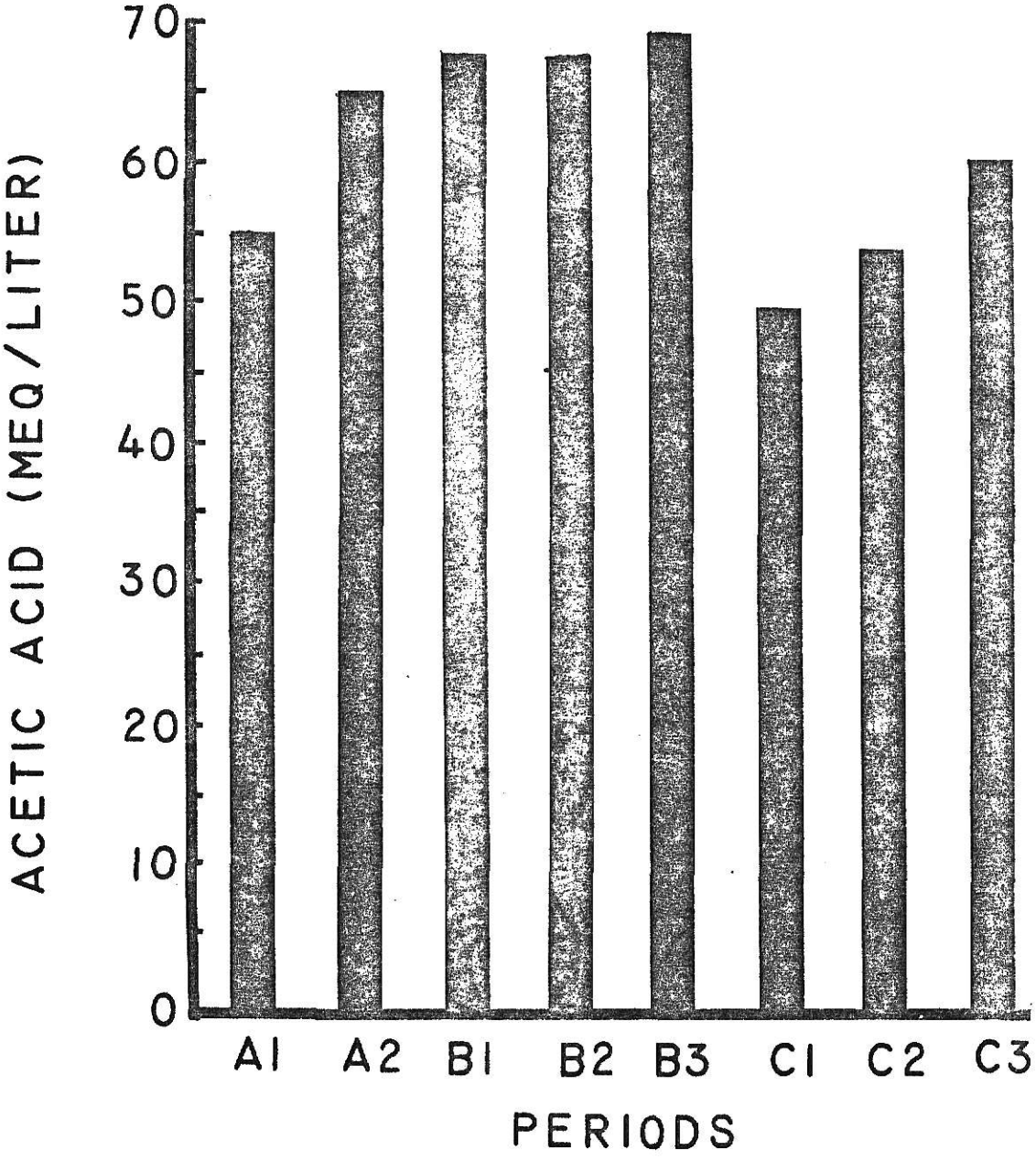

Figure 8. Effect of three ration treatments on mean daily concentration of acetic acid. Rations were: hay and grain (A1, A2), fresh alfalfa (B1, B2, B3), high-grain (C1, C2, C3). Each value is an average of 2 defaunated and 2 faunated cows.

During the hay and grain ration period there was a positive correlation between acetate and pH in the control group and a negative correlation in the defaunated group (Tables 3 and 4). The data from all ration treatments show a negative correlation between acetate and ammonia for the control group and a positive correlation for the defaunated group.

Propionate. The overall mean propionate concentration for all ration treatment periods showed little variation between the animals in either group (Table 1). There was a significant difference between animal 35 (defaunated group) and the three other animals. The mean propionate concentration was highest during the high grain ration treatment periods (C1, C2, and C3) with a significant difference between those three periods. Ration treatments A and B produced the lowest mean propionate concentrations. There was no significant difference between these periods. The overall hourly mean for all animals and ration treatments show little variation. The highest hourly mean was at 6 hour followed by 3, 1.5, and 12 hour. There were no significant differences among the 6, 3, and 1.5 hour samples but the 12 hour sample was significantly different from the 6 and 3 hour samples.

There was a significant relationship between animals and ration treatment periods (Fig. 7). There were only small variations between animals during most ration treatment periods. The relationship between ration treatment periods and sampling times (Fig. 6) shows only a small difference in mean propionate concentrations except for ration treatment A1 where there was a low propionate mean value in the 12 hour sample. There was a negative correlation between propionate and ammonia for the defaunated group and a positive correlation between these for the control group (Tables 3 and 4).

Isobutyrate. The overall mean isobutyric concentration of all ration treatment periods showed no significant difference between animals in the defaunated group but a significant difference existed between animals in the control group (Table 1). There were significant differences between ration treatment periods. The highest concentration of isobutyrate for all animals was during ration treatment B with no significant difference between periods B1, B2, and B3. There was a significant difference of the mean isobutyrate concentration between the 12 and 1.5 hour sampling time. There was no difference between the 3 and 6 hour sampling time.

There was a difference in isobutyrate concentration between animals within ration treatments (Fig. 9). The defaunated animals were similar in mean isobutyric concentration for most ration treatment periods. Figure 10 shows the relationship between ration treatment period and sampling time. The 12 hour sampling time had the highest concentration of mean isobutyrate concentration in periods A2, B1, B3, C1, and C4. The other periods show little difference between the 12 hour and other sampling times.

Butyrate. The mean butyrate concentrations, during all ration treatment periods, show a significant difference between animals in the control group (Table 1). There was no significant difference between animals in the defaunated group. A significant difference exists between the defaunated and control group. Significant differences existed between ration treatment periods. In most instances, the high-grain ration produced the highest mean butyrate concentration while the freshly cut alfalfa ration produced a lower mean concentration. The 6 hour sampling time yielded the highest mean butyrate concentration. There was a significant difference between the 6 and 1.5 hour sampling times while no significant difference existed between 3 and 12 hour

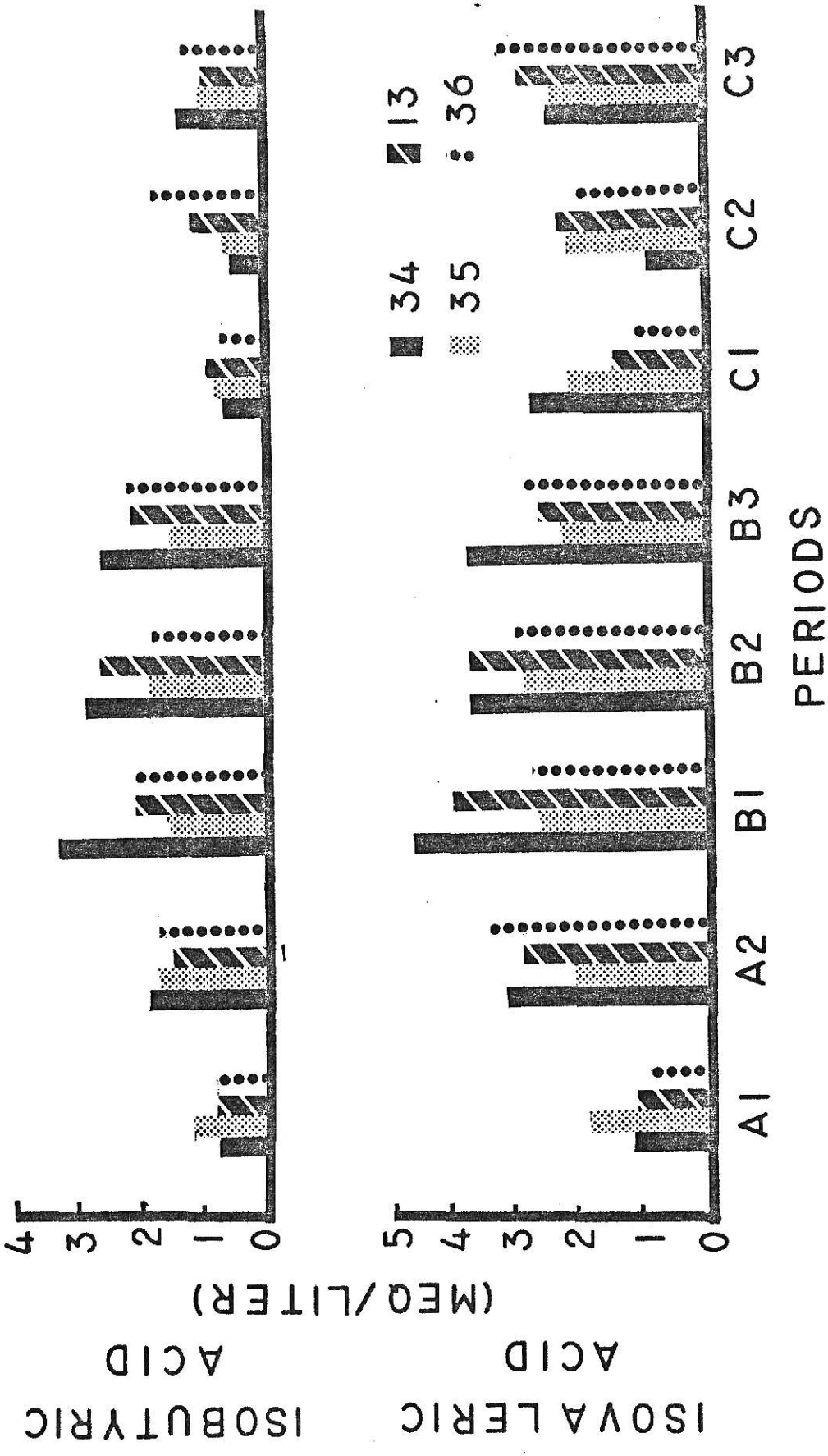

Figure 9. Effect of ratiCn on mean daily concentration of isovaleric acid and isobutyric acid. Rations were: hay and grain (A1, A2), fresh alfalfa (B1, B2, B3), high grain (C1, C2, C3). Defaunated cows 13 and 16, faunated were 34 and 35.

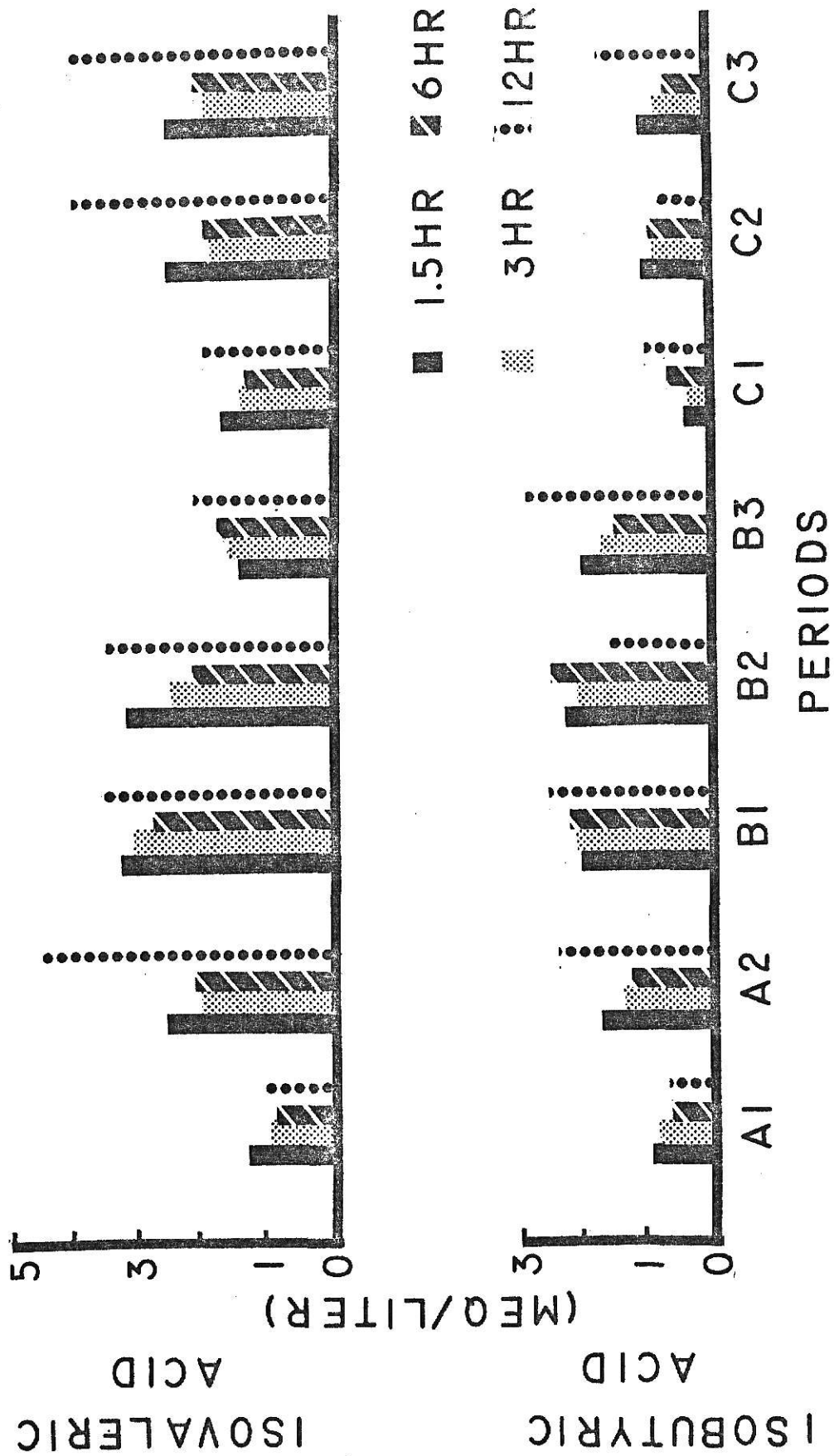

Figure 10. Effect of three ration treatments on the concentration of isovaleric acid and isobutyric acid at 1.5, 3, 6, and 12 hours. Rations were: hay and grain (A1, A2), fresh alfalfa (B1, B2, B3), high grain (C1, C2, C3). Each value is an average of 2 faunated and 2 defaunated cows.

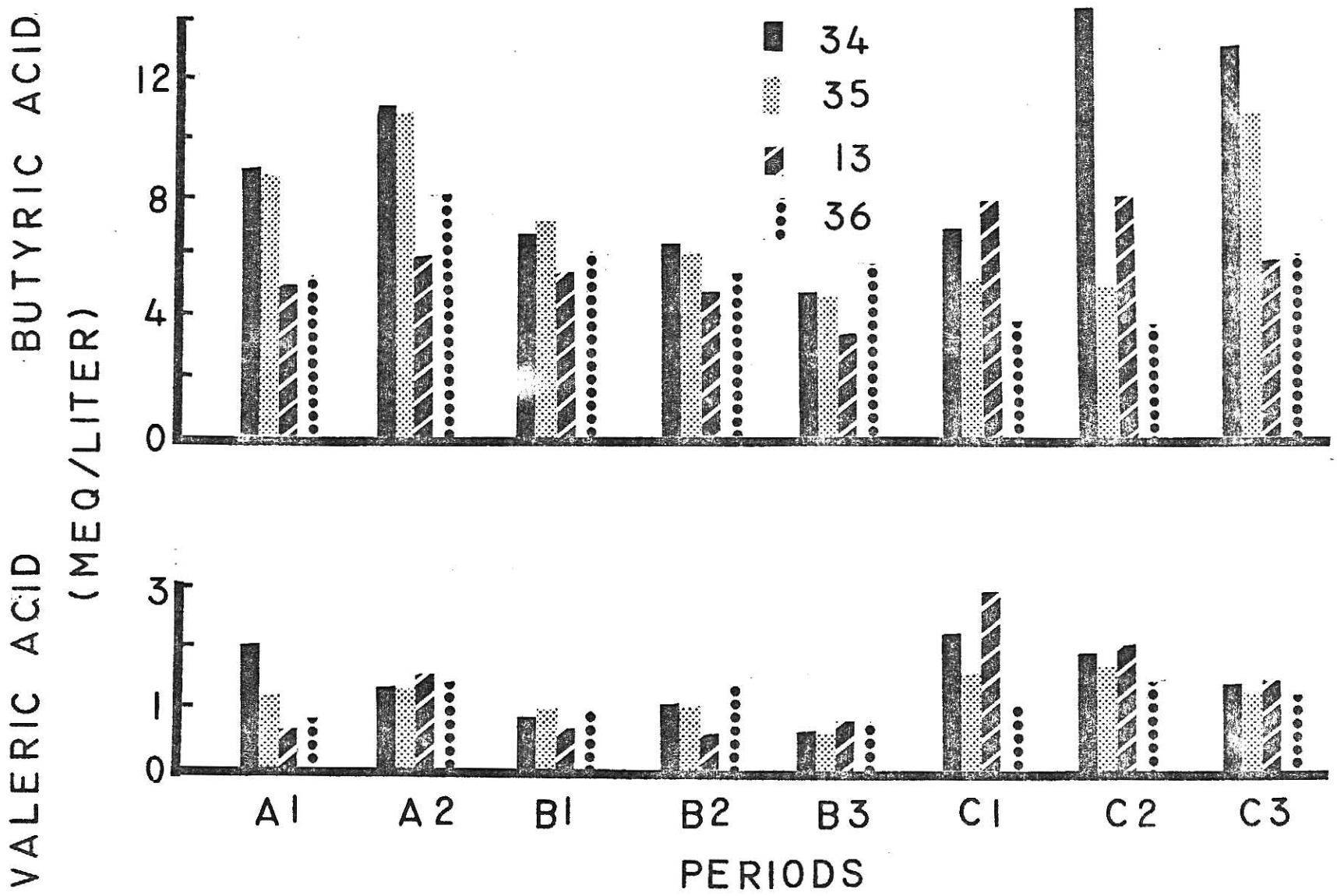

Figure 11. Effect of ration on mean daily concentration of valeric acid and butyric acid. Rations were: ahy and grain (A1, A2), fresh alfalfa (B1, B2, B3), high grain (C1, C2, C3). Defaunated cows were 13 and 16, faunated were 34 and 35.

sampling times.

Differences exist between animals and ration treatment periods (Fig. 11). The greatest differences between control animals existed during the high-grain ration period. The defaunated animals showed their greatest variation in ration treatment periods B1, C1, and C2. Figure 12 shows the differences between ration treatment periods and sampling time. Treatment periods B1, B3, showed more variation and contributed to the significant differences that existed between the 1.5 and 6 hour sampling times.

Isovalerate. There were significant differences between animals in each group but not between groups (Table 1). The overall mean concentration of isovalerate was highest for ration treatment B and lowest for ration treatments A and C. The overall hourly mean for all ration treatments showed that the 12 hour sample had the highest concentration followed by the 1.5, 3, and 6 hour samples. There were significant differences among the 12, 1.5, and 3 hour samples but no significant difference exists between the 3 and 6 hour samples.

Figure 9 shows the differences that exist between animals and ration treatment periods. Variations between animals in each group can be seen in most ration treatment periods. The difference between ration treatment periods and sampling time are shown (Fig. 10). The greatest variation between the 12 and 1.5 hour samples exists in ration treatment periods A1 and C3. There was less variation in the B1, B3, and C2 periods.

Valerate. There was a significant difference between individual animals but not between the two groups (Table 1). There were significant differences among the ration treatment periods. The higher mean valerate concentration was produced with ration treatment C and A. Lowest mean concentrations were

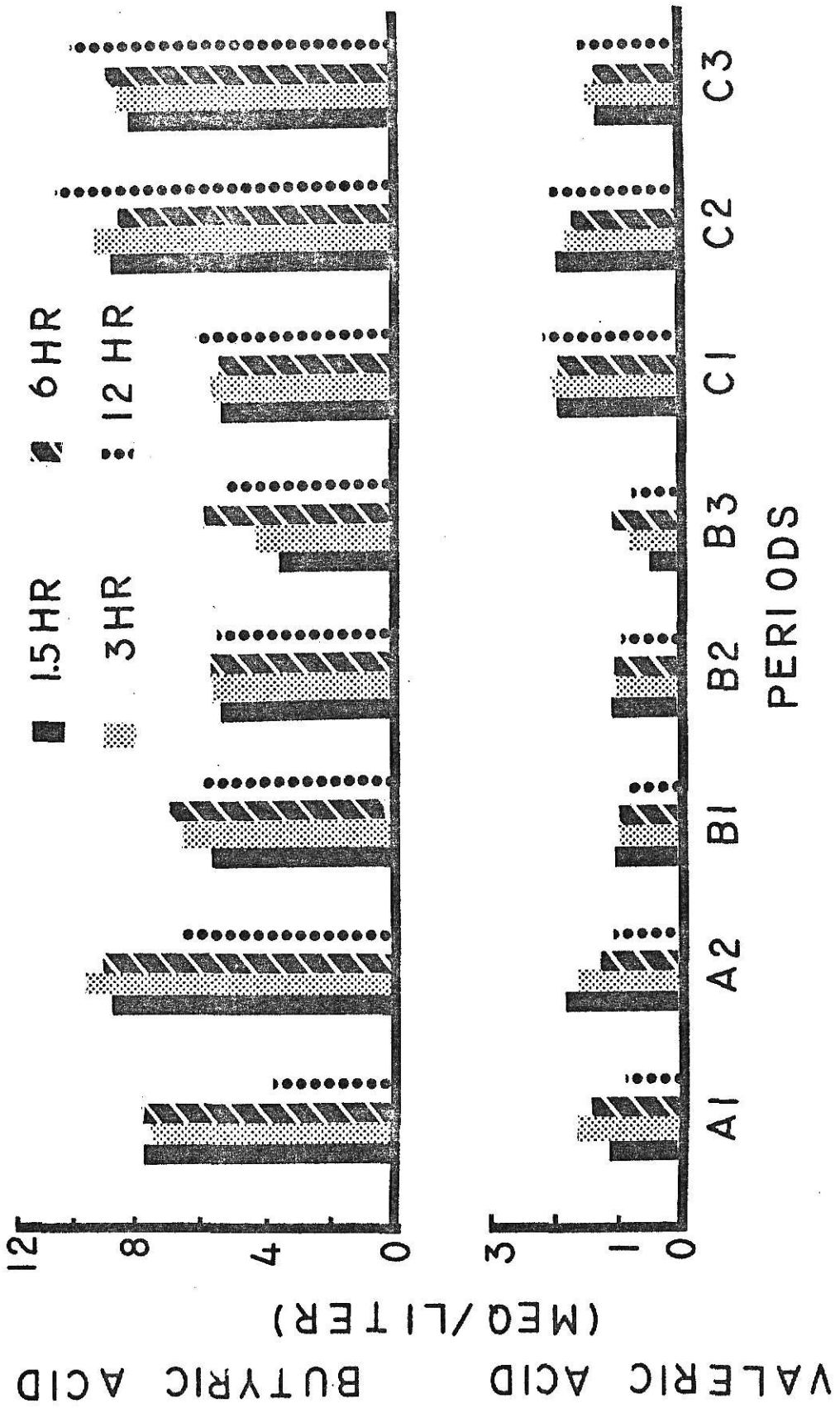

Figure 12. Effect of three ration treatments on the concentration of valeric acid and butyric acid at 1.5, 3, 6, and 12 hours. Rations were: hay and grain (A1, A2), fresh alfalfa (B1, B2, B3), high grain (C1, C2, C3). Each value is an average of 2 faunated and 2 defaunated cows.

produced by ration treatment B. The mean valerate concentration did not vary much in regard to sampling time. The 3 hour sampling time produced the highest mean concentration followed closely by 1.5, 6 and 12 hour. A significant difference existed between the 3 hour (highest) and the 12 hour (lowest). Figure 11 shows the differences that existed between ration treatment periods and animals. Most animal variations occured during ration treatment periods A1, C1, and to a lesser extent B2. Figure 12 shows the difference between ration treatment period and sampling time which are minimal.

Gas production. Significant differences existed in mean gas production between animals but not between groups (Table 1). There was a significant difference between the animals in each group. There were significant differences between ration treatment periods. Ration treatment C produced the highest gas production mean. The lowest gas production means were produced during ration treatment B (freshly cut alfalfa). There was some variation in mean gas production between the four sampling times. The highest mean production was 1.5 hour followed by 3, 6, and 12 hour (lowest). There was no significant difference between the 1.5, 3, and 6 hour sampling times. There was significant difference between the 3 and 12 hour sampling time.

Figure 2 shows that differences existed between ration treatment periods and animals. There was no gas production recorded by certain individual animals during ration treatment periods B1, B3, and C2. There was much variation between animals during ration treatment periods C1, D2, and to a lesser extent between B1, B2, B3, and C3. Figure 13 shows the differences that existed between ration treatment periods and sampling time. The greatest hourly variation occurred during the high grain ration treatment period.

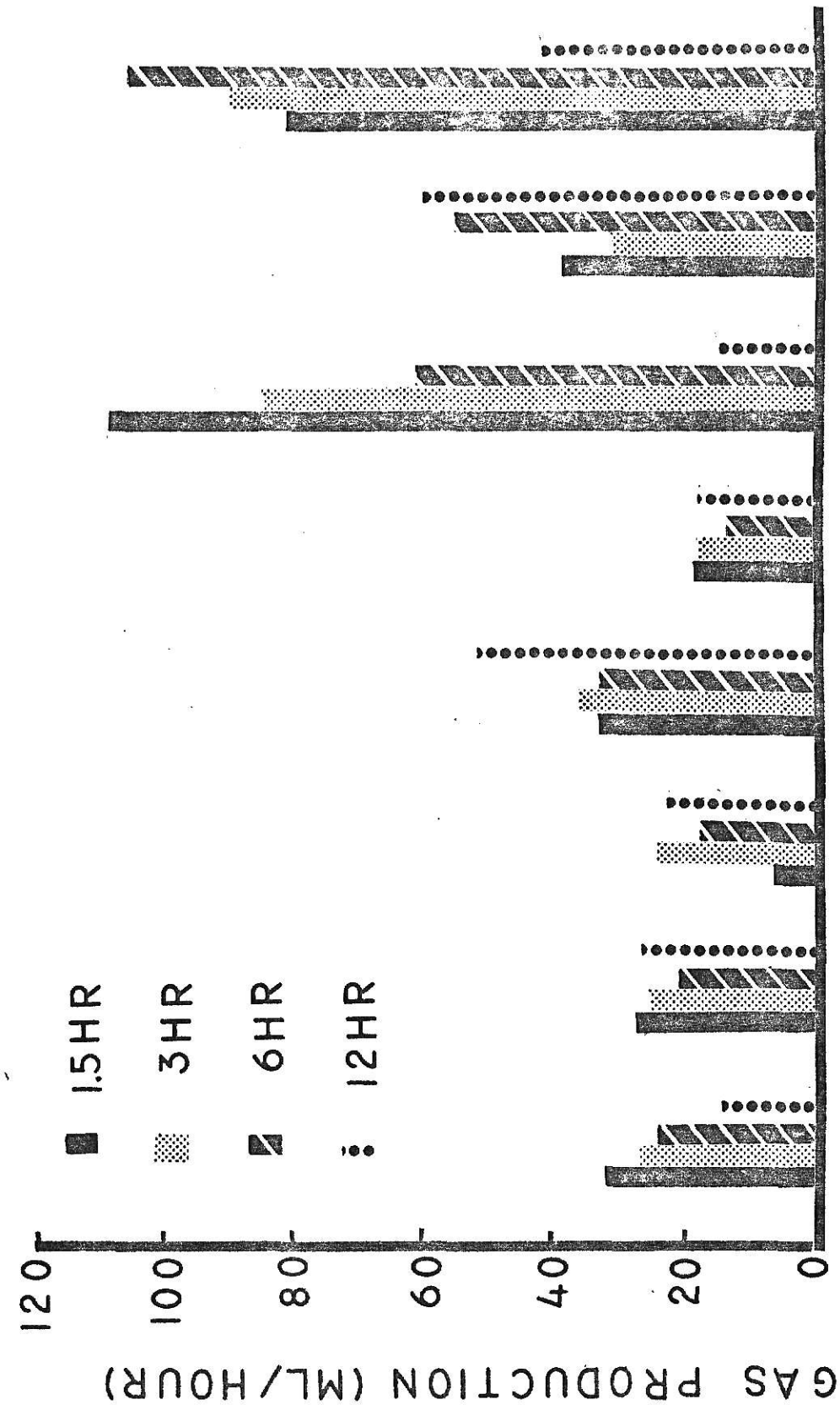

Figure 13. Effect of three ration treatments on gas production at 1.5, 3, 6, and 12 hours. Rations were: hay and grain (A1, A2), fresh alfalfa (B1, B2, B3), high grain (C1, C2, C3). Each value is an average of 2 faunated and 2 defaunated cows.

EXPERIMENT II

INTRODUCTION

This experiment utilized two sets of identical twins in an attempt to reduce the animal variation that existed in Experiment I.

The rumen fermentation measurements made were similar to those of Experiment I. Experiment II differed from Experiment I in that ration treatment remained the same throughout and feed intake was measured and adjusted so that both faunated and defaunated animals had identical daily feed intakes.

EXPERIMENTAL PROCEDURE

Animals. Two sets of yearling identical twin steers were used. One set of twins was Holstein (059-060) and the other set was Angus (048-047). All animals had been fistulated prior to the experiment and were equipped with sampling tubes as described in Experiment I. One member of each set of twins was defaunated while the other served as a control. An isolated barn housed all four animals. Individual pens were constructed for each animal. The two animals to be defaunated were kept in specially constructed stalls that were lined with plywood panels to prevent any direct contact with one another or with other ruminants.

Ration. All animals were fed individually and amounts of feed fed and refused were weighed daily. The ration consisted of a maintenance level of 6 kg of alfalfa-brome hay plus 1.5 kg of ground sorghum grain per animal per day. All animals had free access to trace mineralized salt which was supplied in 3 kg blocks. All animals consumed water ad libitum.

Defaunation Procedure. Aerosol OT was used initially to defaunate animals 059 and 048. Each animal was infused through the sampling tube with 70 g of Aerosol OT. Following the infusion, a liter of warm water was infused through the sampling tube to help disperse the drug and flush the sampling tube. This procedure was continued for four consecutive days. After treatment with Aerosol OT, rumen samples were taken every two days and examined for protozoal activity as described in Experiment I. Whenever protozoa were observed, a retreatment schedule using Aerosol OT would begin. The animals being dosed with Aerosol OT would show decreased feed intake starting about the second day of treatment and would return to original feed intake in 3 to 4 days following the last treatment.

During the latter part of the experiment, a method of defaunation similar to that described by Clark and Reid (1969) was employed. "Emtryl"[1], a soluble powder containing dimetridazole (1,2-dimethyl-5-nitroimidazole), was inserted in the rumen through the fistula at a dosage of 18.2-27.4 g per day of dimetridazole for a period of three consecutive days. The higher dosage level was required to remove Entodinium from the rumen. The temporary side effects as noted with use of Aerosol OT were not observed with dimetridazole.

The animals were cared for by one individual who managed the individual animals in such a way to avoid the least amount of contamination between animals.

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[1]"Emtryl" soluble powder was obtained through Salsbury Laboratories, Charles City, Iowa.

Samples and Analysis. All rumen samples were taken two hours after feeding. Sampling periods occurred only when no protozoa could be observed and when defaunated animals were consuming an amount of feed equivalent to that of the control animals.

The method of collection of rumen fluid was the same as described in Experiment I. About 400 ml. of rumen fluid was collected from each animal. pH was determined using a pH meter with a glass electrode. Ammonia determination was by the method described in Experiment I. Ammonia concentration was expressed as mg NH3/100 ml. Gas production was determined as described in Experiment I with a slight modification. The gas which was liberated from the incubating rumen samples passed through a plastic tube to a water filled graduated glass burette. The gas displaced water in the graduated burette which was read every fifteen minutes.

The amount of dry matter disappearance was determined by using Barnes (1970) in vitro fermentation procedure. The results were calculated and expressed as percentage of in vitro dry matter disappearance (IVDMD). Standard alfalfa hay and standard prairie hay substrates were used for each sample determined.

RESULTS

pH. During all three sampling periods the defaunated animals showed lower pH values than control animals (Table 5).

Ammonia. During the first two sampling periods there was little difference in ammonia concentration between defaunated and control animals. During the third period ammonia levels were lower in the defaunated animals

Table 5. Effect of defaunation on rumen pH, gas production, concentration of ammonia, and in vitro dry matter disappearance.

Sampling period	Animal no.	Treatment	Gas production	pH	Ammonia	In vitro dry matter disappearance	
						Alfalfa	Prairie
			(ml/hr)		(mg/100 ml)	(%)	(%)
I	059	Defaunated	12.45	6.40	3.71	72.49	46.25
	048	Defaunated	20.50	6.20	5.29	70.89	44.52
	047	Control	37.27	6.55	4.23	68.62	40.36
	060	Control	37.45	6.80	4.23	64.97	41.15
II	059	Defaunated	15.60	6.60	8.99	77.67	48.53
	048	Defaunated	8.90	6.50	23.81	73.20	51.48
	047	Control	26.00	6.70	22.22	70.13	55.16
	060	Control	26.25	6.85	22.22	71.59	57.59
III	059	Defaunated	9.50	6.10	5.29	70.14	42.38
	048	Defaunated	10.70	6.25	9.52	65.31	58.86
	047	Control	17.50	6.65	17.46	72.74	48.17
	060	Control	26.40	6.90	17.46	71.71	64.35

than in controls which supports finding from other research reported by Abou Akkada and el-Shazly (1964), Borhami et al. (1967), Christiansen et al. (1965), Chalmers et al. (1968), Klopfenstein (1966), Luther et al. (1966), and Kurihara et al. (1968).

Gas Production. Gas production was reported as milliliters per hour. In all three sampling periods gas production was lower for the defaunated compared to the faunated group.

Invitro dry matter disappearance (IVDMD). There was not any great difference in the IVDMD values between the defaunated and faunated animals with either alfalfa or prairie hay substrates. During sampling periods I and II, the defaunated animals appeared to have a slightly higher IVDMD than the controls with the alfalfa substrate. This may have been due to a bacterial population adapted to alfalfa since alfalfa hay was fed to the animals.

DISCUSSION OF EXPERIMENTS I AND II

In most previous investigations a more active rumen fermentation has existed in faunated than in defaunated animals. This has been evidenced by increased rumen VFA concentration (Abou Akkada and el-Shazly, 1964; Borhami et al., 1967; Christiansen et al., 1965; Luther et al., 1966; and Kurihara et al. 1968). In Experiment I the faunated animals produced significantly higher concentrations of total VFA and butyrate than did the defaunated animals. The other parameters studied in Experiment I did not show any statistically significant difference between the defaunated and control animals because of large variations that existed between animals in each group. However, there was a significant difference between rations fed and among sampling times for all parameters studied. In Experiment II, greater gas production in the faunated animals is further evidence of more active rumen fermentation. However, increased ruminal activity usually resulted in lowered rumen pH. The lower rumen pH observed in defaunated animals is difficult to explain and does not agree with the results of Christiansen et al. (1965). The increased rumen ammonia concentration observed in several instances in Experiment II may explain the higher rumen pH observed.

Increased rumen ammonia concentration observed in faunated animals in some previous studies by Abou Akkada and el-Shazly (1964), Borhami et al. (1967), Christiansen et al. (1965), Chalmers et al. (1968), Klopfenstein (1966), Luther et al. (1966), and Kurihara et al. (1968) may reflect either greater ammonia production from nitrogenous compounds in the feed or poorer conversion of ammonia to microbial protein. That the latter is unlikely is evidenced by the data of Abou Akkada dn el-Shazly (1965) who observed signifi-

cantly higher nitrogen retention in faunated lambs than in those that were defaunated. In Experiment II during the third period, ammonia concentration was higher in the faunated animals. However, in Experiment I no differences existed in ammonia concentration between faunated and defaunated animals.

Previous work (Abou Akkada et al., 1968) showed that Aerosol OT effectively defaunated adult ruminants with only a temporary effect on rumen fermentation. However, since the animals in this study had to be dosed several times to maintain defaunation, there is some question whether differences observed in rumen fermentation were due to the effect of the drug per se or to the absence of protozoa. While methods of chemical defaunation have progressed rapidly in the past two years, further studies are required to insure that the drug effect is not confounding the protozoal effect. It is apparent from the results reported here that it is difficult to remove completely the ciliate protozoa by chemical means. Furthermore, it is difficult to maintain protozoa-free animals for long periods when defaunated by chemical means.

SUMMARY OF EXPERIMENTS I AND II

Experiment I. A study was made of the difference in rumen metabolism between defaunated and faunated ruminants fed three kinds of rations. The ciliate rumen protozoa of two fistulated animals were removed and two other fistulated animals with protozoa served as controls. Rumen fluid samples were obtained at periodic intervals during each sampling period to study the diurnal pattern of rumen fermentation. Two to three sampling periods occurred during each of three ration treatments. Ration treatments consisted of hay and grain (1:1 ratio), freshly cut alfalfa, and high-grain low-roughage. Rumen parameters studied were pH, gas production, acetic:propionic acid ratio, and the concentration of ammonia, lactic acid, total VFA, acetate, propionate, butyrate, isobutyrate, valerate, and isovalerate.

The data collected showed much variation between animals, ration treatments and sampling times. A 3-way analysis of variance was performed. A significant difference existed between the control and defaunated animals in concentration of total VFA and butyrate using the overall means for all ration treatment periods and sampling times. Statistically significant differences between the defaunated and faunated animals for all other parameters studied were non existent because of large differences between animals within groups.

There were significant differences between rations and within rations, for some of the parameters studied. There were significant differences among sampling times for all parameters studied. Significant interactions occurred between animals and ration treatment periods for all parameters except lactate and acetate. There was a significant interaction between ration treatment periods and samples for all parameters except pH, C2:C3 ratio, and

concentrations of lactate, acetate, and propionate.

In control group, pH and acetate were correlated significantly when hay and grain were fed, and ammonia and propionate were correlated for all rations. In the defaunated group, negative correlations existed for the same parameters. The control group had negative correlations between ammonia and C2:C3 ratio (all rations) and acetate (all rations) while the defaunated group showed positive correlations.

The original objective of this study was to determine differences in rumen fermentation between faunated and defaunated animals fed different rations. While rations, regardless of presence or absence of protozoa, significantly affected rumen fermentation, there were no clear cut differences in fermentation between faunated and defaunated animals.

It was evident from clinical observation that the defaunated animal showed an overall decreased feed intake and weight loss when compared with the faunated controls at the termination of this experiment.

Experiment II. Two sets of identical twin yearling steers were used. One member of each set was defaunated while the other member served as a control. Three samples were obtained during the experiment and pH, ammonia, gas production, and in vitro dry matter disappearance were determined. Little difference was noted in ammonia and dry matter disappearance between the defaunated and control animals. Gas production and pH were lower during all sampling periods for the defaunated animals.

ACKNOWLEDGMENTS

The author is deeply indebted to Dr. E. E. Bartley for his assistance, guidance and encouragement during the course of the author's study.

The initial motivation for this study came from Dr. Abou Akkada while he was a National Science Foundation Visiting Professor at Kansas State University. The author greatly appreciates Dr. Akkada's advice and assistance during the course of this investigation.

Appreciation is extended to Dr. RoNel Meyer for her technical assistance in the laboratory and for statistical evaluation of the data.

Gratitude is expressed to Dr. C. L. Norton, Head of Department of Dairy and Poultry Science, for his guidance and critical review of the thesis.

The author appreciates the assistance of Dr. Russell Frey and Dr. J. L. Morrill who kindly served on his committee.

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COMPARATIVE RUMEN FERMENTATION ACTIVITY OF CATTLE WITH AND WITHOUT RUMEN CILIATE PROTOZOA

by

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AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Dairy and Poultry Science

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1970

Studies were conducted to compare rumen metabolic activity between adult cattle free of rumen ciliate protozoa (defaunated by chemical agents) and normal controls.

Experiment I utilized four rumen fistulated Jersey cows. Two cows were defaunated with Aerosol OT and two cows served as controls. Three different ration treatments were fed including hay and grain (1:1 ratio), freshly cut alfalfa pasture, and a high-grain low-roughage ration. Rumen samples were collected at 1.5, 3, 6, and 12 hours after feeding. Changes in rumen fermentation were studied by measuring rumen pH, gas production, acetic:propionic acid ratio, and the concentrations of ammonia, total VFA, acetate, propionate, butyrate, isobutyrate, valerate, isovalerate and lactate.

There were significant differences between animals and ration treatments but no clear cut difference in fermentation between defaunated and faunated animals. Clinically, the defaunated animals showed a decreased feed intake and some weight loss when compared with controls.

Experiment II used two sets of identical twin steers to reduce animal variation that existed in Experiment I. One animal from each set of twins was defaunated while the other served as a control. The ration was kept constant throughout the experiment and feed intakes of both groups were kept constant. Rumen samples were taken two hours after feeding and analyzed for pH, ammonia, gas production, and in vitro dry matter disappearance. Defaunated animals had lower pH and gas production values than did the controls. Ammonia concentration appeared to be higher in faunated than in defaunated animals. There did not appear to be any clear cut difference in in vitro dry matter disappearance between the defaunated and faunated animals.