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THE EFFECT OF FEEDING DIFFERENT RATIOS OF HEATED TO UNHEATED CONCENTRATE MIXTURE ON THE VOLATILE FATTY ACIDS, pH AND AMMONIA CONTENTS OF RUMEN FLUID IN THE COW*

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A Friesian cow with fistulated rumen was used to investigate the effect of feeding a ration of long hay and varios ratios of heated to unheated concentrate mixture upon the volatile fatty acids, pH and ammonia contents of the rumen fluid.

Increasing the percentage of heated concentrate in the daily ration from 0 to 100% increased the total VFAs, on the average, from 9.88 to 11.51 mg. equiv./100 ml. rumen fluid, respectively, and the molar proportion of propionic acid from 17.9 to 19.2%. On the other hand, it decreased the molar proportion of acetic acid from 70.9 to 69.5% and resulted in a narrower acetate to propionate ratio from 3.97 : 1 to 3.62 . 1. In addition, the pH was depressed from 6.71 to 6.38 and the ammonia concentration decreased from 21.4 to 19.7 mg./100 ml.

The differences in the pH and molar proportions of the acetic, propionic and butyric acids, between the different feeding periods of various ratios of heated to unheated concentrate mixture were found to be statistically significant ($P<0.01$) while those of ammonia were not significant. On the other hand, the variations in the concentrations of ammonia, total and individual VFAs, between the sampling hours after feeding time, were found to be statistically significant while those of pH were not significant.

The volatile fatty acids produced during fermentation in the rumen play an important role in the energy metabolism of ruminants. They have been shown to vary in their nutritive properties and to be used with a greater efficiency for fattening when they contain a high proportion of propionic and butyric acids than when they contain a high proportion of acetic acid. (Armstrong and Blaxter, 1957).

Ensor *et al.* (1959), also reported that the fat content of milk of dairy cows as well as the efficiency of gains in steers can be controlled to a remarkable extent by manipulating the ruminal production of volatile fatty acids.

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* This investigation was carried out in "Hoorn" Institute for Animal Nutrition Research, Hoorn, Netherlands.

El-Shazly (1952), made an extensive study of the VFAs in the rumen of sheep fed on a number of different rations and concluded that the amino acids of the protein fed may be the principal source of both the ammonia and the branched-chain acids.

It has been widely recognized that factors other than the chemical composition of the ration are of great importance in relation to the pattern of microbial fermentation in the rumen, as showed by the marked effect of the physical form of the ration (Balch and Rowland, 1957 ; Ensor *et al.*, 1959 ; Van Adrichem, 1962). Grinding the hay and pelleting the ration (King and Hemken, 1962 ; Jorgensen and Schultz, 1963 ; and Jorgensen *et al.*, 1965), and cooking the maize portion of the concentrate (Balch and Rowland, 1957 ; Ensor, 1959 ; Ensor *et al.*, 1959 ; Show *et al.* 1959, 1960 ; and Rook, 1961) have been reported to cause changes in the proportions of the volatile fatty acids formed in the rumen, by decreasing the proportion of acetic acid, increasing the proportion of propionic acid and narrowing the ratio of acetate to propionate as a result of the two previous changes. Accordingly, the nutritive value of the ration can be increased by such methods without changing its chemical composition, due to the relatively smaller heat increments of propionic and butyric acids than that of acetic acid, as found by Armstrong and Blaxter (1957). On the other hand, it is probable that the only disadvantage of a ration which increases propionate and decreases acetate is its tendency to depress the milk fat test. (Jorgensen and Schultz, 1963 ; and Van Soest, 1963). However, such ration will be excellent for dairy cows during the dry and freshening periods since it produces more efficient gains and is more glucogenic than normal rations, and thus aids in the prevention of ketosis as believed by Ensor *et al.* (1959), Shaw *et al.* (1959) and Van Adrichem (1962).

Cardon (1963), reviewed research on grain processing and concluded that steam rolling was advantageous for corn and barley, but not for sorghum grain. He discussed the interrelationship of moisture, temperature and time in processing and attributed the benefits obtained to the gelatinization of starch.

An experiment has, therefore, been undertaken to investigate the effect of feeding different ratios of heated to unheated concentrate mixture on the total and molar proportions of the volatile fatty acids and also on the pH and ammonia contents of the rumen fluid in the cow.

Material and Methods

A Friesian cow with fistulated rumen from the herd of "Hoorn" Institute for Animal Nutrition Research was placed on experiment for 60 days. It was fed daily 7 kg. of grass hay together with 5 kg. of concentrates. The daily ration was given in two equal meals. The hay was given to the cow in the long form. The concentrate mixture contained linseed meal, 43.03%. maize meal. 29.26%. barley meal. 22.38%; dried beet pulp, 3.44% and vitamin and mineral supplement, 1.89%ro%, experimental periods of ten days each 0, 1, 2, 3, 4, and 5 kg. of the daily concentrate ration were fed after being moistened with hot water (1.5 litres/1 kg. conc.), heated in autoclave at 120.°C (under 1 kg. press/cm^2) for 30 minutes to assur complete gelatinization of strach and finally cooled to room temperature.

The scheme of feeding and the daily rations are shown in Table 1.

TABLE 1.—SCHEME OF FEEDING AND DAILY RATIONS

		p.m. a.m.	
1st	period	(10—20 April) :	7 kg. Hay + 5 kg. conc. (unheated) + 0 kg. Conc. (heated).
2nd	,,	(20—30 ,,) :	7 kg. Hay + 4 kg. Conc. (unheated) + 1 kg. Conc. (heated).
3rd	,,	(30 Apr.—10 May:	7 kg. Hay + 3 kg. Conc. (unheated) + 2 kg. Conc. (heated).
4th	,,	(10—20 May) :	7 kg. Hay + 2 kg. Conc. (unheated) + 3 kg. Conc. (heated).
5th	,,	(20—30 May) :	7 kg. Hay + 1 kg. Conc. (unheated) + 4 kg. Conc. (heated).
6th	,,	(30 May—9 June):	7 kg. Hay + 0 kg. Conc. (unheated) + 5 kg. Conc. (heated).

Change of ration : on 10th days (p.m.).

After transitory periods of eight days each on the assigned ration rumen fluid samples were taken during the ninth and tenth days of each of the experimental periods at 8, 10 and 12 O'clock in the morning i.e. 0,2, and 4 hours after the feeding time, respectively. The samples were analysed for PH, ammonia and molar proportions of the volatile fatty acids.

Representative samples of the hay and the concentrate mixture were taken throughout the experiment and analysed. Their proximate composition is given in Table 2.

TABLE 2.—THE PROXIMATE COMPOSITION OF THE FEEDS

Feed	Dry matter	Crude Protein	Ether Extract	Crude Fiber	N-free Extract	Ash
		% of dry matter				
Hay	96.82	9.96	—	35.56	48.53	5.95
Concentrate Mixture	88.34	21.86	5.55	7.63	59.75	5.21

Analytical procedures :

1. *Feeds* : The A.O.A.C. methods (1950), were used in the analysis of the feeds.

2. *Rumen fluid :*

   (*a*) The pH was determined with a glass electrode after the samples had been cooled rapidly to room temperature.

   (*b*) Total and proportions of VFAs : The technique of Erwin, Marco and Emery (1961), was followed using a F & M. gas chromatograph.

   (*c*) Ammonia was determined by distillation.

Results and Discussion

The experimental heated concentrate ration was palatable and readily consumed by the cow without noticeable digestive or physiological disturbances. The pH and ammonia concentration of the rumen fluid samples and their mean values representing the sampling hours throughout the experimental periods are given in Table 3. The pH and NH_3 mean values are also plotted in Fig. 1 and 2, respectively.

pH : The highest pH values were obtained in the first period with the starting ration of hay and unheated concentrate while the lowest values were attained in the last period with the fiinal ration of hay and heated concentrate. The mean pH values in the first period were 6.69, 6.79 and 6.66 in the rumen fluid samples taken 0, 2, and 4 hours after feeding, respectively. The corresponding mean values in the last period were 6.45, 6.36 and 6.32. During the other periods the pH values were intermediate.

It was noticed that the pH dropped after feeding and fell to lowest levels in the rumen fluid samples taken 4 hours after feeding. Fig. 1.

However, there was an abnormal rise in the pH of the samples taken 2 and 4 hours after feeding in the first and fourth periods, respectively which cannot be explained. The fall in the pH values after feeding appeared to be rather slower in the 2nd and 3rd periods than in the last two periods.

The analysis of variance (Table 5) showed that the differences in the pH mean values were significant ($P < 0.01$) between the periods where different proportions of heated concentrate were fed, and not significant ($P > 0.05$) betoween sampling hours after feeding time.

All the pH values obtained in the present investigation were below the range of 6.83 — 7.01 reported by Monroe and Perkins (1939), with normal rations of roughages and concentrates in fair amounts. The comparatively low pH values found in the rumen fluid samples taken during the last two periods, favours rapid absorption of short chain fatty acids, as suggested by Phillipson (1952). However they were higher than the

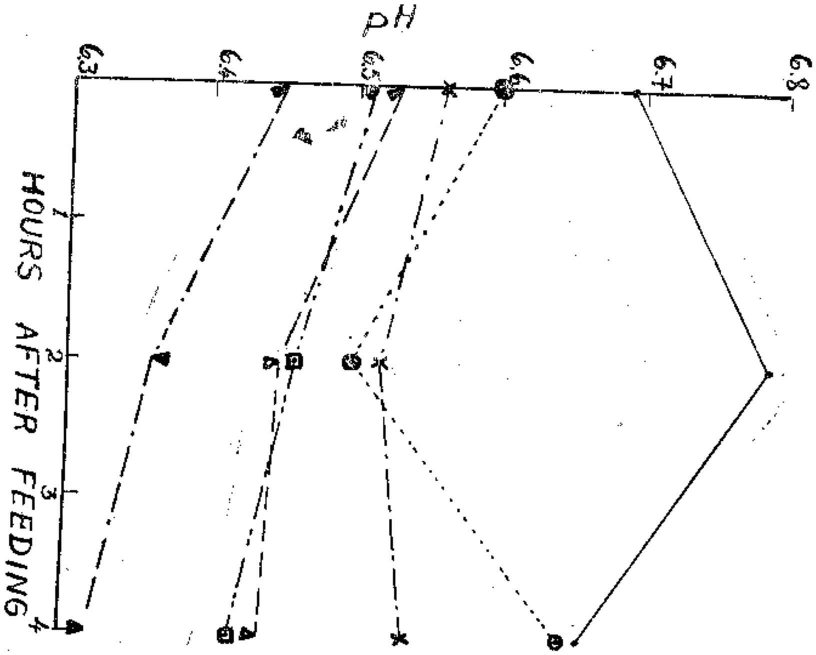

FIG.1 pH IN RUMEN FLUID

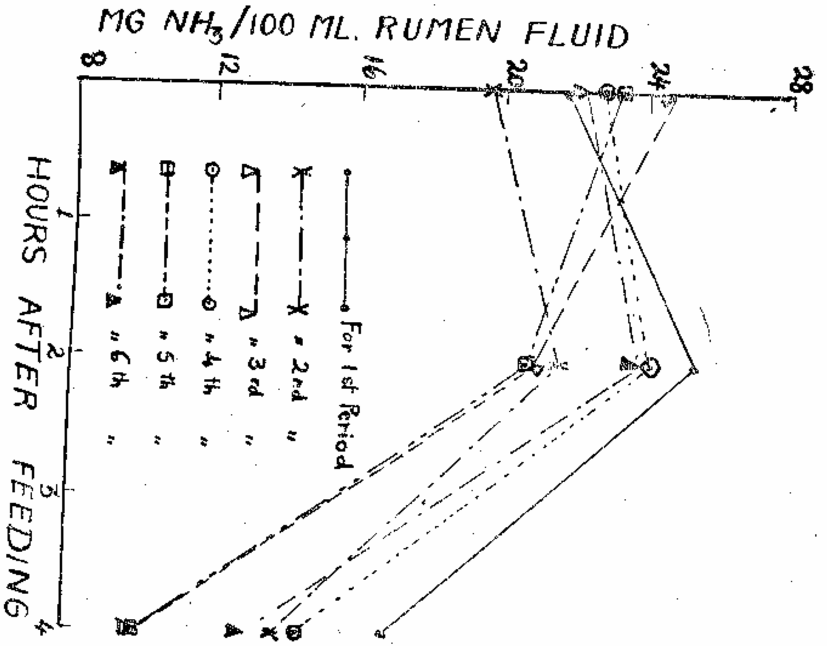

FIG.2. NH_3 IN RUMEN FLUID

TABLE 3.—THE pH AND AMMONIA CONCENTRATION IN CONSECUTIVELY DRAWN SAMPLES OF COW'S RUMEN FLUID

Period	Date of Sampling	Daily Ration (kg)			pH				NH_3 mg/100 ml.			
		Hay	Conc. (unheated)	Conc. (Heated)	Hours after feeding			Period mean	Hours after feeding			Period mean
					0	2	4		0	2	4	
1	19/4/66	7	5	0	6.62	6.75	6.50		21.7	24.8	15.1	
	20/4/66	7	5	0	6.75	6.82	6.82		21.7	26.1	18.7	
					6.69	6.79	6.66	6.71	21.7	25.5	16.9	21.4
2	29/4/66	7	4	1	6.53	6.50	6.40		16.6	22.2	15.4	
	30/4/66	7	4	1	6.58	6.54	6.67		22.6	20.9	12.4	
					6.56	6.52	6.54	6.54	19.6	21.6	13.9	18.4
3	9/5/66	7	3	2	6.53	6.48	6.38		25.5	24.6	11.2	
	10/5/66	7	3	2	6.53	6.42	6.50		23.9	17.3	8.4	
					6.53	6.45	6.44	6.47	24.7	21.0	9.8	18.5
4	19/5/66	7	2	3	6.60	6.50	6.70		22.7	21.9	12.4	
	20/5/66	7	2	3	6.60	6.50	6.60		22.7	26.5	16.6	
					6.60	6.50	6.65	6.58	22.7	24.2	14.5	20.5
5	29/5/66	7	1	4	6.55	6.48	6.43		22.9	20.5	8.8	
	30/5/66	7	1	4	6.47	6.43	6.40		23.5	21.1	10.9	
					6.51	6.46	6.42	6.46	23.2	20.8	9.9	18.0
6	8/6/66	7	0	5	6.52	6.42	6.39		26.4	27.9	15.0	
	9/6/66	7	0	5	6.37	6.30	6.25		18.0	19.9	11.2	
					6.45	6.36	6.32	6.38	22.2	23.9	13.1	19.7
	(Time of sampling) mean				6.56	6.51	6.51		22.4	22.8	13.0	

values obtained by him with diets low in hay and high in flaked maize. A rapid fall in pH was reported by Elsden and Phillipson (1948) ; and Phillipson (1952), with the addition of readily available carbohydrates to the ration. Briggs *et al.* (1957), also found that lactic acid accumulated and rumen pH was lowest when much soluble carbohydrates were fed, and reported that with other diets pH was the result of the balance between volatile fatty acids and ammonia and salivary alkali. In the present investigation, the mean pH values for all the rumen fluid samples taken 0, 2 and 4 hours after feeding were 6.56, 6.51 and 6.51, respectively. Gray (1947), demonstrated that the rumen pH has a profound effect on the rates at which the volatile fatty acids are absorbed, and showed that at a pH of 6.50 propionic acid disappeared more rapidly from the rumen than did acetic acid.

Amonia : Reference to Table 3 shows that the mean values for ammonia concentration in the first period with unheated concentrate were 21.7, 25.5 and 16.9 mg. per 100 ml. in rumen fluid samples taken 0, 2 and 4 hours after feeding, respectively. The corresponding values in the last period with heated concentrate were 22.2, 23.9, and 13.1 mg per 100 ml. It is clear that, in the last period, there was a drop in NH_3 concentration in the rumen fluid sampled 2 and 4 hours after feeding compared with those of the first period, while there was a slight increase in the first sample taken just after feeding time. There were some fluctuations in ammonia concentration during the intermediate periods, on various heated to unheated concentrate ratios. However, the analysis of variance (Table 5) indicated that the differences found in ammonia mean values between the experimental periods were not significant ($P > 0.05$).

It was noticed that the decrease in ruminal ammonia content during the successive experimental periods was associated with decrease in ruminal pH. It seems that the total output of saliva was reduced to some extent by feeding the increased proportions of heated concentrate, and as a consequence the buffering capacity of the rumen contents was reduced resulting in a lower pH. It was reported by McDonald (1952), Chalmers and Synge (1954) ; and Annison *et al.* (1954), that ammonia concentration in the rumen varies with the kind of protein in the ration, but the addition of starch rich feeds such as maize meal or flaked maize to high protein rations depressed considerably the formation of ammonia. In this connection, Phillipson *et al.* (1959), suggested that the decreased ruminal ammonia level obtained with such rations might be partly due to an increase in the numbers of bacteria that can utilize ammonia nitrogen. It is worth noting that the total ration used in the present investigaion was not high in protein content ; it had approximately 15 percent crude protein. This may partly explain the comparatively moderate decrease in ammonia concentration by increasing the percentage of the heated concentrate mixture in the daily ration.

It was noticed in the present investigation, that ammonia concentration rose after feeding and reached the maximum levels after two hours then declined (Fig. 2). This was noted in all the experimental periods except the 3rd and the 5th periods where the highest values were found in the first samples taken just after feeding time. The lowest mean values for ammonia concentration were attained in all the rumen fluid samples taken 4 hours after feeding. The differences in ammonia mean values were found to be statistically significant ($P < 0.01$) between the hours of sampling after feeding time (Table 5).

These observations are generally in accordance with the findings of Butz *et. al.* (1958) and Van Adrichem (1962). The former workers found that ammonia concentration rose after feeding and then declined slowly over several hours. Van Adrichem (1962), reported that the easily fermentable carbohydrates decreased the ammonia content in the rumen and stimulated the synthesis of bacterial protein and that the maximum NH_3 concentration was attained two hours after feeding. A range from 4 to 41 mg.% of ammonia nitrogen was reported by Briggs *et al.* (1957), in rumen fluid of sheep fed a ration of 50% Iucerne and 50% cracked maize. In the prsent investigation a narrower range was found (from 11.2 to 27.9 mg. NH_3 per 100 ml. of rumen fluid) on approximately 58% hay and 42% heated concentrate, in the last period.

Volatile Fatty Acids

The total volatile fatty acid concentration and the molar percentages of the individual acids in the consecutively drawn samples of rumen fluid, representing the experimental periods, are given in Table 4. In addition, the variations in the mean concentrations of the acetice, propionic, butyric and total VF As are also shown in figures 3, 4, 5 & 6, respectively.

Acetic Acid

Acetic acid was present in greatest amount in the rumen fluid and the proportion of propionic acid exceeded that of butyric. On the starting ration of hay and unheated concentrate, acetic acid on average accounted for 71.3, 70.4 and 71.1 per cent of the total volatile fatty acids on molecular basis, in the rumen fluid samples taken 0, 2 and 4 hours after feeding, respectively. The corresponding mean values on the final ration of hay and heated concentrate, in the last period, were 68.3, 69.5 and 70.7%. It was noticed that, by increasing the percentage of the heated concentrate in the daily ration during the successive periods, the acetic acid concentration decreased with some fluctuations (Table 4). The lowest mean values were obtained in the 4th and 5th periods. On the other hand, the rumen fluid samples taken 4 hours after feeding contained the highest proportions of acetic acid in all the experimental periods except the first period, where the last sample contained a slightly lower concentration of acetic acid than the first sample taken just after feeding time, in the same period (Fig. 3). It was also noted, that the mean values for acetic acid proportion in the second period were nearly the same (i.e. 70.2, 70.2 and 70.3%) at different hours of sampling. This was due to the fact that the higher value for an individual sample on a day was compensated by a relatively lower value in the corresponding sample on the other day.

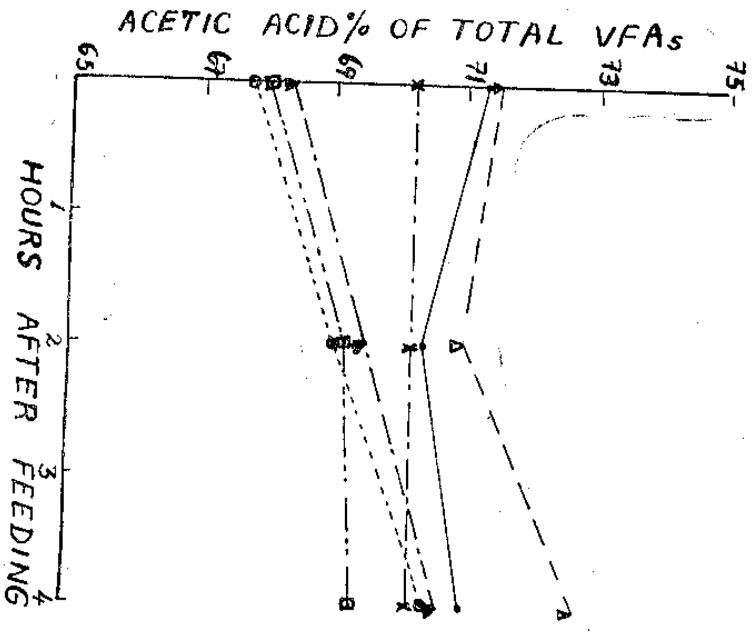

FIG.3 ACETIC ACID% IN RUMEN FLUID

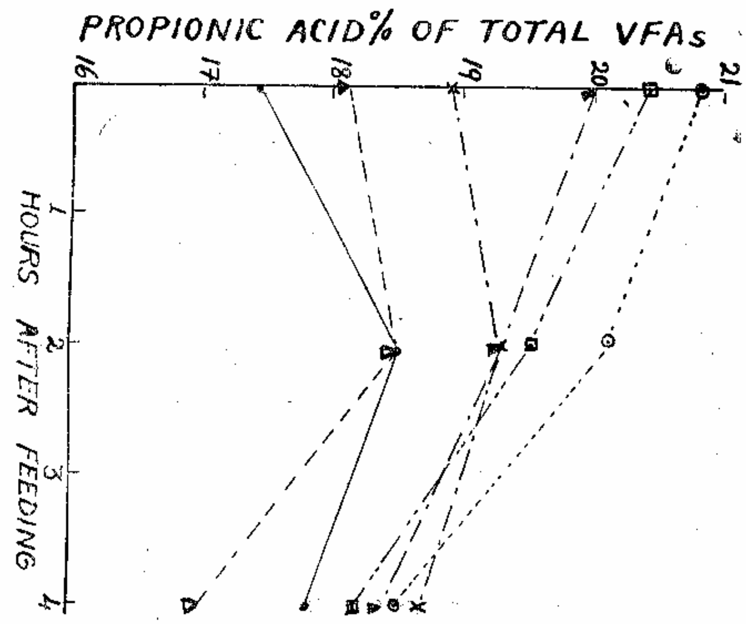

FIG.4 PROPIONIC ACID% IN RUMEN FLUID

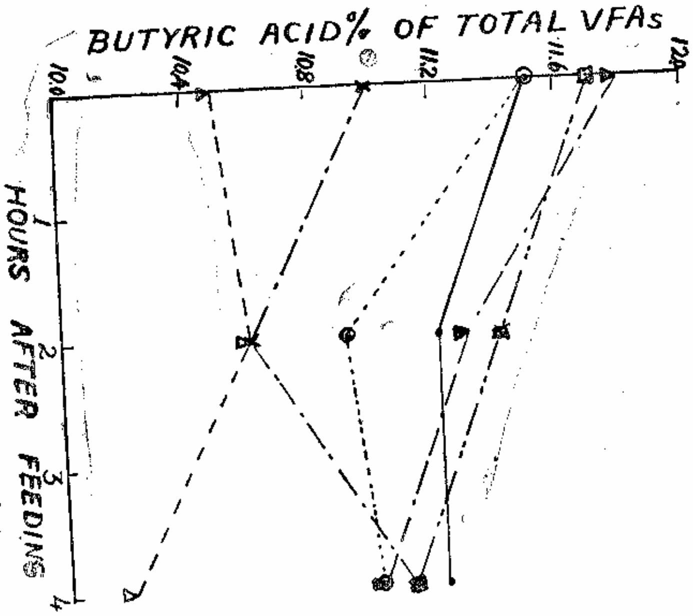

FIG.5 . BUTYRIC ACID% IN RUMEN FLUID

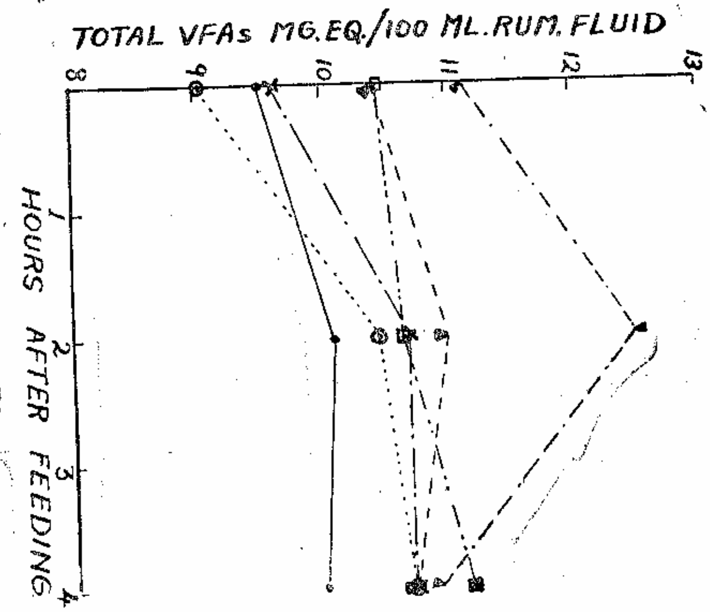

FIG.6 TOTAL VFAs IN RUMEN FLUID.

TABLE 4.—MOLAR PROPORTIONS OF VOLATILE FATTY ACIDS IN CONSECUTIVELY DRAWN SAMPLES OF COW'S RUMEN FLUID.

Period	Date of sampling	Daily ration (Kg)			Acetic Acid %				Propionic Acid %				Acetate : Propionate (: 1)			
		Hay	Conc. (unheated)	Conc. (heated)	Hours after feeding			period mean	Hours after feeding			period mean	Hours after feeding			period mean
					0	2	4		0	2	4		0	2	4	
1	19/4/66	7	5	0	70.6	71.1	71.5		18.1	18.1	17.9		3.90	3.93	3.99	
	20/4/66	7	5	0	71.9	69.7	70.6		16.6	18.8	17.7		4.33	3.71	3.99	
					71.3	70.4	71.1	70.9	17.4	18.5	17.8	17.9	4.10	3.81	3.99	3.97
2	29/4/66	7	4	1	71.2	70.0	69.6		18.5	19.9	19.3		3.85	3.52	3.61	
	30/4/66	7	4	1	69.1	70.4	70.9		19.2	18.6	18.1		3.60	3.78	3.92	
					70.2	70.2	70.3	70.2	18.9	19.3	18.7	19.0	3.71	3.64	3.76	3.70
3	9/5/66	7	3	2	70.4	70.6	73.1		18.9	18.6	16.6		3.72	3.80	4.40	
	10/5/66	7	3	2	72.5	71.3	72.4		17.3	18.3	17.4		4.19	3.90	4.16	
					71.5	71.0	72.8	71.8	18.1	18.5	17.0	17.9	3.95	3.84	4.28	4.02
4	19/5/66	7	2	3	67.7	69.9	70.9		20.8	19.7	18.2		3.25	3.55	3.90	
	20/5/66	7	2	3	67.7	68.1	70.0		20.8	20.4	18.8		3.25	3.34	3.72	
					67.7	69.0	70.5	69.1	20.8	20.1	18.5	19.8	3.25	3.43	3.81	3.50
5	29/5/66	7	1	4	67.7	69.2	69.9		20.2	19.3	18.6		3.35	3.59	3.76	
	30/5/66	7	1	4	68.0	69.2	68.8		20.6	19.6	17.7		3.30	3.53	3.89	
					67.9	69.2	69.4	68.8	20.4	19.5	18.2	19.4	3.33	3.55	3.81	3.56
6	8/6/66	7	0	5	68.1	69.8	71.5		20.2	19.1	17.4		3.37	3.65	4.11	
	9/6/66	7	0	5	68.5	69.1	69.8		19.8	19.4	19.4		3.46	3.56	3.60	
					68.3	69.5	70.7	69.5	20.0	19.3	18.4	19.2	3.42	3.60	3.84	3.62
(Time of sampling) mean					69.5	69.9	70.8		19.3	19.2	18.1		3.63	3.65	3.92	

TABLE 4. *(contd.)*—MOLAR PROPORTIONS OF VOLATILE FATTY ACIDS IN CONSECUTIVELY DRAWN SAMPLES OF COW'S RUMEN FLUID.

Period	Date of sampling	Daily ration (Kg)			Butyric Acid %				Total VFAs mg. eq/100 ml. rumen fluid.			
		Hay	Conc. (unheated)	Conc. (heated)	Hours after feeding			period mean	Hours after feeding			period mean
					0	2	4		0	2	4	
1	19/4/66	7	5	0	11.3	10.9	10.6		10.16	10.15	10.51	
	20/4/66	7	5	0	11.6	11.5	11.7		8.85	10.07	9.53	
					11.5	11.2	11.2	11.3	9.51	10.11	10.02	9.88
2	29/4/66	7	4	1	10.3	10.1	11.1		10.10	10.79	11.05	
	30/4/66	7	4	1	11.7	11.0	11.0		9.04	10.58	10.27	
					11.0	10.6	11.1	10.9	9.57	10.69	10.66	10.31
3	9/5/66	7	3	2	10.8	10.8	10.2		10.34	11.02	10.49	
	10/5/66	7	3	2	10.2	10.4	10.2		10.41	11.01	10.83	
					10.5	10.6	10.2	10.4	10.38	11.02	10.66	10.69
4	19/5/66	7	2	3	11.5	10.3	11.0		9.01	10.58	10.64	
	20/5/66	7	2	3	11.5	11.5	11.0		9.01	10.29	10.79	
					11.5	10.9	11.0	11.1	9.01	10.44	10.72	10.06
5	29/5/66	7	1	4	12.0	11.5	11.5		10.81	10.46	10.30	
	30/5/66	7	1	4	11.4	11.2	10.7		10.08	10.84	12.03	
					11.7	11.4	11.1	11.4	10.45	10.65	11.17	10.76
6	8/6/66	7	0	5	11.7	11.1	11.1		11.31	12.09	10.61	
	9/6/66	7	0	5	11.8	11.5	10.9		10.96	12.86	11.21	
					11.8	11.3	11.0	11.4	11.14	12.48	10.91	11.51
(Time of sampling) mean					11.3	11.0	10.9		10.01	10.90	10.69	

The analysis of variance (Table 5) showed that the differences in the mean molar proportions of acetic acid in the rumen fluid were significant between the periods ($P < 0.01$) and also between the sampling hours ($P < 0.05$).

Propionic Acid

The data in Table 4 show that in the first period, the mean values for propionic acid molar proportions were 17.4, 18.5 and 17.8% in the rumen fluid samples taken 0·2 and 4 hours after feeding time, respectively. The corresponding values, in the last period were 20.0, 19.3 and 18.4%. It was noticed that the molar proportions of propionic acid in the rumen fluid increased gradually, with some fluctuations, by increasing the percentage of the heated concentrate in the daily ration. The highest values were attained in the fourth period, where 60% of the concentrate was given after it had been heated. However, the proportion of propionic acid in the rumen fluid sample taken 4 hours after feeding in this period, was slightly below that of the corresponding sample in the second period. A little decrease was noticed in the molar proportion of propionic acid in the rumen fluid samples taken during the last two periods in comparison with those of the fourth period.

Concerning the concentration of propionic acid throughout the feeding cycle, it was found that the highest values were obtained in the rumen fluid samples taken 2 hours after feeding in the first three periods, and just after feeding during the last three periods (Fig. 4). The minimum values for propionic acid molar proportion were obtained in the rumen fluid samples taken 4 hours after feeding time. This was noted in all the experimental periods except the first period.

The difference in the mean molar proportions of propionic acid in the rumen fluid were found to be statistically significant ($P < 0.01$) between the periods and also between the sampling hours after feeding time ($P < 0.01$) Table 5).

Acetate to Propionate Ratio

Reference to Table 4 shows that, with the unheated concentrate in the first period, the mean ratios of acetic acid to that of propionic on molecular basis were 4.10 :1, 3.81 : 1 and 3.99 : 1 in the rumen fluid samples taken 0·2 and 4 hours, after feeding, respectively. The corresponding mean ratios on the completely heated concentrate, in the last period were 3.42 : 1, 3.60 : 1 and 3.84 : 1. By increasing the percentage of heated concentrate in the daily ration throughout the intermediate experimental periods, the molar proportion of acetic acid decreased while that of propionic increased.

As a consequence, the ratio of acetate to propionate decreased and became narrower gradually with some fluctuations. However, there was an abnormal increase in the ratio of acetate to propionate in the rumen fluid

TABLE 5.—ANALYSIS OF VARIANCE

Source of Variation	Degrees of freedom	pH			Ammonia			Acetic Acid			Propionic Acid		
		Sum of squares	Mean squares	F	Sum of squares	Mean squares	F	Sum of squares	Mean squares	F	Sum of squares	Mean squares	F
Periods	5	0.20	0.04	13.33†	27.15	5.43	1.22	19.53	3.91	8.00†	9.59	1.92	5.68†
Hours after feeding	2	0.01	0.005	1.67	367.42	183.71	41.29†	5.46	2.73	5.63*	5.15	2.58	7.63†
Experimental error	10	0.03	0.003		44.49	4.449		4.85	0.485		3.38	0.338	
Total	17	0.24			439.06			29.84			18.12		

Source of Variation	Degrees of freedom	Acetate : Propionate			Butyric Acid			Total VFAS		
		Sum of squares	Mean squares	F	Sum of squares	Mean squares	F	Sum of squares	Mean squares	F
Periods	5	0.71	0.14	6.67†	2.06	0.41	9.11†	5.20	1.04	5.12*
Hours after feeding	2	0.32	0.16	7.62†	0.55	0.28	6.22*	2.59	1.30	6.40*
Experimental error	10	0.21	0.021		0.45	0.045		2.03	0.203	
Total	17	1.24			3.06			9.82		

* Significant at level of 5%.
† Significant at level of 1%.

samples representing the third period. The narrowest acetate to propionate mean ratios were found in the last three periods in the rumen fluid samples taken 0 and 2 hours after feeding where the molar proportions of acetic acid reached the minimum values and the molar proportions of propionic acid reached the maximum.

The differences noted in the ratios of acetate to propionate in the rumen fluid were found to be statisticaly significant between the periods ($P < 0.01$) and also between the sampling hours after feeding time ($P < 0.01$) Table 5.

It was interesting to find that the values for acetic and propionic acid proportions varied inversely. This agrees with the observations of Balch and Rowland (1957) ; and McCallough (1966). The latter found a negative correlation of (— 0.867) between the molar percentage of acetic acid and that of propionic in the rumen fluid. On the other hand the increase and the decrease in the molar proportions of acetic and propionic acids, respectively, showed in Figs. 3 and 4 in different sampling hours after feeding are generally in accordance with the findings of Rhodes and Woods (1962), who noted that with most rations fed to lambs, acetate increased with time to reach the peak approximately 4 hours after feeding while propionate decreased. However, the present observations differ from those reported by Reid *et al.* (1957).

It can be concluded that increasing the percentage of heated concentrate in the daily ration of the cow decreased the molar proportion of acetic acid and increased the molar proportion of propionic acid, and made narrow the acetate to propionate ratio in the rumen fluid. These results are generally in agreement with the findings of Phillipson (1952), with lambs, Eusebis *et al* (1959) with Holstein heifers and Balch and Rowland (1957) with dairy cows, all using high proportions of flaked maize.

Butyric Acid

The data in Table 4 chow that, in the first period with hay and unheated concentrate, the mean molar proportions of butyric acid were 11.5, 11.2, and 11.2% in the rumen fluid samples taken 0,2 and 4 hours after feeding. The corresponding mean values on the final ration of hay and completely heated concentrate were11.8, 11.3, and 11.0%. It is evident that the differences between the values in the two periods are rather small. It was noticed that the concentraions of butyric acid in the rumen fluid samples decreased gradually by increasing the percentage of the heated concentrate in the ration till the third period, where the lowest values were attained. Then there was an increase in butyric acid concentration during the last three periods. The analysis of variance (Table 5) indicated that the differences noted during the successive experimental periods between the mean values of butyric acid molar proportions were significient ($P < 0.01$). However, the range of variation was rather small (10.4—11.4). It has been reported by Phillipson (1952), that low values of butyric acid were found in the rumen fluid of lambs fed a diet high in flaked maize. Eusebis *et al.* (1959), also noted the same observation with Holstein heifers but when the ration of flaked corn was supplemented with linseed oil meal, the molar proportion of butyric acid increased markedly. In the light of these findings, the present results can be explained that the presence of relatively high percentage of linseed oil meal (43%) in the concentrate mixture would have hindered the depressive effect of the heated maize meal on the molar proportion of butyric acid. On the other hand, Bath and Rook (1963), found that when hafl the hay was replaced by flaked maize the molar proportions of propionic, butyric and valeric acids increased and that of acetic acid decreased.

Concerning the cocentration of butyric acid throughout the feeding cycle, it was found that the rumen fluid samples taken just after feeding contained the highest concentrations of butyric acid in all the experimental periods except the 2nd and3rd periods where they contained nearly the same proportions of those taken 4 and 2hours after feeding, respectively. On the other hand, the lowest mean concentrations of butyric acid were obtained in the rumen fluid samples taken 4 hours after feeding in the 3rd, 5th and 6th periods, and two hours earlier in the 1st, 2nd and 4th periods. The differences in the mean butyric acid molar proportions between sampling hours after feeding time were found to be statistically significant, $P < 0.05$ (Table 5). The present results differ from those reported by Rhodes and Woods (1962), that butyric acid remained fairly constant after feeding time.

Total Volatile Fatty Acids

The lowest concentrations of total VFAs were attained i nthe first period with the unheated concentrate, with the exception of the first sample which contained more total VFAs than the corresponding sample in the fourth period (Table 4). By increasing the percentage of the heated concentrate in the daily ration, the concentration of the total VFAs in the rumen fluid samples increased gradually wih some fluctuations in the successive periods to reach the maximum in the last period, where the completely heated concentrate was fed. However, the rumen fluid sample taken 4 hours after feeding in this period contained slightly lower concentration of total VFAs than the corresponding sample in the fifth period. The mean values were 9.51, 10.11 and 10.02 mg. equiv.per 100ml. in he first period, and 11.14, 12.48 and 10.91 mg. equiv./100ml in the last period, in the rumen fluid samples taken 0, 2 and 4 hrs. after feeding, resplctively. The differences found in the mean concentrations of total VFAs in the rumen fluid due to the change of the heated to unheated concentrate ratios were statistically significant ($P < 0.05$) Table 5.

The rumen fluid samples taken just after feeding contained the lowest concentrations of total VFAs in all experimental periods except in the last period where the first samples contained slightly higher content of VFAs than the third sample taken 4 hours later in the same period. It was noticed that the concentration of total VFAs increased after feeding (Fig. 6) due to increased fermentation and subsequently a decrease took place as fermentation declined. The highest concentrations of total VFAs were attained in the rumen fluid samples taken 2 hours after feeding in the experimental periods 1, 2, 3 and 6 and in the samples taken 4 hours after feeding in the periods 4 and 5. The differences noted in the mean concentrations of total VFAs in the rumen fluid between the hours of sampling after feeding were found to be statistically significant ($P < 0.05$) Table 5.

The mean concentrations of total VFAs found in the rumen fluid in the last period with 7 kg. hay and 5 kg heated concentrate (*i. e.* 11.14, 12.48 and 10.91 mg. equiv/100 ml.) were higher than han the average value of 10.73 ± 0.32 mg. equiv/100 g. reported by Van Adrichem (1962), with cows fed 4 kg. of cooked maize meal in addition to hay and concentrates. It was interesting to notice that the concentration of the total VFAs in the rumen fluid fluctuated inversely with the pH, the lowest mean VFA values found in the first period coincided with thl highest pH mean values obtained in this period, and the opposite was noticed in the last period. (Tables 3 and 4). Similar observations were reported by Balch and Rawland (1957), with dairy cows on variety of rations and by Van Adrichem (1962), who found a negative correlation of −0.94 between the pH and the total VFAs formed in the rumen.

In can be concluded that increasing the percentage of heated concentrate in the daily ration, in the successive experimental periods resulted in a decrease in the pH, ammonia and the molar proportion of acetic acid in the rumen fluid. At the same time, there was an increase in the molar proportion of propionic acid and in the total VFAs. The response of the molar proportion of butyric acid was different, it decreased during the first three periods then increased during the rest periods.

Though the differences in the pH and individual VFAs between the experimental periods were statistically significant ($P < 0.01$) yet the ranges of variation were rather small. These might have been due mainly to the following facts :

1. The hay was given in sufficient quantity, (7 kg. daily) and in the long form. Ensor *et al.* (1959), reported that the effect of heated maize on the fat content of milk and on thl ruminal VFAs was reduced greatly when long hay was fed in addition.

2. The concentrates were fed in a moderate quantity (5 kg. daily) and contained moderate percentage of starch-rich ingredients (Maize and barley, 51.6%). Moreover, the concentrates were neither pelletted nor finely ground.

The significant differences found, in the present investigation, in the total and molar proportions of volatile fatty acids in the rumen fluid, between the hours of sampling after feeding time, are generally in agreement with the findings of Bath and Rook (1963) ; Martin and Wing (1966). It is interesting that the last two workers found a significant linear change in the molar proportions of the VFAs produced in the rumen after feeding time. However, Shaw (1961), reported that the variations in the molar proportions of the individual volatile fatty aids throughout a feeding cycle are in general small and he considers that a single sample of rumen fluid drawn at any time throughout a feeding cycle is usually adequate to characterize a diet. However, Bath and Rook (1963), did not agrle with Shaw in this respect as they observed a range from 66.9 to 73.0 and from 60.9 to 67.4 for the molar proportion of acetic acid in the rumen fluid on rations of hay and of hay and concentrates, respectively, given in two feeds daily at 12 hours intervals. For this reason, they prefer the sampling of the digesta to be carried out at regular intervals throughout a feeding cycle in order to avoid gross error. In the present work, the range of variation in acetic acid molar proportion, in the rumen fluid after feeding time, was much smaller than those reported by Bath and Rook (1963). Sampling the rumen fluid at regular intervals after feeding seems to be more important for ammonia than for VFA determinations, according to the present findings.

It is recommedend to carry out more research in the U.A.R. to investigate the effects of local feed stuffs and feeding practices on the ammonia and volatile fatty acids in the rumen fluid of sheep and dairy cattle due to their great importance upon animal performance.

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تأثير التغذية بنسب مختلفة من المخلوط المركز المعامل وغير المعامل بالحرارة على محتويات سائل الكرش فى البقرة من الأحماض الدهنية الطيارة والأمونيا ورقم الاس الايدروجينى *

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الملخص

استعملت بقرة فريزيان بفتحة كرش صناعية لدراسة تأثير التغذية بعليقة من الدريس غير المقطع مع نسب مختلفة من مخلوط مركز معامل وغير معامل بالحرارة على محتويات سائل الكرش من الأحماض الدهنية الطيارة والأمونيا ورقم الاس الأيدروجينى .

وقد وجد أنه بزيادة نسبة المخلوط المركز المعامل بالحرارة من صفر الى ١٠٠٪ فى العليقة اليومية قد زادت جملة الأحماض الدهنية الطيارة فى المتوسط من ٩ر٨٨ الى ٥١ر١١ مللجرام مكافىء/١٠٠ سم^{٣} من سائل الكرش على التوالى كما ارتفعت نسبة حمض البربيونيك من ٩ر١٧ الى ٢ر١٩٪ بينما انخفضت نسبة حمض الخليك من ٩ر٧٠ الى ٥ر٦٩٪ وضاقت نسبة الخليك : البربيونيك من ٩٧ر٣ : ١ الى ٦٢ر٣ : ١ وبالاضافة الى ذلك انخفض رقم الاس الأيدروجينى من ٧١ر٦ الى ٣٨ر٦ كما انخفضت الأمونيا من ٤ر٢١ الى ٧ر١٩ مللجرام/١٠٠ سم^{٣} .

وقد وجدت الاختلافات فى رقم الاس الأيدروجينى وفى نسب حمض الخليك والبروبيونيك والبيوتريك بين فترات التغذية على النسب المختلفة من المخلوط المركز المعامل وغير المعامل بالحرارة وجدت مؤكدة احصائيا (تحت احتمال ١٪) بينما وجدت الفروق فى الأمونيا غير مؤكدة احصائيا . ومن الناحية الأخرى وجدت الاختلافات فى الأمونيا وفى الأحماض الدهنية الطيارة فردية وجملة بين ساعات أخذ العينات بعد وقت التغذية وجدت مؤكدة احصائيا بينما كانت الفروق بينها بالنسبة لرقم الاس الأيدروجينى غير مؤكدة احصائيا .

(*) أجرى هذا البحث فى معهد « هورن » لأبحاث تغذية الحيوان فى هورن بهولنده .