

PHOSPHORUS DEFICIENCY METABOLISM AND FOOD UTILIZATION IN BEEF HEIFERS ¹

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1. INTRODUCTION

The respiration trials reported in this paper were carried out in order to determine the influence of phosphorus deficiency on the efficiency of food utilization. The term 'efficiency of food utilization' may have several different meanings and is often used rather vaguely. It is necessary therefore to define the term.

Weight efficiency relates gain in body weight to weight of the food consumed. In this paper we are mainly dealing with energy efficiency, a relation between the chemical energy of the animals' products and the chemical energy in the food. By energy in the animals' product, or net energy N, we mean the heat of combustion of the gained body substance (or the heat of combustion of the produced milk plus the heat of combustion of the gained body substance etc.). The net energy is expressed in kilogram calories or in therms (1 therm = 1000 kg. calories).

The food energy may be expressed as heat of combustion of the food, as digestible energy or as metabolizable energy. The following formulation is applicable to any of these three bases for measuring food energy. The calculations of our

¹This report is part of an investigation on the relation of nutrition to reproduction which became cooperative with the United States Bureau of Animal Industry, July 1, 1929.

results (tables 8, 9, 10) are based on metabolizable energy but table 5 furnishes the data necessary for expressing the results also in terms of digestible food energy or heat of combustion of the food. The unit for the food energy is the same as that for the net energy.

Two main types of efficiency should be distinguished, the total efficiency and the partial efficiency.

The total efficiency is the net energy per unit of the energy (heat of combustion or digestible energy or metabolizable energy) in the total food, thus:

$$\text{Total efficiency} = \frac{\text{Net energy}}{\text{energy in total food}} = \frac{N}{U} \quad (1)$$

At a certain food level (maintenance level) the net energy will be zero, $N = 0$ (that does not mean that nothing is produced but that the sum of the energy in the product is zero, a milk cow, for example, may continue to yield milk but at the cost of her own body substance). The total efficiency at the maintenance level is zero. If the food ration is smaller than the maintenance requirement, the net energy, and consequently also the total efficiency are negative. The total efficiency characterizes the economy of animal production. It does not characterize the nutritive value of the food. In order to measure the food value it is necessary to eliminate the influence of the maintenance requirement of the animal. This can be done by feeding the animal at two different levels and determining the difference in net energy resulting from the difference in the food energy; thus one measures the effect of a part of the food energy and may formulate:

$$\text{Partial efficiency} = \frac{\text{Difference in net energy}}{\text{Difference in food energy}} = \frac{N_1 - N_2}{U_1 - U_2} \quad (2)$$

Below maintenance both N_1 and N_2 will be negative and the partial efficiency will, as a rule, be positive even though the total efficiency is negative.

If the partial efficiency is independent of the plane of nutrition ($\frac{N_1 - N_2}{U_1 - U_2} = k$) then the net energy N will be proportional to the difference of the total food energy U and the maintenance requirement M .

$$N = k (U - M) \tag{3}$$

In this case the following equation shows the relation between partial efficiency k and total efficiency $\frac{N}{U}$.

$$\frac{N}{U} = k \left(1 - \frac{M}{U}\right) \tag{4}$$

In order to formulate the relation of the total efficiency to the fasting katabolism B instead of the maintenance requirement M it may be considered that for maintenance $U_1 = M$ and $N_1 = 0$ and for fasting $U_2 = 0$ and $N_2 = -B$

$$k = \frac{N_1 - N_2}{U_1 - U_2} = \frac{0 - (-B)}{M - 0} = \frac{B}{M} \tag{5}$$

If this result ($M = \frac{B}{k}$) is introduced into equation (4) one obtains for the total efficiency

$$\frac{N}{U} = k - \frac{B}{U} \tag{6}$$

The result of this equation may be stated as follows:

If the partial efficiency is independent of the plane of nutrition then the total efficiency is the difference between the partial efficiency and the quotient $\frac{\text{Fasting katabolism}}{\text{Total energy intake}}$.

The reciprocal of this quotient, namely $\left(\frac{U}{B}\right)$, is a measure for the level of food intake (relative food level) indicating how many times as much energy the animal takes in as it spends during fasting. If the animal can eat as much as it wants then the quotient $\frac{U_a}{B}$ characterizes the appetite of the animal.

The presupposition of a constant partial efficiency is strictly applicable only in special cases. The general formulation of the total efficiency (with variable partial efficiency)

is more complicated.² The definition of the terms by equation (6) is, however, sufficiently precise for the discussion in this paper.

An influence of phosphorus deficiency on the efficiency of food utilization has been observed by Theiler in his classical investigation on aphosphorosis in South Africa. Theiler ('33) concluded that animals given sufficient phosphorus utilize their food better and gain more per unit of food consumed than do animals on a phosphorus-low diet. Theiler thus observed that phosphorus deficiency lowers the total weight efficiency of the animals.

Eckles and Gullickson ('27) concluded from their experiments that cows on a phosphorus-low diet needed at least 20% more digestible nutrients to maintain their live weight than was indicated by Morrison's feeding standard.

Riddell, Hughes and Fitch ('34) studied the oxygen consumption of cows kept alternately at normal and low phosphorus intake. From the 6-minute records, obtained by

²In general the partial efficiency varies with variations in the plane of nutrition. It is often higher for submaintenance than for super-maintenance feeding. If k_p stands for the partial efficiency above maintenance and k_m for the partial efficiency below maintenance then the total efficiency may be expressed as follows:

$$\frac{N}{U} = k_p - \frac{k_m B}{k_m U} \quad (6')$$

If the partial efficiency varies even within the submaintenance and super-maintenance levels as indicated by the result of Forbes et al. ('28), Wiegner and Ghoneim ('30), Mitchell et al. ('32) and confirmed by the experiments reported in this paper (p. 150) then the best characteristic for the nutritive value of the food would be the differential efficiency $\frac{dN}{dU} = \eta$ and in this case the total efficiency may be formulated as follows:

$$\frac{N}{U} = \frac{\int_0^U \eta dU}{U} - \frac{B}{U} \quad (6'')$$

The term $\left(\frac{\int_0^U \eta dU}{U}\right)$ may be classified as the mean partial efficiency for the entire food level and the result summarized as follows:

In general the total efficiency is the difference between the mean partial efficiency of the entire food and the quotient $\frac{\text{Fasting katabolism}}{\text{Total energy intake}}$.

Brody's muzzle method 12 hours after the last food was given, they selected those that were most uniform and showed the lowest oxygen consumption. These authors concluded that the addition of phosphorus to a phosphorus-low diet lowers the general metabolism. The main criticism applying to their result is that the metabolism of cows 12 hours after the last feeding is neither a true fasting katabolism nor representative of the average daily metabolism of the fed animal.

The results reported in the present paper are based on thirty-four complete metabolism trials conducted with three pairs of beef heifers fed all the same diet low in phosphorus but one heifer of each pair receiving a supplement of phosphate. Each metabolism trial lasted 2 weeks. The fasting katabolism was determined in sixteen respiration trials of 48 hours duration during the fourth and fifth day of fasting.

The results of our experiments confirm the observation of previous investigators that phosphorus deficiency lowers the efficiency of food utilization for gain in weight, more specifically the total weight efficiency. The new information supplied by our work is the influence of phosphorus deficiency on the gain or loss of body substance in terms of chemical energy, the energy efficiency, and the differentiation of the total energy efficiency in the two components: partial efficiency and appetite, the appetite being determined as the quotient

$$\frac{\text{Intake of food energy at abundant feeding}}{\text{fasting katabolism}}$$

2. METHOD

Animals used. Six beef heifers were selected and matched into pairs, their weights, their gains from birth, and also the weights of their ancestors being considered. Animals 18, 27 and 29 were pure bred Herefords; nos. 10 and 51 were pure bred Aberdeen-Angus; and no. 32 was a crossbred Aberdeen-Angus-Shorthorn. All the animals had been raised in the university herd. Only four were kept under observation throughout the entire experimental period. The animals were 14 to 15 months of age when started on the experiment.

Food and feeding. The guiding principle in selecting rations was to secure one deficient in phosphorus, yet complete with regard to other nutrient constituents. This is accomplished only with difficulty, particularly in providing adequate protein, when a roughage good in protein and low in phosphorus is not available. The rations used, together with the chemical composition, are shown in table 1.

TABLE 1
Composition of food per 100 gm. as fed

	RATION 1 CONTROL	RATION 2 LOW-PHOS- PHORUS	RATION 3 CONTROL	RATION 4 LOW-PHOS- PHORUS	RATION 5 CONTROL	RATION 6 LOW-PHOS- PHORUS
	gm.	gm.	gm.	gm.	gm.	gm.
a. Food mixture						
Alfalfa molasses meal	30.0	30.0	30.0	30.0		
Dried beet pulp	33.0	30.0	30.0	30.0	62.0	62.5
Cornstarch	25.0	25.6	31.9	32.6	30.0	30.0
Casein	2.5	2.5	1.5	1.5	1.5	1.5
Corn gluten meal	7.5	7.5				
Cottonseed oil	1.5	1.5	3.0	3.0	2.5	2.5
Cod liver oil	0.5	0.5	0.5	0.5	1.0	1.0
Calcium carbonate	1.0	2.4	1.0	2.4	1.0	2.5
Calcium acid phosphate	2.0		2.1		2.0	
b. Chemical analyses						
Moisture	10.20	10.00	13.20	12.20	13.40	13.20
Crude protein	13.38	13.97	8.27	8.84	6.43	6.45
Fat	1.75	1.79	2.53	2.57	0.75	0.80
Ash	6.08	5.12	6.17	5.62	4.88	4.30
Crude fiber	11.72	12.06	11.80	11.05	9.94	10.54
N. F. E.	56.87	57.06	58.03	59.72	64.60	65.31
Calcium	1.57	1.43	1.48	1.52	1.28	1.29
Phosphorus	0.51	0.13	0.45	0.09	0.41	0.068
Carbon	37.68	38.20	38.40	38.00	38.20	39.20
Calories per gram	3.88	3.92	3.78	3.79	3.75	3.68

Cottonseed oil was used in the rations to reduce the dryness and dustiness caused principally by the cornstarch and to make the physical characteristics of the rations acceptable to the animals. The oils were first mixed with the beet pulp or beet pulp and alfalfa meal in a power mixer; then the cornstarch and other ingredients were added and mixed. The finer components adhered to the alfalfa meal and to the beet pulp so that the mixture remained uniform.

All animals were started on ration 1 on January 16, 1932, and continued until February 27, 1932, when no. 32 was changed to ration 2 (low in phosphorus). The remaining five animals continued on ration 1 throughout respiration trials 1 to 6 inclusive. Animals 51 and 27 were changed to low-phosphorus ration 2 on May 14th and May 28th, respectively, and were on this ration during respiration trials 7 to 9, inclusive. During July and August, 1932, control animals 10, 18 and 29 were changed to ration 3, and the low-phosphorus animals 27, 32 and 51 were changed to ration 4, the animals being on these rations during respiration trials 13 to 16, inclusive, and remaining until September 27, 1933. On this date heifers 18 and 29 were changed to control ration 5, and heifers 27 and 32 to phosphorus-low ration 6. The animals remained on these rations until July 26, 1934, when they were slaughtered. Shortly after nos. 18 and 29 were changed to ration 5, a monocalcium phosphate especially prepared for animal feeding was substituted for dicalcium phosphate. Soon thereafter the animals went off feed and did very poorly for about 6 weeks. It was discovered that something in the mineral supplement, presumably ferrous compounds, caused the fats in the ration to become rancid within 24 hours after mixing. As soon as dicalcium phosphate feeding was resumed, the appetite of the animals returned to normal.

Animals 10 and 51 were taken out of the experiment on July 18 and September 28, 1933, respectively.

The animals were fed individually twice each day during most of the experimental period. Feeding thrice daily was tried for a time to see whether or not a significantly higher feed intake could be attained. Except at feeding time during the early part of the experiment the animals all ran together in a small paved corral adjacent to their stalls. During the latter part of the experiment the controls were entirely separated from the low-phosphorus animals to prevent the latter from consuming phosphorus-rich feces of the controls.

The amounts of dry matter consumed per day during each metabolism trial are presented in table 5. During the first

nine trials the two pair mates were fed equal amounts of food at approximately one-half, one and one and one-half maintenance levels. After 1 year of experiment the animals on the P deficient diet would not even eat enough for maintenance (trials 13 to 16) equal amounts of food were still fed to the pair mates at the one-half maintenance level.

After 2 years of the P-low regimen the difference in weight between the animals of one pair became so great that equal amounts of food for both no longer represented the same food level. Food levels are not comparable when they are expressed in kilograms or calories per animal or per kilogram of the animals' weight but are comparable when given in terms of the energy requirement (for example one-half maintenance, maintenance, etc.). The maintenance requirement is approximately proportional to the fasting katabolism of the animal (see equation (5), p. 123) consequently the food level may also be expressed in terms of the fasting katabolism (equation (6)). The fasting katabolism in turn is proportional to the $\frac{3}{4}$ power of body weight (Kleiber, '32), therefore the food level may also be expressed in terms of the $\frac{3}{4}$ power of body weight and two animals on the same diet may be regarded as being on the same relative food level if their food intakes per kg.³ are equal.³ Based on this consideration we made the rations in trials 22 and 23 so that the pair mates were given approximately the same amount of food per kg.³. The higher rations were measured according to the same principle but the phosphorus deficient animals ate only a fraction of these rations.

Methods of analysis. Composite samples of the feed used were taken for chemical analysis from a large batch of the mixed feed as the daily rations were being weighed out for the respiration trials. These composites were then run through a Wiley mill, reduced in size by quartering, and finally ground to pass a 40-mesh sieve. In the first six trials the total feces voided each day were dried in a large

³The symbol kg.³ is used throughout this paper as unit of the $\frac{3}{4}$ power of body weight, $W^{\frac{3}{4}}$, where W is expressed in kilograms.

electric oven at 90° to 95°C. The dry residues were weighed and added together until the end of the 12-day trial. The total dry matter voided was thus determined. The dry feces were then run through a laboratory hammer mill, and samples obtained for chemical analysis.

When, later, it was found that this method of drying feces caused a loss of some carbon and nitrogen, a different procedure was adopted. The moist feces from each 24 hours were carefully mixed, and an aliquot was taken for total nitrogen and for moisture and volatile carbon compounds. This procedure has been described by Kleiber, Caldwell and Johnson ('36). The remaining fresh feces were then dried as before in the air oven at 70°C. and a composite dry sample was prepared for analysis. Corrections were then applied for losses of C during drying. The value used for the total nitrogen in the feces was that obtained from the analysis of the wet feces samples taken daily.

All feed and feces samples were analyzed for crude protein, fat, ash, crude fiber and moisture, according to Methods of Analysis, Association of Official Agricultural Chemists, 1930 edition. Calcium was determined on the ash by McCrudden's method ('10, '11). Phosphorus was determined colorimetrically by the method of Fiske and Subarrow ('25), modified for ash materials. The accuracy of this method for feed and feces phosphorus was repeatedly checked by the official volumetric method of the Association of Agricultural Chemists ('30).

Blood calcium was determined by the Clark-Collip ('25) method, and inorganic serum phosphate by the Fiske and Subarrow ('25) method.

The fuel value of feeds and feces was determined in an Emerson fuel calorimeter. Carbon was determined after each combustion by allowing the CO₂ to escape from the bomb through an absorbing tower containing BaCl₂—Ba (OH)₂ solution. Titration of the excess Ba(OH)₂ was made with a mixed indicator, described in detail by Kleiber ('35).

Carbon determination on urine was carried out by a wet-combustion process as described by Mohlman and Edwards ('31). Instead of using a mixture of H_3PO_4 and H_2SO_4 and a solution of chromic acid, a mixture of $\text{K}_2\text{Cr}_2\text{O}_7$ and NaH_2PO_4 was made up. The proportions used were 7 gm. $\text{K}_2\text{Cr}_2\text{O}_7$ to 25 gm. NaH_2PO_4 . These amounts are the quantities used per determination of approximately 100 mg. carbon. In place of the mixture of H_3PO_4 and H_2SO_4 , 90 cc. of concentrated H_2SO_4 were added. The method was tried on benzoic acid, sulphanilic acid and sodium formate. It produced 98.0, 99.1 and 99.0% recovery of the C, respectively.

The analyses of the bones for CaCO_3 were carried out on samples of dry, fat-free bones; for the determination of Ca and phosphorus the bone samples were ashed.

Respiration trials: Procedure. In order to determine the partial efficiency of energy utilization one must determine the energy balance at two different levels of food intake (see equation (2) of introduction). One of these levels may be at complete fasting. Earlier observations of Forbes and co-workers ('28) as well as Mitchell and collaborators ('32) have indicated that the partial efficiency of energy utilization in cattle may depend on the food level. It seemed desirable, therefore, to run trials with food levels below as well as above maintenance. The appetite of our animals on phosphorus-low rations, however, was so poor that the plan of studying the energy utilization at high food levels had to be abandoned. We have not even been successful in every case in keeping the low-phosphorus animals at a maintenance level.

The history of the 2½-year series of respiration trials with the beef heifers is shown in table 5. Each respiration trial for the animals except those on basal metabolism studies lasted 2 weeks, of which time two 5-day periods constituted uninterrupted respiration experiments. The average coefficient of variation of the daily O_2 consumption within one trial amounted to $\pm(4.4 \pm 0.8)\%$ for the phosphorus deficient and $\pm(4.2 \pm 0.6)\%$ for the control heifers. The corresponding figures for the CO_2 production are $\pm(4.5 \pm 0.91)\%$ for

the phosphorus deficient and $\pm(1.8 \pm 0.25)\%$ for the control heifers. The average standard deviation of the daily R.Q. within one trial was $\pm(0.029 \pm 0.006)$ for the phosphorus deficient and $\pm(0.028 \pm 0.007)$ for the control heifers. The measurements of the fasting katabolism were made in a 48-hour experiment during the fourth and fifth day after the last food.

The technic of the respiration trials followed at this station has been described in detail by Kleiber ('35).

3. SYMPTOMS OF PHOSPHORUS DEFICIENCY

Gain in weight. The weight curves of the animals essentially confirm the earlier observations concerning the effect of phosphorus deficiency on growth. During the first 6 months after changing to phosphorus-low rations 2 and 4, heifers 27, 32 and 51 continued to gain at nearly the same rate as their controls on rations 1 and 3. At the end of this time the low-phosphorus heifers ceased to gain; their weights remained about constant for a period of approximately a year. During this time the controls continued to gain. After the change to rations 5 and 6 the control animals maintained their weight while the weight of the low-phosphorus animals declined. In the following discussion the period of the first 6 months (apparently no influence of phosphorus-deficient food on growth) is termed the first period of the experiment. The following year (cessation of growth in phosphorus-deficient animals) is classified as the second, and the last half-year (decline in weight of the phosphorus-deficient animals) as the third period of the experiment. The difference between the initial and final weight of control animal 18 was 203 kg. that of control animal 29 was 190 kg., while the corresponding differences between initial and final weights of the phosphorus-deficient animals 32 and 27, was only 36 and 6 kg., respectively. Some evidence of limitation of skeletal growth is afforded by the fact that control animals 18 and 29 increased in height at withers 9.5 and 11 cm., respectively, while their low-phosphorus mates increased but 6.5 and 8.5 cm. in height, respectively.

Concentration of phosphorus in the blood serum. The data on serum phosphorus are presented in table 2. Most of the data are based upon composite samples collected on each of 2 or 3 successive days. A few determinations based upon single samples are included. The control animals maintained high inorganic phosphorus content of the blood serum

TABLE 2
Content of inorganic phosphorus in blood serum¹

DATE	MILLIGRAMS PHOSPHORUS PER 100 CC. BLOOD SERUM					
	Control heifers			Low-phosphorus heifers		
	No. 10	No. 29	No. 18	No. 32	No. 27	No. 51
2-25-32	8.9 (1)	(1)	10.1 (1)	9.02 (1)	(1)	9.6 (1)
3-12-32	8.4	8.3		6.1 (2)	11.1	
3-19-32	7.9			6.8		
3-26-32	6.7		9.4	6.0		8.1
4- 2-32	9.1			7.0		
4- 7-32	9.3	9.2		6.7	10.4	
4-28-32			10.0			8.8
5-12-32	7.9	8.6		6.1	8.8 (2)	
6- 3-32	8.2			4.7		
6- 9-32	8.2		10.3	7.8		7.2 (2)
6-18-32	9.2			8.2		
7- 6-32	10.9 (3)	9.7 (3)	11.2 (3)	7.8 (4)	6.2 (4)	7.5 (4)
7-23-32	7.6	9.8	10.5	6.5	7.4	7.4
8- 6-32	7.9			7.3		
8-20-32	8.1	8.4	11.2	5.7	5.6	7.2
9- 2-32				4.4	4.2	5.1
9-10-32	7.8	8.7		5.1	5.5	5.5
9-17-32	8.4	9.3	8.2	6.5	4.7	6.6
10- 6-32	6.7	8.6	9.8	5.1	4.8	5.7
11-17-32	8.8	8.9	10.3	4.6	4.4	6.9
1-12-33	7.8	8.0	9.6	5.5	3.9	5.6
3-25-33	7.5	8.6	10.0	4.3	4.5	5.3
4-15-33	8.3	8.9	9.7	4.8	4.2	5.1
6-16-33		8.5	9.5	5.1	4.9	4.9
9-27-33		(5)	(5)	3.6 (6)	3.1 (6)	
12-19-33				4.6	4.4	
2-12-34		6.9	8.6	2.3	2.8	
3-24-34		7.9	8.7	3.5	3.7	
5-11-34		6.4	8.1	5.3	4.8	
7-25-34		9.4	11.3	3.5	3.9	
Average	8.27	8.56	9.79			

¹ The figures in parenthesis in the table indicate the ration fed and the date on which the change in ration was made.

throughout the experiment, the range being from 6.4 to 11.3 mg. phosphorus per 100 cc. of serum. Considering the age of the animals, the average values of 8.27, 8.57 and 9.8 mg. phosphorus per 100 cc. serum for nos. 10, 29 and 18, respectively, are somewhat higher than the 'normal' figures usually found (Palmer, Cunningham and Eckles, '30), (Guilbert, unpublished data) and presumably reflect the high phosphorus content of the control rations.

After the change from ration 1 to phosphorus-low ration 2, the blood phosphorus of animal 32 dropped from 9 to an average of 6.77 mg. phosphorus per 100 cc. of serum; after the change to ration 4 the average value was 5.42 mg. phosphorus; and during the period of ration 6 the average value dropped to 3.83 mg. phosphorus per 100 cc. The average values for animal 27 during the periods on rations 4 and 6 were 5.05 mg. phosphorus and 3.95 mg. phosphorus per 100 cc. of serum, respectively. The values for animal 51 during the period on ration 4 averaged considerably higher than those for the other animals on the same feed. The consistently high values found for control animal 18 are also noteworthy.

Although the serum phosphorus values decreased in relation to the phosphorus content of the rations fed, only a few of the determinations during the latter part of the experiment would be indicative of phosphorus deficiency on the basis of the criteria of Palmer, Cunningham and Eckles ('30). The vitamin D from the cod liver oil fed to our animals throughout the experiment may have prevented a more conspicuous fall in blood phosphorus. The range in blood-phosphorus values of dairy heifers on phosphorus-low rations reported by Van Landingham, Henderson and Bowling ('35) are somewhat similar to ours.

Pica. All the low-phosphorus animals began showing depraved appetites about December, 1932, coincident with cessation of increase in weight. It was manifested largely by coprophagy, chewing mangers and the chains by which the animals were tied at feeding time. After coprophagy started,

the stalls and corral were swept three times daily and washed with water frequently to prevent the accumulation of dried feces from the control animals, from which the low-phosphorus animals could obtain a significant addition to their phosphorus intake. Notwithstanding these precautions the low-phosphorus animals may at times have obtained added phosphorus in this manner, and this accident may have caused some of the variations found in the serum phosphorus values.

By April, 1933, heifers 32 and 27 showed marked osteophagia when tested with sun-bleached bones. Animal 51 at this time manifested only mild interest, while none of the controls would touch the bones. At this time the serum phosphorus was from 4.20 to over 5.0 mg. phosphorus per 100 cc. of serum. Heifer 51 exhibited definite osteophagia in August, 1933. Animals 32 and 27 were then still in good and animal 51 in excellent condition. As the experiment progressed, pica became more pronounced. All the low-phosphorus animals eagerly licked up blood spilled on the cement floor when blood samples were being taken.

No depraved appetite was noted in the control animals until after the change to ration 5. Wood chewing and occasional licking of urine from the concrete pavement were noted. In other experiments at this station wood chewing has been observed in connection with 'no-roughage' rations regardless of the completeness of the ration in other respects. The licking of urine associated with low-protein intake, has been observed also in other experiments by one of us (H. R. Guilbert).

Lack of appetite. Lack of appetite described by earlier workers (see for example, Huffman, Duncan, Robinson and Lamb, '33) was one of the most conspicuous symptoms in our phosphorus deficient animals.

Table 3 shows the maximum of the weekly food intake per kg.³ during the preliminary period when all animals received a phosphate supplement and during each of the subsequent periods when animals 27 and 32 were on phosphorus-low rations. The reason for expressing the appetite as food intake per unit of the $\frac{2}{3}$ power of weight has been discussed before (p. 128).

The phosphorus-deficient animals decreased the maximal food intake per kg.³ consistently as the phosphorus in the food decreased. From the first to the second period the low-phosphorus animals lost about one-fifth of their appetite, while the appetite of the control animals increased slightly.

During the last period of the experiment the maximal food intake per kg.³ of the two low-phosphorus animals approximated a level one-half of that of the first period. Only a small part of this drastic loss in appetite may be accounted for by a change in the composition of the food aside from its phosphorus content, as indicated by a 10% loss in appetite

TABLE 3
Appetite of phosphorus-deficient and control animals

ANIMALS	MAXIMAL INTAKE OF AIR-DRY FOOD PER WEEK PER UNIT OF BODY SIZE			
	Preliminary period	Time of phosphorus deficiency		
		0-6 months	6-18 months	18-24 months
	kg./w ^{3/4} ¹	kg./w ^{3/4}	kg./w ^{3/4}	kg./w ^{3/4}
Phosphorus-deficient animals				
No. 27	0.437	0.470	0.404	0.260
No. 32	0.465	0.490	0.364	0.234
Control animals				
No. 29	0.481	0.467	0.526	0.367
No. 18	0.407	0.480	0.485	0.473

¹ Heading reads: "kg. of food divided by W to the 3/4 power."

of the control animals. Thus our results confirm the observation of earlier workers (Theiler, Riddell) that phosphorus deficiency depresses the appetite of the animals.

Oestrus cycles. During the period January 15, 1932, to January 7, 1933 control animals 18 and 29 exhibited fifteen and sixteen periods of oestrus, respectively. During the next year and a half these animals were for the most part kept in separate quarters from the low-phosphorus animals and were not under so close observation as before. Moreover, they were not particularly demonstrative during oestrus, so that doubtless some periods were not recorded. Seven and nine periods, respectively, were recorded during this period. The occurrence of oestrus appeared somewhat irregular after change

to ration 5, in which the protein level was rather low. At autopsy, however, the ovaries of both animals were found to be normal, and they contained recent corpora. The third control animal, 10, had thirteen periods of oestrus during the first 9 months on the experiment. At the end of this time she bred, conceived, and in due time gave birth to a normal calf. This was considered to be evidence that the control rations 1 and 3 were reasonably adequate.

Animal 27 had, between January, 1931, and May, 1932, six periods of oestrus. At the end of this period she was changed to the phosphorus-low ration. Only three periods of oestrus were observed during the next 7 months, and only two additional periods during the next year and a half of the experiment. At autopsy she was found to have one small, quiescent ovary and one cystic ovary. Low-phosphorus animal 32 came into oestrus ten times during the first 9 months. At the end of this time (September 2, 1932) she was bred but did not become pregnant. A bloody discharge from the vagina was noted on January 21, 1933, and oestrus was recorded on October 18, 1933 and January 3, 1934. These three occasions were the only manifestations of oestrus during the entire period of the experiment, after breeding on September 2, 1932. On autopsy the ovaries of this animal were found to be small and quiescent. The cessation of oestrus in both these low-phosphorus animals coincided closely with cessation of gains. Low-phosphorus animal 51 continued to have more or less regular cycles throughout the entire period she was on the experiment. This animal always had a more thrifty appearance than 27 and 32, a better appetite, a higher content of inorganic phosphate in the blood serum. She was removed from the experiment in September, 1933, and was never placed on phosphorus low ration 6.

Cessation or irregularity of oestrus has been associated with phosphorus deficiency by a number of workers. The literature was reviewed by Eckles, Palmer and associates ('35). The experiments reported in their paper cast some doubt as to whether the interference with oestrus is caused

by phosphorus deficiency per se, as their animals on phosphorus-low rations had normal oestrous cycles. From the standpoint of blood and bone analysis and other symptoms, their animals were deficient in phosphorus; but apparently the intake of phosphorus was varied so that total food consumption was not significantly reduced.

Bone analysis. According to Neal and Palmer ('31) the ribs may be considered as the most reliable single samples that represent the condition of the skeleton with regard to its chemical composition. The analysis of the dry, fat-free rib bones of our heifers is presented in table 4.

TABLE 4
Composition of rib bone of phosphorus-deficient and control heifers

CONSTITUENTS	CONTENT PER 100 GM. OF FAT FREE DRY BONES			
	Phosphorus-deficient heifers		Control heifers	
	No. 27	No. 32	No. 18	No. 29
Ash	55.4	58.1	63.0	65.4
Ca	21.6	22.5	24.1	25.0
P	9.49	10.05	11.32	11.75
Mg	0.29	0.32	0.54	0.48
CaCO ₃	8.25	8.25	6.15	7.00
Ca ₃ (PO ₄) ₂	46.20	48.90	54.30	56.70
Ratio:				
$\frac{\text{Ca}_3(\text{PO}_4)_2}{\text{CaCO}_3}$	5.6	5.9	8.8	8.1

The bones of the phosphorus-deficient heifers 27 and 32 had a lower ash content and a higher content of CaCO₃ than the bones of their mates, 18 and 29, on the phosphate-supplemented diet. The ratio Ca₃(PO₄)₂:CaCO₃ is considerably lower in the bones of the control heifers. This result confirms the earlier findings of Neal, Palmer, Eckles and Gullickson ('31). In our trials the effect seemed somewhat more pronounced than in the trials of these authors.

4. RESULTS OF METABOLISM TRIALS

The results of the respiration trials carried out with the six beef heifers are summarized in chronological order in table 5.

TABLE 5
Phosphorus-deficiency and metabolism chronological summary

TRIAL NO.	DATE	ANIMAL NO.	WEIGHT OF ANIMAL kg.	W ² kg.	FEED EATEN IN GRAMS DRY MATTER PER DAY	P LEVEL: GRAMS P. PER 100 GM. DRY FOOD	P BALANCE: GRAMS P. PER DAY	DAILY ENERGY BALANCE (THERMS)						
								Feed	Digested	Metabo- lizable	Net	Heat		
	1932													
1	Feb. 29 to Mar. 12	18 51	317.1 308.0	75.0 73.5	1207 1207	0.608 0.608	+ 2.08 + 3.81	5.27 5.27	4.14 4.19	3.08 3.48	— 2.57 — 2.78	5.65 6.31		
2	Mar. 14 to Mar. 26	29 27	323.4 362.4	76.0 83.5	1207 1207	0.608 0.608	+ 1.78 — 0.77	5.27 5.27	3.97 4.08	3.04 2.97	— 3.69 — 3.83	6.73 6.80		
3	Mar. 28 to Apr. 9	18 51	313.4 315.7	74.0 74.0	2395 2395	0.576 0.621	+ 6.19 + 6.84	10.45 10.46	8.47 8.56	6.96 6.95	— 0.10 — 0.18	7.06 7.13		
4	Apr. 11 to Apr. 23	29 27	327.0 360.6	77.0 83.0	2395 2395	0.576 0.621	+ 6.66 + 5.27	10.45 10.46	8.59 8.41	7.11 6.89	— 0.30 — 0.68	7.41 7.57		
5	May 2 to May 14	18 51	341.1 328.4	79.0 77.5	3895 4100	0.579 0.592	+ 8.52 + 5.62	16.93 17.73	13.61 14.63	11.65 12.29	+ 3.08 + 3.29	8.57 9.00		
6	May 16 to May 28	29 27	351.5 385.6	81.0 87.5	4100 4100	0.579 0.592	+ 5.91 + 8.21	17.76 17.73	14.21 14.19	12.34 12.33	+ 3.01 + 3.25	9.33 9.08		
7	June 6 to June 18	10 32	338.8 354.3	79.0 82.0	4474 4425	0.535 0.137	+ 3.9 + 1.0	19.33 19.29	15.25 15.63	13.31 13.33	+ 1.05 + 1.72	12.26 11.61		
8	July 5 to July 16	10 32	342.5 356.1	80.0 82.0	3720 3764	0.500 0.138	+ 3.3 + 0.3	10.76 10.90	8.81 8.83	7.27 7.29	— 0.36 — 0.46	7.63 7.75		
9	July 25 to Aug. 6	10 32	328.9 347.9	77.0 80.8	1241 1239	0.497 0.124	+ 3.3 + 0.42	5.37 5.39	4.51 4.77	3.63 3.81	— 2.23 — 2.37	5.86 6.18		
10	Dec. 16 to Dec. 18	18 51	453.6 427.7	93.0 94.0								6.59 8.26		
11	Dec. 19 to Dec. 21	29 27	490.7 530.7	104.0 111.0								8.32 8.78		

PHOSPHORUS DEFICIENCY AND FOOD UTILIZATION

13	1953 Mar. 20 to Apr. 1	29 27	521.2 523.0	109.0 110.0	4331 2814	0.514 0.104	-1.3 -0.44	18.87 12.55	14.92 10.00	12.45 8.09	+2.06 -1.64	10.39 9.73
14	Apr. 17 to Apr. 29	10 32	474.9 414.6	102.0 92.0	4331 2640	0.514 0.104	-2.2 -0.59	18.87 11.89	14.85 9.81	12.71 8.54	+1.88 -0.33	10.83 8.87
15	May 1 to May 13	29 27	514.8 505.3	108.0 107.0	1600 1634	0.571 0.112	+1.2 -0.16	6.83 6.95	5.50 5.42	4.38 4.32	-3.35 -4.39	7.73 8.71
16	May 15 to May 27	10 32	464.0 416.4	100.0 92.0	1600 1634	0.571 0.112	+0.8 -0.1	6.83 6.95	5.50 5.83	4.28 4.58	-4.74 -2.45	9.02 7.03
17	May 28 to May 29	29 27	494.0 493.5	104.8 104.7								7.54 7.36
18	May 30 to May 31	18 51	517.1 473.1	108.4 101.4								8.41 8.04
19	June 1 to June 2	10 32	442.7 396.4	96.5 88.9								9.91 5.89
20	1934 Mar. 12 to Mar. 24	29 27	565.2 445.4	116.0 97.0	2756 1656	0.469 0.078	+0.18 -0.48	11.62 7.02	9.56 6.12	8.31 4.93	-1.09 -2.94	9.40 7.87
21	Mar. 26 to Apr. 7	18 32	561.1 382.4	115.0 86.0	2800 1179	0.483 0.077	+0.88 -0.25	11.77 5.04	9.49 4.36	8.29 3.47	-0.50 -2.62	8.79 6.09
22	Apr. 9 to Apr. 21	29 27	535.3 430.5	111.0 94.0	1410 1209	0.468 0.073	+0.59 -0.69	5.97 5.14	5.09 4.33	4.15 3.37	-3.80 -3.76	7.95 7.13
23	Apr. 23 to May 5	18 32	529.0 369.6	110.0 84.0	1401 1203	0.468 0.074	+1.47 -0.52	5.93 5.11	4.72 4.33	3.62 3.44	-4.30 -2.30	7.92 5.74
24	May 7 to May 9	29 27	521.2 413.7	109.1 91.7								7.916 7.067
25	May 9 to May 11	18 32	516.6 355.9	108.3 81.9								6.568 5.189

Phosphorus deficiency and digestibility. The digestibility of the energy of the experimental food was high and uniform throughout the experiment. The standard deviation of the single experiment is $\pm 3.1\%$ for the deficient animals and $\pm 2.5\%$ for the controls. The average digestibility of the food energy in eight respiration trials with the phosphorus-deficient animals in the second and third periods of the experiment is $83 \pm 1.1\%$. The average digestibility of the food energy in the corresponding experiments in the control animals is $81 \pm 0.9\%$. No influence of phosphorus deficiency on the digestibility of protein could be observed. The apparent protein digestibility in the phosphorus-deficient animals was $46.1 \pm 5.6\%$; that of the control animals $46.4 \pm 2.2\%$.

Phosphorus deficiency and urine excretion. The phosphorus-deficient animals excreted on the average slightly less nitrogen in the urine per day than did the controls. The difference, which is insignificant, may be partly related to the somewhat higher food intake of the normal animals. In the fasting katabolism trials the urine was collected during the fourth and fifth day of fasting, and one-half the amount of the nitrogen excretion thus found was regarded as the daily nitrogen excretion during fasting. The daily nitrogen loss during fasting was higher than the nitrogen loss of the animals during food intake. The daily average nitrogen loss for the phosphorus-deficient animals during fasting was 34.5 gm. nitrogen. The normal animals lost on the average 38.1 gm. nitrogen per day of fasting. There was thus no increase in protein katabolism during fasting of the phosphorus-deficient animals as compared with their mates.

In the urine of the animals receiving food the $\frac{C}{N}$ ratio ($\frac{\text{grams C}}{\text{grams N}}$) showed a tendency to be increased with increasing plane of nutrition, ranging from 1.5 to 2.4 in the urine of the phosphorus-deficient animals and from 1.3 to 2.6 in the urine of the controls. The average urinary $\frac{C}{N}$ ratio for the phosphorus-deficient animals is slightly below that of the controls. The difference is, however, negligible.

During fasting the $\frac{C}{N}$ ratio in the urine dropped to an average of 1.08 for both groups of heifers, a fact indicating that in starvation the composition of the urine of ruminants approaches that of carnivorous animals.

The phosphorus-deficient animals used the digested protein less efficiently for sparing body protein than the control animals. This result follows from the data in table 6, where the partial relative protein katabolism, namely the increase in urinary nitrogen divided by the corresponding increase in digested nitrogen, is calculated for the change from the one-

TABLE 6
Partial relative protein katabolism ($\frac{\Delta N \text{ in urine}}{\Delta N \text{ digested}}$) for one-half maintenance to maintenance level

TRIAL NO.	PHOSPHORUS-DEFICIENT ANIMALS						CONTROL ANIMALS					
	Animal	N digested	N in urine	ΔN digested	ΔN in urine	$\frac{\Delta N \text{ in urine}}{\Delta N \text{ digested}}$	Animal	N digested	N in urine	ΔN digested	ΔN in urine	$\frac{\Delta N \text{ in urine}}{\Delta N \text{ digested}}$
13	27	27.0	24.5	21.0	11.4	0.55	29	35.6	28.8	22.8	4.3	0.19
15	27	6.0	13.1				29	12.8	24.5			
14	32	26.9	22.2				10	32.0	20.7	18.6	-0.7	-0.04
16	32	16.1	16.3	10.8	5.9	0.55	10	13.4	21.4			
20	27	10.7	15.5				29	13.6	10.8			
22	27	3.9	10.9	6.8	4.6	0.68	29	6.7	9.8	6.9	1.0	0.15
21	32	6.3	10.6				18	11.9	12.7	5.8	0.6	0.10
23	32	4.2	8.6	2.1	2.0	0.95	18	6.1	12.1			

half maintenance to the maintenance food level. The average of this quotient for the second and third periods of phosphorus deficiency amounts to 0.68 ± 0.10 gm. nitrogen katabolized per gram nitrogen digested for the phosphorus-deficient animals and to only 0.10 ± 0.05 gm. nitrogen katabolized per gram nitrogen digested for the control animals.

CH₄ production. The amounts of CH₄ produced during the second and third periods of the experiment ranged from seventy-two to 161 liters per day for the control animals.

The rate of CH₄ production is strongly influenced by the plane of nutrition. The correlation coefficient for food intake

and CH_4 production amounts to 0.72 for the phosphorus-deficient and 0.92 for the control animals. The effect of food intake on CH_4 production in our trials may be summarized by the following regression equations for the phosphorus-deficient animals:

$$CH_4 = 37 + 32.6 F$$

for the control animals:

$$CH_4 = 29 + 32.9 F$$

where CH_4 = amount of CH_4 produced daily in liters at standard conditions, F = amount of dry matter of food consumed daily in kilograms.

The metabolizability of the food energy is the factor by which the amount of total food energy (heat of combustion as measured in the calorimetric bomb) is to be multiplied in order to calculate the amount of energy available for meat, fat or heat production. The average metabolizability of the food energy in the phosphorus-low rations of $67.0 \pm 1.1\%$ is practically equal to the average metabolizability of $66.6 \pm 1.3\%$ for the energy in the control rations.

CO₂ production. Table 7 presents the results on the respiratory exchange of the phosphorus deficient and the control animals during the second and third period of our experiment. The effect of phosphorus deficient food on the respiratory exchange should be compared with the effect of the phosphorus supplemented food. For this comparison it is necessary to summarize the effect of food on the respiratory exchange in all the trials with phosphorus deficient as well as in the trials with the control animals, since the original plan of having a comparison within each pair trial was defeated by the low appetite of the phosphorus-deficient animals. In order to summarize the effect of food intake on the respiratory exchange one may assume a linear relation between the two variables, such as formulated in equation (7)

$$y = y_0 + r x \quad (7)$$

where y = respiratory exchange, y_0 = respiratory exchange without food, x = food intake, r = a constant characterizing the effect of food.

TABLE 7
Respiratory exchange of phosphorus-deficient and control animals

TRIAL NO.	PHOSPHORUS-DEFICIENT ANIMALS						CONTROL ANIMALS					
	Animal No.	Food dry consumed per day gm.	CO ₂ produced per day liters (s) ¹	O ₂ consumed per day liters (s)	R. Q.	Animal No.	Food dry consumed per day gm.	CO ₂ produced per day liters (s)	O ₂ consumed per day liters (s)	R. Q.		
13	27	2814	1883 ± 29	2015 ± 30	0.958 ± 0.013	29	4331	2222 ± 14	2201 ± 45	1.042 ± 0.018		
14	32	2640	1714 ± 40	1705 ± 44	1.013 ± 0.033	10	4331	2282 ± 10	2188 ± 32	1.035 ± 0.005		
15	27	1634	1538 ± 24	1812 ± 27	0.871 ± 0.015	29	1600	1441 ± 11	1691 ± 24	0.874 ± 0.007		
16	32	1634	1307 ± 25	1468 ± 40	0.915 ± 0.021	10	1600	1633 ± 12	1956 ± 36	0.871 ± 0.013		
17	27	0	1129	1834	0.616	29	0	1128	1620	0.711		
18	51	0	1237	1783	0.694	18	0	1259	1819	0.709		
19	32	0	964	1284	0.704	10	0	1484	2170	0.700		
20	27	1656	1513 ± 7	1648 ± 11	0.919 ± 0.007	29	2756	1880 ± 9	1960 ± 14	0.959 ± 0.003		
21	32	1179	1158 ± 36	1247 ± 35	0.928 ± 0.013	18	2800	1819 ± 13	1891 ± 29	0.974 ± 0.006		
22	27	1209	1310 ± 9	1516 ± 8	0.863 ± 0.003	29	1410	1452 ± 8	1697 ± 14	0.856 ± 0.004		
23	32	1203	1100 ± 12	1222 ± 11	0.898 ± 0.003	18	1401	1442 ± 11	1630 ± 27	0.887 ± 0.016		
24	27	0	1088	1536	0.708	29	0	1232	1730	0.712		
25	32	0	888	1210	0.734	18	0	1075	1478	0.727		

¹ Liters (s) = liters at 0°C., 760 mm. Hg pressure and dry.

If y_0 and r are constant they may be calculated from any number of experiments with varying x and y . The respiratory exchange without food, y_0 , can, however, not be regarded as a constant since it depends on the body size of the animals, which varied during the experiment. In order to eliminate the influence of body size we divide the entire equation (7) by the $\frac{2}{3}$ power of body weight and formulate

$$\frac{y}{w^{\frac{2}{3}}} = \frac{y_0}{w^{\frac{2}{3}}} + r \frac{x}{w^{\frac{2}{3}}} \quad (8)$$

Since the fasting katabolism in calories per $\text{kg.}^{\frac{2}{3}}$ is constant and since the respiratory exchange at a given R. Q. is proportional to the heat production, the term $\frac{y_0}{w^{\frac{2}{3}}}$, the fasting gas exchange per $\text{kg.}^{\frac{2}{3}}$, may also be regarded as a constant. The method of least squares (Ezekiel, '30, p. 55) has thus been applied to the relation of gas exchange per $\text{kg.}^{\frac{2}{3}}$ and food consumption per $\text{kg.}^{\frac{2}{3}}$.

The following regression equation resulted from twelve respiration trials with the phosphorus-deficient animals (27 and 32) during the second and third periods of the experiment (table 7):

$$C = 10.7 + 0.248 f \quad (9)$$

where C = liters CO_2 produced daily per $\text{kg.}^{\frac{2}{3}}$, f = grams dry matter of food consumed daily per $\text{kg.}^{\frac{2}{3}}$.

The corresponding equation for the two control animals 29 and 18 based on ten respiration trials reads

$$C = 10.6 + 0.233 f \quad (10)$$

The difference in the regression coefficients seems to indicate that the food increases the katabolic processes in the phosphorus-deficient animals to a greater extent than in the control animals. This difference in the regression coefficients in our trials, namely 0.015 ± 0.0244 is, however, not statistically significant, the probability that it occurred by random being between 50 to 60% (Fisher, '30, table 14). To prove this difference according to statistical rule, 105 pair trials would have to be carried out if the standard deviation of a

determination would remain the same as in our experiments and if the mean results did not change.

O₂ consumption. The data for O₂ consumption during the second and third period of our experiment are presented in table 7. During the fourth day of fasting the phosphorus deficient animals consumed on the average daily 15.3 ± 0.71 liters O₂ per kg.³. Practically the same result was observed with the control animals namely 15.4 ± 0.23 liters O₂ per kg.³. In the averages for the phosphorus-deficient animals one result with animal 27 has been omitted since an R. Q. of only 0.62 showed that it was erroneous. The conclusion that phosphorus deficiency did not materially affect the O₂ consumption during fasting is, however, justified, whether or not this erroneous result is included in the calculation. It is interesting to note that animal 27 did not decrease its O₂ consumption when the food intake was reduced from the one-half maintenance level to fasting; it showed even a slight increase from 16.1 liters O₂ per day per kg.³ at one-half maintenance to 16.8 liters O₂ per day per kg.³ during fasting which, however, is statistically not significant.

Assuming a linear relation between O₂ consumption per kg.³ and food intake per kg.³ in order to give an approximate summary of our results (as explained for CO₂) the following regression equations have been calculated by the method of least squares applied to the data obtained in respiration trials 13 to 25: for phosphorus-deficient animals

$$O = 14.89 + 0.108 f$$

For the control animals

$$O = 15.04 + 0.067 f$$

The terms in these equations are defined as follows: O = liters O₂ consumed per day per kg.³. f = grams dry matter of food eaten per day per kg.³.

The regression coefficients for O₂ consumption on food intake (0.108 and 0.067) are considerably lower than the regression coefficient for CO₂ production on food consumption (0.248 and 0.233). This result is to be expected if the food

contains mainly carbohydrates. If the animal has an R. Q. of 0.7 during fasting and subsequently raises the R. Q. to 1.0 by ingestion of carbohydrates, then, theoretically, the CO₂ production may increase 40% without a rise in the O₂ consumption.

The regression coefficient of O₂ consumption on food consumption is higher in the phosphorus-deficient animals than in the controls. This relation parallels that for CO₂. The difference is likewise not statistically significant. The probability that the difference is a matter of random is 20 to 30%. At least forty-nine additional pair trials with the same results as those discussed here would be necessary to prove with statistical significance the increase in the stimulating effect of food consumption on O₂ consumption as a result of phosphorus deficiency.

R. Q. During the second and third periods the R. Q. of the phosphorus-deficient animals varied from 0.708 to 1.013 (maximum food intake) (table 7). The corresponding range for the control animals was from 0.709 to 1.042.

The R. Q. of both groups of animals is essentially influenced by the plane of nutrition. The correlation coefficient of R. Q. and the food intake per kg.³ amount to 0.96 for the phosphorus-deficient and to 0.98 for the control animals.

The linear regression equations calculated by the method of least squares are as follows:

For the phosphorus deficient animals

$$R. Q. = 0.731 + 0.10 f$$

For the control animals

$$R. Q. = 0.730 + 0.009 f$$

The term *f* stands, as in the previous equations, for grams dry matter of food consumed daily per kg.³.

During the fourth day of fasting the average R. Q. of the phosphorus-deficient heifers amounted to 0.715 ± 0.009 (three trials); the R. Q. of the control animals to 0.716 ± 0.004 (four trials).

The results of the respiration trials during the second and third periods of the experiment lead thus to the conclusion

that phosphorus deficiency had no influence on the R.Q. of the fasting animals. The slight increase in the R. Q. of the phosphorus-deficient animals with food over that of the control animals is insignificant.

Phosphorus deficiency and fasting metabolism. The calorific value of CO₂ produced by our beef heifers during the fourth and the fifth day of fasting amounted to 6.52 kg. calories per liter. This result has been obtained by calculating the energy loss on the basis of the C and N balances in

TABLE 8
Heat increment and partial energy efficiency. Example of calculation

ANIMAL NO.	TRIAL NO.	FOOD ENERGY METABOLIZABLE PER DAY PER kg. ³ $\frac{\Delta Q}{U}$	HEAT PRODUCED PER DAY PER kg. ³ Q	INCREASE IN METABOLIZABLE FOOD ENERGY ΔU	INCREASE IN HEAT PRODUCTION ΔQ	RELATIVE HEAT INCREMENT $\frac{\Delta Q}{\Delta U}$	PARTIAL ENERGY EFFICIENCY $1 - \frac{\Delta Q}{\Delta U}$
		kg. cal.	kg. cal.	kg. cal.	kg. cal.	%	%
Phosphorus deficient 27	17	0.0	67.8	40.3	13.6	33.7	66.3
	15	40.3	81.4	33.4	7.1	21.3	78.7
	13	73.7	88.5	35.8	- 1.2	- 3.4	103.4
	24	0.0	77.1	15.0	5.2	34.7	65.3
	22	35.8	75.9				
	20	50.8	81.1				

trials 24 and 25. If this figure is used in order to calculate the heat production of the animals during the fifth day of fasting in the second and third periods, one derives an average daily fasting katabolism per kg.³ of (69.3 ± 3.0) kg. calories for the phosphorus-deficient animals (four trials) and (70.3 ± 5.0) kg. calories for the controls (three trials).

Calorigenic action of the food and partial energy efficiency. Table 8 gives an example of the calculation of the relative heat increment, that is, the heat increment or calorigenic action of the food in per cent of the metabolizable energy. The table also shows how the partial-energy efficiency is derived from the relative heat increment.

Fifteen out of twenty comparisons between different food levels in our trials confirm earlier observations of Forbes ('28) and Mitchell and co-workers ('32) that the calorogenic action per unit of food energy in cattle is increased with increasing plane of nutrition.

Thus in eight comparisons of heat production of our heifers at normal phosphorus intake there was an average calorogenic action of $11.8 \pm 3.4\%$ of metabolizable food energy for the change from fasting to one-half maintenance, while the corresponding calorogenic action in changing from one-half maintenance to full maintenance amounted to $26.6 \pm 3.9\%$.

Since the partial efficiency is thus affected by the plane of nutrition the comparison between the efficiency of phosphorus-deficient and control animals should be made at the same food level.

Two animals are assumed to be at the same food level if they take in equal amounts of metabolizable energy of a given food mixture per kg.³ (p. 128). The results of two heifers have been calculated to an intake of 70 kg. calories metabolizable food energy per kg.³. The method used is illustrated in table 9.

The results of these calculations are summarized in table 10. The partial efficiency thus derived from our results is high throughout compared with the efficiency for fattening which would be expected on the basis of the composition of the food. Using Kellner's figures (Kellner, '19, appendix, table 1) one may calculate for example that the 'Wertigkeit' (value) of ration 1 was 82.7%, that of ration 5, 86.5%. Kellner's further data (Kellner and Köhler, '00) for the metabolizable energy in digestible protein (4.96 kilocalories per gram) digestible fat (8.82 kilocalories per gram) digestible crude fiber (3.65 kilocalories per gram) and starch (3.76 kilocalories per gram) together with the partial efficiency of the metabolizable energy for fattening (namely, 45% for protein, 56% for fat, 63% for fiber and 59% for starch), lead to the calculation of a partial efficiency of 46.4% of the metabolizable energy for our ration 1 and 50.7% for ration 5.

The 'Wertigkeit' mentioned above has been used for this calculation.

If the net energy for these two rations is calculated according to Armsby's method (Armsby, '22, p. 674) a partial efficiency of 61% of the metabolizable energy results for ration 1 and 64% for ration 5. The fact that Armsby's values are higher than those of Kellner and our actual results still higher than the values obtained from Armsby's calculation is not surprising. Kellner's data are strictly fattening values for

TABLE 9
Calculation of partial efficiency at a food level of 70 kg. cal. metabolizable energy per kg.³

<i>Animal 27</i>	
Heat production at an intake of 73.7 kg. cal. metabolizable food energy per kg. ³ (trial 13)	88.5 kg. cal. per kg. ³
Heat increment per calorie metabolizable energy between an intake of 40.3 kg. cal. (trial 15) and 73.7 kg. cal. per kg. ³ (trial 13): 0.21 kg. cal.	
Heat increment for a difference of 3.7 kg. cal. metabolizable energy	0.8 kg. cal. per kg. ³
Interpolated heat production at an intake of 70 kg. cal. per kg. ³	87.7 kg. cal. per kg. ³
Heat production during fasting (trial 17)	67.8 kg. cal. per kg. ³
Calculated heat increment for increase of food energy from fasting to 70 kg. cal. per kg. ³	19.9 kg. cal. per kg. ³
Heat increment in per cent of increase in metabolizable food energy: $\frac{19.9}{70} \times 100 =$	28.5%
Partial energy efficiency between fasting and intake of 70 kg. cal. metabolizable food energy: $100 - 28.5 =$	71.5%

adult steers, Armsby's method is based on experiments at supermaintenance as well as submaintenance levels with steers ranging from 9 to 60 months of age (Armsby and Fries, '15, p. 438) and the partial efficiency in our trials was measured at submaintenance of young animals.

Kellner's as well as Armsby's method of calculating net energy leads to the conclusion that the partial efficiency of food B (particularly ration 5) should be higher than that of food A (particularly ration 1). Our data presented in table 10 are in agreement with this prediction.

During the preliminary period, when both heifers received dicalcium phosphate as a supplement to their phosphorus-deficient diet, the partial-energy efficiency was practically the same. During the second period of our trial, when animal 27 was changed to the phosphorus-low diet and developed symptoms of phosphorus deficiency, its partial-energy efficiency was decreased, while its mate that was still receiving dicalcium phosphate utilized the food energy with the same partial efficiency as in the preliminary period.

TABLE 10

Partial energy efficiency of phosphorus-deficient and control heifers. Calculated to a food level of 70 cal. metabolisable food energy per kg.¹

	ANIMAL 27		ANIMAL 29	NO. 32		NO. 18
	Phosphorus deficient	Normal phosphorus intake	Normal phosphorus intake	Phosphorus deficient	Normal phosphorus intake	Normal phosphorus intake
	%	%	%	%	%	%
Food A rations (1 to 4)						
Preliminary period trials 2, 4 and 11		83.1	81.4		77.3	73.3
Second period of phosphorus deficiency trials 13, 15, 17	71.5		83.3	69.6		
Food B (rations 5 and 6)						
Third period of phosphorus deficiency trials 20, 22, 24	84.7		88.7			78.0

The partial efficiency of the phosphorus-deficient animal during the third period is again lower than that of its pair mate on the same diet supplemented with phosphate.

The partial efficiency of the Angus heifer, 32, at the start of the experiment was slightly below that of the two Hereford heifers. Phosphorus deficiency in this heifer also lowered the partial-energy efficiency, namely, from 77.3 to 69.6% during the second period of our experiment, thus confirming the observation with the Hereford heifer, 27. During the third period of the experiment heifer 32 had unfortunately lost its appetite to such a degree that it would not eat more than a one-half maintenance ration.

The decrease in partial-energy efficiency goes parallel with the decrease in the efficiency of protein utilization. The increase in the katabolism of protein in the phosphorus-deficient animals alone cannot, however, account for the entire increase in the calorogenic action of the phosphorus-deficient food.

5. SUMMARY

Two beef heifers fed a phosphorus-deficient diet containing only 0.13% of phosphorus ceased to grow after 6 months of the low-phosphorus regimen, maintained their body weight during the next year (with 0.09% phosphorus in the food), and finally when fed a diet still lower in phosphorus (with 0.068% phosphorus) lost weight. Two control heifers fed with the same food but supplemented with dicalcium phosphate so that the phosphorus content was above 0.4% increased their weight while their phosphorus-deficient pair mates ceased to grow.

The phosphorus-deficient animals developed bone-chewing and coprophagia. The inorganic phosphorus content in their blood dropped from 9.0 to 3.9 mg. of phosphorus per 100 cc. of blood serum while that of the controls remained at the initial level of about 9 mg. phosphorus per 100 cc. of serum.

Phosphorus deficiency had no effect on body temperature, digestibility and metabolizability of the food energy, R. Q. and fasting katabolism.

Phosphorus deficiency increased slightly the regression of CO₂ production and O₂ consumption on food intake (not proved with statistical significance). It decreased the partial efficiency of energy utilization, the efficiency of food protein for sparing body protein, and the appetite of the animals.

Phosphorus deficiency lowers the total efficiency of energy utilization ($\frac{\text{Energy in the product}}{\text{Total food energy}} = \text{mean partial efficiency} - \frac{\text{Total intake of food energy}}{\text{Fasting katabolism}}$) mainly by lowering the appetite and secondly by lowering the partial efficiency whereas it does not seem to influence the fasting katabolism.

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