

The effect of the plane of energy nutrition of the cow on the secretion in milk of the constituents of the solids-not-fat fraction and on the concentrations of certain blood-plasma constituents

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An increase in the plane of energy nutrition of the cow is known to increase both the yield and the solids-not-fat (S.N.F.) content of milk: a decrease in fat content also occurs but there is little effect on the yield of fat (for review of literature, see Burt, 1957*a*). Though small depressions in milk lactose content occur with underfeeding (Rowland, 1946), any change in S.N.F. content is largely due to a change in the protein content of the milk, and thus an increase in the net energy supply to the cow gives rise to a specific increase in the synthesis of protein by the mammary gland. The metabolic pathways by which this effect and the increase in milk yield are achieved are, however, as yet unknown.

The experiments described here were designed to investigate the effect of the plane of energy nutrition of the cow on the amounts of the individual proteins in milk and on the concentrations of certain blood-plasma constituents that are possibly of importance in the synthesis of proteins within the mammary gland.

Two experiments have been made. In the first, the variations in plane of energy nutrition imposed were within the range of feeding levels normally experienced in practice, whereas in the second extreme differences in plane of energy nutrition were compared in an attempt to exaggerate any changes in blood composition associated with change of level of feeding.

EXPERIMENTAL

Design of experiments

Expt 1. Six Friesian cows in their 2nd–3rd month of lactation and yielding from $4\frac{1}{2}$ to 6 gal milk/day were selected from the Institute herd. The experimental design was a complementary set of two 3×3 Latin squares with periods of 28 days. Each animal was fed at three planes of energy nutrition, and the allocation of animals to the feed treatments in the first period was random. Before the experiment the cows had been rationed according to yield on hay, grass silage and a mixture of barley, bran and decorticated groundnut cake prepared on the farm, and they were introduced to the experimental rations over a period of 3 days.

The three levels of feeding were:

(a) a 'low-energy' ration, providing 2.5 lb starch equivalent (S.E.) less than the

standard recommended by Woodman (1957) of 7.0 lb s.e. for maintenance and 2.5 lb s.e./gal milk. The daily ration for maintenance was based on hay (8 lb) and brewer's grains (30 lb) and for production on a mixture of dredge corn (3 parts), dried distiller's grains (1 part), bran (1 part) and decorticated groundnut cake (1 part) prepared on the farm (4 lb for each gallon of milk after the first);

(b) a 'normal-energy' ration as for (a) together with 3 lb flaked maize, providing the standard of Woodman (1957);

(c) a 'high-energy' ration as for (a) together with 9 lb flaked maize, providing 5 lb s.e. more than the standard of Woodman (1957). The basal ration had a protein content sufficient to meet the protein requirements of the cows at all the levels of feeding. The normal lactational decline in milk yield was allowed for, throughout the course of the experiment, by decreasing the amount of farm-mixed concentrates by 2 lb at the end of each period.

Expt 2. Four Friesian cows, in their 4th–5th month of lactation, and two dry Shorthorn cows were selected from the Institute herd. They were allotted at random to two groups, each of two milking cows and one dry cow, and the groups were fed according to the following experimental plan:

Group 1

Animals: Adelaide 3, Valient, Winnie 32 (dry)
Feeding: Period 1, weeks 1–4, a high-energy ration
Period 2, weeks 5–6, a low-energy ration

Group 2

Animals: Adelaide 2, Begonia 2, Champion 36 (dry)
Feeding: Period 1, weeks 1–2, a low-energy ration
Period 2, weeks 3–6, a high-energy ration

Before the experimental period all the cows were rationed on hay, grass silage and a mixture of barley, bran and decorticated groundnut cake prepared on the farm, and were transferred to the experimental rations over a period of 3 days. The rations were as follows:

Low-energy ration: For milking cows, the ration offered was oat straw (10 lb), hay (8 lb), a mixture of barley (3 parts), bran (1 part) and decorticated groundnut cake (1 part) prepared on the farm (1 lb plus an additional 4 lb for each gallon of milk produced in excess of 3 gal), 2 lb decorticated groundnut meal and 1 lb linseed cake. For dry cows, the ration consisted solely of oat straw (15 lb).

High-energy ration: For all cows, the ration offered was hay (8–12 lb), farm-mixed concentrates (10 lb) and flaked maize (14 lb). At the end of the 1st week on this ration an additional 4 lb/day of a mixture of equal weights of farm-mixed concentrates and flaked maize was offered to all the cows.

At the end of the 6-week experimental feeding period the two dry cows were removed from the experiment and the four milking cows were transferred to a ration of 20 lb oat straw/day for 2 days (period 3). The calculated planes of energy nutrition of the cows during each of the experimental periods are given in Table 1.

Sampling and methods of analysis

Samples of milk were taken at each milking over the last 8 days of each main treatment period in both experiments, and weighted composite samples representing successive 2-day periods were made for each cow. In the period of straw feeding in Expt 2, samples were taken at each milking and analysed individually. Samples were analysed for fat by the Gerber method (British Standards Specification, 1955) and for total solids gravimetrically (British Standards Specification, 1951), S.N.F. contents being obtained by difference. The values for fat and S.N.F. were corrected for systematic errors in the determination of fat by the Gerber method according to the tables provided by Crocker, Jenkins, Provan, Macdonald, Rowland & White (1955). Over the last 4 days of each main treatment period and throughout the straw-feeding period in Expt 2 samples were analysed also for lactose by a modification of the method of Hinton & Macara (1927), and for total nitrogen and N distribution by the method of Aschaffenburg & Drewry (1959).

Table 1. *Expt 2. Planes of energy nutrition of the six cows at the end of each experimental feeding period expressed as a percentage of the theoretical requirements for maintenance and production calculated according to the standards of Woodman (1957)*

Cow	Period		
	1	2	3
Adelaide 2	55	130	30
Adelaide 3	—*	85	30
Begonia 2	85	145	30
Valient	175	90	35
Campion 36	35	210	—
Winnie 32	250	35	—

* Towards the end of period 1, Adelaide 3 scoured badly and went off her feed.

Samples of blood were taken with a minimum of excitement from the jugular vein of all the animals and from the internal iliac artery, by puncture through the rectal wall, of three animals in one treatment block in Expt 1 and of all the animals in Expt 2, at approximately 2, 5 and 8 h after feeding on the last day of the main treatment periods, and at 2 h after feeding each day in period 3 of Expt 2. Plasma samples of arterial blood were analysed for total volatile fatty acids (Annison, 1954) and plasma samples of venous blood for glucose (Nelson, 1944) and α -amino N (Hamilton & Van Slyke, 1943).

RESULTS

Milk composition

The treatment mean values, with a standard error, for milk yield and composition in Expt 1 are given in Table 2. The results show the changes in milk composition typically associated with an increase in the plane of energy nutrition, namely a slight fall in fat content and an increase in S.N.F. content, the latter resulting mainly from an increase in milk protein content. The increase in protein N content was largely

Table 2. *Expt 1. Mean values with a standard error, for the yield and composition of milk of the six cows at the three planes of energy nutrition*

Ration	Yield* (lb/day)	Fat* (%)	s.n.f.* (%)	Lactose† (anhydrous) (%)	Total N† (mg/100 g)	Casein N† (mg/100 g)	Total albumin N† (mg/100 g)	β-Lacto- globulin N† (mg/100 g)	Residual albumin N† (mg/100 g)	Proteose N† (mg/100 g)	Globulin N† (mg/100 g)	Non- protein N† (mg/100 g)
High-energy	49.8	3.68	8.85	4.59	518	394	79	50	29.0	11.0	10.2	26.5
Medium-energy	47.8	3.79	8.67	4.58	500	375	77	49	27.3	10.5	8.5	30.0
Low-energy	46.2	3.91	8.51	4.53	483	361	74	47	26.9	9.8	8.5	31.5
s.e. of differences between two means	± 0.95	± 0.12	± 0.10	± 0.06	± 9	± 9	± 1.2	± 1.4	± 1.1	± 1.1	± 1.0	± 1.1
Probability that dif- ferences as large as the observed dif- ferences would have arisen by chance	< 0.05	> 0.05	< 0.05	> 0.05	< 0.05	< 0.05	< 0.01	> 0.05	< 0.05	> 0.05	> 0.05	< 0.01

* Based on results for the last 8 days of each treatment period.

† Based on results for the last 4 days of each treatment period.

Table 3. *Expt 1. Mean values for the yields (lb/day) of the major milk constituents of the six cows at the three planes of energy nutrition calculated from the values given in Table 2*

Ration	Fat	s.n.f.	Lactose (anhydrous)	Casein*	Total albumin*
High-energy	1.83	4.41	2.29	1.25	0.25
Medium-energy	1.81	4.14	2.19	1.14	0.23
Low-energy	1.81	3.93	2.09	1.07	0.22

* N × 6.38.

accounted for by casein N, but a statistically significant increase occurred also in total albumin N (β -lactoglobulin N + residual albumin (mainly α -lactalbumin) N). Small and not statistically significant increases in globulin and proteose N content were also observed but changes in the concentrations of the minor proteins must necessarily be interpreted with caution since they are each determined as a difference between two much larger values and errors of analysis may be high. The changes in lactose content with plane of energy nutrition were small (less than 1.5% of the mean value) and the greater part of this change was due to a slight fall in lactose content when the cows were on the low-energy ration. It has previously been shown that a ration providing energy in excess of the standards of Woodman (1957) has no effect on milk lactose content (Rook, 1953) whereas a ration deficient in energy produces a small depression (Rowland, 1946).

These changes in milk composition occurred together with changes in the yield of milk, and the variations in the yields of the major milk constituents are given in Table 3. The treatment mean values for fat, lactose, casein and total albumin obtained during the period of high-energy feeding expressed as a percentage of those obtained during the period of low-energy feeding were, respectively, 102, 110, 117 and 115. Thus, an increase in the plane of energy nutrition had a negligible effect on fat synthesis but increased the synthesis of lactose and the major milk proteins, the proportionate increase being greater for the proteins than for lactose.

Table 4. *Expt 2. Mean values for the yields (lb/day) of milk and milk constituents of the four cows on the various rations*

Ration	Yield	Fat	S.N.F.	Lactose (anhydrous)	Total protein*	Casein*	Total albumin*
High-energy†	27.7	1.04	2.43	1.17	0.94	0.73	0.120
Low-energy†	21.5	0.93	1.79	0.93	0.67	0.49	0.086
Straw							
Preceding 4 days	21.7	0.88	2.02	0.98	0.77	0.59	0.094
Day 1	18.9	0.94	1.70	0.84	0.66	0.51	0.088
Day 2	14.2	0.77	1.22	0.61	0.48	0.36	0.062

* $N \times 6.38$.

† Values for Adelaide 3 for these rations were not used in the calculation of mean values.

The pattern of change in milk yield and composition in Expt 2, periods 1 and 2, was in all cows similar to that reported for Expt 1 in spite of the fact that, because of the advanced stage of lactation of the cows used, the effects of feeding would be confounded to some extent with effects due to stage of lactation. The mean values for the differences in milk yield and composition between the high and the low planes of energy nutrition for Adelaide 2, Begonia 2 and Valient were: yield, 6.2 ± 3.3 lb/day; fat, -0.52 ± 0.24 %; S.N.F., 0.49 ± 0.13 %; casein N, 54 ± 17 mg/100 g; total albumin N, 5 ± 2 mg/100 g; lactose, 0.01 ± 0.13 %. The widest contrast in plane of nutrition was achieved with Valient, and the change from the high-energy to the low-energy plane produced a fall in S.N.F. from 8.89 to 8.18%. The concomitant changes in casein N, total albumin N and lactose contents were from 440 to 376 mg/100 g, 83 to 73 mg/100 g and 4.14 to 3.88%, respectively.

The transfer to a ration of straw produced a marked and immediate fall in milk yield in all the cows (see Table 4). The effects on milk composition were, however, small and not consistent from cow to cow with the exception of an increase in fat % (mean value 1.21 ± 0.47) associated with the fall in yield. The effects of the long-term changes in the plane of nutrition of the cow on the S.N.F. content of milk were not immediate but were established progressively over a period of 2-3 weeks (see p. 111).

The treatment mean values for the yields of the major milk constituents in Expt 2 are recorded in Table 4. The percentage increases with increase in plane of nutrition in the mean values for the yields of the various constituents were: fat, 10; lactose, 25; casein, 50; total albumin, 40. Thus, as in Expt 1, the increase in the synthesis of proteins was proportionately much greater than the increase in lactose synthesis. With the short-term change to a ration of straw, however, the falls in the yields of lactose and proteins were similar, being about 40% by the 2nd day.

Blood-plasma composition

Glucose. The treatment mean values for plasma glucose in Expt 1 (Table 5) showed a small and consistent increase with an increase in the plane of energy nutrition, but the differences were significant ($P < 0.05$) only for the samples taken 8 h after feeding. At all planes of nutrition there was a tendency for the plasma glucose content to decrease with an increase in the interval after feeding. In Expt 2 no consistent differences in plasma glucose contents with plane of nutrition were observed, in spite of the more extreme differences in feeding imposed. The treatment mean values for the three milking cows for the high-energy and low-energy rations, respectively, were 64.9 ± 3.0 and 64.8 ± 3.0 mg/100 ml blood plasma. With the two dry cows also, glucose levels were unaffected by a gross change in plane of energy nutrition. The transfer of the milking cows to a ration of straw produced a mean increase of 16.4 ± 12.3 mg/100 ml (four animals) in plasma glucose concentration on the 1st day but the mean level returned to its previous value on the 2nd day.

Blood glucose concentrations in the ruminant are known to be little affected by level of feeding but underfeeding can produce a fall in blood-sugar levels in pregnant ewes in which foetal requirements for glucose are high (Reid, 1950). The small effect of plane of nutrition on plasma glucose concentration observed in Expt 1 may thus have arisen from a partial breakdown in homeostatic mechanisms due to the high requirement for glucose for synthesis of milk lactose by the high-yielding animals used in that experiment. A cow yielding 5 gal milk/day requires roughly 1 kg glucose for the synthesis of lactose.

Total volatile fatty acid. The moderate differences in plane of energy nutrition imposed in Expt 1 had no measurable effect on the plasma content of total volatile fatty acid (Table 5) but, with the wider differences in plane of energy nutrition in Expt 2, values for the milking cows were considerably higher on the high-energy than on the low-energy ration in all cows 2 h after feeding, in two of three cows 5 h and in one cow 8 h after feeding (Fig. 1). The transfer of the milking cows to an all-straw ration invariably produced a marked fall in volatile fatty-acid concentration. With the two dry cows the extreme differences in the plane of energy nutrition provided by the

two experimental rations gave rise to marked differences in plasma volatile fatty-acid concentration throughout the day.

The total volatile fatty acid present in peripheral blood is accounted for largely by acetate (see Annisson, 1954) and undoubtedly the observed changes in total volatile fatty-acid concentration reflect changes in plasma acetate concentration.

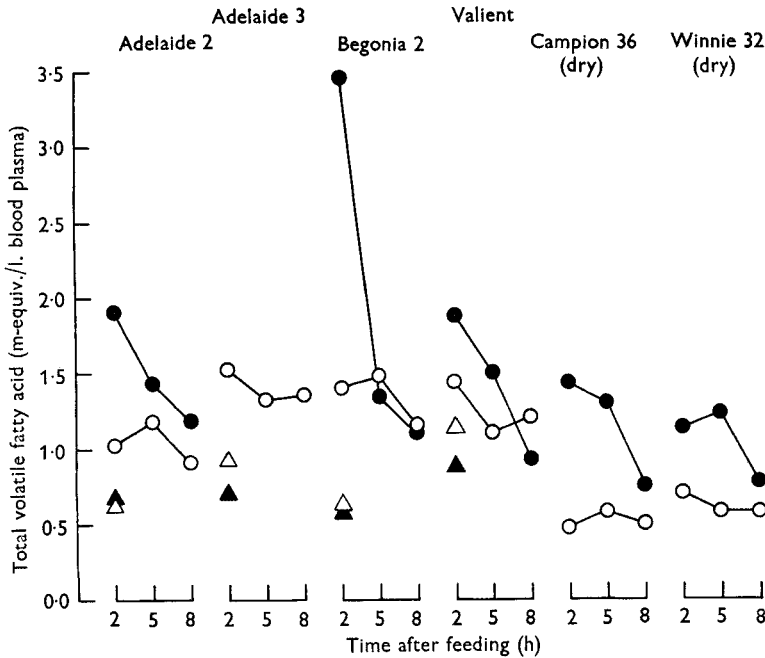


Fig. 1. Expt 2. Variations with plane of energy nutrition of the cow in the total volatile fatty-acid concentration of arterial blood plasma. ●, high plane of energy nutrition; ○, low plane of energy nutrition; Δ, straw feeding, 1st day; ▲, straw feeding, 2nd day.

Table 5. Expt 1. Mean values for plasma glucose, total volatile fatty-acid and amino-acid concentrations in the six cows at the three planes of energy nutrition for samples taken at 2, 5 or 8 h after feeding

Ration	Glucose (mg/100 ml of jugular venous-blood plasma)			Total volatile fatty acid (m-equiv./l. of arterial-blood plasma)			α-Amino N (mg/100 ml of jugular venous-blood plasma)		
	2 h	5 h	8 h	2 h	5 h	8 h	2 h	5 h	8 h
High-energy	76.9	75.3	73.4	1.34	1.44	0.85	3.33	3.30	3.21
Medium-energy	75.8	74.0	72.7	1.40	1.36	1.03	3.06	3.08	3.33
Low-energy	73.5	73.8	65.0	1.27	1.41	1.29	3.07	3.24	3.04
S.E. of differences between two means	± 1.69	± 4.28	± 3.70	± 0.12	± 0.08	± 0.37	± 0.20	± 0.23	± 0.54
Probability that differences as large as the observed differences would have arisen by chance	> 0.05	> 0.05	< 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

α-Amino N. The treatment mean values for plasma α -amino N concentration in Expt 1 (Table 5) showed on average a small increase with an increase in plane of nutrition but the differences observed were not statistically significant. With the wider differences in plane of nutrition in Expt 2 (Fig. 2) increases in the average α -amino N concentration with an increase in plane of nutrition were observed in all three milking cows, the increase being particularly marked with Valient for which the greatest change in milk protein content was observed. The effect of plane of nutrition was, however, much less marked with samples taken 2 h after feeding than with those taken 5 or 8 h after feeding. The two dry cows failed to show a consistent relationship between plasma α -amino N concentration and plane of energy nutrition, a fact which may be related to the much lower requirement for amino acids of the dry animal than of the milking cow: a cow producing 5 gal milk daily will secrete in its milk the equivalent of some 700 g of amino acids.

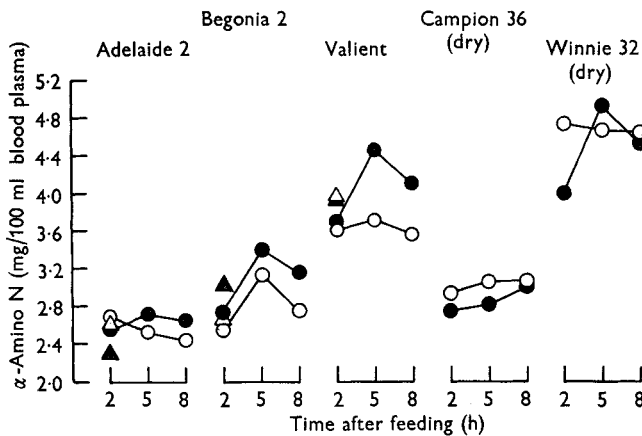


Fig. 2. Expt 2. Variations with plane of energy nutrition of the cow in the α -amino nitrogen concentration of jugular venous-blood plasma. ●, high plane of energy nutrition; ○, low plane of energy nutrition; △, straw feeding, 1st day; ▲, straw feeding, 2nd day.

DISCUSSION

The effect of the plane of energy nutrition of the cow on the s.n.f. content of milk, as demonstrated in this and earlier work (Burt, 1957*b*; Dijkstra, 1942; Holmes, Reid, MacLusky, Waite & Watson, 1957; Rook, 1953; Rowland, 1946), is represented in Fig. 3. The changes in s.n.f. content were largely changes in the contents of the main milk proteins, casein, β -lactoglobulin and α -lactalbumin, and, consistent with the law of diminishing returns, the changes were more marked with underfeeding than with overfeeding; with underfeeding, small changes in lactose content also occurred. In terms of the synthesis of milk constituents, however, an increase in the plane of energy nutrition of the cow produced a marked increase in the synthesis of both protein and lactose.

The observations on the changes in the plasma concentrations of glucose, total volatile fatty acid and α -amino N associated with these changes in milk composition

were much less definite. This is understandable since for obvious technical reasons plasma samples may be taken for analysis only at infrequent intervals, and for metabolites that show marked variations in plasma concentration with interval after feeding the plasma samples analysed may provide a poor indication of the mean plasma levels throughout a 24 h period. With the particular experimental approach adopted, it is, moreover, difficult to exaggerate the changes in blood composition, since to achieve wide differences in plane of energy nutrition a considerable degree of undernutrition is necessary at the lower level of feeding. In the milking cow gross underfeeding produces a marked fall in milk yield, and the consequent reduction in the uptake of metabolites from the plasma offsets to a large extent the effects of the reduced uptake

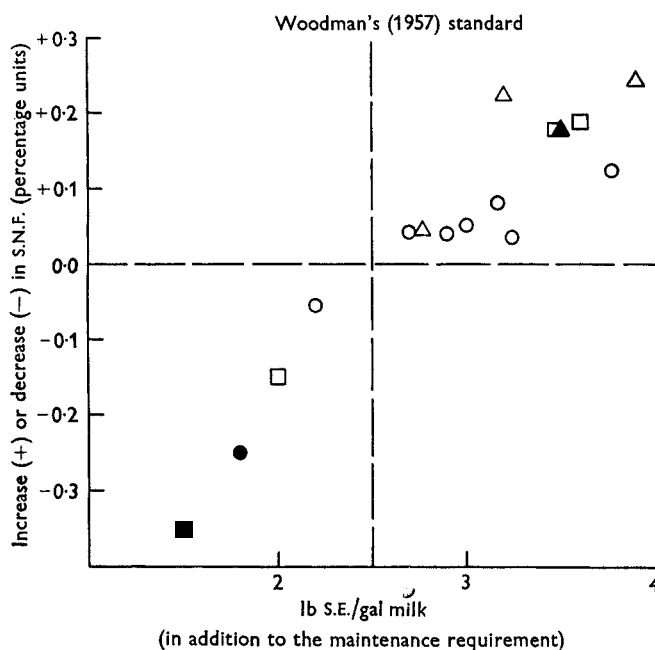


Fig. 3. Summary of the results of published experiments demonstrating the effect of the plane of energy nutrition of the cow on the solids-not-fat content of milk. (The results of experiments in which it was not possible to calculate the effect of plane of energy nutrition from the base-line of Woodman's (1957) standard have been excluded.) O, Burt (1957a); ●, Dijkstra (1942); △, Holmes *et al.* (1957); ▲, Rook (1953); □, this work; ■, Rowland (1946).

of nutrients. Such considerations do not apply to the dry cow, but here, for certain metabolites such as glucose and possibly also amino acids, the absence of the high demand for nutrients associated with milk production permits an effective functioning of homeostatic mechanisms at all levels of feeding.

Though it is necessary to interpret the observed changes with caution, they do suggest that important variations in plasma acetate and amino-acid concentrations occur with variations in plane of energy nutrition, and the possible significance of such changes in relation to the observed changes in milk secretion will now be discussed.

In general, the rate of synthesis of a material within the body is dependent on three

factors, the concentrations of precursor and product and the supply of energy. The rates of mammary synthesis of lactose and protein are likely, therefore, to be affected by the supply of adenosinetriphosphate, which in turn would be determined by the amounts of plasma acetate or glucose available for oxidation within the mammary gland, and by the plasma concentrations of the known precursors of lactose and milk proteins, glucose and amino acids, respectively. Any hypothesis should conform, therefore, with these generalizations, with the observed effects of plane of energy nutrition on plasma glucose, volatile fatty-acid and amino-acid concentrations, and also with current knowledge of energy metabolism in the ruminant where a large part of an animal's requirement for energy is provided by the volatile fatty acids, acetic, propionic and butyric, produced by the fermentation of food materials within the rumen. Of these acids, propionic and butyric are metabolized almost wholly in the rumen wall or in the liver, whereas acetic acid is metabolized to only a small extent in the rumen wall and liver and a large part of the acetate absorbed from the rumen enters the peripheral circulation (see Annison, Hill & Lewis, 1957).

As a basis for future work, the following hypotheses are put forward.

(a) An increase in the plane of energy nutrition increases the amount of acetate absorbed from the rumen and, consequently, the plasma acetate concentration in peripheral blood. This change in turn increases the rate of mammary synthesis of both lactose and protein. To maintain isotonicity of the mammary secretion with blood plasma, an increase in the synthesis of lactose will produce an increase in the output of water and therefore in the yield of milk (see Rook & Wood, 1959). Since the percentage increases in the yields of lactose, protein and water will be of the same order, no significant change in the milk content of lactose or protein will occur.

(b) An increase in the plane of energy nutrition increases the amount of propionate absorbed from the rumen, which in the milking cows gives rise to a small increase in plasma amino-acid concentration. This in turn produces a small specific increase in protein synthesis, additional to that mentioned above, and therefore an increase in the protein content of the milk secreted. The control of plasma amino-acid levels by the portal blood supply of propionate could be achieved in the liver through the intermediary of the Krebs's cycle (see Rook, 1959).

The preliminary observations (Rook & Balch, 1959) in experiments now in progress in which additions of individual volatile fatty acids, as continuous intraruminal infusions, are being made to the rations of dairy cows to determine their effects on milk yield and composition support the above hypotheses.

SUMMARY

1. A study has been made of the effect of the plane of energy nutrition of the cow on the yield of milk and on the contents of fat, solids-not-fat (S.N.F.), lactose and proteins in the milk and on the concentrations of glucose, total volatile fatty acid and α -amino nitrogen in the blood plasma. Two experiments have been done: in the first, the differences in plane of energy nutrition were within the range experienced in practice; in the second, differences were more extreme.

2. In both experiments an increase in the plane of energy nutrition of the cow increased the yield of milk, decreased the fat content and increased the S.N.F. content. The last effect was almost wholly due to an increase in protein content, increases occurring in the concentrations of all three of the major milk proteins, casein, β -lactoglobulin and α -lactalbumin. In terms of the yield of milk constituents there was a negligible effect on fat yield, but the yields of both lactose and protein were increased, the increase in the yield of protein being proportionately much greater than in that of lactose.

3. The small differences in plane of energy nutrition studied in the first experiment did not affect significantly the blood plasma concentrations of glucose, total volatile fatty acid or α -amino N, but with the wider differences in the second experiment significant increases in total volatile fatty-acid and α -amino N concentrations with an increase in plane of nutrition were demonstrated.

4. Hypotheses are put forward to explain the observed effects of the plane of energy nutrition of the cow on the yield of milk and the yields of lactose and protein.

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