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VOLATILE FATTY ACID METABOLISM IN SHEEP. 2. CORRELATION BETWEEN THE VOLATILE FATTY ACID PRODUCTION AND CONCENTRATION IN THE RUMEN DURING THE COURSE OF A FEEDING CYCLE

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ABSTRACT

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Four German Merino wethers were maintained on 1 600 g of lucerne hay fed twice daily. A dilute solution of a labelled volatile fatty acid (VFA) marker was infused over a period of 22 h and 6 discrete rumen fluid samples were withdrawn at 2 h intervals over the last 12 h of infusion (08h00–20h00).

Total VFA production varied over the period of the feeding cycle, rising 89% from a basal rate of 3,32 moles/12 h to a peak of 6,38 moles/12 h at 4,5 hours after feeding. The concomitant rise in VFA concentration (39%) was not as great as that of the production and the disparity was ascribed to the dilution of the VFA pool by the intake of fodder and saliva into the rumen during the period of maximum production. VFA production rate cannot, therefore, be predicted with any confidence during this period from the VFA levels present in the rumen.

Pooled rumen fluid samples were constituted from equal aliquots of the 6 discrete samples and analysed together with the above individual samples. No statistical difference ($P < 0,9$) was found between the average net total VFA production rate of 4,43 moles/12 h calculated from the pooled sample data and the arithmetic mean of 4,6 (range 3,32–6,28) moles/12 h derived from the discrete sample data.

Résumé

MÉTABOLISME DES ACIDES VOLATILES GRAS CHEZ LE MOUTON. 2. RAPPORT ENTRE LA PRODUCTION DES ACIDES VOLATILES GRAS ET LEUR TAUX DANS LE RUMEN AU COURS D'UN CYCLE D'ALIMENTATION

Une solution diluée d'un marqueur d'acides volatiles gras (VFA) a été infusée au cours d'une période de 22h à 4 moutons de race Merinos allemand maintenus sur 1 600 g de foin de luzerne dispensé deux fois par jour. Pendant les dernières 12h de l'infusion (08h00–20h00), 6 échantillons du liquide du rumen ont été prélevés toutes les deux heures.

La production totale des VFA a varié au cours du cycle d'alimentation en augmentant 89% d'un taux de base de 3,32 moles/12h à un maximum de 6,38 moles/12h de 4,5h après l'alimentation. L'élévation concomitante de la concentration des VFA (39%) était inférieure à celle de la production. On a attribué cette disparité à la dilution de l'ensemble des VFA par l'ingestion dans le rumen du foin et de la salive au moment de la production maximale, période pendant laquelle on ne peut pas prévoir exactement l'importance de la production des VFA d'après le taux des VFA dans le rumen.

Six parties égales des échantillons prélevés ont été rassemblées et soumises à l'analyse en même temps que les autres. Aucune différence statistique ($P < 0,9$) n'a pu être mise en évidence d'après l'importance de la production moyenne nette et totale des VFA (4,43 moles/12h) évaluée sur l'échantillon composé et la moyenne de 4,6 (3,32–6,28) moles/12h appréciée sur les échantillons individuels.

INTRODUCTION

The considerable problems associated with the measurement of a valid, average, daily VFA production rate in the rumen were extensively reviewed by Warner (1964). These problems arise, *inter alia*, from the continuously changing milieu of the rumen which is consequently unable to maintain a state of equilibrium for any length of time. Leng & Brett (1966), Leng, Corbett & Brett (1968) and Weston & Hogan (1968) approached these problems by introducing a continuous feeding regimen to encourage a near state of equilibrium in the course of which all necessary parameters were measured. Despite the errors inherent in the extrapolation of such short term data to daily average values, the VFA production rates so obtained were shown to be directly proportional to the magnitude of the ruminal VFA pool when the sheep were fed a high roughage diet having adequate nitrogen levels (6% crude protein).

On the other hand, various workers (Gray, Weller, Pilgrim & Jones, 1967, and Weller, Pilgrim & Gray, 1969) have since shown that valid, daily, average VFA production rates may be obtained in sheep fed normally, provided that adequate time is allowed for the infused marker to equilibrate with the VFA pool prior to the sampling period. The latter must also coincide with a complete cycle of the feeding regimen. This enables an average VFA specific activity, representative of the complete feeding cycle, to be used for the calculation of an average total VFA production rate, despite the considerable cyclic variations in the VFA levels found in such a feeding regimen (Van der Walt & Briel, 1976). The production rates so obtained are equivalent to those measured in sheep fed continuously and have the same direct correlation with average ruminal VFA levels.

It follows from all the above results that since daily, average VFA production rates, and hence the metabolizable energy available to the sheep on a high roughage diet, can be predicted from the mean, daily ruminal VFA level, the need for costly and time-consuming labelled VFA turnover studies in any ration evaluation programme can be eliminated. Furthermore, it would appear feasible to predict relative changes in VFA production rate over the course of a feeding cycle from the parallel cyclic variation in their levels previously noted in sheep fed twice daily (Van der Walt & Briel, 1976). However, the experiment described here indicates that the period of maximum fermentation in the rumen is associated with a change in the relation between VFA concentration and production and, consequently, changes in the VFA production rate will be underestimated when calculated from the corresponding change in VFA level.

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METHODS

Animals and feeding

The 4 German Merino wethers (P53, P57, P60 & P64) used for this experiment were maintained on the same daily ration (viz. 1 600 g of lucerne hay fed in 2 equal portions of 800 g each at 08h00 and 20h00, supplemented with 15 g of a trace element and salt mixture* and 1 g Vitamin A† weekly) described previously (Van der Walt & Briel, 1976). They were allowed free access to water at all times.

Experimental procedure

The technique, previously described by Van der Walt & Briel (1976), was modified in the following way. A series of 6 manually-collected rumen fluid samples (30 ml each) replaced the pooled sample previously collected automatically. The sheep were sampled initially at 08h30 (0,5 h after feeding) and every 2 h thereafter till 18h30 (10,5 h after feeding). Great care was taken to sample only from the ventral, anterior region of the rumen and at least 3 separate aliquots of rumen contents were strained through a double layer of cheese cloth to make up each 30 ml sample.

A manually-pooled sample for each sheep was prepared by mixing together 10 ml aliquots from the 6 individual 30 ml samples collected over the 12 h period.

Analytical methods

The methods described in the previous study (Van der Walt & Briel, 1976) were used without modification. Total VFA concentrations and specific activities, determined from the analysis of the individual samples, were used to calculate total VFA production rates at different periods of the feeding cycle. An analysis of the manually-pooled sample yielded values for total concentrations, relative molar compositions, average daily total VFA production, percentage interconversion between individual acids and, consequently, net individual production rates.

RESULTS

The rumen fluid samples that were collected at 2 h intervals throughout the experiment yielded on analysis the total VFA levels and projected daily net VFA production rates listed in Table 1.

The variation in both the VFA levels and the projected VFA production rates is clearly shown in Fig. 1 where the average values given in Table 1 have been graphically represented. Although both parameters reached maximum levels at 4,5 h after feeding, the percentage increase in the VFA production rate over basal values (89%) was greater than the corresponding increase in the ruminal VFA concentration (39%).

A regression analysis of the individual values listed in Table 1 gave an equation:

$y=0{,}040x + 1{,}22$ with a correlation coefficient of $r^2=0{,}74$ where y=VFA production rate (moles/12 h)
and x=VFA concentration (milli-equivalents/l)

after exclusion of the 2,5 and 4,5 h data together with one 6,5 h result. This relationship is graphically represented in Fig. 2 and, with reference to Table 1, it can be seen that the 4 sheep exhibited a wide range of fermentation responses to the lucerne hay diet. The consistently low values obtained from sheep P53 were ascribed to a slow feeding pattern that resulted in an inability to finish the 800 g ration within the available 12 h. For this reason, none of the overall mean estimates included sheep P53 even though the total VFA production rates correlated well with the corresponding total VFA levels obtained from the amount eaten (Fig. 2). Despite this low fermentation rate, sheep P53 still showed an anomalous disparity between the VFA concentration and production at 2,5 h and 4,5 h, described in Fig. 1 for the whole group. The greatest disparity was found in sheep P64 which routinely finished its ration within an hour as, in this case, the 6,5 h production and concentration values still showed an anomalous correlation. Only sheep P57 attained a maximum rate of fermentation at 2,5 h after feeding, but this returned rapidly to basal levels and even the 4,5 h estimates for production and concentration lie on the regression line shown in Fig. 2.

TABLE 1 The total VFA concentrations in the rumen are shown at 2 h intervals throughout the 12 h feeding cycle together with the associated total VFA net production rates projected from the specific activity data of 3 separate experiments where Na–1–^{14}C acetate, Na–2–^{14}C propionate and Na–1–^{14}C butyrate, respectively, were infused

Sheep	Sampling time (hours after feeding)	Total VFA concentration (meq/l)	Projected net VFA production rate (moles/12 h)
P53.....	0,5	73,1	1,71
	2,5	80,3	2,89
	4,5	88,5	2,92
	6,5	77,7	1,69
	8,5	70,2	1,57
	10,5	66,0	1,57
	Average	76,0	2,06
P57.....	0,5	107,3	3,28
	2,5	150,2	7,02
	4,5	151,8	4,50
	6,5	134,1	3,10
	8,5	119,3	3,54
	10,5	84,0	2,05
	Average	124,5	3,92
P60.....	0,5	95,5	3,25
	2,5	138,1	4,91
	4,5	137,7	5,55
	6,5	124,3	3,01
	8,5	100,3	2,70
	10,5	108,7	2,27
	Average	117,4	3,61
P64.....	0,5	120,6	3,43
	2,5	119,7	6,61
	4,5	160,8	8,79
	6,5	151,4	8,05
	8,5	131,9	4,60
	10,5	135,7	5,43
	Average	136,7	6,15
Mean of P57 +P60+ P64	0,5	107,8	3,32
	2,5	136,0	6,18
	4,5	150,1	6,28
	6,5	136,6	4,72
	8,5	117,2	3,61
	10,5	109,5	3,25
	Average	126,2	4,56

* Kimtrafos 25, Kynoch Feeds, Kimberley, RSA.
† Vitamin A, Peter Hand Panvet, Johannesburg, RSA.

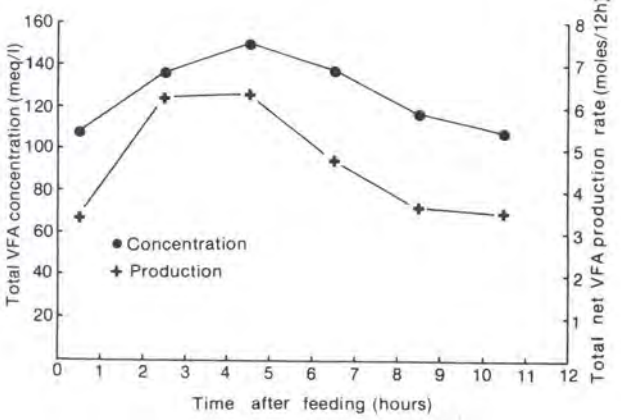

FIG. 1 The variation in the ruminal concentration of the total VFA pool over the 12 h feeding cycle compared to the projected net total VFA production rates obtained from an analysis of the individual samples. Each value represents the average of 3 experiments

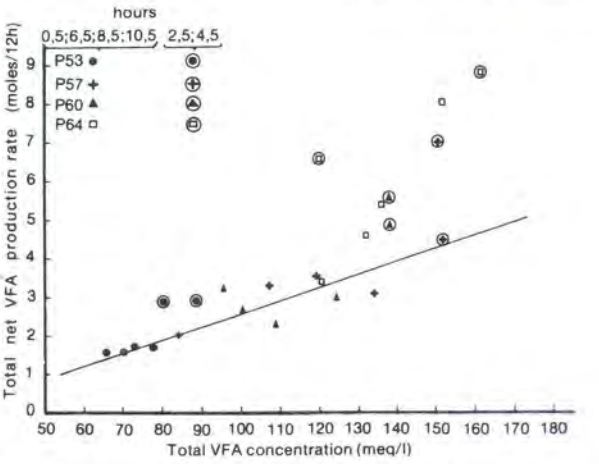

FIG. 2 Derivation of the correct straight line relation between the ruminal VFA concentrations and the net total production rates projected from the analysis of the individual samples. Each value represents the average of 3 experiments, where Na–1–^{14}C acetate, Na–2–^{14}C propionate and Na–1–^{14}C butyrate, respectively, were infused

TABLE 2 The net total and gross individual VFA production rates are listed together with the respective total VFA concentrations obtained from an analysis of the manually-pooled rumen fluid samples

Sheep	Acid infused*	Total VFA Concentration meq/l	Net total VFA Production moles/12 h	Gross production of		
				Acetic acid moles/12 h	Propionic acid moles/12 h	Butyric acid moles/12 h
P53.....	A	88,6	2,42	1,80	—	—
	P	79,1	2,01	—	0,52	—
	B	59,2	1,73	—	—	0,39
	Average	75,6±15,0	2,05±0,35	1,80	0,52	0,39
P57.....	A	139,7	3,52	3,02	—	—
	P	142,4	3,94	—	0,96	—
	B	110,1	3,31	—	—	0,98
	Average	130±18,0	3,59±0,32	3,02	0,96	0,98
P60.....	A	137,3	3,43	2,90	—	—
	P	127,5	3,44	—	1,02	—
	B	86,1	2,75	—	—	0,78
	Average	117,0±27,2	3,21±0,32	2,90	1,02	0,78
P64.....	A	218,1	8,13	6,66	—	—
	P	161,5	5,75	—	1,30	—
	B	141,3	5,63	—	—	2,04
	Average	173,6±32,5	6,50±1,15	6,66	1,30	2,04
Overall mean†....		140,4±36,0	4,43±1,73	4,19±2,15	1,09±0,18	1,26±0,68

* A = Na–1–^{14}C acetate, P = Na–2–^{14}C propionate and B = Na–1–^{14}C butyrate
† Sheep P53 not included in overall mean

An analysis of the pooled rumen fluid aliquots gave VFA turnover estimates similar to those previously reported from automatically-collected rumen fluid (Van der Walt & Briel, 1976).

Table 2 shows clearly that the net total VFA production rate may be derived from the infusion of any single labelled acid and that the overall mean total net VFA production rate of 4,43±1,73 moles/12 h was statistically indistinguishable from the value of 4,52±1,01 moles/12 h previously obtained (Van der Walt & Briel, 1976).

Also shown in Table 2 are the gross production rates for the individual VFA which were corrected for the interconversion taking place in the rumen (Leng & Leonard, 1965), and listed in Table 3 as net average VFA production rates.

TABLE 3 Comparison between the average relative concentration and production rate of each VFA in the rumen

	Concentration		Production rate	
	meq/l	%	moles/12 h	%
Acetic.....	80,7	58	2,56	58
Propionic....	33,0	23	1,00	23
Butyric.....	17,2	12	0,58	13
Other†.....	9,5	7	0,29	6
Total.....	140,4	100	*4,43	100

* From Table 2
† For composition of "other" VFA, see Table 4

TABLE 4 Composition of VFA in the manually-pooled rumen fluid samples

†Sheep No.	Total VFA concentration meq/l	Relative molar** percentage of total						Individual VFA concentration (meq/l)					
		A	P	IB	B	IV+ 2MB	V	A	P	IB	B	IV+ 2MB	V
P53	75,7	57,5	18,0	3,5	12,0	6,5	2,5	43,6	14,6	2,7	9,1	4,9	1,9
P57	130,7	57,0	22,0	2,5	14,0	2,5	2,0	74,5	28,7	3,2	18,3	3,2	2,6
P60	117,0	58,0	23,0	2,5	12,0	2,5	2,0	67,9	26,9	2,9	14,1	2,9	2,3
P64	173,4	57,5	25,0	2,5	11,0	2,0	2,0	99,7	43,3	4,3	19,1	3,5	3,5
*Average	140,4	57,5	23,5	2,5	12,2	2,3	2,0	80,7	33,0	3,5	17,2	3,2	2,8

* P53 not included in the average

†Average of 3 experiments

** A=acetic acid
P=propionic acid
IB=iso-butyric acid
B=butyric acid
IV+2MB=iso-valeric plus 2-methyl butyric acid
V=valeric acid

The percentage molar composition of the VFA pool in the rumen given in Table 3 was derived from the gas liquid chromatographic data given in Table 4. The close correspondence between the VFA pool composition and the contribution of each acid to the total, daily, net VFA production rate can be clearly seen. Furthermore, the relation between the total net VFA production rates derived from analysis of the pooled samples and the corresponding VFA levels in the rumen is linear (see Fig. 3) and is defined by the equation:

$$y = 0,040x - 1,11$$

with $r^2 = 0,88$

where y = VFA production rate in moles/12 h and x = VFA concentration in milli-equivalents/1.

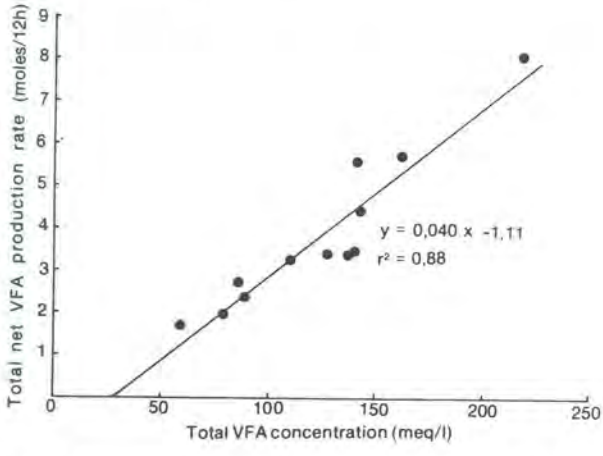

FIG. 3 The straight line correlation between the levels of the total VFA pool in the rumen and the net production rates obtained from an analysis of the pooled samples

Discussion

Despite the considerable problems associated with the measurement of VFA production in the rumen, an analysis of the pooled rumen fluid samples gave results similar to those previously reported from automatically-collected rumen fluid (Van der Walt & Briel, 1976). The net total VFA production rate shown in Table 1 (4,43±1,73 moles/12 h) corresponded closely to the mean of 4,52+1,05 moles/12 h previously found. Likewise, the differences between the present and previously reported individual gross production rates of the individual VFA were insignificant ($P<0,9$, Student's t test). The average net production rates of acetic, propionic and butyric acids, calculated from the gross rates and the percentage interconversion figures, according to Leng & Leonard (1965), again corresponded closely to the relative composition of the VFA pool in the rumen.

Subsequent work (unpublished observation) revealed the presence of propionic acid in the butyric acid peak. The net propionic acid production rate was therefore underestimated (0,86 moles/12 h) and, when corrected (1,00 moles/12 h) for this contamination, corresponded exactly (23%) to its molar percentage concentration.

The relationship between the average total net VFA production rate and the corresponding average total VFA concentration of the manually-pooled samples is represented by the equation:

$y = 0,040x - 1,11$ with $r^2 = 0,88$.

This confirmed the relationship previously found (Van der Walt & Briel, 1976) when analysis of the data collected from the automatically-pooled samples yielded an equation

$y = 0,034x + 0,16$ with $r^2 = 0,83$.

Furthermore, a specific production rate of 0,835 moles VFA per 100 g of digestible organic matter (DOM) was derived from the data in similar fashion to the previously calculated value of 0,85 moles/100 g DOM.

The above comparisons show clearly that the process of manually pooling equal aliquots of the 6 discretely-collected rumen fluid samples yielded results statistically indistinguishable ($P<0,9$) from those previously reported for continuously collected samples (Van der Walt & Briel, 1976). Therefore, the considerable changes in production rate found over the 12 h period (range 3,32–6,28 moles/12 h) were felt to reflect the reality of the VFA production cycle.

The VFA concentrations associated with the 2,5 and 4,5 h samples were therefore anomalously low and were ascribed to the increase in rumen volume caused by the ingestion of 800 g lucerne hay together with the secretion of a considerable volume of saliva.

The observation of Weston & Hogan (1968) that the VFA pool gave a better correlation with the VFA production rate than did the VFA concentration supports this assumption. A further contributing factor could be the increased portal blood flow associated with increased VFA production in the rumen reported by Bensadoun & Reid (1962). This would lead to a greater absorption of VFA from the rumen and so tend to limit the increase in the ruminal VFA levels. It is apparent, therefore, that VFA production rate patterns cannot be predicted with any accuracy from the associated changes in VFA concentration patterns over the period of a feeding cycle. Similarly, total net VFA production rates cannot be safely extrapolated from values obtained during short term infusions encompassing only a portion of the feeding cycle.

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