extract, not exceeding 4 ml. were submitted to the procedure described above. As the extracts of the whole blood or of the plasma gave a small but significant colour (equivalent to about 2 mg./100 ml.) with dinitrophenylhydrazine, it was necessary to determine the intensity of this colour in a control experiment, so that a correction could be made. Table 4 gives 10 representative determinations. The standard error of percentage deviation in concentrations from 2·5 to 200 mg./100 ml. of blood or plasma was found by us to be 3½ %.

Liver, kidney and muscle. The tissues obtained from normal guinea-pigs, the added diketogulonic acid and 2 parts by weight of 10 % trichloroacetic acid were ground with sand in a mortar, squeezed through muslin, reground, and centrifuged in the usual way. The sensitivity of the determination in this case was conditioned by the fact that the use of too much extract produced turbidity after the addition of the alkali. We nevertheless found that it was possible to determine 0·15 mg./g. of tissue with a standard error of percentage deviation of 4¼ % after correcting for the blank. Results chosen at random are given in Table 5. The approximate equivalents of diketogulonic acid yielded by the blanks were, muscle 0·1 mg./g.; kidney 0·15 mg./g.; liver 0·2 mg./g.

Table 5. *Determination of diketogulonic acid in liver, muscle and kidney*

Liver mg./g.		Muscle mg./g.		Kidney mg./g.	
Added	Found	Added	Found	Added	Found
0·15	0·14	0·15	0·15	0·15	0·16
0·20	0·21	0·20	0·21	0·20	0·21
0·25	0·26	0·25	0·26	0·25	0·23
0·50	0·51	0·50	0·52	1·00	1·04
0·75	0·75	0·65	0·64	1·00	0·98
1·00	1·02	1·00	0·95	1·50	1·56
1·00	0·98	1·00	1·04	2·00	1·98
1·25	1·30	1·25	1·30	2·00	2·09
1·50	1·60	1·50	1·52	3·00	3·00
1·50	1·60	2·00	1·94	3·00	3·15

Urine. The diketogulonic acid was added to freshly voided human urine and the determination was carried out directly on the urine without previous extraction. When necessary a correction was made for the blank. This was never higher than the equivalent of 5 mg. of diketogulonic acid per 100 ml. of urine. Table 6 gives some results obtained with concentrations 2·5–40 mg./100 ml. of urine. The standard error of percentage deviation of all the urine determinations carried out by us was 3 %.

Table 6. *Determination of diketogulonic acid in urine*

Added, mg./100 ml.	2·5	5·0	10·0	10·0	15·0
Found, mg./100 ml.	2·5	5·0	10·2	9·6	15·2
Added, mg./100 ml.	20·0	20·0	25·0	30·0	40·0
Found, mg./100 ml.	20·0	19·4	24·7	29·0	40·0

We should like to take this opportunity of expressing our gratitude to Dr R. G. Hatton, Director of East Malling Research Station, for laboratory accommodation. Thanks are also due to Messrs Roche Products Ltd. for a gift of ascorbic acid. One of us (J. R. P.) is indebted to the Medical Research Council for a whole time grant.

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Urea as a Partial Protein Substitute in the Feeding of Dairy Cattle

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(*Received 29 September 1942*)

The belief that certain simple nitrogenous compounds such as urea are capable of supplying to the ruminant a significant proportion of its nitrogen requirement is many years old. The theory most commonly advanced to account for the utilization of these simple compounds is that they are built up into protein by certain micro-organisms of the rumen which use them as a source of nitrogen for their own multiplication. The protein so formed is subsequently broken down in the small intestine to amino-acids which are absorbed into the blood stream of the host along with those resulting from the digestion of the diet.

This theory has never been fully substantiated. Many feeding trials were carried out, mainly on the Continent, in the first three decades of the present century, but insuperable difficulties are encountered when an attempt is made to interpret the results of most of the experiments. In some instances the periods in which the non-protein nitrogen was fed were too short to enable reliable conclusions to be drawn. In others the periods were longer but the body-weights of the animals were not recorded, and again in others the controls were inadequate. These difficulties were emphasized by Krebs [1937] who, in reviewing the literature up to 1936, cited over 100 references and stressed the highly controversial nature of the results.

Since 1936 further publications have appeared suggesting that at least a portion of the dietary protein of ruminants can be replaced by substances such as urea. Hart, Bohstedt, Deobald & Wegner [1939] found that urea and ammonium bicarbonate, when added to a diet poor in protein and fed to growing calves, caused gains of live weight which were larger than those produced by the untreated diet, although the increases were slightly smaller than those obtained by the addition of the same amount of nitrogen in the form of casein. Bartlett & Cotton [1938] found no significant differences when one-third of the protein in the ration of young dairy heifers was replaced by urea, though they believed that the urea diet was slightly inferior to the protein diet. These publications, together with several others, have been discussed recently by Benesch [1941] and reviewed by Owen [1941]. It may be noted that they are concerned mainly with the effect of feeding on the growth of young stock or sheep, and that none of the work published in recent years has dealt with the value of these simple nitrogenous compounds for milk production.

The controversial nature of results and the wartime shortage of feeding-stuffs appeared to justify a thorough reinvestigation of the dietary value of urea for milk production. The general plan of attack and the objects in view have been outlined briefly by Owen, Smith & Wright [1941]. One section of the work was to consist of metabolism trials with lactating cows, in which urea would be compared with blood meal as a source of one-third of the nitrogen of the production ration. In the second section use was to be made of a steer with a large rumen fistula to determine by both in vivo and in vitro methods whether protein synthesis could actually be observed in the contents of the rumen and, if so, to study the factors affecting the magnitude and efficiency of such synthesis.

The present communication is limited to an account of the metabolism trials and their results.

OUTLINE OF THE EXPERIMENTS

Seven Ayrshire cows of average milk yield were used for the experiments. They were first fed a production ration in which a third of the nitrogen was supplied by blood meal, a product which has been shown by Morris & Wright [1933*a*] to contain a protein of first-class biological value for milk production. After the animals had been receiving this diet for a few weeks, the blood meal was replaced by its nitrogen equivalent of urea plus its energy equivalent as pure starch. With the first two cows the urea was fed for a period of 6 weeks and then replaced by blood meal. With the remaining five cows a period of about a fortnight without either urea or blood meal was inserted immediately after the first urea period. Each dietary change from one period to another was made gradually over 5 days. Further details as to the dates of the various periods and their duration are given in Table 1.

The animals were kept in special stalls in the metabolism house which has already been described by Morris & Wright [1933*b*]. The stalls were so fitted that by means of a continuously moving belt the urine and faeces of the cows could be collected separately with only slight mutual contamination. The faeces were weighed and sampled every 2 days, but the vessels for collecting urine were emptied each day and the urine sampled. Toluene was used to minimize bacterial action in these vessels. Composite 2-day samples of urine were then made for analysis. The slight contamination of the urine with faeces imparted to the urine a marked 'urease activity', with the result that part of the excreted urea was liable to be converted to ammonia in the collecting vessels. This rendered the estimation of urea itself relatively valueless and it was found necessary to estimate urea and ammonia nitrogen together. Preliminary tests showed, however, that this conversion of the urea did not result in any loss of nitrogen from the urine.

The milk yields were recorded daily, and with the first five cows composite 2-day samples were analysed for all the major constituents. With the remaining two cows the analysis was carried out at less frequent intervals. Urea and non-protein nitrogen were also determined at suitable times throughout the experiments in both the blood serum and the milk.

Two of the animals were weighed at the beginning and end of the experiment, and the remaining five at the beginning and end of each period.

The basis of rationing for maintenance was 5·5 lb. starch equivalent and 0·5 lb. protein equivalent per 1000 lb. live weight. For milk production the allowance was 2·5 lb. starch equivalent and 0·45 lb. protein equivalent per gal. The production ration per gal. of milk consisted of 0·25 lb. blood meal, 2 lb. oats, 1½ lb. bran, 0·25 lb. treacle and 0·5 lb. starch.

In Exp. 1 the maintenance ration for cows A and B consisted of 28 lb. swedes, 14 lb. straw and 2 lb. oats per day. It was found in this experiment that swedes were technically undesirable since they were not always completely consumed by the animals and since they contained a somewhat large proportion of non-protein nitrogen of which the nutritional value cannot at present be accurately assessed. In the experiment with cows C and G, a maintenance ration consisting of 13·5 lb. hay (6 % crude protein content) and 2·12 lb. potato starch per day was therefore fed, and the bran in the production ration was reduced by 0·5 lb. to compensate for the resulting slight increase of protein equivalent. In Exps. 1 and 3 a reduction was made in the production ration during the course of the work to parallel the decrease in milk yield. The point at which this change was made is shown in Fig. 1. In Exp. 2 the production ration was fed at the same level throughout.

The constituents of the production ration and the oats or starch from the maintenance ration were weighed each day and then thoroughly mixed in a mechanical mixer, the urea being dissolved in the molasses plus an equal volume of water. This procedure avoided any risk of localized concentrations of urea. The mixture was fed twice daily. There were no refusals of food during the urea periods, which indicates that the mixture was quite palatable. In Exp. 2 the hay was sampled and analysed at frequent intervals to ensure that the nitrogen intake was maintained at the correct level. The hay in Exp. 3 and the mixed diet in Exps. 2 and 3 were analysed every 2 days.

In all Kjeldahl determinations a mixed catalyst of potassium and copper sulphates and powdered selenium was used. Sulphur in the urine was determined by a modification of the method of Wolf & Osterberg [1910]. After having been boiled with conc. HNO_3 under a reflux condenser, the urine was subjected to a second oxidation with Denis's [1910] copper nitrate reagent. The total sulphur was then determined gravimetrically as $BaSO_4$. This method gave results agreeing well with those obtained by oxidation with a mixture of sodium carbonate and sodium peroxide. Urea and ammonia in the urine were determined by the urease method of Van Slyke and Cullen as described by Peters & Van Slyke [1932]. Before the determination of ammonia the samples of faeces were subjected to the action of urease.

RESULTS AND DISCUSSION

Milk yields

The milk yields for 2-day period for all seven cows are shown in Fig. 1, while the average yields in each period are recorded in Table 2. It will be seen from Fig. 1 that on feeding urea to cow A there was a decrease in yield from some 35 kg. to about 29 kg. during the first 16 days, a fall of 17 %. This decrease was, however, followed by a slight recovery, with

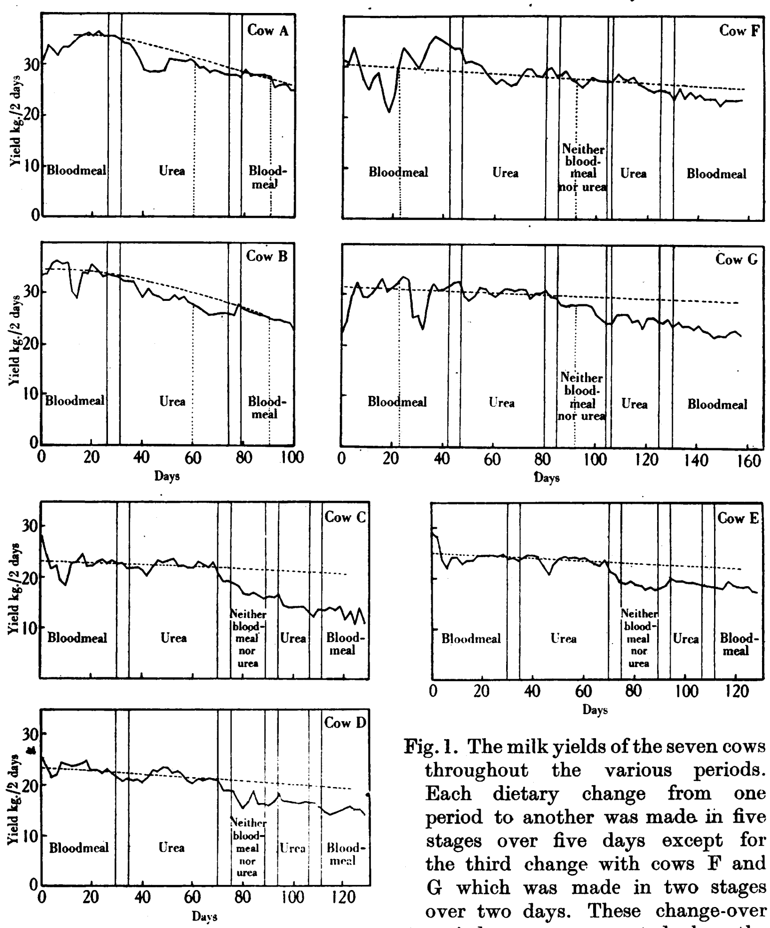

Fig. 1. The milk yields of the seven cows throughout the various periods. Each dietary change from one period to another was made in five stages over five days except for the third change with cows F and G which was made in two stages over two days. These change-over periods are represented by the pairs of vertical lines. In accordance with the decrease in milk yields, an appropriate reduction was made in the production ration fed to cows A, B, F and G at the points denoted by the vertical dotted line.

Table 1. *Plan of experiments*

Exp. no.	Period no.	Duration days	Description
1	1	26	Blood meal
Cows A and B	2	44	Urea in place of blood meal
6. xii. 39 to	3	28	Blood meal
19. iii. 40			
2	1	30	Blood meal
Cows C, D and E	2	36	Urea in place of blood meal
15. iv. 40 to	3	12	Neither blood meal nor urea
22. viii. 40	4	14	Urea replaced in the diet
	5	18	Blood meal
3	1	43	Blood meal
Cows F and G	2	29	Urea in place of blood meal
3. iii. 41 to	3	17	Neither blood meal nor urea
7. viii. 41	4	17	Urea replaced in the diet
	5	25	Blood meal

Table 2. *The average milk yields during the various periods (kg./2 days)*

	Cow						
Period	A	B	C	D	E	F	G
1	34·4	34·0	22·8	23·3	24·6	30·3	29·6
2	30·6	31·5	22·3	21·5	23·6	28·7	30·4
3	27·0	25·0	17·6	17·3	18·7	27·9	26·9
4	—	—	14·9	16·8	19·2	26·9	25·3
5	—	—	13·3	15·0	18·2	24·1	23·1

the result that during the last 3 weeks of urea feeding the yield approached closely to a value which might reasonably have been recorded as normal had blood meal been fed throughout. In fact the averages quoted for cow A in Table 2 suggest that urea did not result in any very serious decrease in yield, if the normal fall in lactation is taken into account. The urea feeding period in this experiment coincided with the exceptionally cold spell in the early part of 1940, and it appears that the temporary fall in yield may have been due to adverse weather conditions. With cow B no such initial decrease took place and the efficiency of urea for milk production appears to have approached very closely to that of blood meal.

The diagrams for cows C, D, E and G and the averages in Table 2 all show that milk production during the 4 or 5 weeks of urea feeding was practically as high as that obtained with blood meal, and that on removing urea from the diet a marked decrease in milk yield at once ensued. Thus with these four cows, yields of some 22, 21, 23 and 30 kg. at the end of period 2 were rapidly reduced to 17, 17, 19 and 27 kg. by the withdrawal of urea from the diet. These decreases amount to between 10 and 20%. With cow F the milk yield in period 1 was so variable that it is difficult to estimate the effect of urea with any certainty, but this animal appears to have behaved like cow A in that there was an initial decline in yield followed by a partial recovery. On withdrawing urea from the diet, however, the yield in this instance did not decline appreciably. It will be shown later that of the two cows F and G which were observed at the same time under identical conditions, the wastage of the ingested urea was much greater with cow F than with cow G. This fact may explain why urea did not seem to be as effective with cow F as it was with G.

It will be observed from Fig. 1 and Table 2 that for cows C, D, E and G the replacement of urea, and subsequently of blood meal, in the diet, after the period in which neither was fed, did not cause the milk yield to return to its expected (normal lactation) value. This is not an unusual experience, for if the milk yield is once allowed to decrease through inadequate feeding it is most difficult, particularly in the latter half of the lactation, to increase it again merely by an improvement in the diet.

So far as milk yields are concerned it may therefore be tentatively concluded that of the seven cows, five were able to utilize urea for milk production with reasonable efficiency, though the results for the sixth cow (F) were not clear-cut. With the remaining cow (A) there was an initial fall in yield at the outset of urea feeding, but this only proved to be temporary, and was probably due to the severe weather conditions. It has been suggested [Kay, 1942] that the feeding of urea in more than very limited quantities may have a toxic effect. It is clear that in the present experiments no such effect occurred. Indeed, with four of the seven cows the yield was practically maintained at its initial level in spite of advancing lactation.

Body weights

The body weights, which are recorded in Table 3, were obtained by weighing each animal three times on one day and averaging the values so obtained.

Table 3. *Body weights in kg.*

	Cows				
	C	D	E	F	G
At beginning of 1st blood meal period	464	473	488	483	478
At end of 1st blood meal period	450	425	450	446	428
Change	−14	−48	−38	−37	−50
At end of 1st urea period	450	455	478	445	413
Change during this urea period	None	+30	+28	−1	−15
At end of period when neither urea nor blood meal was fed	448	436	454	435	402
Change during this period	−2	−19	−24	−10	−11
At end of 2nd urea period	449	464	457	434	410
Change during this period	+1	+28	+3	−1	+8
At end of 2nd blood meal period	479	489	479	419	402
Change during this period	+30	+25	+22	−15	−8

Cows A and B were weighed only at the beginning and the end of Exp. 1. Their initial weights were 442 and 410 kg. respectively and their final weights were 394 and 409 kg.

The three weighings were made at 6 a.m., noon and 6 p.m. This precaution was taken to ensure that the average weight recorded for any one day was affected as little as possible by the ingestion of food and water or by the excretion of faeces and urine. Except on one occasion the differences between any of the three weighings on one day never exceeded 17 kg. and were usually much less.

It will be seen that the weights of all five cows in Exps. 2 and 3 decreased slightly during the first blood meal period, but that during period 2, when urea was substituted for blood meal, this tendency was checked, and in two instances (D and E) the decrease was converted to an increase. When, however, urea and blood meal were both withheld (period 3), the weights of cows D and E again showed a tendency to decrease in spite of the fact that the period was short and that it was accompanied by a reduction in milk yield. When urea feeding was resumed in period 4 these decreases in weight were again checked, and in period 5, except in the case of cows F and G, they were converted to gains.

It may be concluded that the urea feeding did not cause any significant loss of body weight, and that in certain instances gains were observed during the urea periods.

Nitrogen excretion

At 2-day intervals throughout the experiments composite samples of urine and faeces were analysed for total nitrogen and urea + ammonia nitrogen, and for creatinine and creatine. In the case of cows A and B urinary sulphur was also determined. Owing to the need for economy in space it is impossible to present here the large number of results obtained in this section of the work. Details of two typical examples are given in Fig. 2. Cow A gave similar results to cow B, while the results for cow C were representative of the remaining five animals. The average values for the intake and output of nitrogen and for the urea + ammonia excretion for Exps. 2 and 3 are shown in Table 4. It will be relevant to discuss at this point the metabolic data.

Nitrogen balances

It will be seen from Table 4 that, except in period 3, when both urea and blood meal were withheld, the animals appeared to be in a state of positive nitrogen balance to a somewhat remarkable degree. This was particularly true for cows C, D and E, the apparent positive balances attaining in some instances a figure of 90 g./2-day period. In view of the relatively small changes in the body weights of the animals (Table 3)

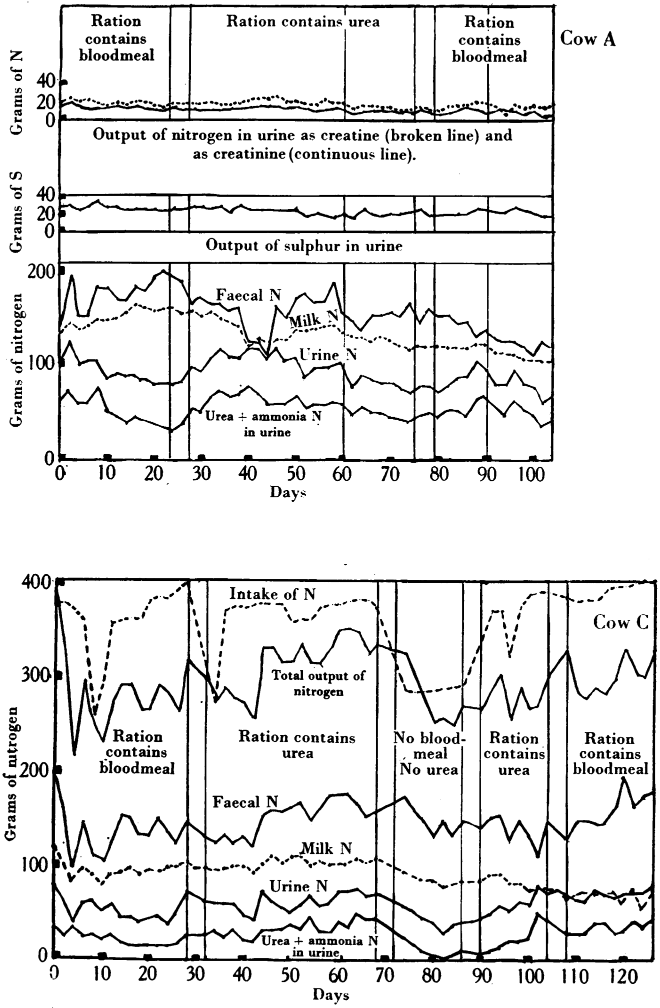

Fig. 2. Output of total nitrogen in milk, faeces and urine, output of nitrogen as urea + ammonia in urine, and total intake of nitrogen.

it is difficult to regard these values as true indications of the precise state of nitrogen balance. We are unable to explain the tendency for our technique apparently to indicate such large positive balances. Each step of the procedure has been examined in detail with a view to solving this problem, but so far no solution has been found. It may be noted that in the work of Morris & Wright [1933*b*] and of Morris, Wright & Fowler [1936], in which the same technique was used, positive balances of as much as 50 and over 70 g. nitrogen per 2-day period were encountered in extreme instances, whereas negative balances occurred infrequently and were usually very small.

We are, however, of the opinion that the figures recorded for the balance in any one period can be compared in a relative sense with the corresponding figures for other periods. Thus with cow C the figures of +76·9, +53·2 and +11·4 for periods 1, 2 and 3 indicate that a probable positive nitrogen balance when blood meal was fed was slightly reduced when the blood meal was replaced by urea and was very much further reduced (if not rendered negative) when neither urea nor blood meal was ingested. It seems reasonable to apply a similar method of interpretation to all the nitrogen balance figures in Table 4.

It was again restored, however, when urea feeding was resumed.

The fact that the nitrogen balance was so much more positive in the two main urea periods than in the intervening nitrogen-deficient period indicated either that the urea-nitrogen was retained and anabolized by the animals or that the presence of urea in the diet in some way rendered the other nitrogenous constituents of the food more readily available for the animals' use. In this latter connexion a study of the values recorded in Table 4 for the faecal nitrogen of periods 1 and 2 does not suggest that the amount of nitrogen absorbed by the animal for metabolic purposes from the other constituents of the food was greater during urea feeding than it was with blood meal. In other words the apparent utilization of urea cannot be ascribed solely to an increase in the digestibility of the other constituents of the urea ration.

Table 4. *Nitrogen excretion (g. nitrogen/2 day period)*

Period	Urine	Faeces	Milk	Total	Nitrogen intake	Nitrogen balance	Urea + NH_3-N in Urine	Urea + NH_3-N in Faeces	Urea + NH_3-N in Total	Creatinine g./2 days	Creatine g./2 days
Cow C											
1	52·0	136·1	94·9	283·0	359·9	+76·9	21·4	10·3	31·7	11·1	10·0
2	61·5	150·6	101·8	313·9	367·1	+53·2	32·9	15·6	48·5	10·3	5·8
3	42·7	147·6	84·1	274·4	285·8	+11·4	10·7	7·0	17·7	12·2	2·2
4	61·2	139·4	80·8	281·4	370·2	+88·8	25·3	10·5	35·8	13·3	5·8
5	68·8	161·8	67·4	298·0	390·4	+92·4	35·0	11·1	46·1	13·0	2·2
Cow D											
1	36·8	167·5	112·1	316·4	405·0	+88·6	18·3	17·9	36·2	6·2	7·8
2	71·1	172·5	109·1	352·7	404·0	+51·3	49·3	22·4	71·7	7·5	10·8
3	43·9	152·3	93·1	289·3	287·7	− 1·6	10·6	12·2	22·8	12·3	9·2
4	72·9	138·8	95·0	306·7	398·8	+92·1	45·9	12·2	58·7	9·0	12·5
5	76·1	164·9	88·0	329·1	418·8	+89·7	48·9	14·3	63·2	8·6	7·8
Cow E											
1	57·8	166·5	103·0	327·3	422·1	+94·8	30·4	19·5	49·9	11·7	8·7
2	90·3	166·8	108·3	365·4	406·6	+41·2	57·6	18·1	75·7	12·6	10·1
3	51·9	152·2	85·5	289·6	301·8	+12·2	11·4	9·0	20·4	16·1	7·3
4	102·2	132·2	92·8	327·2	424·0	+96·8	63·2	11·0	74·2	16·3	9·9
5	98·0	162·2	91·4	351·6	450·0	+98·4	60·8	12·8	73·6	12·7	5·4
Cow F											
1	96·0	162·0	120·9	378·9	388·1	+ 9·2	50·1	12·5	62·6	15·3	11·1
2	143·6	146·8	120·9	411·3	433·9	+22·6	95·8	15·5	111·3	14·9	15·9
3	59·4	127·2	115·0	301·6	298·2	− 3·4	24·8	6·9	31·7	14·5	10·3
4	88·1	133·6	117·8	339·5	356·5	+17·0	52·9	11·2	64·1	15·1	16·3
5	72·7	165·4	105·9	344·0	368·9	+24·9	34·8	9·4	44·2	14·3	11·3
Cow G											
1	67·4	174·0	131·9	373·3	398·3	+25·0	40·3	19·4	59·7	9·3	10·2
2	93·1	162·9	139·4	395·4	436·0	+40·6	57·1	21·9	79·0	9·1	12·8
3	44·2	139·6	123·7	307·5	302·9	− 4·6	15·7	9·8	25·5	8·5	10·6
4	71·3	138·3	122·8	332·4	365·0	+32·6	39·2	16·6	55·8	11·8	16·4
5	45·3	175·8	112·8	333·9	364·7	+30·8	16·9	14·3	31·2	9·6	8·6

If this method of interpretation is valid, two main conclusions may be drawn. (1) With three cows (C, D and E) the positive nature of the balance in the first urea period was significantly less than in the first blood meal period. In the case of the other two cows (F and G) the reverse was true. On the other hand, with all five animals the positive nature of the balance was very considerably reduced during period 3, when neither urea nor blood meal was fed.

Krebs [1937] has stated that an apparent retention of urea such as has been found here might merely be due to a swamping of the tissues with urea and not to a conversion of urea to protein. But if this were true one would expect urea to be equally effective in the diet of both the ruminant and the nonruminant animal, whereas it has been well established that in the latter ingested urea is of no nutritional value. Moreover, as a simple calculation will show, it is unreasonable to suppose that the large retention of urea-nitrogen observed in the present experiments could ever be explained by a swamping of the tissues with urea. If it be assumed that the amount of water in which urea can dissolve in the body of the cow is 75 % of its body weight and that the concentration of urea in this water is the same as that in the blood serum, for which the maximum value found in this work never exceeded 15 mg. urea-N/100 ml. serum, then a 500 kg. cow would retain only about 120 g. urea. But in the whole of the second period of these experiments the total amount of urea fed to each cow was of the order of 3000 g. Even if the level of urea in the tissues could rise well above that in the blood, it is difficult to believe that it could ever attain a figure which would be sufficient to explain the observed findings, nor would such an hypothesis be capable of accounting for the maintenance of the milk yield and body weights.

Creatinine and creatine

Judging from recent publications by Bloch & Schoenheimer [1941], who find that urea is not a precursor of creatine, and by Beard, Espenan & Pizzolato [1939], who claim that it is a precursor, any variations which might be found in the amount of creatinine and creatine excreted in the urine of the ruminant, either on a blood meal or urea diet, would be difficult to interpret. It was, however, felt that a study of the creatinine-creatine excretion might give some useful information regarding the metabolism of the animals ingesting urea. The urinary excretion of creatinine and creatine was therefore estimated for all seven animals by the usual colorimetric procedure of Folin. For the first two cows the coloured solutions were compared with the standard in a visual colorimeter. For the others a 'Spekker' photo-electric absorptiometer was used. The results for cow A, which were very similar to those for cow B, are shown in Fig. 2. The average values for cows C to G are recorded in Table 4. It is impossible to draw any definite conclusion from the results, for the variations from one 2-day period to another were usually too large to enable inter-period variations to be detected. Nor was there any appreciable change when both urea and blood meal were withheld. It seems probable that really substantial inter-period changes in these values can only be observed with cows subjected to relatively severe inanition such as that described by Morris & Ray [1939]. Further work on this subject is, however, clearly desirable, since the results do not fall into line with those published earlier by Morris & Wright [1933 *a*, *b*].

Diuresis

Since urea is a diuretic agent it is important to determine whether urea-feeding with ruminants is liable to cause excessive and harmful diuresis. The average weight of urine excreted per 2-days in the various periods is shown for all seven cows in Table 5.

Table 5. *Average weight of urine excreted in the various periods* (*kg./2 days*)

	Cows						
Period	A	B	C	D	E	F	G
1	15·36	9·63	6·28	5·25	5·25	12·89	8·62
2	12·99	10·55	7·11	11·96	9·62	12·65	9·68
3	12·75	8·32	7·31	9·01	7·54	8·73	7·55
4	—	—	9·05	12·51	14·35	7·83	8·22
5	—	—	8·88	10·28	9·34	7·80	6·72

With cows A and F there was no diuresis when urea replaced blood meal in the diet. With the other cows, however, in which the weight of urine was initially much less, increased excretion did occur during the urea periods. This was most marked with cow E, in which a value of 5·25 kg. in period 1 rose to 9·62 kg. in period 2. Again, the value of 7·54 kg. in period 3, when both blood meal and urea were withheld, increased to 14·35 kg. in period 4, when urea feeding was resumed. On the other hand, it should be noted that the figure of 9·62 kg. for the first urea period did not differ materially from that of 9·34 kg. for the final blood meal period, nor does the highest value of 14·35 kg. represent an abnormal excretion for a cow in 2 days. Smaller amounts were excreted in period 1 by cows A and F and again in period 5 by cow A. It therefore seems unlikely that urea, if used in a prepared production ration in which it accounted for some 30 % of the nitrogen, would induce any harmful diuresis.

The non-utilization of a portion of the ingested urea

The results recorded in Table 4 show that the total amount of nitrogen excreted in the urine each 2 days during the urea periods was greater than that excreted during the blood meal periods, and that this excess could be accounted for mainly by an increase in the excretion of urea + ammonia-nitrogen. With cow D, for example, a value of 36·8 g. for the total urinary nitrogen during the blood meal period increased to 71·1 g. during the urea period, an increase of 34·3 g., the corresponding values for urea + ammonia-nitrogen in the urine being 18·3 and 49·3, a difference of 31·0 g. If, however, it were assumed that urea was quantitatively converted to protein in the rumen and that this protein became available to the animal to the same degree as blood meal, the excretion of urinary nitrogen would be expected to remain the same in the urea periods as it was in the blood meal periods. It seems probable therefore that

Table 6. *The proportion of ingested urea which does not appear to be utilized by the animal compared with the level of urea in the blood*

Cow	Urea-N ingested in period g./2 days		The amount by which the urinary N in period 2 exceeded that in period 1		The amount by which the urinary N in period 4 exceeded that in period 5		% of ingested urea which appeared to be wasted in period		Average value for urea-N in the blood serum mg. N/100 ml. during period		% increase
	2	4	Total urinary N g./2 days	Urea + NH_3-N g./2 days	Total urinary N g./2 days	Urea + NH_3-N g./2 days	2	4	1	2	
A	85·7	—*	13·0	13·7	—	—	15–16	—	4·5	8·3	84
B	91·0	—	23·0	14·1	—	—	15–25	—	6·3	10·5	67
C	85·9	93·7	9·5	11·5	− 7·6	− 9·7	11–13	None	4·7	5·8	23
D	93·7	93·8	34·3	31·0	− 3·2	− 3·0	33–36	None	4·5	7·3	62
E	99·6	99·7	32·5	27·2	+ 4·2	+ 2·4	27–33	2–4	6·2	9·0	45
F	99·4	81·6	47·6	45·7	+15·4	+18·1	46–48	19–22	—†	—	—
G	99·8	83·6	25·7	16·8	+26·0	+22·3	17–26	27–31	—	—	—

* There were only three periods with cows A and B.
† No blood analysis was carried out for cows F and G.

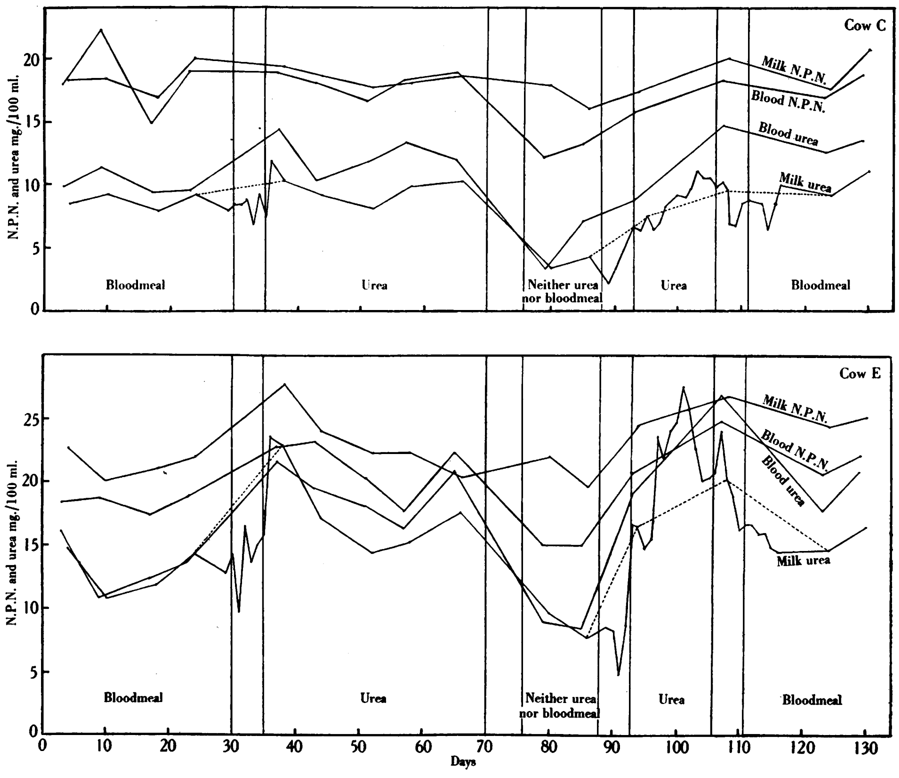

Fig. 3. The concentration of N.P.N. and urea in the blood and milk. For the five animals for which these data are available, cow C showed the least and cow E the greatest variation on replacing blood meal with urea. For this reason the results for the two cows have been chosen for illustration. The broken lines bring out the general parallelism between the concentration in the blood and that in the milk.

this increase in the nitrogen excretion when urea is fed may be taken as a measure of the amount of ingested urea which passes through the animal without acting as a protein substitute. In Table 6 the approximate amount of this unused urea is calculated. It should be noted that the value obtained for any one cow is roughly the same whether the total urinary nitrogen or only that excreted as urea + ammonia is used as the basis of calculation.

If the above reasoning is sound it may be concluded that the proportion of urea utilized varies greatly with different animals. With the seven cows in the present work the proportion of urea passing through the animal unused in period 2 varied from 12 to 47 % and averaged 25 %. Moreover, it is important to observe that cow C, which showed the smallest wastage of urea, also showed the greatest benefit from the feeding of this substance, for the milk yield of this animal was maintained at as high a level in period 2 as in period 1, though removal of both blood meal and urea resulted in an abrupt decline. Again, cow F, which showed the least benefit in milk yield from urea feeding, also showed the greatest degree of wastage.

One further point should be noted. For cows C, D and E the values calculated for the wastage of urea in period 2, which followed a blood meal period, was very much higher than those calculated for period 4, in which urea feeding followed immediately after a period of 2 weeks during which both blood meal and urea were withheld. In fact, with cows C and D the nitrogen of urea appeared to be better utilized during period 4 than was that of blood meal fed in equivalent amounts during period 5. It appears from this fact that the proportion of the dietary urea which is wasted may depend to some extent on the nutritional state of the animal prior to urea feeding, a phenomenon which is not uncommonly encountered in other types of nutritional study. The same feature was observed with cow F, though not with cow G. It should be pointed out that these wastage values have been calculated on the assumption that the nitrogen of blood meal itself is completely utilized; they are therefore relative and not absolute.

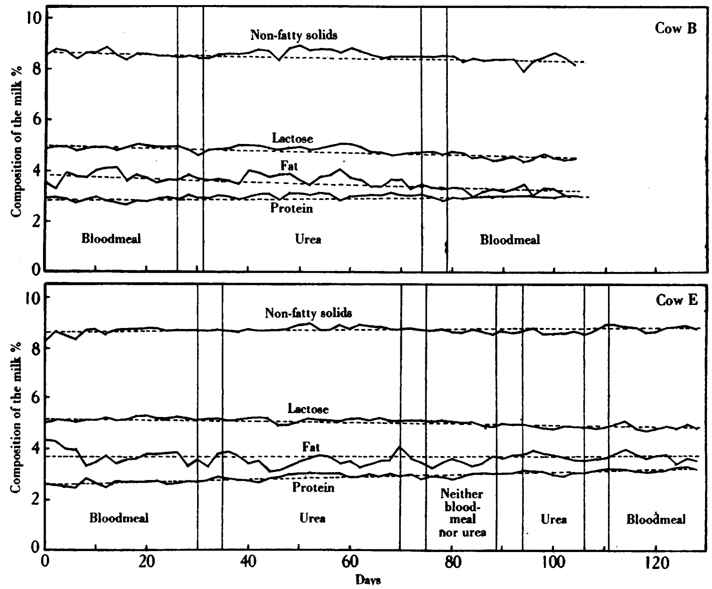

Fig. 4. The level of the major constituents of the milk throughout the various periods. The results for cow A were very similar to those obtained for cow B. The results for cow E are typical of those obtained for all the other five animals. In each instance the average values for the first and last periods have been plotted at points mid-way through these periods. The broken lines join these points.

Urea in the blood and milk

At intervals during the experiments urea and non-protein nitrogen were determined in the blood serum and milk of cows A to E and also in the milk of cows F and G. Typical results (those for cows C and E) are shown in detail in Fig. 3. It will be seen that the changes brought about by alterations in the diet were much smaller with cow C than with cow E. The average values of the blood urea for cows A to E during the first two periods are recorded in Table 6. During the urea period these exceeded those found during the blood meal period by amounts varying from 23 to 84 %. When both urea and blood meal were withheld from the diet a marked decrease in blood urea occurred (Fig. 3), and this was again followed by a substantial increase when urea feeding was resumed.

In all instances in which analyses of this type were obtained, there was a general parallelism between the concentration of N.P.N. in the blood and that in the milk, while the concentration of urea showed a similar relationship. It may be noted that the blood samples were taken within a period of minutes whereas the milk samples were representative of a complete 24 hr. secretion. The results confirm the earlier finding of Peskett [1934] that the urea content of cows' milk is almost identical with that of the blood.

In addition to these analyses, which were carried out at 5–10-day intervals, urea was also estimated on composite daily samples of the milk just before, during and after the change-over from period 1 to period 2, and also throughout period 4. Typical results are shown in Fig. 3. When urea was included in the diet immediately after blood meal feeding, the urea in the milk reached a maximum after 2–3 days. On the other hand, after period 3, when the diet was deficient in nitrogen, the time required for the same maximum to be attained was 8–10 days after the resumption of urea feeding.

Of the five cows for which blood urea values are available, the one which showed the least increase in the level of urea in period 2 (cow C) also showed the least apparent wastage, and therefore the greatest utilization of this substance (Table 6). As regards milk yield (Fig. 1 and Table 2), this cow also benefited most from the inclusion of urea in its diet and suffered most from its withdrawal. For the other animals no definite correlation could be detected between blood urea levels and the apparent wastage of urea.

The composition of the milk

To both the producer and the consumer of milk it is important that data should be available to show whether the composition of the milk is adversely affected by the ingestion of urea. In the present experiments the highest concentration of urea in the milk was roughly 28 mg./100 ml., or 0·16 g. per pint (cow D, period 4); with the remaining cows it was very considerably below this level throughout most of period 2. Such an amount of urea is without effect on the taste of the milk and could not have any adverse effect on the consumer.

The principal constituents in composite 2-day samples of the milk were also determined for the first five cows, while for the remaining two similar determinations were made at less frequent intervals throughout the experiment. Two examples, which are typical of all the results, are shown in Fig. 4. They demonstrate clearly that urea-feeding did not bring about any alteration in the general composition of the milk.

SUMMARY

1. The value of urea as a partial substitute for dairy cows has been investigated by feeding it to seven lactating animals in an amount equivalent to 33% of the nitrogen in the production ration or to 25% of the total nitrogen intake. Data for milk yield, body weight, nitrogen balance and milk composition obtained while feeding this diet have been compared with the corresponding data obtained when the animals were receiving (*a*) blood meal and no urea and (*b*) neither blood meal nor urea.

2. The milk yields of five of the seven animals were well maintained when blood meal was replaced by urea. With four of the five cows under test a rapid and significant decrease in milk yield took place when urea was removed from the food. For one of the remaining two cows the graph of the milk yield was difficult to interpret owing to an initial decrease during the first few days of urea feeding. This decrease may have been due in part to the exceptional weather conditions then prevailing. The seventh cow did not appear to benefit to any significant extent from the inclusion of urea in its food.

3. Changes in body weights were not marked at any stage of the experiment, but on the whole they seemed to be better maintained on urea than on blood meal.

4. The nitrogen balance and excretion data show that, although urea was partially retained by all the animals, its retention was not complete. Compared with blood meal, amounts varying from some 12 to 47% and averaging 25% of the ingested urea apparently passed through the animal without being utilized. This apparent wastage was much reduced when urea feeding was preceded by a period in which the diet had been deficient in total nitrogen. The quantitative importance of this failure to utilize all the dietary urea is discussed.

5. The urea content of the blood tended to increase at the outset of the urea period and then to return towards its initial value as the urea feeding progressed. The urea content of the milk closely approximated that of the blood and never exceeded 28 mg./100 ml. (0·16 g. per pint), an amount which would not have any deleterious effect on the consumer.

One cow showed very little change in the urea content of its blood and milk at any time during the experiment. This animal also appeared to utilize the ingested urea to the greatest degree and to benefit most from its inclusion in the diet.

6. The ingestion of urea had no measurable effect on the percentage of protein, fat, lactose or total solids in the milk.

The authors wish to record their thanks to Dr M. P. Applebey and Prof. A. C. Chibnall, F.R.S., for their interest and encouragement during the course of the work, and to Imperial Chemical Industries Limited for a grant towards the expenses.

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