

## **The Amino Acid Deficiencies of Certain Plant Proteins and the Supplementary Effect between Plant Proteins as measured by means of their Biological Values.**

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In previous work by the authors (1938-9) the amino acid deficiencies of different plant proteins were established by means of the paired feeding method. As was pointed out then, the paired feeding method, which measures the response caused, by the inclusion of certain amino acids in terms of growth, does not supply any information regarding the metabolic effect of these minute amounts of amino acids on the utilizability of the nitrogen complex contained in the plant proteins. In order to obtain this desired information, as well as additional proof of the amino acids limiting the nutritive value of the proteins, the biological values of the supplemented plant proteins were determined. From the combined data it is not only possible to obtain a detailed picture of the changes in nitrogen metabolism, which are caused by the absence or presence of certain indispensable amino acids, but also to apply this information in the economical nutrition of farm animals. Thus it may be known that lucerne is deficient in cystine, but unless the nitrogen conservation through the addition of cystine, and the extent to which other feeds in practice can supplement this cystine deficiency, are known, the results remain of purely academic value. For these reasons a separate study on the biological utilization of the supplemented plant proteins was undertaken which, in conjunction with the results of the paired feeding tests, will afford a scientific basis for the future selection and balancing of protein feeds in rations for livestock.

In this study the supplementary effect of methionine on lucerne, lysine on whole oats seed, cystine and methionine on peanutmeal, cystine on linseedmeal and the supplementary relationship between whole yellow maize and lucerne and soyabeans have been investigated.

That methionine has a supplementary effect on the proteins of lucerne has already been shown by the authors (1940) by means of the paired feeding method. Similarly McCollum and Simmonds (1916) and Mitchell and Smuts (1932) obtained evidence of a lysine deficiency of oats. The supplementary relationship between gelatin

and oats has been explained by McCollum and co-workers (1917) as due to the lysine content of the gelatin. Mitchell (1924), however, could not establish any supplementary relationship between oats and gelatin mixed in the proportions of 3:1 by means of their respective biological values.

A methionine deficiency has been ascribed by Beach and White (1937) and Baernstein (1938) to arachin, one of the protein components of the peanut. In paired feeding experiments by the authors (1938-39) no cystine or methionine deficiencies could be established for the proteins of peanutmeal. Indications of slight improvements when supplementing with the two amino acids were, however, observed, but were so small as to be insignificant. In the same year evidence was obtained of the cystine deficiencies of linseed and soyabean meals. Notwithstanding the amino acid deficiencies of lucerne and yellow maize Kellermann (1935) obtained normal growth on rats with a mixture of yellow maize and lucerne proteins. Since this protein mixture was not improved by the addition of 0.15 per cent. l-cystine it must be concluded that the proteins supplemented each other in respect to cystine.

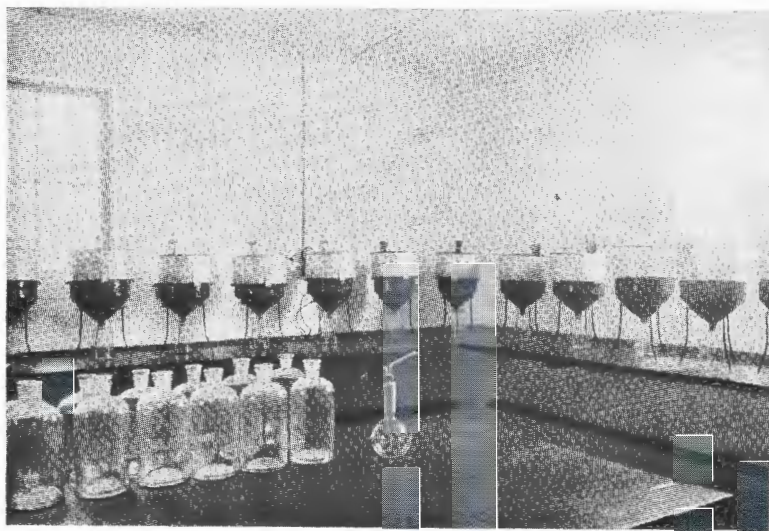


Fig. 1.

#### EXPERIMENTAL.

In determining the supplementary effect of the amino acids on the different proteins the method described by Mitchell (1924) was adopted. Young male rats were used throughout, only one biological value was determined on a series of six rats. Specially constructed earthenware metabolism cages, as illustrated (Fig. 1) were used. The rats stand on a horizontal wire screen (Fig. 2) with a  $\frac{3}{8}$  inch mesh which allows the faeces to drop through on to a second wire gauze (Fig. 3) on which it is then collected.

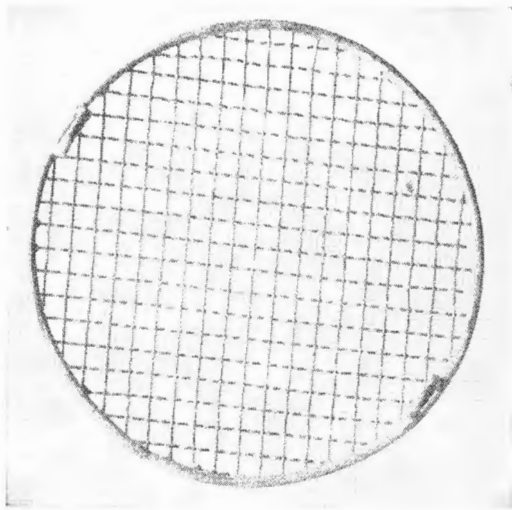


Fig. 2.

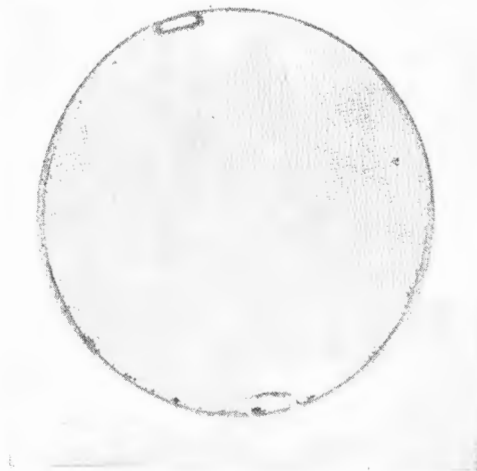


Fig. 3.

#### AMINO ACID DEFICIENCIES OF PLANT PROTEINS.

The urine and faeces were collected daily. The daily faeces collections, after careful removal of adhering hair, were digested according to the usual Kjeldahl method. The week's digests were made up to volume and analysed for nitrogen. For the urine collections the metabolism cages were washed out daily with 0.5 per cent. tartaric acid solution. The daily urine collections were kept in dilute  $H_2SO_4$  and at the end of the collection period made up to volume and a suitable aliquot digested for the nitrogen determination. To distinguish between faeces of the preliminary and collection periods  $Fe_2O_3$  was used as a marker. The collection periods were of seven days' duration. The nitrogen low period was conducted either prior to or after the protein periods. Six to seven days were allowed on the nitrogen low ration to establish constant nitrogen excretion. For the protein periods at least 10 days were allowed.

To prevent food wastages a special food basin (Fig. 4) was used.



Fig. 4.

The rations were made up so as to contain approximately 8 per cent. crude protein. The rations were stored in an ice chest to prevent deterioration. The composition of the rations is given in Table 1.

TABLE 1.  
Percentage Composition of the Rations.

	N—Low.	N—Low.	Lucerne + Methionine.	Oats + Lysine.	Peanut Cystine.	Peanut Methionine.	Linseed + Cystine.	Yellow Maize + Lucerne.	Yellow Maize + Soyabean.
Lucerne meal.....	—	—	39.3	68.2	—	—	—	20.5	—
Whole oats seed.....	—	—	—	—	—	—	—	—	—
Peanut meal.....	—	—	—	—	15.3	14.2	22.5	—	—
Linseed meal.....	—	—	—	—	—	—	—	42.1	42.1
Whole yellow maize meal.....	—	—	—	—	0.2	—	0.2	—	10.2
Soyabean meal.....	—	—	—	—	—	—	—	—	—
l-cystine.....	—	—	0.2	—	—	—	—	—	—
dl-methionine.....	—	—	—	0.2	—	—	—	—	—
d-lysine-di-hydrochloride.....	—	—	—	—	—	—	—	—	—
Whole egg white (1).....	3.8	—	—	—	—	—	—	—	—
Whole egg (2).....	—	3.8	—	—	—	—	—	—	—
Sucrose.....	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Butterfat (3).....	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Yeast Extract (4).....	10.0	—	—	—	10.0	—	—	—	—
Harris Yeast (5).....	—	2.0	2.0	2.0	—	2.0	2.0	2.0	2.0
Cod Liver Oil.....	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Salt mixture (Osborne & Mendel) (6).....	4.5	—	—	—	4.5	—	—	—	—
Salt mixture (Hübbel, etc.) (7).....	—	2.0	2.0	2.0	—	2.0	2.0	2.0	2.0
Starch (dextrinized).....	5.87	69.2	35.2	6.6	49.0	60.6	52.3	12.4	22.7
NaCl.....	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Agar.....	2.0	2.0	—	—	—	—	—	—	—
TOTAL.....	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
PERCENTAGE N.....	0.65	0.63	1.45	1.45	1.34	1.44	1.47	1.58	1.56

(1) The whole egg white has been dried on a waterbath and extracted with ether.

(2) The whole egg has been dried on a waterbath and extracted with ether.

(3) The butterfat has been filtered through a coarse filter paper to remove casein.

(4) A yeast extract prepared according to the method of Itter, S., Orent, E. R. and McCollum, E. V. (1935), *J. Biol. Chem.*, Vol. 108, pp. 571-577.

(5) Harris Yeast a preparation of The Harris Laboratories, Tuckahoe, New York.

(6) A modified Osborne and Mendel Salt mixture described by Hawk, P. B. and Osler, B. L. (1931), *Science*, Vol. 74, pp. 369.

(7) A new salt mixture described by Hübbel, R., Mendel, J. B. and Wakeman, A. J. (1937), *J. Nutr.*, Vol. 14, pp. 273-285.

## RESULTS.

From the experimental data given in Table 2, it will be seen that the biological values for lucerne +0.2 per cent. dl-methionine, whole oats seed +0.2 per cent. d-lysine-di-hydrochloride, peanutmeal +0.2 per cent. l-cystine, peanutmeal +0.2 per cent. dl-methionine, linseedmeal +0.2 per cent. l-cystine, yellow maize +lucerne and yellow maize+soyabeans are  $90 \pm 0.68$ ,  $86 \pm 1.00$ ,  $66 \pm 1.81$ ,  $78 \pm 1.53$ ,  $86 \pm 0.68$ ,  $80 \pm 0.61$  and  $75 \pm 2.77$  respectively. According to Smuts and Malan (1938) the biological values for lucernemeal, peanutmeal and linseedmeal are  $60 \pm 1.49$ ,  $72 \pm 1.61$  and  $78 \pm 1.92$  respectively, while the biological values of whole oats seed, whole yellow maize and soyabeans are according to Smuts and Marais (1938-40)  $83 \pm 2.04$ ,  $67 \pm 0.98$  and  $55 \pm 1.72$  respectively. It is, therefore, clear that the supplementation of lucerne with 0.2 per cent. dl-methionine improved the nitrogen utilization of the lucerne proteins by  $30 \pm 1.64$  per cent. This improvement is very nearly the same as that obtained previously (1938) for the supplementation with 0.2 per cent. l-cystine, namely  $28 \pm 2.46$  per cent. This enhancement in the biological value of the supplemented protein verifies the previous results (1939) obtained by means of the paired feeding method.

The supplementation of the proteins of oats seed with 0.2 per cent. d-lysine-di-hydrochloride gave only a slight increase of  $3 \pm 2.27$ , which signifies no significant improvement and the lysine deficiency of oats must be regarded as only of very little importance. The biological value of peanutmeal supplemented with cystine definitely proves that the protein of peanutmeal is not deficient in cystine, since supplementation even lowered the biological value. The result of the methionine supplementation on the other hand indicates that the proteins of peanutmeal may be deficient in methionine as a  $6 \pm 2.22$  per cent. increase in the nitrogen utilization was obtained. This would seem to be in agreement with the findings of Beach and White (1937) and Baernstein (1938) on the methionine deficiency of arachin.

The increased nitrogen utilization of  $8 \pm 2.04$  per cent., caused by the supplementation of linseedmeal with 0.2 per cent. l-cystine agrees very well with the results of Smuts and Marais (1938) by means of the paired feeding method and definitely establish the cystine deficiency of linseedmeal.

If no supplementary relationship existed between the proteins of yellow maize and lucerne and yellow maize and soyabeans, the mean biological values of the protein mixtures would have been  $64 \pm 1.26$  and  $61 \pm 1.40$  respectively. It will be seen, however, that the biological values are actually  $80 \pm 0.61$  and  $75 \pm 2.77$  for the protein mixtures of yellow maize+lucerne and yellow maize+soyabeans. The differences obtained namely  $16 \pm 1.40$  and  $14 \pm 3.10$  definitely prove that there exists a supplementary relationship between these proteins. The supplementations are perfectly in harmony with the respective amino acid deficiencies of the proteins. The proteins of yellow maize are deficient in lysine and tryptophane whereas the proteins of lucerne and soyabeans are markedly deficient

in cystine. It is, therefore, clear that the maize proteins can supplement the cystine deficiency of lucerne and soyabeans, while the proteins of lucerne and soyabeans can again supplement the lysine-tryptophane deficiency of the maize proteins.

It is evident that the present data on the amino acid deficiencies of the different proteins may serve as a basis for predicting any possible supplementary relationship between mixed proteins.

#### SUMMARY AND CONCLUSIONS.

The supplementary effect of methionine on lucerne, lysine on oats seed, cystine and methionine on peanutmeal, cystine on linseedmeal as well as the supplementary relationship between yellow maize and lucerne and of yellow maize and soyabeans have been determined by means of their biological values. From the results obtained it is concluded:—

1. That 0.2 per cent. dl-methionine increased the nitrogen utilization of the lucerne proteins by  $30 \pm 1.64$  per cent.
2. Cystine has no supplementary effect on peanutmeal, while methionine improves the protein to a slight extent.
3. Cystine supplementation increases the nitrogen utilization of linseedmeal by  $8 \pm 2.04$  per cent.
4. Lysine supplementation has no significant effect on the proteins of whole oats seed.
5. The proteins of whole yellow maize and lucerne and of whole maize and soyabeans supplement each other in a marked and significant manner.

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TABLE 2.  
*Nitrogen Metabolism Data and the Calculation of*  
*N-low period.*

Rat Number.	Initial Weight.	Final Weight.	Average Weight.	Daily Food Intake.	Daily N Intake.	Daily Faecal N.	METABOLIC N.	
							Per Gram Food.	Per Day.
	Gm.	Gm.	Gm.	Gm.	Mgm.	Mgm.	Mgm.	Mgm.
1.....	115	124	120	9.9	—	34.2	3.45	—
2.....	95	96	96	8.0	—	27.2	3.40	—
3.....	87	87	87	7.7	—	24.2	3.14	—
4.....	116	112	114	8.1	—	29.0	3.58	—
5.....	90	90	90	5.9	—	18.6	3.15	—
6.....	92	92	92	6.3	—	22.0	3.49	—

*Lucerne + 0.2 per cent. dl-methionine period*

1.....	153	163	158	12.7	184.2	78.4	3.45	43.8
2.....	134	146	140	12.2	176.9	70.8	3.40	41.5
3.....	125	137	131	13.0	188.5	56.8	3.14	40.8
4.....	149	161	155	13.1	190.0	74.0	3.58	46.9
5.....	132	148	140	13.1	190.0	74.8	3.15	41.3
6.....	125	141	133	13.1	190.0	70.4	3.49	45.7

*N-low period.*

1.....	100	104	102	9.7	—	30.4	3.13	—
2.....	128	132	130	12.0	—	41.0	3.42	—
3.....	140	142	141	10.7	—	39.8	3.72	—
4.....	127	129	128	10.9	—	32.6	2.99	—
5.....	135	133	134	11.9	—	43.0	3.61	—
6.....	140	138	139	10.7	—	39.4	3.68	—

*Oats seed + 0.2 d-lysine-di-hydrochloric period*

1.....	100	104	102	11.0	159.5	38.0	3.13	34.4
2.....	115	135	125	13.6	197.2	54.0	3.42	46.5
3.....	132	152	142	14.9	216.1	59.2	3.72	55.4
4.....	112	134	123	13.2	220.4	47.6	2.99	45.4
5.....	125	135	130	12.9	187.1	50.4	3.61	46.6
6.....	127	142	135	14.3	207.4	53.6	3.68	52.6

233-234b →

TABLE 2.  
*and the Calculation of the Biological Value.*  
*N-low period.*

Food N in Faeces.	Absorbed N.	Daily Urinary N.	ENDOGENOUS N.		Food N in Urine.	N Retained.	N Balance.	Biological Value.
			Per 100 Gram Weight.	Per Day.				
Mgm.	Mgm.	Mgm.	Mgm.	Mgm.	Mgm.	Mgm.	Mgm.	—
—	—	21.0	17.5	—	—	—	—	—
—	—	18.3	19.1	—	—	—	—	—
—	—	20.5	23.6	—	—	—	—	—
—	—	19.0	16.7	—	—	—	—	—
—	—	18.3	20.3	—	—	—	—	—
—	—	22.8	24.8	—	—	—	—	—

*methionine period (1.45 per cent. N).*

34.6	149.6	40.8	17.5	27.7	13.1	136.5	+65.0	91
29.3	147.6	40.5	19.1	26.7	13.8	133.8	+65.6	91
16.0	172.5	47.0	23.6	30.9	16.1	136.4	+84.7	91
27.1	162.9	47.0	16.7	25.9	21.1	141.8	+69.0	87
33.5	156.5	42.7	20.3	28.4	14.3	142.2	+72.5	91
24.7	165.3	47.6	24.8	33.0	14.6	150.7	+72.0	91
								90

*N-low period.*

—	—	24.4	23.9	—	—	—	—	—
—	—	28.4	21.8	—	—	—	—	—
—	—	27.6	19.6	—	—	—	—	—
—	—	27.6	21.6	—	—	—	—	—
—	—	28.8	21.5	—	—	—	—	—
—	—	24.4	17.6	—	—	—	—	—

*hydrochloric period (1.45 per cent. N).*

3.6	155.9	52.4	23.9	24.4	28.0	127.9	+69.1	82
7.5	189.7	55.6	21.8	27.3	28.3	161.4	+87.6	85
3.8	212.3	56.0	19.6	27.8	28.2	184.1	+100.9	87
2.2	218.2	52.0	21.6	26.6	25.4	192.8	+120.8	88
3.8	183.3	50.0	21.5	28.0	22.0	161.3	+86.7	88
1.0	206.4	47.6	17.6	23.8	23.8	182.6	+106.2	88
								86

← 233-234a

TABLE 2 (A).

*Nitrogen Metabolism Data and the Calculation of  
N-low period.*

Rat Number.	Initial Weight.	Final Weight.	Average Weight.	Daily Food Intake.	Daily N Intake.	Daily Faecal N.	METABOLIC N.	
							Per Gram Food.	Per Day.
	Gm.	Gm.	Gm.	Gm.	Mgm.	Mgm.	Mgm.	Mgm.
7.....	82	82	82	6.7	—	26.4	3.94	—
8.....	103	100	102	7.0	—	23.4	3.34	—
9.....	102	102	102	6.7	—	25.2	3.76	—
10.....	85	90	88	6.6	—	24.8	3.76	—
11.....	90	88	89	7.0	—	25.2	3.60	—
12.....	95	89	92	8.3	—	21.6	2.60	—

*Linseedmeal + 0.2 per cent. l-cystine (1.*

7.....	109	111	110	7.8	114.7	32.6	3.94	30.7
8.....	132	142	137	10.8	158.8	51.5	3.34	36.1
9.....	139	149	144	10.9	160.2	48.3	3.76	41.0
10.....	120	124	122	8.9	130.8	39.9	3.76	33.5
11.....	130	136	133	8.8	129.4	44.1	3.60	31.7
12.....	125	133	129	10.9	160.2	49.7	2.60	28.3

235-236b



TABLE 2 (A).

and the Calculation of the Biological Value.

N-low period.

Food N in Faeces.	Absorbed N.	Daily Urinary N.	ENDOGENOUS N.		Food N in Urine.	N Retained.	N Balance.	Biological Value.
			Per 100 Gram Weight.	Per Day.				
Mgm.	Mgm.	Mgm.	Mgm.	Mgm.	Mgm.	Mgm.	Mgm.	—
—	—	13.3	13.3	16.2	—	—	—	—
—	—	15.0	14.7	—	—	—	—	—
—	—	13.0	12.7	—	—	—	—	—
—	—	13.5	15.4	—	—	—	—	—
—	—	13.0	14.6	—	—	—	—	—
—	—	14.8	16.1	—	—	—	—	—

cent. l-cystine (1.47 per cent. N).

1.9	112.8	30.8	16.2	17.8	13.0	99.8	+ 51.3	88
5.4	143.4	42.4	14.7	20.1	22.3	121.1	+ 64.9	84
7.3	152.9	41.0	12.7	18.3	22.7	130.2	+ 70.9	85
6.4	124.4	33.3	15.4	18.8	14.5	109.9	+ 57.6	88
2.4	117.0	36.1	14.6	19.4	16.7	100.3	+ 49.2	86
21.4	138.8	42.0	16.1	20.8	21.2	117.6	+ 68.5	85
								86

← 235-236a

TABLE 2 (1)  
*Nitrogen Metabolism Data and the Calculated*  
*N-low period*

Rat Number.	Initial Weight.	Final Weight.	Average Weight.	Daily Food Intake.	Daily N Intake.	Daily Faecal N.	METABOLIC N.	
							Per Gram Food.	Per Day.
	Gm.	Gm.	Gm.	Gm.	Mgm.	Mgm.	Mgm.	Mgm.
1	88	88	88	5.5	—	15.9	2.89	—
2	87	83	85	6.0	—	21.4	3.57	—
3	97	92	95	6.8	—	23.5	3.46	—
4	98	92	95	5.3	—	20.2	3.81	—
5	95	91	93	5.6	—	21.0	3.75	—
6	90	92	91	7.3	—	26.8	3.67	—

*Whole Yellow Maize meal + Lucerne meal*

1	103	111	107	8.2	129.6	35.4	2.89	23.7
2	111	124	118	11.1	175.4	47.8	3.57	39.6
3	116	130	123	11.1	175.4	49.4	3.46	38.4
4	112	128	120	11.1	175.4	54.0	3.81	42.3
5	111	130	121	11.6	183.3	50.3	3.75	43.5
6	123	138	131	12.3	194.3	57.7	3.67	45.1

*N-low period*

1	114	111	113	7.9	—	30.0	3.80	—
2	120	120	120	6.2	—	22.4	3.61	—
3	113	114	114	9.6	—	36.0	3.75	—
4	116	110	113	6.7	—	29.6	4.42	—
5	110	108	109	7.5	—	28.0	3.73	—
6	123	121	122	10.4	—	38.0	3.65	—

*Whole Yellow Maize meal + Soyabean meal*

1	120	118	119	5.5	85.8	23.6	3.80	20.9
2	123	129	126	6.1	95.2	27.6	3.61	22.0
3	112	119	116	10.0	156.0	53.6	3.75	37.5
4	113	115	114	5.8	90.5	30.4	4.42	25.6
5	114	115	115	7.5	117.0	34.8	3.73	28.0
6	118	121	120	8.9	138.8	53.6	3.65	32.5

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TABLE 2 (B).

*Data and the Calculation of the Biological Value.**N-low period.*

Food N in Faeces.	Absorbed N.	Daily Urinary N.	ENDOGENOUS N.		Food N in Urine.	N Retained.	N Balance.	Apparent Digestibility.	True Digestibility.	Biological Value.
			Per 100 Gram Weight.	Per Day.						
Mgm.	Mgm.	Mgm.	Mgm.	Mgm.	Mgm.	Mgm.	Mgm.			
—	—	13·6	15·5	—	—	—	—	—	—	—
—	—	14·0	16·5	—	—	—	—	—	—	—
—	—	16·1	16·9	—	—	—	—	—	—	—
—	—	16·9	17·8	—	—	—	—	—	—	—
—	—	11·9	12·8	—	—	—	—	—	—	—
—	—	14·8	16·3	—	—	—	—	—	—	—

*meal + Lucerne meal period (1·58 percent. N).*

11·7	117·9	42·0	15·5	16·6	25·4	92·5	+ 52·2	73	91	78
8·2	167·2	54·1	16·5	19·5	34·6	132·6	+ 73·5	73	95	79
11·0	164·4	50·5	16·9	20·8	29·7	134·7	+ 75·7	72	94	82
11·7	163·7	52·3	17·8	21·4	30·9	132·8	+ 69·1	69	93	81
6·8	176·5	48·2	12·8	15·5	32·7	143·8	+ 84·8	73	96	81
12·6	181·7	57·2	16·3	21·4	35·8	145·9	+ 79·4	70	94	80
								72	94	80

*N-low period.*

—	—	18·8	16·6	—	—	—	—	—	—	—
—	—	17·2	14·3	—	—	—	—	—	—	—
—	—	21·6	18·9	—	—	—	—	—	—	—
—	—	25·6	22·7	—	—	—	—	—	—	—
—	—	14·4	13·2	—	—	—	—	—	—	—
—	—	23·2	19·0	—	—	—	—	—	—	—

*meal + Soyabean meal period (1·56 per cent. N).*

2·7	83·1	40·4	16·6	19·8	20·6	62·5	+ 21·8	73	97	75
5·6	89·6	38·2	14·3	18·0	20·2	69·4	+ 29·4	71	94	77
16·1	139·9	69·6	18·9	21·9	47·7	92·2	+ 33·0	66	90	66
4·8	85·7	39·1	22·7	25·9	13·2	72·5	+ 21·0	66	95	85
6·8	110·2	39·4	13·2	15·2	24·2	86·0	+ 42·8	70	94	78
21·2	117·6	59·3	19·0	22·8	36·5	81·1	+ 25·9	61	85	69
								68	93	75

← 237-238a

237-238b