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ON A METHOD OF ESTIMATING NUTRIENT REQUIREMENTS IN METABOLISM STUDIES ON ANIMALS

By P. V. Sukhatme, S. S. Prabhu and V. N. Amble

Indian Council of Agricultural Research, New Delhi

A number of metabolism experiments have been carried out in this country primarily with the object of estimating nutrient requirements but the statistical studies that have been carried out on such data do not usually go beyond the recording of the balances. It is the object of this note to attempt a statistical analysis of data from one such study with a view to (*a*) indicating a statistical method of estimating nutrient requirements and (*b*) suggesting possible modifications in the planning of these experiments. The study has been carried out on the calcium metabolism data collected under the Coimbatore Animal Nutrition Scheme conducted under the auspices of the Indian Council of Agricultural Research during the period 1935–43.

2. Four series of experiments namely A, B, C and D were conducted under the scheme. Experiments in Series A were conducted on five crossbred cows, B on six Kangayam heifers, C on six crossbred heifers and D on five of the animals in C after they had attained maturity. Series A consisted of 5 experiments, B of 23, C of 10 and D of 11. The method used for collection of data was the familiar 'balance' technique. Before the commencement of each experiment the animals were put through a preliminary period of seven days during which they received the experimental rations. Green fodder and straw were allowed *ad lib*, while the concentrates were adjusted according to body weight and condition of individual animals. Each experiment consisted of recording for each animal the amounts of feed consumed, the excreta

voided, and in the case of a milking cow quantity of milk delivered on each of four consecutive days. The feed, the excreta and the milk were chemically analysed to obtain the amounts of intake and outgo of calcium and phosphorus for each day. The excess or deficit of daily intake over the corresponding outgo constituted the balance. As has already been observed the present study has been confined to calcium data alone.

3. Before proceeding with the statistical analysis of the data it will be useful to examine the general features and limitations of the plan of experimentation. Table I shows the brand numbers and the breed of the animals used in each series of experiments, the date of commencement of each four-day experiment, the condition of the animals at the time of each experiment and the average age of the animals. The animals selected for each series of experiments were more or less of the same age but not necessarily of the same breed. Whereas the heifers in the series of experiments B were all of one breed, *viz.*, the Kangayam, the animals in the other series included several crossbreds. Again the interval between successive four-day experiments within a period in the life cycle of the animals, such as gestation, lactation or dry period was not regular. It is seen to vary from twenty days to over half a year. Lastly the roughage in the feed, while remaining unchanged within a given experimental period of four days, varied from experiment to experiment. Space does not permit us to reproduce the whole of the feed table, but we reproduce below two portions of the table to illustrate the point:—

SERIES B			SERIES C		
Experiment No.	Type of green fodder	Type of dry fodder	Experiment No.	Type of green fodder	Type of dry fodder
1	Ragi		6	Guinea grass	Cholam straw
2	{Guinea grass		7	Thellai	Cholam straw
	{Silage		8	Cholam	Cholam straw
3	Guinea grass		9	Maize	Cholam straw
4	Maize		10	Cholam	Cholam straw
5	Maize	Paddy straw			
6	Cholam				

It may be added, however, that the supplement in the form of the concentrate mixture consisting of groundnut cake, cotton seed, rice bran, dhall husk, and a mineral mixture with salt remained practically the same throughout the series of experiments. The implications of these observations will become clear as we proceed with the study.

4. In calculating the calcium requirements from the intake and outgo figures we have proceeded on the basis of the definition of requirement as given by Leitch and Duckworth (1937), *viz.*, that the requirement is that amount of the mineral which is just sufficient to keep an animal which is in normal health in mineral equilibrium under stated conditions of experimentation. In other words, requirement is that value of the intake which gives the least positive—ideally zero balance under the specified experimental conditions. The last proviso is essential as the requirement is known to depend among other things upon the condition of the animal and the nature and quantity of feed. This point will however be discussed at length at a later stage.

The average outgo itself is often taken as the requirement. This cannot be taken to be correct *a priori* since it ignores the obvious fact that the outgo depends on the intake. On the other hand, a direct determination of the value of the requirement, in the sense defined above, by means of experimentation, by contriving to make the balance zero is also not feasible, as all the balances are subject to uncontrollable fluctuations, due in part to endocrine activity. The consideration, however, that the outgo bears a relationship to the intake and is yet subject to fluctuations can be utilised to offer a solution of the problem of estimating the requirement. The relationship can be looked upon as a statistical one and can be approximated to by means of a regression of outgo on intake based on repeated balance studies. The regression curve having been determined the requirement can be found as the minimum value of the intake for which it is equalled by the regression estimate of the value of the outgo.

If, for instance, the regression is linear, it may be expressed by the usual equation

$$Y = \bar{y} + b(x - \bar{x}), \tag{1}$$

where y denotes the outgo, x the intake, $\bar{y}$ the average outgo, $\bar{x}$ the average intake and b, the regression coefficient of y on x. The line of equal intake and outgo is

$$y = x \tag{2}$$

The requirement R is the point of intersection of (1) ana (2) and is therefore given by

$$R = \frac{\bar{y} - b\bar{x}}{1 - b} \tag{3}$$

The value of the requirement so arrived at is only an estimate and is therefore subject to errors of random sampling. An idea of the magnitude of its standard error is consequently necessary for judging its accuracy. It can be shown that an estimate of the variance of R is given by the formula:

$$V(\mathrm{R}) = \left\{\frac{1}{n(1-b)^2} + \frac{(\bar{y}-\lambda)^2}{S(x-\bar{x})^2 \cdot (1-b)^4}\right\} s^2 \qquad (4)$$

where s^2 denotes the residual variance of y after adjustment for regression, and n the number of pairs of observations of intake and outgo. The square root of (4) gives an estimate of the standard error of the requirement. In the case of the Coimbatore data, the assumption involved in the above method of estimating the requirement, *viz.*, that the observations of intake are independent, is perhaps justifiable as the straw was fed *ad lib.* At any rate, we found no evidence of significant correlation between successive observations in any four-day experimental period. The regression of outgo on intake being approximately linear, the formulæ (3) and (4) were utilised in obtaining estimates of calcium requirements per diem for each animal during the various four-day experimental periods and their standard errors. These are shown in Table II. The figure in the first line of each cell gives the estimated requirement and that in the second line gives its percentage standard error; the figures in *italics* give alternative estimates of requirement described as cubic estimates obtained by a method explained later on in this paper.

5. The figures in the table indicate the extent to which calcium requirements vary not only from experiment to experiment but also from animal to animal. An unhappy feature of the table, however, is the fact that in a number of cases it was not possible to obtain satisfactory estimates of requirement. For some experiments, it was not possible at all to work out the estimates of requirement. This was the case with some experiments like Nos. 4, 17, 18 on heifer No. 351, and Nos. 8, 17 and 18 on heifer No. 353 in Series B as the reported values of the intakes of calcium were identical on all the days of the experiments. Since in such cases no study of the variation of outgo with intake could be made, the data could not be utilised in any manner to obtain estimates of requirement by the regression method. In the remaining cases in which requirement could not be estimated, as for example in experiment No. 3 on animal No. 285 in Series A and in experiment No. 2 on animal No. 382 in Series D, the regression method outlined above yielded either unreasonably large positive values or sometimes negative values of the estimates, and thus rendered them devoid of any physical meaning. There were other experiments again for which, although the numerical data admitted of estimation by the linear regression method, the estimates obtained were of such poor

precision, as can be seen from the high values of the corresponding percentage standard errors of requirements, that it became superfluous to examine whether the estimates happened to be in conformity with expectations or were far wide off them. Experiment No. 10 on animal No. 354 and experiments No. 2, 8, 16 and 20 on animal No. 355 in Series B, experiments No. 2, 7, and 9 on animal No. 687 in Series C, and experiment No. 4 on each of animals No. 381, 382 and 385 in Series D may be cited as instances in point. In these cases it is obvious that the failure to obtain satisfactory estimates of requirement must be attributed to the non-validity of some of the assumptions made in employing the linear regression method of estimation. The possibility of errors of measurement and recording being responsible in some instances for these discrepancies cannot, however, be ruled out.

Another point which should be considered in this connection is the fact that in large animals, particularly the ruminants, the process of digestion of ingested food takes several days, and consequently the outgo on a particular day may depend not only on the intake of that day but also on those of the previous days. It may therefore be more appropriate to fit the regression of outgo on the intake of the corresponding day as well as those of the previous days. Such a study would throw light on the gain in efficiency of the partial regression method on the procedure adopted here. It could be undertaken, however, only if the values of the previous daily intakes were available and the observations were numerous enough to permit the fitting of the regression line with a fair degree of precision. Obviously the four pairs of observations available per experiment were too few to permit us to examine this point. The adequacy or otherwise in the present study of the method of linear regression of outgo on the single independent variable, *viz.*, the corresponding intake can be judged by pooling the data together and examining (*a*) the extent of variation removed by regression and (*b*) the percentage of experiments in which the residual variance was at all less than the total variance. The results are given in the table below. It will be seen that in the case of milking animals alone has there been a significant reduction in variance due to regression and that only in about 40 per cent. of the experiments was the method emplcyed successful in reducing the variance. In the absence of more extensive data no attempt could be made to see if a more satisfactory result could have been achieved by employing as independent variates not only the intake of the corresponding day but those of previous days also.

Table showing the extent of reduction in variance due to linear regression

		D.F.	M.S.	F	Percentage of expts. where residual variance ⩽ Total variance
Heifer ..	Regression	54	22·81	1·24	43·6
	Deviation	107	18·34		
Milking ..	Regression	52	139·07	1·49*	42·1
	Deviation	104	93·15		
Milking & Pregnant	Regression	4	9·28	..	25·0
	Deviation	8	22·21		
Dry ..	Regression	68	42·11	..	30·4
	Deviation	136	104·62		
Dry & Pregnant	Regression	57	31·72	1·16	42·5
	Deviation	114	27·45		

* Indicates significance at 5 per cent. level of probability.

6. An alternative course was to employ a regression curve of a higher degree. As the fitting of a second degree parabola would have left us with only a single degree of freedom for estimating the precision of the estimated requirement it was decided to ignore altogether the daily variation in the observations arising out of random causes and to estimate the requirements in these cases by fitting with the four pairs of observations, the outgo (y) as a cubic of the intake (x) and finding the point of intersection of the cubic so fitted,

$$y = a' + b'x + c'x^2 + d'x^3 \qquad (5)$$

with the line of equal intake and outgo:

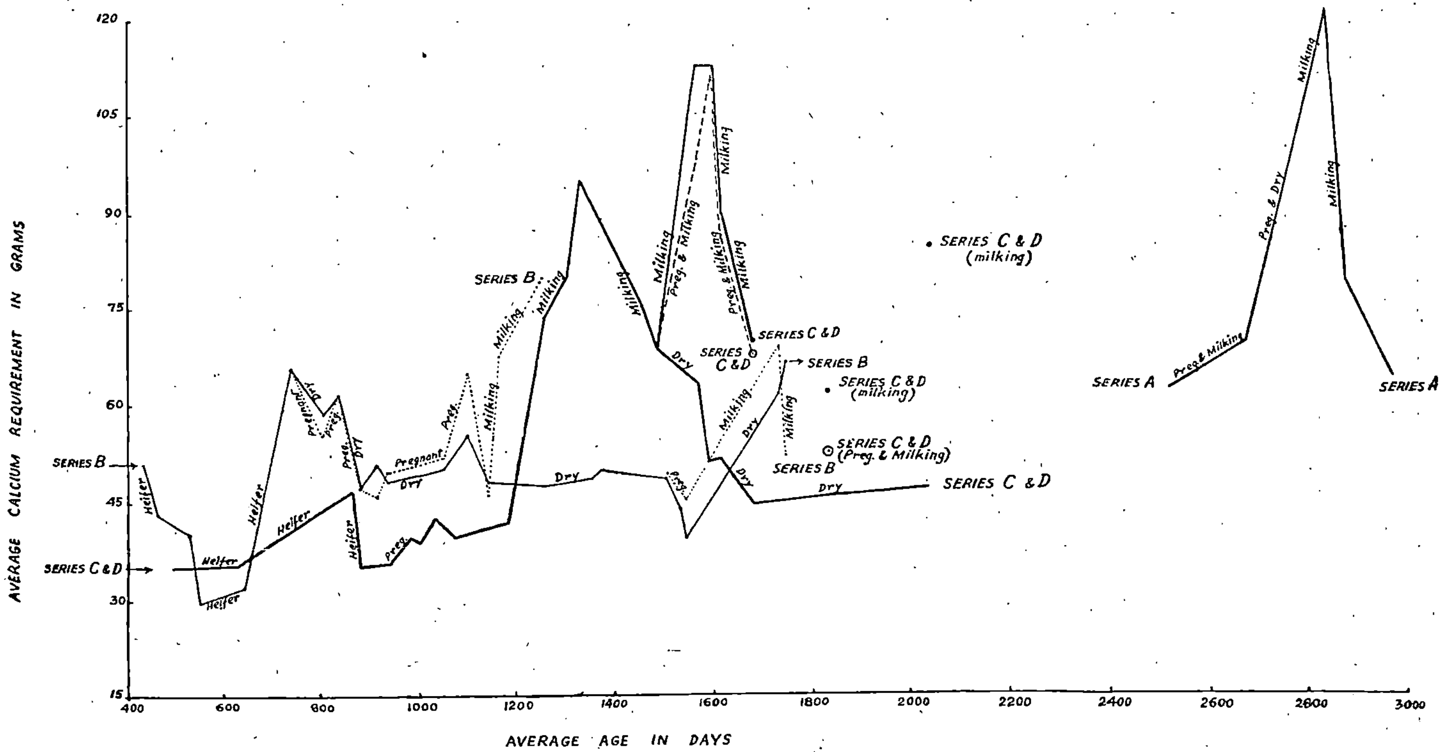
$$y = x.$$

In case there happened to be three points of intersection, that point which gave the least positive value was taken to correspond to the estimated requirement in accordance with the definition of requirement already given. These 'cubic' estimates have been shown in *italics* in Table II.

These estimates were found to be closer to the values of outgo than the corresponding linear regression estimates, and for this reason were considered to be more satisfactory. For, one would expect that if animals are in normal condition and are fed on balanced diet, as they were in these experiments, the requirements would not deviate largely from the values of outgo. The method naturally suffers from the defect that it takes no account of the sampling fluctuations in the day to day observations and it fails altogether in cases wherein even two of the intake values happen to coincide as in experiment No. 10 on Kangayam heifer No. 354 in Series B. This is inevitable because the assumptions underlying the method amount to visualising a mathematical and not statistical relationship between intake and outgo. The method has, however, the merit of using all the information at one's disposal in estimating the requirement and in this sense is superior to the usual method of taking the average outgo as the estimated requirement. We do not recommend this method for general adoption; it has been adopted here solely in the compelling circumstance that only four pairs of observations were available for each experiment. Had the experiments been conducted for a greater number of days, say ten, the regression method (not necessarily linear) would have been applicable in all cases.

7. An examination of Table II shows that although the estimated requirements vary for animals in the same series and the same phase of life-cycle, the trend of the estimated requirement with the phase of life-cycle is broadly similar. In Table II are shown the average values of the estimated requirement separately for the several phases of life-cycle for each series. The average values were calculated from the linear regression estimates of requirement where they appeared to be satisfactory and from the cubic estimates in the remaining cases. These have been plotted in the appended diagram against the average ages of the animals concerned at the dates of commencement of the experiments. Since the experiments in Series D were conducted on the very animals which were earlier employed in Series C the curves for the two series have been together. The curves indicate how the calcium requirements of dairy stock go on changing with the change in their 'condition' and the general trend of the curves is in conformity with facts already known, *viz.*, that the requirement increases appreciably with the commencement of milking reaching a maximum sometime between the third and the fifth month of lactation and then falls off as the lactation advances and is perhaps the least in the dry period. The early phase of gestation does not cause any appreciable change in requirement but in its last phase the amount of calcium needed increases.

8. While the overall picture thus presented is in accord with common observations, it is not possible to draw specific conclusions regarding the actual calcium requirements of the dairy stock at different well-marked stages in their life-cycle. The main reason for this is the fact brought out already in an earlier paragraph that the feed of the stock has varied from experiment to experiment. The utilisation of dietary calcium is known to be influenced by a number of factors, such as type of feed, condition of animal, breed, season, etc. Even if some of these factors which render the estimates from successive experiments non-


AVERAGE CALCIUM REQUIREMENT IN GRAMS
AVERAGE AGE IN DAYS
120
105
90
75
60
45
30
15
400
600
800
1000
1200
1400
1600
1800
2000
2200
2400
2600
2800
3000
SERIES B
SERIES C & D
Helfer
Helfer
Helfer
Helfer
Helfer
Pregnant
Preg.
Preg.
Dry
Pregnant
Dry
Preg.
Milking
Milking
Milking
Milking
Milking
Preg. & Milking
Preg. & Milking
Milking
Dry
Dry
Dry
Milking
Dry
Milking
Dry
SERIES B
SERIES C & D
SERIES C & D
SERIES B
SERIES C & D (milking)
SERIES C & D (milking)
SERIES C & D (Preg. & Milking)
SERIES B
SERIES C & D
SERIES A
Preg. & Milking
Preg. & Dry
Milking
Milking
SERIES A

comparable were ignored, the variation in type of feed, caused by the change in roughage, appears to be too great to be overlooked (*vide* experiments No. 3 to 6 in Series B in Table II B). It appears therefore that the requirement can be estimated only in the context of the type of feed given. If, on the other hand, the variation of calcium requirement with changes in feed were desired to be investigated, the proper layout would be what is commonly known as the switchover trial wherein each set of animals is given, in the course of a series of experiments, all the different types of feed under study in a cyclic order, different sets being given different orders of the cycle. This design, if employed with due precautions such as making allowance for the carry-over effect of the previous feed by having an adequate preliminary period of experimental feeding, could be effectively utilised in eliminating from the errors of the feed comparisons, the component variation due to the inherent differences existing between the sets of animals employed.

9. The next point in a study of the present nature would be to consider how best the individual estimates of calcium requirements may be combined to obtain the average values of requirements of calcium for animals of a given breed, on a specified feed, at different stages of their life-cycle. There are two distinct ways of estimating the average values. We may pool the observations (of daily intake and outgo) over all animals for the same four-day experimental period, calculate the pooled estimate of regression of outgo on intake and thence estimate the average requirement. Alternatively, we may find first the individual calcium requirements for different animals and take an appropriate weighted mean of these to obtain the average value. The first method or the second would be appropriate according as the pooling of observations is justifiable or not. Before proceeding, therefore, to find the average value, we have to test for the homogeneity of the sets of observations made on different animals.

For the purpose of illustrating these points, the group of experiments No. 12 to No. 23 on Series B of Kangayam cows has been chosen because it was the only long set of successive experiments during which the feed (dry cholam straw together with the usual concentrate mixture) remained unaltered in type, and in addition, the animals were all of the same breed (Kangayam), more or less of the same age, and in the stage of life-cycle (dry period). An attempt was made accordingly to test the significance of the departure of the observations from the hypothesis that all the k samples (each consisting of 4 pairs of observations of daily intake and outgo) corresponding to the k animals employed in the experiment came from one and the same normal population of y's (the values of the outgo) distributed about their expectations (given by the linear regression of y on x, the intake value) with the same residual variance (*cf.* Welch, 1935). The test failed to reveal heterogeneity between the samples for any of the experiments under study. The observations on different animals were therefore pooled and the linear regression of outgo on intake was estimated after eliminating the differences between animals by means of analysis of covariance. The value of the regression coefficient so obtained was used along with the pooled average values of intake and outgo to estimate the 'pooled' average calcium requirement per diem for each experiment. The standard errors of these estimates were also worked out in the manner described already. These values are given in Table III.

For the sake of comparison and in order to illustrate the procedure to be followed in case the sets of observations for different animals turned out to be heterogeneous, the weighted average estimates of calcium requirement were also obtained for the same group of experiments. The individual estimates of requirement were weighted with the reciprocals of the estimates of their variances to obtain the average value. In other words, if $R_1, R_2 \ldots . R_k$ denoted the linear regression estimates of requirement of the k animals employed in an experiment, and $V(R_i)$ or V_i denoted the variance of R_i, the weighted average $\overline{R}_w$ was found from the formula

$$\overline{R}_w = \frac{\sum_{i=1}^{k} \left(\frac{R_i}{V_i}\right)}{\sum_{i=1}^{k} \left(\frac{1}{V_i}\right)}. \tag{6}$$

These values also are given in Table III. An estimate of the standard error of such an average can be had from the formula for the variance given below:

$$V(\overline{R}_w) = \frac{1}{\left(\sum_{i=1}^{k} \frac{1}{V_i}\right)} + \frac{1}{\left(\sum_{i=1}^{k} \frac{1}{V_i}\right)^4} \cdot \sum_{i=1}^{k} \left[\left\{ \frac{1}{V_i^4} \left(\sum_{j=1}^{k} \left(\frac{R_i - R_j}{V_j} \right)^2 \right\} \right. \right.$$
$$\left. \left. \times \left\{ \frac{2V_i^2}{n_i - 2} + \frac{4V_i s_i^2}{A_i (1 - b_i)^2} \left(4V_i - \frac{3s_i^2}{n_i (1 - b_i)^2} \right) \right\} \right] \tag{7}$$

where for the ith sample: n_i denotes the size of sample, $\overline{x}_i$ and $\overline{y}_i$ denote the means of x and y, b_i the regression of y on x, s_i^2 the residual variance of y, R_i the estimated requirement, V_i^2 the estimate of the variance of R_i given by (4), and $A_i = S(x_{ij} - \overline{x}_i)^2$.

The first term on the right-hand side of the formula would have given us the value of the variance of the weighted average had the true weights been known, these being used in place of the reciprocals of V_i's in the first term. The additional term represents the approximate value of the correction which is needed owing to the fact that the weights employed have themselves been estimated from the data and are therefore subject to sampling fluctuations. As is readily seen from the nature of the formula the calculation of these standard errors is rather cumbersome. Moreover, the formula is not strictly applicable to our data because the V_i's are found to vary very widely and the smallest value of V_i dominates the standard error as estimated from the formulæ. These standard errors are not therefore shown in the table. It is however interesting to note that the weighted estimates of average requirements agree well with those from the pooled data.

Summary

A method of evaluating nutrient requirements in metabolism studies on animals, based on the concept of statistical regression of outgo on intake, has been described, and discussed with reference to the calcium data on dairy stock collected under the Coimbatore Animal Nutrition Scheme during 1935–43. Difficulties in applying this method to these and similar data have been brought out and suggestions have been made regarding certain precautions to be observed and essential features to be kept in view in designing such experiments for obtaining satisfactory estimates of requirement. Lastly the question of combining the results of such experiments on a number of animals for estimating the average requirements has also been considered.

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TABLE I A

Showing the particulars regarding the animals in Series 'A'

Expt. No.	Date of commencement of experiment	ANIMAL NUMBERS AND BREED				
		279 Ayr. Sindhi	281 Ayr. Sindhi	285 Hol. Ayr. His. Sindhi	285-L.J. Ayr. Montgomery	289 Ayr. Sahiwal
		Condition of animals				
1	16-3-36	Pregnant and Milking	Pregnant and Milking	Pregnant and Milking	Pregnaut and Milking	X
2	17-8-36	Pregnant and Dry	Pregnant and Dry	Pregnant and Dry	X	Pregnant and Dry
3	24-1-37	In lactation	In lactation	In lactation	In lactation	X
4	7-3-37	X	,,	,,	,,	In lactation
5	13-6-37	X	,,	,,	,,	,,

* Average age at the commencement of the series of experiments: 6 yrs. 10 months. X—Animal not included in the experiment.

Ayr. = Ayrshire ; Hol. = Holstein ; His. = Hissar.

TABLE I B

Showing the particulars regarding the animals in Series 'B'

Expt. No.	Date of commencement of experiment	ANIMAL NUMBERS AND BREED					
		348 Kangayam	351 Kangayam	853 Kangayam	354 Kangayam	355 Kangayam	356 Kangayam
		Condition of Animals					
1	17-10-37	Heifer	Heifer	Heifer	Heifer	Heifer	Heifer
2	15-11-37	do	do	do	do	do	do
3	25-1-38	do	do	do	do	do	do
4	15-2-38	do	do	do	do	do	do
5	16-5-38	do	do	do	do	do	do
6	21-8-38	do	do	do	do	do	do
7	25-10-38	Dry but not pregnant	do	Dry but not pregnant	do	Pregnant	Pregnant
8	20-11-38	do	do	do	X	do	do
9	8-1-39	do	Pregnant	do	Dry but not pregnant	do	do
10	12-2-39	do	do	do	do	do	Dry but not pregnant
11	5-3-39	do	do	do	do	do	do
12	25-6-39	do	do	Pregnant	do	X	do
13	14-8-39	do	do	do	do	Dry	do
14	25-9-39	do	do	do	do	do	do
15	16-10-39	X	Milking	X	X	X	X
16	16-1-40	Dry but not pregnant	do	X	Dry but not pregnant	Dry	Dry but not pregnant
17	16-4-40	do	do	Dry	do	do	do
18	19-5-40	do	Dry	Dry and pregnant	do	do	do
19	22-9-40	do	do	do	do	X	X
20	20-10-40	do	do	do	do	Dry	Dry but not pregnant
21	19-11-40	do	do	do	do	do	do
22	4-5-41	do	do	Milking	do	do	do
23	8-6-41	do	do	do	do	do	do

Average age at the commencement of the series of experiments: 1 yr. 2 months. X—Animal not included in the experiment.

TABLE I C

Showing the particulars regarding the animals in Series 'C'

Expt. No.	Date of commencement of experiment	ANIMAL NUMBERS AND BREED					
		687	688 Hol. Ayr. His. Sindhi	691 Ayr. Mont. Sindhi	692 Ayr. Mont. Sindhi	697 Ayr. Sah. Sindhi	699 Ayr. Sah. Sindhi
		Condition of Animals					
1	10-2-36	Heifer	Heifer	Heifer	Heifer	X	X
2	5-7-36	do	do	do	do	X	X
3	23-2-37	do	do	do	do	X	X
4	11-3-37	do	do	do	do	X	X
5	16-5-37	Pregnant	Pregnant	Pregnant	Pregnant	Pregnant	Pregnant
6	26-6-37	do	do	do	do	do	do
7	13-7-37	do	do	do	do	do	do
8	15-8-37	do	do	do	do	do	do
9	19-9-37	do	do	do	do	do	do
10	7-1-38	do	do	X	do	do	do

Average age at the commencement of the series of experiments: 1 yr. 4 months. X—Animal not included in the experiment.

Hol. = Holstein; Ayr. = Ayrshire; His. = Hissar; Mont. = Montgomery.

TABLE I D

Showing the particulars regarding the animals in Series 'D'

Expt. No.	Date of commencement of experiment	ANIMAL NUMBERS AND BREED				
		381 (688)*	382 (691)*	383 (692)*	384 (697)*	385 (699)*
		Condition of Animals				
1	25-3-38	Milking	Milking	Milking	Milking	X
2	8-5-38	do	do	do	do	X
3	5-6-38	do	X	do	do	X
4	9-10-38	do	Milking	do	do	Milking
5	9-11-38	do	do	do	do	do
6	23-1-39	do	Dry	X	Dry	Pregnant and Milking
7	20-2-39	do	do	Milking	do	do
8	12-3-39	do	do	do	do	do
9	15-5-39	do	do	do	do	do
10	16-10-39	Pregnant and Milking	do	Pregnant and Milking	do	Milking
11	5-5-40	Milking	do	Milking	do	X

Average age at the commencement of the series of experiments: 3 yrs. 5 months. X—Animal not included in the experiment.

* The number in ( ) brackets refers to the brand No. of the same animal as a calf in series C.

TABLE II A

Showing the estimated calcium requirements in grams per diem for animals in Sèries 'A'

Expt. No.		Animal Number					Average values	Remarks
		279	281	285	285 L.J.	289		
1	R	64·4	66·8	52·8	66·3	X	62·5 (4)	Pregnant and Milking
	S.E. %	16·3	15·8	8·4	8·1			
2	R	77·8 *57·7*	75·9	65·6	X	85·4	71·2 (4)	Pregnant and Dry
	S.E. %	32·2	2·9	8·5		4·7		
3	R	120·0	114·1	† *115·9*	133·2	X	121·0 (4)	Milking
	S.E. %	1·9	11·2	†	1·5			
4	R	X	89·4	66·5	82·4	79·0	79·3 (4)	,,
	S.E. %		7·5	2·7	0·4	1·7		
5	R	X	66·1	50·7 *52·2*	66·6	78·9 *72·3*	64·3 (4)	,,
	S.E. %		10·2	23·7	0·9	55·7		

R denotes the estimated calcium requirement.

S.E.% denotes the percentage standard error of the regression estimate.

X denotes that the animal was not included in the experiment.

† denotes that the estimation was not possible.

The figure in *italics* gives alternative estimate of requirement described as 'cubic estimate' in the paper.

The figure in ( ) brackets gives the number of the animals on which the averages are based.

TABLE II B

Showing the estimated calcium requirements in grams per diem for animals in Series 'B'

Expt. No.		Animal Numder						Average values		
		348	351	353	354	355	356	Not milking Not pregnant	Pregnant and dry	In lactation
1	R S.E. %	56·0 13·6	67·7 *45·1* 132·7	49·4 3·3	139·9 *58·0* 628·6	42·8 4·6	41·3 *53·3* 45·3	50·8 (6)		
2	R S.E. %	33·9 25·0	48·2 *43·5* 15·8	45·8 0·4	49·0 *43·5* 19·9	31·7 *44·8* 173·4	50·4 *45·2* 17·0	43·6 (6)		
3	R S.E. %	26·1 *43·5* 275·9	43·1 0·6	42·7 1·9	36·3 1·0	35·8 17·3	40·1 1·2	40·3 (6)		
4	R S.E. %	25·3 *27·4* 48·0	† †	31·4 0·6	22·3 *29·2* 432·0	28·5 4·8	31·6 0·2	29·6 (5)		
5	R S.E. %	31·2 8·6	32·0 1·2	34·8 7·6	23·5 *30·4* 25·2	31·0 1·7	30·1 3·0	31·6 (6)		
6	R S.E. %	94·8 *55·1* 201·4	66·6 4·1	156·3 *72·8* 41·5	55·5 9·3	76·0 6·2	79·3 7·3	65·9 (6)		
7	R S.E. %	56·8 4·2	58·0 0·2	57·5 0·3	62·3 10·3	58·3 1·6	52·7 14·9	58·7 (4)	55·5 (2)	
8	R S.E. %	61·1 1·7	62·0 0·3	† †	X	49·7 *61·2* 340·5	62·0 0·5	61·6 (2)	61·6 (2)	
9	R S.E. %	† †	47·2 20·0	47·8 6·6	† †	† †	† †	47·8 (1)	47·2 (1)	
10	R S.E. %	47·3 40·7	49·4 0·8	48·8 2·7	56·9φ 69·9	43·4 29·5	† †	51·0 (3)	46·4 (2)	
11	R S.E. %	43·9 17·4	† †	48·7 1·1	50·1 1·3	49·6 0·3	65·3 *51·1* 103·6	48·5 (4)	49·6 (1)	
12	R S.E. %	59·0 4·0	51·9 0·5	52·6 0·2	44·3 24·5	X	48·7 0·2	50·7 (3)	52·3 (2)	
13	R S.E. %	57·2 3·3	71·9 0·7	58·0 3·4	52·7 1·8	55·6 0·3	57·2 0·6	55·7 (4)	65·0 (2)	
14	R S.E. %	61·9 *48·8* 3·4	43·2 18·6	49·1 0·4	47·8 1·4	47·4 0·6	49·5 7·1	48·4 (4)	46·2 (2)	

TABLE II B—(*Contd.*)

Expt. No.		Animal Number						Average values		
		348	351	353	354	355	356	Not milking Not pregnant	Pregnant and dry	In lactation
15	R S.E. %	X	67·8 16·1	X	X	X	X	X		67·8 (1)
16	R S.E. %	44·3 23·6	80·7 0·4	X	48·9 2·0	114·4 *50·4* 3350·0	† †	47·8 (3)		80·7 (1)
17	R S.E. %	76·6 *54·9* 155·0	† †	† †	47·1 4·7	45·2 9·2	† †	49·1 (3)		
18	R S.E. %	53·3 0·8	† †	† †	46·0 3·2	52·6 *50·0* 48·1	53·0 5·3	50·6 (4)		
19	R S.E. %	52·2 7·4	48·9 1·2	49·7 6·5	45·9 5·2	X	X	49·0 (3)	49·7 (1)	
20	R S.E. %	46·3 0·8	47·2 0·1	47·0 0·5	41·3 8·2	60·5 *42·0* 116·4	44·9 3·4	44·4 (5)	47·0 (1)	
21	R S.E. %	42·8 0·5	42·4 2·0	46·0 21·7	37·5 15·4	40·3 1·4	41·0 0·7	40·8 (5)	46·0 (1)	
22	R S.E. %	120·8 *70·0* 333·2	47·2 1·4	69·4 4·2	60·5 1·0	61·7 12·7	70·7 1·2	62·0 (5)		69·4 (1)
23	R S.E. %	76·7 7·2	50·5 16·9	51·6 12·3	62·7 1·0	73·8 5·5	72·0 1·6	67·2 (5)		51·6 (1)

R denotes the estimated calcium requirement.

S.E.% denotes the percentage standard error of the regression estimate.

X denotes that the animal was not included in the experiment.

† denotes that the estimation was not possible.

ϕ denotes that the estimation by alternative method was not possible.

The figure in *italics* gives alternative estimate of requirement described as 'cubic estimate' in the paper.

The figure in ( ) brackets gives the number of the animals on which the averages are based.

TABLE II C

Showing the estimated calcium requirements in grams per diem for animals in Series 'C'

Expt. No.		Animal Number						Average	Remarks
		687	688	691	692	697	699		
1	R	37·8	38·6	26·7	35·8	X	X	34·7 (4)	Heifer
				†					
	S.E. %	10·9	6·9	92·9	8·9				
2	R	18·8	41·4	24·5	28·6	X	X	35·1 (4)	,,
		33·9		*36·5*					
	S.E. %	327·0	8·4	183·0	14·0				
3	R	44·9	49·2	46·2	46·1	X	X	46·6 (4)	,,
	S.E. %	2·3	7·7	19·0	2·5				
4	R	34·2	41·9	25·1	4·7	X	X	35·1 (4)	,,
				32·2	*32·2*				
	S.E. %	2·0	4·1	290·8	3087·1				
5	R	39·9	36·4	35·4	35·7	37·3	32·7	35·4 (6)	Pregnant
		35·0							
	S.E. %	65·0	5·9	16·8	1·1	4·5	0·3		
6	R	36·6	43·0	40·2	40·6	41·4	51·6	40·1 (6)	,,
							39·0		
	S.E. %	13·1	3·5	0·9	5·6	5·3	123·1		
7	R	36·0	†	39·2	37·1	40·3	37·9	39·4 (6)	,,
		40·6	*41·1*						
	S.E. %	93·8	†	1·0	0·8	1·3	1·7		
8	R	47·2	42·4 (54·9)	41·9	40·5	44·6	41·3	43·0 (6)	,,
			42·4						
	S.E. %	1·9	180·3	1·6	14·9	5·7	1·0		
9	R	34·6	39·9	36·7	44·0	43·6	37·4	40·1 (6)	,,
		39·0							
	S.E. %	126·2	2·6	1·5	41·2	14·1	11·5		
10	R	36·1	45·2	X	42·6	44·7	42·8	42·3 (5)	,,
	S.E. %	0·3	1·1		1·1	15·3	3·9		

R denotes the estimated calcium requirement.

S.E.% denotes the percentage standard error of the regression estimate.

X denotes that the animal was not included in the experiment.

† denotes that the estimation was not possible.

The figure in *italics* gives alternative estimate of requirement described as 'cubic estimate' in the paper.

The figure in ( ) brackets gives the number of the animals in which the averages are based.

TABLE II D

Showing the estimated calcium requirements in grams per diem for animals in Series 'D'

Expr. No.		Animal Number					Average values		
		381	382	383	384	385	Milking	Dry	Pregnant & Milking
1	R	80·9	88·6 *70·3*	†	70·8	X	74·0 (3)		
	S.E. %	2·3	77·1	†	11·8				
2	R	83·1	† *73·1*	85·3	81·5	X	80·8 (4)		
	S.E. %	1·0	†	4·9	0·9				
3	R	95·7	X	91·9	97·2	X	94·9 (3)		
	S.E. %	2·3		0·9	2·2				
4	R	62·4 *72·5*	60·3 *77·1*	71·5 *70·6*	79·1	2323·1 *78·5*	75·6 (5)		
	S.E. %	214·4	167·6	24·2	3·7	V. high			
5	R	70·9	64·1	68·2	65·8	74·4	68·7 (5)		
	S.E %	3·1	3·0	0·3	2·5	18·9			
6	R	112·9	60·7	X	66·7	98·2	112·9 (1)	63·7 (2)	98·2 (1)
	S.E. %	6·3	4·5		7·0	10·4			
7	R	108·0	50·2	116·9	53·0	110·3	112·5 (2)	51·6 (2)	110·3 (1)
	S.E. %	8·6	1·2	0·7	1·9	17·9			
8	R	104·1 *82·2*	49·6	98·3	54·1	86·7	90·3 (2)	51·9 (2)	86·7 (1)
	S.E. %	20·8	1·3	3·3	0·3	0·8			
9	R	106·7 *71·2*	45·3	54·1 *69·9*	45·0	68·3	70·6 (2)	45·2 (2)	68·3 (1)
	S.E. %	95·4	14·2	144·5	0·5	5·3			
10	R	58·4 *70·2*	45·6	46·6	46·6	62·3	62·3 (1)	46·1 (2)	53·4 (2)
	S.E. %	152·0	0·4	0·1	0·2	2·7			
11	R	70·3	45·3	100·1	49·7	X	85·3 (2)	47·5 (2)	
	S.E. %	8·7	0·4	0·7	1·0				

R denotes the estimated calcium requirement.

S.E.% denotes the percentage standard error of the regression estimate.

X denotes that the animal was not included in the experiment.

† denotes that the estimation was not possible.

The figure in *italics* gives alternative estimate of requirement described as 'cubic estimate' in the paper.

The figure in ( ) brackets gives the number of the animals on which the averages are based.

TABLE III

Showing the estimates of average calcium requirements obtained by different methods for dry Kangayam cows on dry cholam straw feed

Experiment Number	Date of commencement of experiment	Number of cows averaged	Regression Estimate from pooled data (in gm.)	Weighted Mean of Regression Estimates (in gm.)	% Standard Error of Pooled Estimate
12	23-6-39	3	47·4	48·8	8·0
13	14-8-39	4	57·7	55·9	7·8
14	25-9-39	4	44·5	47·7	10·1
15	16-10-39	X	X	X	X
16	16-1-40	3	46·2	48·8	13·2
17	16-4-40	*	46·6 (4)	46·7 (3)	9·8
18	19-5-40	4	50·4	52·8	1·0
19	22-9-40	3	50·3	48·8	5·0
20	20-10-40	5	62·7	47·1	17·9
21	19-11-40	5	41·6	42·1	1·2
22	4-5-41	5	59·0	58·3	2·1
23	8-6-41	5	56·5	64·9	27·6

X No experiment conducted on dry Cows.

* Number of cows indicated in brackets.