

The Two Hundred and Ninety-first Scientific Meeting of the Nutrition Society (One Hundred and Seventeenth of the Scottish Group) was held in the Hugh Nisbet Building, Heriot-Watt University, Riccarton, Currie, Edinburgh, on Thursday and Friday, 25 and 26 March, 1976, at 10.15 hours, when the following papers were read:

Acetyl groups in dietary polysaccharides. By J. S. D. BACON, A. H. GORDON and A. J. HAY, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The fact that the digestibility of straw is improved by treatment with alkali directs attention to the presence of a number of alkali-labile ester linkages in the plant cell wall. A familiar example is the methylation of the acidic groups in pectins. Less well known are various esters of the neutral sugar residues; in monocotyledons, cell-wall polysaccharides are esterified with phenolic acids, such as ferulic acid (Hartley, 1972), which may indicate the nature of lignin-polysaccharide bonding, and the xylose residues in hardwood hemicellulose are acetylated.

We have recently drawn attention to the presence of acetyl groups in cell walls of a variety of plants and have suggested that these groups may depress the digestion of the hemicellulose fraction in the rumen (Bacon, Gordon, Morris & Farmer, 1975).

Using a gas-liquid chromatograph (GLC) method for determination of acetyl groups (Bethge & Lindstrom, 1973), and simultaneous measurement of cell-wall sugars by GLC of their alditol acetates (Sloneker, 1971), we have examined the quantitative relationship between acetyl groups and the individual sugars in various leaves and roots. Waite & Gorrod (1959) gave analyses of holocellulose fractions from maturing grass samples which indicated a fairly constant molar ratio, xylose:acetyl, of about 2.5; this we confirm with whole dried grass. However, ethanol-insoluble residues from expanding leaves of beech (*Fagus sylvatica*) showed no such constancy: xylose 2.4, 4.3 and 7.4%, acetyl 1.1, 2.3 and 2.1%.

The explanation is probably that acetyl groups are present not only in the hemicellulose but also in the pectin. Following Ehrlich & Sommerfeld (1926) the pectins from sugar-beet root and many fruits have been shown to have high acetyl contents (perhaps up to 100 mg/g); apart from the adverse effect on gelling properties, the significance of these observations seems to have been ignored.

When ethanol-insoluble residues from radish root tissue were extracted with the neutral-detergent reagent of Van Soest & Wine (1967) a substantial part of the arabinose, galactose and acetyl content was removed, but none of the xylose. This latter sugar then had a molar ratio, xylose:acetyl, of 1.8 in the residues from both young and old roots.

Thus it will be important in considering the possible effects of acetyl groups upon the digestion of cell-wall polysaccharides first to establish their distribution between the pectic and hemicellulose fractions.

We thank Miss Morag Adam, Mrs Glenda Edwards and Mrs Elaine McIntosh for technical assistance.

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Digestion of acetyl groups and cell-wall polysaccharides of grasses in the rumen. By E. JANE MORRIS and J. S. D. BACON, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Samples (4 g) of dried grass, ground in a hammer mill without screen to a size representative of rumen digesta, were placed in nylon bags (200×90 mm, 60–70 µm pore size) in the rumen of fistulated sheep fed on dried grass. Bags were removed at intervals, and the dry matter (DM) digestion measured. The material undigested at 18 h (when digestion was advanced but not complete) was analysed for acetyl content, and for cell-wall sugars determined as their alditol acetates, as described by Bacon, Gordon & Hay (1976). The digestibility of each component was as follows (values for two sheep): DM 0.75, 0.74; arabinose 0.80, 0.82; xylose 0.50, 0.52; galactose 0.83, 0.91; glucose 0.59, 0.61; acetyl 0.44, 0.45.

Samples (0.5 g) of cell walls isolated from leaves of *Lolium perenne* were placed in nylon bags (200×90 mm, 5 µm pore size, permitting 7% of the DM to escape) in the rumen of a grass-fed sheep for 3, 6, 12 and 24 h (Table 1).

Table 1. *Digestion of grass cell walls in nylon bags in the rumen of sheep (mg/g lost from bag)*

Time in rumen (h)	Dry matter	Arabinose	Xylose	Galactose	Glucose	Acetyl
3	100	160	100	100	120	90
6	420	570	340	520	440	340
12	730	880	670	840	790	610
24	870	940	840	930	930	790
Composition of grass cell walls (mg/g)		47	177	17	430	14

In both experiments arabinose and galactose were the most digestible sugars, presumably because both are constituents of pectin. Glucose (a measure of

cellulose) is more digestible than xylose (a measure of hemicellulose), which confirms the findings of Gaillard (1962), Waite, Johnston & Armstrong (1964), Dekker, Richards & Playne (1972) and others.

Although it had seemed possible that acetyl groups would be readily removed by rumen enzymes, the digestibility of acetyl was in fact lower than that of any of the measured sugars. The undigested residue was more acetylated than the starting material. By analogy with hardwoods, it seems likely that acetyl groups are located on xylose residues in the hemicellulose. If this is so, the grass cell walls contain one acetyl group in every four xylose residues, and the 24 h undigested residue one in three. These findings are compatible with the hypothesis that acetyl groups impede digestion of hemicelluloses (Bacon, Gordon, Morris & Farmer, 1975).

These results with nylon bags have been confirmed by a separate experiment in which the food and faeces from standard digestion trials on a hay and a dried grass were analysed similarly.

We thank Dr E. R. Ørskov for his help and the Agricultural Research Council for the award of a studentship to E.J.M.

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The effect of the condensed tannins of sainfoin (*Onobrychis viciaefolia*) on the release of soluble leaf protein into the food bolus of cattle. By J. L. MANGAN, R. L. VETTER*, D. J. JORDAN and P. C. WRIGHT, *Biochemistry Department, Institute of Animal Physiology, Babraham, Cambridge CB2 4AT*

When herbage is consumed by cattle a high proportion of the soluble leaf constituents is released during mastication (Reid, Lyttleton & Mangan, 1962). The release of soluble protein is of interest in the nutrition of cattle because of rapid fermentation and ultimate production of excessive amounts of ammonia; also because a leaf protein (F1) or ribulosediphosphate carboxylase (EC 4.1.1.39) has been implicated as the active foaming agent in pasture 'bloat' in cattle (McArthur & Miltimore, 1966).

Using cattle with large rumen fistulas (125 mm) closed with flexible PVC cannulas (Harrison, 1961; F. A. Harrison & J. L. Mangan, unpublished results), food boluses were collected at the cardia by hand while the animals consumed several species of fodder plants. Released nutrients were washed out with potassium-free synthetic saliva and the bolus residue extracted by maceration into ice-cold synthetic saliva, using an Ultra Turrax T45 homogenizer. K release was

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Table 1. Release of plant cell constituents into the food bolus of cattle

Food (bolus weight (g) in parentheses)	Treatment	Potassium		Chlorophyll		Ribulosediphosphate carboxylase (EC 4.1.1.39)		Total soluble nitrogen		Soluble protein-N	
		mmol	(%)*	mg	(%)*	$\mu\text{C}^{14}\text{CO}_2$ fixed	(%)*	mg	(%)*	mg	(%)*
Lucerne (177)	BE	8.7	(56.6)	25.0	(14.7)	55.4	(46.2)	159.5	(29.4)	99.7	(24.3)
	BR	6.7		145.0		64.6		381.9		311.4	
Ryegrass (157)	BE	9.4	(58.4)	26.7	(18.5)	56.7	(41.4)	126.6	(31.5)	85.6	(27.9)
	BR	6.7		117.4		80.2		275.0		222.0	
Red clover (183)	BE	5.1	(49.5)	9.6	(11.8)	2.1	(16.4)	77.7	(24.7)	50.3	(20.4)
	BR	5.2		71.8		10.7		237.5		196.7	
Sainfoin (223)	BE	7.2	(63.7)	3.0	(3.5)	0.3	(16.7)	38.7	(39.5)	0	(0)
	BR	4.1		83.3		1.5		59.4		30.9	

BE, bolus extract obtained by washing bolus with synthetic saliva; BR, washed bolus residue extracted by maceration.

*Percentage of constituents released into BE.

considered to give a simple measure of the release of small-molecular-weight constituents, and as shown in Table 1, all species showed a high level of plant-cell rupture. Lucerne, ryegrass and red clover released large amounts of soluble protein, ribulosediphosphate carboxylase, total soluble nitrogen and chlorophyll. Sainfoin, however, released virtually no protein material and little chlorophyll. Sainfoin contains condensed tannins (Bate-Smith, 1954) which have been shown in vitro (Jones & Mangan, 1976) to form complexes with F1 protein, reversible with polyethylene glycol (molecular weight 4000). Table 2 shows that bolus material from a sainfoin-fed cow contains this type of reversible complex.

Table 2. *Release by polyethylene glycol (PEG) of chlorophyll and nitrogen from the food bolus of cattle fed on sainfoin*

Constituent*	PEG concentration (mg/ml)			
	0	1	10	50
Chlorophyll	2.7	6.3	7.0	4.0
Total soluble N	18.6	41.0	43.2	32.8
Soluble protein-N	0.9	15.6	17.7	12.5

*As mg in total extract; 50 g bolus in 100 ml medium.

We are grateful to Dr F. A. Harrison for preparing the cattle.

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The energy value for sheep of three successive harvests of lucerne when artificially dried and fed in the chopped and pelleted forms. By J. S. SMITH, F. W. WAINMAN and P. J. S. DEWEY, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Three successive harvests of lucerne were artificially dried and fed to sheep in the chopped and pelleted forms, *ad lib.* and at about maintenance level. Measurements of energy balance were made during the final 4 d of each 4-week feeding period using closed-circuit respiration chambers, and fasting metabolism was measured for each sheep at the beginning and end of the experiment. The metabolizable energy (ME) value (Q) of the three cuts is shown in Table 1. Pelleting reduced Q for cut 1 only. The efficiency of utilization of ME below maintenance (k_m) tended, as expected, to decline with decreasing Q and was not markedly affected by pelleting. However, values for k_m and for k_f , the efficiency of utilization of ME above maintenance, were both much lower than predicted from previously observed relationships between Q, k_m and k_f for grasses and mixed diets (Blaxter, 1973), although pelleting increased k_f values for cuts 1 and 3 by about 40%.

The second cut, which had been harvested at a later stage of maturity than the other two, was too poor in quality to promote positive energy retention when fed *ad lib.* in the chopped form. Table 1 also indicates the extent to which the improvement in energy retention at *ad lib.* intake due to pelleting could be attributed to increased intake and to increased net energy. These results were discussed in relation to other published measurements of the energy values of hay and dried grass.

Table 1. *Energy balance measurements for sheep given dried lucerne from three successive harvests*

	Harvest					
	1		2		3	
	Chopped	Pelleted	Chopped	Pelleted	Chopped	Pelleted
Crude fibre (g/kg)	196	187	268	270	222	203
ME* (MJ/kg DM)	11.08	10.59	8.60	8.66	10.50	10.44
k	0.679	0.695	0.605	0.600	0.681	0.668
k_f	0.339	0.475	—	0.282	0.308	0.441
Energy retention, <i>ad lib.</i> (kJ/kg W ^{0.75} per d)	88.5	156.8	—	48.3	83.5	157.4
Increase due to pelleting (kJ/kg W ^{0.75} per d)						
Total	68.3		—		73.9	
Due to intake	19.2		—		25.3	
Due to net energy	49.1		—		48.6	

ME, metabolizable energy; DM, dry matter; k_m , efficiency of utilization of ME below maintenance; k_f , efficiency of utilization of ME above maintenance; W, body-weight.

*Measured at about maintenance.

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The intake, digestibility and retention time of roughage diets by red deer (*Cervus elaphus*) and sheep. By R. N. B. KAY and E. D. GOODALL, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Red deer (*Cervus elaphus*) have been found to digest experimental diets a little less fully than sheep, perhaps due to more rapid movement of the food through the digestive tract (Maloiy & Kay, 1971). To obtain further information two chopped roughages, grass hay and dried grass, were given to two mature hinds and two mature ewes, each weighing about 58 kg. Four levels of intake were offered, low, medium, high and *ad lib.*, the medium level corresponding nearly to maintenance. Supporting evidence was obtained from two other pairs of deer and sheep at *ad lib.* intake. The rations were given in two equal meals at 09.00 and 16.00 hours. Mean retention time (MRT) was estimated as described by Coombe & Kay (1965);

50 g ration, stained with basic fuchsin, was given at 08.50 hours and stained particles were counted in faeces samples collected subsequently.

Table 1 shows that digestibility of dry matter (DM) shows a weak tendency, and MRT a stronger tendency, to decline with DM intake. At intakes similar either in terms of g/d or relative to maintenance, the deer consistently showed lower digestibility and shorter MRT than the sheep. At the restricted intakes the deer digested DM and cellulose about 4 and 5% units, respectively, less fully and they retained stained hay and grass for 31 and 25 h, respectively, less than the sheep. At *ad lib.* intakes the difference in MRT between deer and sheep was only about 8 h. Stained particles first appeared in the faeces some 8 h earlier in the deer than in the sheep, suggesting more rapid propulsion through the intestines as well as through the rumen.

Table 1. *Intake, digestibility and mean retention time (MRT) by deer and sheep of roughages at various levels of intake (low, medium, high and ad lib.)*

	DM intake (g/d)	DM digestibility	MRT (h)	DM intake (g/d)	DM digestibility	MRT (h)
Hay						
		Low			Medium	
Deer	693	0.60	74	949	0.59	59
Sheep	433	0.61	119	690	0.63	85
		High			<i>Ad lib.</i>	
Deer	1212	0.58	61	1372	0.54	54
Sheep	943	0.62	81	1302	0.58	62
Dried grass						
		Low			Medium	
Deer	696	0.72	60	960	0.71	57
Sheep	435	0.77	99	698	0.77	84
		High			<i>Ad lib.</i>	
Deer	1219	0.72	54	1770	0.71	56
Sheep	958	0.75	64	1368	0.76	64

DM, dry matter.

The results support the hypothesis that deer retain food in their digestive tract for less time than sheep, and that this leads to rather poorer digestion but better appetite.

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Measurements over 5 d of the flow of dry matter and of chromic oxide at the duodenum of cattle. By J. D. SUTTON, F. G. YOUSSEF* and J. D. OLDHAM, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

With few exceptions, recovery of markers in digesta collected for 24 h from re-entrant cannulas in the proximal duodenum of ruminants has been about 90% or less. It has generally been assumed that the incomplete recovery of markers reflected a depression in flow of digesta due to the interference with the animal during sampling and further that adjustment of the 24 h flow to 100% recovery of marker provided an acceptable estimate of 'normal' flow.

Four dry Friesian cows with a cannula in the rumen and a re-entrant cannula in the proximal duodenum were given 2.2 kg hay and 6.6 kg dairy cubes (a total of 7.6 kg dry matter (DM)) daily. Each cow was given the foods at two frequencies, every 1 h and every 12 h. One cow ate only 73% of the ration when fed at 12 h intervals. The cows were given 40 g chromic oxide paper, containing 13.4 g chromic oxide, daily via the rumen fistula at 06.00 and 18.00 hours. Automatic equipment (Corse, 1974) was used to measure flow of digesta and to retain about 50 g/kg for analysis. Collections lasted for 5 d. There was no effect of frequency of feeding on the flow of digesta or of chromic oxide so the results shown in Table 1 are pooled and are the means from eight collections.

Table 1. *Daily mean flow of chromic oxide and of dry matter (DM) at the duodenum of cattle relative to mean flow over 5 d*

Day	Cr ₂ O ₃ recovery (% of daily dose)	Cr ₂ O ₃ recovery (% of 5 d mean)	DM flow	Adjusted DM flow (% of 5 d unadjusted mean)	Cumulative DM flow
1	89.0	93.4	92.0	104.1	92.0 ± 1.1
2	95.6	100.2	103.9	111.7	98.0 ± 1.2
3	94.0	98.7	102.6	110.1	99.7 ± 0.6
4	98.1	103.5	99.3	102.6	99.5 ± 0.6
5	99.1	104.0	102.2	103.7	100 —
SEM*	3.9	4.1	1.7	4.8	—

*For a single day.

Mean recovery of chromic oxide over the 5 d was $95.2 \pm 2.7\%$ and mean DM flow was 4.12 ± 0.16 kg/d. Flow of both DM and chromic oxide was lowest on day 1 but whereas flow of chromic oxide increased steadily over the 5 d, flow of DM was fairly constant after the first day so that adjustment of DM flow for chromic oxide recovery only partially reduced day-to-day variation in mean flow. Much higher standard errors were associated with adjusted DM flows than with unadjusted flow due to the variability in the flow of chromic oxide. It was concluded that it was

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preferable to avoid adjustment for chromic oxide recovery and that little was to be gained by extending collections beyond 3 d.

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Effect of dietary proportions of roughage and concentrate on rate of digestion of dried grass and cellulose in the rumen of sheep. By A. M. CHIMWANO, E. R. ØRSKOV and C. S. STEWART, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

When rapidly fermented concentrates are added to a diet of roughages, the rate of cellulose digestion is commonly reduced. As a result, intake of roughage falls, sometimes by an amount equal to the concentrate supplement (see e.g. Lonsdale, Poutiainen & Tayler, 1971; Ørskov & Fraser, 1975).

Our experiment was done to find the proportion of concentrate that could be included in a diet without seriously reducing the rate of cellulose digestion. The concentrate consisted of a pelleted mixture (7.4 mm die) of rolled barley-protein-mineral mixture (9:1, w/w). Four Suffolk cross sheep fitted with rumen cannulas were used. Diets containing either 0, 260, 500, 750 or 1000 g concentrate/kg, and dried grass, were given once daily at a feeding level of 60 g/kg^{0.75} for periods of 14 d. Two sheep were at first fed on grass alone and were later given increasing proportions of the concentrate. The other two sheep were started on concentrate and then given increasing proportions of the grass. During the last 2 d of each period, dried grass was incubated in the rumen in Dacron bags and cellulose (cotton) threads were suspended within the rumen digesta (Balch & Johnson, 1950). The weight loss of these materials during incubation is shown in Table 1.

Table 1. *Effect of composition of a barley-based-concentrate-dried-grass diet on the disappearance of dried grass or cotton threads from Dacron bags incubated in the rumen of sheep*

Proportion of concentrate in diet (g/kg)	Dried grass: loss in wt (mg/g) after:				Cotton thread: loss in wt (mg/g) after 24 h
	6 h	12 h	18 h	24 h	
1000	267	398	427	461	0
750	353	421	482	553	33
500	366	425	505	658	134
250	400	536	683	754	262
0	464	638	737	784	334
SE of treatment means	31	25	29	31	52

The proportion of concentrate fed greatly influenced the disappearance of dried grass from the Dacron bags, particularly at longer times of incubation. While the linear depression in both dried-grass and cellulose disappearance was significant in

all instances ($P < 0.001$), the effect of giving 250 g concentrate/kg diet was very small particularly when it was incubated for the whole feeding cycle of 24 h. This work suggests that feeding a processed concentrate once daily even at a reasonably low level will reduce rate of digestion of cellulosic material. If higher levels are used, it may be advantageous to increase the feeding frequency of the processed concentrate to more than once daily or, alternatively, to give sheep unprocessed barley (Ørskov & Fraser, 1975).

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The effect of processing before wafering on the nutritive value of first and second harvest artificially dried grass from the same sward. By F. W. WAINMAN and J. S. SMITH, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

First and second harvest artificially dried grasses from the same sward were each prepared in four different forms: chopped (C), chopped and wafered (CW), milled and wafered (MW) and a 50:50 (w/w) mixture of chopped and milled then wafered (C/MW). The wafering was carried out using a Glomera press and each of the eight forms was offered to three or four adult wether sheep at the maintenance and *ad lib.* levels in energy balance trials.

Table 1. *Dry matter (DM) intake and energy balance data (/kg body-weight^{0.75}) for sheep fed on dried-grass diets either chopped (C), chopped and wafered (CW) or milled and wafered (MW), or an equal mixture of chopped and milled, then wafered (C/MW)*

	1st Harvest				2nd Harvest			
	C	CW	MW	C/MW	C	CW	MW	C/MW
Efficiency of utilization of ME for maintenance	0.72	0.77	0.71	0.73	0.71	0.75	0.71	0.71
DM intake (g) <i>ad lib.</i>	92.1	83.6	81.0	91.9	78.1	81.4	93.4	86.4
DM intake (g) above maintenance	58.7	52.1	47.5	58.4	38.1	43.0	52.2	46.1
Retention (kJ) above maintenance	+406.1	+367.5	+293.5	+399.0	+149.0	+172.5	+209.6	+214.0
Net energy (kJ) above maintenance	6.92	7.05	6.18	6.83	3.91	4.01	4.02	4.64
Efficiency of utilization of ME above maintenance	0.53	0.53	0.54	0.56	0.41	0.41	0.50	0.51

ME, metabolizable energy.

The efficiencies of utilization of the metabolizable energy (ME) for maintenance of the C, MW and C/MW forms of each harvest were similar whilst those of the CW forms were higher (Table 1). Wafering the chopped first harvest grass increased its net energy for maintenance from 8.53 to 9.08 MJ/kg dry matter (DM) and similarly for the second harvest from 7.16 to 7.45 MJ/kg DM. Thus at the maintenance level of feeding, wafering the chopped grasses increased their energy value to sheep but milling had no effect.

For the first harvest grass the *ad lib.* intakes of the C and the C/MW were higher than the other two forms and they promoted higher energy retentions. The *ad lib.* intake of the sheep was lowest for the MW form of the first harvest grass and this resulted in the smallest energy retention. This was unexpected in view of earlier results, but all the wafers from the first harvest grass were extremely hard and this may have depressed the appetite of the sheep.

Processing of the second harvest grass produced increases in intake and energy retention which conformed to expectation. Appetite was lowest for the C and greatest for the MW forms. The CW, MW and C/MW forms promoted energy retentions which were respectively 23.5, 60.6 and 65.0 kJ/kg body-wt^{0.75} greater than the C form. Increased intake accounted for 93% of the increase for the MW form and 7% was due to the improved energy value, whilst for the C/MW only 57% was related to intake and the other 43% to increased energy value.

These results indicate that wafers composed of mixtures of chopped and milled forages may have advantages if they are not so hard as to inhibit appetite.

Carbon metabolism in sheep fed on poor-quality hill herbage. By J. C. MACRAE, S. WILSON and J. A. MILNE, *Hill Farming Research Organization, Bush Estate, Penicuik, Midlothian EH26 0PY*

Carbon metabolism was measured within and between the rumen and plasma metabolite pools of four Blackface wethers. The sheep, prepared with a rumen cannula and two jugular catheters, were fed by constant feeders a range of intakes (300–500 g dry matter (DM)/d) of frozen, poor-quality *Agrostis-Festuca* herbage. NaH¹⁴CO₃ was continuously infused, intravenously and intraruminally, and the irreversible losses of bicarbonate-C from each pool were calculated from the plateau specific activities obtained 12–20 h after commencement of infusion (see Nolan & Leng, 1974). These data were used in a series of simultaneous equations (J. V. Nolan, personal communication; simplified version of appendix to Nolan, Norton & Leng, 1976) to produce the model given in Fig. 1.

Data from further experiments with this herbage indicate that the amounts of CO₂ (see Fig. 1) and volatile fatty acids produced in the rumen and amounts of hexose fermented anterior to the duodenum agree with calculations based on stoichiometric relationships (Leng, 1970).

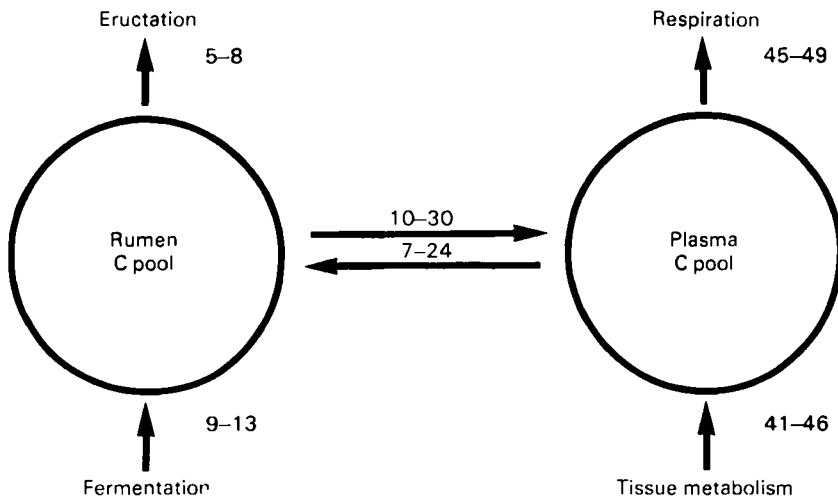


Fig. 1. A model of movement of carbon dioxide between the rumen and blood CO₂ pools in the sheep: movements are given as g carbon/d.

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Intestinal digestion of tropical starches by the preruminant calf. By B. E. ASSAN and P. THIVEND, *INRA Centre de Recherches Zootechniques et Vétérinaires de Theix, 63110 Beaumont, France*

Our previous work (Toullec, Thivend & Mathieu, 1971; Thivend & Toullec, 1973) showed that preruminant calves can digest a high proportion of starch products (100–150 g starch/kg dry matter (DM) of the milk-substitute from birth to 8 weeks; 250–300 g/kg DM later). The utilization of the starch energy is very satisfactory (Vermorel, Bouvier, Thivend & Toullec, 1973) but we did not know the relative roles of the small and large intestine in the digestion process and the nature of the end-products.

To provide information on this aspect, eight 20-d-old calves were fitted with ileocaecal re-entrant cannulas and single colon cannulas. Two of them received only cow's milk (control diet). The others (two for each experimental diet) were fed with milk-substitute containing 170 g sweet-potato, cassava or banana starch/kg DM. The calves were fed by pail, twice daily, according to their metabolic weight. The experiment was carried out during four successive periods. For each period (3 weeks), we have determined the digestibility of organic matter and starch (5 d) and the digestion of the diet by sampling the intestinal contents every 2 h for 20 or 24 h.

The digestibility of the tropical starches was satisfactory (0.65–0.79; Table 1) but less than the digestibility of cereal starches. It improved for cassava and sweet potato starch as the calves grew older; on the other hand, that of the banana starch fell with increasing age.

Table 1. *Intestinal digestion of starch by the preruminant calf when different starches replace 170 g/kg dry matter in milk-substitute diets*

Added Starch	Apparent digestibility	Disappearance (% digested starch) in:		pH		Lactic acid (mmol/l)	
		Small intestine	Large intestine	Ileum	Colon	Ileum	Colon
		Cassava	0.695	68.8	31.2	7.0	4.6
Sweet potato	0.788	41.6	58.4	7.3	5.2	12.3	42.1
Banana	0.649	39.4	60.6	7.0	4.9	4.2	17.9
Control (milk only)	—	—	—	8.3	6.6	1.4	0.6

The digestion of starch takes place, partly in the small intestine and partly in the large intestine (Table 1). Cassava starch is digested mainly in the small intestine, in contrast with sweet-potato or banana starch. In the small intestine, the amylolytic activity increases with the age of calves but there seems to be a shortage of maltase and oligo-1,6-glucosidase, as in lambs (Mayes & Ørskov, 1974). The microbial digestion in the caecum and colon induces a decrease of pH, a high production of lactic acid and volatile fatty acids. These end-products can account for 30–60% of the digestible energy of starch. Ammonia is very satisfactorily used by the microflora of the large intestine. The microbial digestion is very constant probably because the supply of energetic substrate in the caecum is steady.

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The value of processing barley in a mixed diet offered to cattle. By
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Previous experiments made with growing cattle showed that the digestibility of a diet containing predominantly whole barley (860 g dry matter (DM)/kg) was 10% lower than when the barley was rolled (MacLeod, Macdearmid & Kay, 1972). Experiments made with whole rather than pelleted cereals offered to sheep have shown advantages in terms of improved animal health while digestibility was essentially unaltered (Ørskov, Fraser & Gordon, 1974). Sheep are known to ruminate when offered whole cereals whereas this does not appear to be so with cattle. The purpose of this experiment was to measure the effect of processing barley on food intake and digestibility of a mixed diet. A mixed diet was used so that the cattle would ruminate. The diet contained barley offered twice daily as a supplement to silage.

Twelve Friesian steers weighing approximately 300 kg were used in a trial of Latin-square design to measure the intake and digestibility on diets consisting of two silage-barley mixtures (65:35 and 35:65, w/w) and two forms of barley (whole or rolled). The silage and barley contained approximately 185 and 860 g DM/kg respectively. During each period rumen pH was measured at hourly intervals after the barley feed. The main results are given in Table 1.

Table 1. *Food intake and digestibility by cattle of mixed diets containing whole or rolled barley*

Diet . . .	Silage-barley (65:35, w/w)		Silage-barley (35:65, w/w)		SE
	Whole	Rolled	Whole	Rolled	
Treatment of barley . . .					
DM intake (kg/d)	5.69	5.80	6.38	6.92	0.12
Silage DM (kg/d)	3.76	3.74	2.36	2.24	0.06
DM digestibility	0.631	0.740	0.608	0.755	0.018
Acid-detergent fibre digestibility	0.712	0.702	0.642	0.586	0.020
Rumen pH	6.92	6.77	6.87	6.76	0.07

DM, dry matter.

Processing the barley did not affect the amount of silage DM eaten. DM digestibility was lower for the diets containing whole barley than for those containing rolled barley and the difference was greater with the higher proportion of barley in the diet. The digestibility of acid-detergent fibre was significantly better with whole than with rolled barley in the diet containing 650 g barley/kg.

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A protein allowance for lactating beef cows. By S. A. ZAHRA, J. H. TOPPS and T. B. MILLER, *School of Agriculture, 581 King Street, Aberdeen AB9 1UD*

Cattle sustaining a relatively low level of production and provided with adequate dietary energy may well require less protein than that recommended in accepted standards. The existence of such an effect and its potential usefulness have been examined in lactating beef cows.

Eight Hereford × Friesian cows in their first lactation were allocated at random to groups of four and given, from the 29th day of lactation, (kg/d) either 8 hay, 1 barley, 1.5 dried sugar-beet pulp and 1 soya-bean meal (97 MJ metabolizable energy (ME) and 840 g digestible protein/d) or 8 hay, 2 barley and 1.5 dried sugar-beet pulp (97 MJ ME and 500 g digestible protein/d). Each cow suckled its calf and

weight changes, milk yield, milk consumption and hay consumption by the calves were measured weekly for 12 weeks. During the last week the nitrogen balance of the cows was determined.

The results (given in Table 1) show no difference between treatments in milk yield and calf growth but the cows receiving the higher amount of protein gained a considerable amount of weight, secreted more milk protein and excreted appreciably more N and urea in their urine.

Table 1. Mean weight changes, milk yield, milk composition and nitrogen balance data of suckled Hereford×Friesian cows given two levels of dietary protein

	High protein		Low protein		Significance of difference
	Mean	SE	Mean	SE	
Weight change of cow (g/d)	+301	—	-12	—	NS
Milk yield (kg/d)	7.5	0.23	8.1	0.25	NS
Milk protein (g/kg)	34	0.30	31	0.40	•
Weight gain of calf (kg/d)	0.87	0.15	0.81	0.07	NS
Hay intake of calf (kg/d)	1.03	0.15	1.16	0.09	NS
N retention of cow (g/d)	19.6	—	15.4	—	NS
Urinary N (g/d)	74.4	—	27.2	—	••
Urinary urea (g/d)	125	—	48	—	••
DM digestibility	0.686	—	0.619	—	•

NS, not significant; DM, dry matter.

• $P < 0.05$, •• $P < 0.01$.

It appears that Hereford×Friesian cows given approximately 75% of accepted requirements for protein utilize protein more efficiently in order to maintain an adequate milk supply for their calves.

Measurement of flow of intestinal contents in sheep by an electromagnetic method. By C. PONCET, ELENA DIMOVA, C. DARDILLAT and P. THIVEND, INRA Centre de Recherches Zootechniques et Vétérinaires de Theix, 63110 Beaumont, France

A quantitative study of food digestion in the intestine requires the use of markers. In spite of recent improvements which certain authors have proposed (cf. review by MacRae, 1975), this technique remains limited (by the animals' adaptation period, the feeding of markers, sampling, and quantitative determinations) and sometimes uncertain. We thought that it could be advantageous to replace it by an electromagnetic flow-meter which is commonly used to determine blood-flow (Rérat, 1971). Singleton (1961) had already proposed this method, but with an apparatus that did not allow its utilization under normal, physiological conditions.

Three adult sheep were fitted with re-entrant duodenal cannulas. An extracorporeal electromagnetic probe was placed at the outgoing part of the proximal cannula at the time of measurement. The probe was connected to a recording flow-meter. A valve was placed just before the probe to prevent the

digesta from flowing back into the abomasum. After crossing the probe, the contents were taken into a measuring cylinder and we compared the values obtained in this way to those supplied by the flow-meter. The contents were reintroduced manually. The measures were carried out over periods varying in duration from 4 to 8 h. The animals were given hay-concentrates (7:3, w/w), or silage, on the basis of 900–950 g dry matter (DM)/sheep per d.

The recorded flow (Y) shown by the electromagnetic probe was, for the twenty-three determinations carried out, very near to and not significantly different from that obtained by direct measurement (X) (Table 1). The two variables are related by the regression equation $Y=1.047^{***}X (\pm 0.025)-21.0^*$ (*not significant, $***P<0.001$; $r\ 0.994$). Variations in DM content and in the resistivity of digesta do not affect the probe's reaction. Nonetheless the electromagnetic apparatus used habitually to measure blood-flow should be adapted to the measurement of the flow in intestinal contents, because this latter appears in the form of gushes with a rather regular frequency but in varying volumes. Among other things, the apparatus used should take into account the reflux movements of the digesta into the cannula which correspond to a volume that must be subtracted from the total quantity of the recorded contents.

Table 1. *Comparison of duodenal flow (ml/h) in sheep by direct measurement and by the electromagnetic method*

Diet . . .	Hay-concentrate			Silage	
	Sheep 1	Sheep 2	Sheep 3	Sheep 1	Sheep 2
No. of determinations	3	4	4	8	4
Manual method	615	626	487	649	634
Electromagnetic method	604	618	495	671	636

This method is particularly suitable for the study of digestive physiology. Also, it should be able to replace marker techniques so long as the methods of sampling and of reintroduction do not disturb the flow of digesta.

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The effect of frequency of feeding and of the dietary energy source upon microbial protein synthesis in the rumen of sheep. By A. AL ATTAR, R. A. EVANS and R. F. E. AXFORD, *Department of Biochemistry and Soil Science, University College of North Wales, Bangor, Gwynedd LL57 2UW*

Four 35 kg Welsh Mountain wether sheep were fitted with re-entrant cannulas at the proximal duodenum, from which representative samples of the digesta

passing were obtained by an automated procedure (Axford, Evans & Offer, 1971). The sheep were fed once/d for 14 d periods, in accordance with a Latin-square design. The four rations, supplying 14 MJ gross energy/d, were made up of 350 g grass nuts/d to which was added a supplement consisting of milled barley and sucrose as shown in Table 1. Soya-bean meal was added to adjust the total nitrogen intake to 18 g/d. The experiment was repeated with the ration split up into equal portions fed at 2 h intervals. Digesta samples were collected for the last 4 d of each period, and their energy, total N and amino acid content determined. Microbial protein was estimated from the amino acid profiles by the method of Evans, Axford & Offer (1975), and showed correlation ($r+0.87$ based on thirty-two samples) with the diaminopimelic acid content of the digesta. Table 1 shows the daily flows of microbial protein, and the energetic efficiency of its synthesis in relation to the gross energy disappearing across the rumen (corrected for the energy content of the microbial mass). The results show that microbial protein synthesis is promoted by continuous feeding, in agreement with the findings of Hungate, Reichl & Prins (1971). The energetic efficiency of synthesis is also greater when continuous feeding is employed. The use of high levels of sucrose supplementation results in a suppression of microbial protein synthesis, and the energetic efficiency of synthesis is much reduced.

Table 1. *Flow of microbial protein (g/d), and energetic efficiency of protein synthesis (g/MJ) in the rumen of sheep fed once daily or continuously*

(Mean values with their standard errors for four sheep)

		Diet supplement (g/d)				SE
		0	76	152	228	
Sucrose . . .						
Barley . . .		315	210	105	0	
Fed once daily	Microbial protein	20.0	25.5	24.2	7.9	2.4
	Energetic efficiency	3.1	4.3	3.7	1.0	0.4
Fed continuously	Microbial protein	39.1	30.1	35.5	22.4	2.2
	Energetic efficiency	6.1	4.2	5.4	2.8	0.4

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Absorption and recycling of nitrogenous compounds in the digestive tract of sheep. By J. V. NOLAN, *Department of Biochemistry and Nutrition, University of New England, Armidale, NSW 2351, Australia* and J. C. MACRAE, *Hill Farming Research Organization, Bush Estate, Penicuik, Midlothian EH26 0PY*

A 'digesta diversion' technique has been used with intraruminal infusions of ^{15}N -labelled ammonium sulphate to measure ammonia loss from the rumen by its direct absorption, as distinct from its incorporation into bacterial cells that leave the rumen and are subsequently digested and absorbed from the small intestine of sheep.

The study was made with two Merino wethers each prepared with a rumen cannula, with re-entrant cannulas at the proximal duodenum and terminal ileum and with jugular vein catheters. The sheep were fed hourly on a pelleted, low-quality roughage diet (42 g/h; 16 g N/d). In the first part of the study (Expt 1) each received a continuous infusion of $(^{15}\text{NH}_4)_2\text{SO}_4$ and ^{103}Ru -phenanthroline for 36 h. All ^{15}N -labelled digesta leaving the fore-stomachs were diverted out of the body via the duodenal cannula and replaced with equal quantities of unlabelled 'donor' digesta previously collected from the same animal. In the second part of the experiment (Expt 2), 1 week later, the freeze-stored ^{15}N -labelled duodenal digesta were reintroduced into the duodenal cannula of the same animals at flow rates similar to those observed during Expt 1. Thus in Expt 1, direct absorption of labelled material was possible only from the forestomachs, while in Expt 2 direct absorption was possibly only from the intestines.

Duodenal and ileal digesta flow rates, together with 'plateau' ^{15}N enrichments of rumen $\text{NH}_3\text{-N}$, rumen bacterial N, duodenal and ileal $\text{NH}_3\text{-N}$ and non- $\text{NH}_3\text{-N}$, and plasma urea-N were measured at intervals between 24 and 36 h after the start of each experiment. The results indicate that despite quite low rumen NH_3 concentrations in both animals (117 and 68 mg N/l respectively), 53 and 62% of the 14 and 10 g $\text{NH}_3\text{-N/d}$ lost irreversibly from the rumen was absorbed, probably as NH_3 , from the forestomachs. On average, 60% of rumen bacterial N was derived from rumen NH_3 and, from the mean $^{15}\text{N}:^{103}\text{Ru}$ ratio in duodenal and ileal digesta, it was calculated that 60–70% of microbial non- $\text{NH}_3\text{-N}$ produced in the rumen was apparently digested in the small intestine (see Salter & Smith, 1974); other data obtained in the experiment suggest that this value is probably an underestimate of the true digestibility of microbial protein.

There was a gain of 2–6 g non- $\text{NH}_3\text{-N/d}$ across the forestomachs. This may have been due, in part, to recycling of endogenous urea N, but probably was due also to secretion of endogenous protein.

The technique will be useful as a means of estimating digestibility of digesta fractions and of partitioning various routes of absorption and recycling of N in various parts of the digestive tract for modelling purposes.

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The effect of time upon responses to intraperitoneal injections of DL-methionine in sheep fed on silage diets. By T. N. BARRY* and R. J. WILKINS, *Grassland Research Institute, Hurley, Maidenhead, Berks.*

Intraperitoneal injections of methionine have been shown to increase intake and wool growth in sheep fed on silages (Barry, Fennessy & Duncan, 1973). Two experiments were made to examine the effects of time on the responses to methionine.

Expt 1. Precision-chopped, direct-cut perennial ryegrass silage (177 g dry matter (DM)/kg, NH_3 -nitrogen 19% of total N, pH 4.2) was fed *ad lib.* to four wethers for six consecutive 9 d periods. Intraperitoneal injections (2.4 g methionine/2 d) were given to pairs of animals, with a changeover after 27 d. Voluntary DM intake (57.4 ± 1.74 g/kg $W^{0.75}$ per d) and apparent DM digestibility (0.779 ± 0.0067) were unaffected by methionine supplementation or time. N retention was increased by methionine supplementation ($P < 0.10$) and the methionine \times time interaction was significant ($P < 0.05$), the response to methionine increasing with time.

Expt 2. Red clover-dominant herbage was direct-cut with a flail harvester and ensiled with and without 80 g formaldehyde/kg crude protein ($N \times 6.25$) applied as formalin. Untreated (UT) and treated (FT) silages contained 179 and 196 g DM/kg, 14.5 and 5.5% of total N as NH_3 -N, and pH values were 5.1 and 5.6. A constant amount of hay was fed to thirty-two young sheep for 28 d and wool growth measured. Four groups of eight, balanced for live weight and wool growth, were then allocated to UT and FT silages with and without methionine (2.0 g/2 d) for 42 d. Apparent DM digestibility (UT 0.602, FT 0.585; SEM 0.0067) was unaffected by the methionine injections.

Methionine supplementation increased voluntary DM intake on FT silage in weeks 1 ($P < 0.001$) and 2 ($P < 0.01$) and on UT silage in weeks 4 and 5 ($P < 0.05$), giving a significant methionine \times weeks \times silages interaction ($P < 0.001$). Wool growth (g/d), measured over days 21–42, was increased by methionine administration ($P < 0.001$), the values being 2.4 and 4.5 for UT and FT silages without methionine and 5.3 and 7.0 with methionine.

Responses to methionine which increase with time probably reflect time required for the host ruminant tissues to adjust to a changing pattern of amino acid supply and protein synthesis. Pre-feeding periods of 21–28 d are desirable when evaluating treatments which increase postruminal amino acid supply and this conclusion is supported by the work of Barry (1976) with formaldehyde-treated hay. The present results and those of Kelly & Thomas (1975) show responses to methionine in sheep fed all four silages. Intake was increased only for the silage which had the lowest digestible DM intake (UT, Expt 2; 29.1 g/kg $W^{0.75}$ per d). Direct (Expt 1) and indirect measures of N retention (wool growth, Expt 2; rate of plasma methionine increase, Kelly & Thomas (1975)) indicate that methionine availability limited tissue protein synthesis on all four silages. Expt 2 with FT

*On leave of absence from Invermay Agricultural Research Centre, Mosgeil, New Zealand. Expt 2 was carried out at Invermay.

silage indicates that where there is a change in diet which involves an increase in intake, the increase may be delayed due to a temporary amino acid deficiency probably caused by the time required for adjustment in rumen microbial protein production.

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The effect of fasting in the non-lactating cow on hepatic lipid content and ultrastructure. By I. M. REID, R. A. COLLINS and G. D. BAIRD, *ARC Institute for Research on Animal Diseases, Compton, Newbury, Berks. RG16 0NN*

The fatty liver in the dairy cow fasted in early lactation is characterized chemically by an increase in hepatic triglyceride and cholesteryl ester content (Brumby, Anderson, Tuckley, Storry & Hibbitt, 1975) and ultrastructurally by an increase in both large lipid droplets and liposomes (Reid, 1973). The present study was designed to determine the effect of a 6 d fast on the hepatic lipid content and ultrastructure of the non-lactating cow.

Ten non-lactating cows were used, four as fed controls; the remaining six were fasted completely for 6 d but allowed unlimited access to water. Blood for lipid analysis was obtained by jugular venepuncture and a sample of liver was removed by laparotomy for lipid analysis and ultrastructural morphometric analysis.

The serum concentration of free fatty acids (FFA) increased to 2 mmol/l during the 6 d fast but there was no change in serum concentrations of total lipid, triglyceride or cholesterol. The hepatic lipid content doubled in the fasted, non-lactating cows (Table 1); the increase was mainly due to an 8-fold increase in triglyceride content, with smaller increases in cholesterol, cholesteryl ester and

Table 1. *Liver lipid content (g lipid/kg wet weight) in fed and fasted non-lactating cows*

	Fed cows		Fasted cows		Significance of difference
	Mean	SE	Mean	SE	
Triglyceride	3.35	0.34	27.22	5.64	$P < 0.025$
Cholesterol	1.12	0.19	1.81	0.07	$P < 0.05$
Cholesteryl ester	0.30	0.15	0.65	0.08	$P < 0.05$
Free fatty acid	1.37	0.15	5.75	0.07	$P < 0.001$
Phospholipid	17.27	0.93	18.00	1.83	NS
Total lipid	25.27	1.21	53.32	4.09	$P < 0.01$

NS, not significant.

FFA content. The composition of liver lipids changed, with triglyceride making up 50% of the total lipid content. However, unlike the fatty liver in the fasted, lactating cow (Brumby *et al.* 1975), there was no change in the proportion of total lipids contributed by cholesterol or cholesteryl ester. At the ultrastructural level, there was an increase in the volume of hepatic cytoplasm occupied by lipid droplets in fasted, non-lactating cows but, unlike the fasted, lactating cow (Reid, 1973), there was no change in the volume of hepatic cytoplasm occupied by liposomes or rough endoplasmic reticulum.

Thus, fasting in the non-lactating cow resulted in a fatty liver which was chemically and morphologically different from the fatty liver in the fasted, lactating cow. The two types of fatty liver provide an ideal model for nutritional fatty liver in the dairy cow and are being actively investigated.

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Antiketogenic effect of glucose in fasted, lactating cows. By G. D. BAIRD and R. J. TREACHER, *ARC Institute for Research on Animal Diseases, Compton, Newbury, Berks. RG16 0NN*

When dairy cows are fasted for 6 d (144 h) in early lactation they become hyperketonaemic and hypoglycaemic, and the free fatty acid (FFA) content of the blood is increased. The hyperketonaemia is probably due to increased hepatic ketogenesis, the source of the ketone bodies being blood FFA. In the livers of these fasted cows, the content of various intermediates of the citric acid cycle and the gluconeogenic pathway is much decreased below that in fed cows, while the extent of cytosolic reduction is much increased (Baird, Heitzman & Hibbitt, 1972).

In our experiments, cows fasted in this manner each received an intravenous infusion of a solution of 500 g glucose/l at the constant rate of 1.5 ml/min (0.75 g glucose/min) for the final 48 h of the fast. Over this 48 h period blood ketone-body concentrations fell almost to zero. Blood FFA concentrations also decreased, but remained 2–3-fold higher than in fed cows. We conclude that the glucose infusion suppressed hepatic ketogenesis entirely, partly by decreasing the availability of blood FFA and partly by eliciting antiketogenic mechanisms within the liver.

In the livers of these glucose-treated, fasted cows the content of citrate and 2-oxoglutarate, both intermediates of the citric acid cycle, rose higher even than in fed cows, while the extent of cytosolic reduction decreased below that in the fed state. The increase in citrate and 2-oxoglutarate content could arise as the result of a partial inhibition of hepatic gluconeogenesis by the glucose load (West & Passey, 1967), while the decrease in cytoplasmic reduction could be due either to a decrease in FFA oxidation or to a glucose-induced increase in lipogenesis (Ballard, Filsell &

Jarrett, 1972) or both. These metabolic changes are likely to lead to diversion of acetyl-CoA from ketone-body formation. Thus, the increases in citrate and 2-oxoglutarate content, together with the redox change, are consistent with an overall increase in hepatic oxaloacetate content. More oxaloacetate might therefore be available for acetyl-CoA oxidation. Furthermore, any increase in lipogenesis would involve utilization of acetyl-CoA for fat synthesis.

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