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# JOURNAL OF AGRICULTURAL RESEARCH

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## A COMPARISON OF DIRECT AND INDIRECT CALORIMETRY IN INVESTIGATIONS WITH CATTLE<sup>1</sup>

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### INTRODUCTION

From the time of the construction of the first respiration calorimeter by Lavoisier (9, p. 355)<sup>2</sup> until Rubner (10 p. 135) demonstrated the applicability of the law of conservation of energy to the vital processes of the animal body, the interest of experimenters with animal calorimeters centered mainly in the question as to whether the products of respiration fully represent the production of heat by the animal.

With the establishment of the validity of this conception began a new era in respiration calorimetry, which has been marked by the perfecting of apparatus and the standardization of methods. Since, however, the respiration calorimeter is a complicated and costly apparatus, both in construction and use, methods have been evolved for the indirect determination of the heat production, by the use of a respiration apparatus, without the calorimetric features.

For the purpose of indirect heat estimation there are now in use two principal methods, with modifications of detail, to meet special conditions as to kind of animal, length of experiment, equipment available, accuracy sought, and the particular problem in hand.

Briefly and generally, one of these methods (2) involves the estimation of the amounts of protein, carbohydrates, and fat oxidized in the body, and the computation of the heat production from the calorific values of these nutrients.

The amounts katabolized are computed from the oxygen consumed and carbon dioxide produced, and the nitrogen, carbon, oxygen, and hydrogen of the urine, these computations being based on the respiratory quotients (pro-

portions of carbon dioxide produced to oxygen consumed) for the nutrients oxidized, these quotients being approximately 0.7 for fat, 0.8 for protein and 1.0 for carbohydrates.

The other method depends on the balances of nitrogen and carbon in the animal body, and the determination of the potential energy of the feed and excreta. By this method the heat production is measured by deducting from the gross energy of the feed the potential energy of the excreta and the energy equivalent of the body tissue gained (or adding the energy equivalent of tissue lost).

The more fundamental considerations as to energy metabolism having been established, a question of much importance is the comparative accuracy of the expensive and time-consuming direct-heat estimation by means of the respiration calorimeter and the relatively simple indirect-heat estimation by means of the two general procedures outlined.

As a contribution to the understanding of this problem the writer presents the following consideration of the possibilities of error in each, and the extent to which these errors affect results, the comparisons being based on the experimental work of this institute.

The respiration calorimeter at the Institute of Animal Nutrition, which has been used for more than 20 years in metabolism experiments with cattle, is, as the name implies, both a calorimeter and a respiration apparatus. In each experiment heat production has been determined directly, and also, for comparison, by computation from the balance of matter and energy, as in the second of the indirect methods referred to above. This method is essentially the same as that used by Kellner (?) in his extensive researches on cattle.

<sup>1</sup> Received for publication June 11, 1924; issued May, 1925. This study was undertaken at the suggestion of Dr. E. B. Forbes, to whom the writer is especially indebted for kindly criticism and assistance in revising the manuscript. He is also under obligations to the other members of the staff of the Institute for many valuable suggestions in connection with this work.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 406.

A comparison of the observed and computed heat production in experiments with steers, conducted in the years prior to and including 1909, has been reported by Armsby (6). Since the year 1909 a number of experiments have been made on steers, and on cows as well, and upon these the present paper is based.

### OBSERVED AND COMPUTED HEAT PRODUCTION

Before entering upon a discussion of the direct and indirect methods of determining the heat production by animals let us compare the results obtained by the two methods. Tables I and II present the results of experiments with steers and cows, the steer experiments comprising 35 periods and the cow experiments 36. Each period was of 48 hours' duration (except in experiment 221a and 221d, 886-I, where the periods were 24 hours).<sup>3</sup> All results have been computed to a 24-hour basis.

The results of the steer experiments show that in 21 cases out of the 35 the computed heat production is higher than the observed; in 10 cases the computed heat is lower than the observed, and in 4 cases the difference between the two is so small as to be negligible.

Considering the differences in the individual trials, and arranging them in groups according to magnitude, the results are as follows: In 21 cases, or 60.0 per cent of the total number, the difference exceeds 1 per cent, of which 13 are plus (that is, show the computed heat production the larger); in 14 cases, or 40.0 per cent of the total number, the difference exceeds 2 per cent, of which 10 are plus; in 6 cases, or 17.1 per cent of the total number, the difference exceeds 3 per cent, of which 5 are plus; in 2 cases, or 5.7 per cent of the total number, the difference exceeds 4 per cent, of which both are plus; and in 2 cases, or 5.7 per cent of the total number, the difference exceeds 5 per cent, of which both are plus.

TABLE I.—Observed and computed heat production of steers

Experiment No.	Animal	Period No.	Gain by animal	Heat production in 24 hours		Difference	
				Computed	Observed		
			<i>Calories</i>	<i>Calories</i>	<i>Calories</i>	<i>Calories</i>	<i>Per cent</i>
210	Steer D	I	-845.7	9,262.9	9,460.6	-197.7	-2.1
		II	-1,141.5	8,026.1	8,186.0	-159.9	-1.9
		III	-2,153.8	6,942.0	7,110.4	-168.4	-2.4
211	do	I	-883.2	12,139.1	11,546.9	+592.2	+5.1
		II	-496.9	9,813.8	9,596.5	+217.3	+2.3
		III	+7,663.8	13,815.6	13,937.2	-121.6	-0.9
211	Steer G	IV	-2,462.1	9,227.4	9,196.5	+30.9	+0.3
		V	-4,968.6	8,010.9	7,952.9	+58.0	+0.7
		I	+327.9	11,642.1	11,710.8	-68.7	-0.6
211	Steer G	II	-1,565.5	8,229.9	8,197.0	+32.9	+0.4
		III	+6,133.6	12,807.3	13,290.2	-482.9	-3.6
		IV	-2,981.5	8,942.6	8,936.5	+6.1	+0.1
212	Steer H	V	-4,154.1	6,894.8	6,882.5	+12.3	+0.2
		I	+2,241.2	11,130.7	11,022.9	+107.8	+1.0
		II	+2,529.3	10,690.4	10,903.4	-213.0	-1.9
212	Steer H	III	+840.4	9,712.9	9,723.1	-10.2	0.1
		IV	+859.9	9,805.8	9,701.6	+104.2	+1.1
		V	-739.6	7,296.0	7,304.9	-8.9	-0.1
216	Steer J	VI	-409.8	6,950.4	6,773.1	+177.3	+2.6
		I	+5,111.5	15,931.6	15,541.2	+390.4	+2.5
		II	-1,046.5	9,836.3	9,547.2	+289.1	+3.0
216	Steer J	III	+596.7	11,420.2	10,865.8	+554.4	+5.1
		IV	-2,672.9	8,186.4	8,020.4	+166.0	+2.1
		V	+2,299.7	12,820.9	12,827.5	-6.6	0.1
217	do	VI	+907.3	11,062.8	10,838.2	+224.6	+2.1
		VII	-1,708.8	8,501.5	8,398.4	+103.1	+1.2
		I	+927.2	11,240.9	10,896.8	+344.1	+3.2
217	do	II	+8,493.1	16,934.8	16,671.7	+263.1	+1.6
		III	+6,463.3	21,416.0	21,204.9	+211.1	+1.0
		IV	-559.6	14,716.0	14,224.4	+491.6	+3.5
220	Steer K	I	-626.0	11,881.5	12,123.6	-242.1	-2.0
		II	-2,270.5	10,108.7	10,288.1	-179.4	-1.7
		III	+464.5	10,809.7	10,754.2	+55.5	+0.5
220	Steer K	IV	+4,644.5	13,524.3	13,844.0	-319.7	-2.3
		V	-2,951.5	10,136.3	10,055.8	+80.5	+0.8
Totals and averages, 35 periods				379,868.6	377,535.2	+2,333.4	+0.6

<sup>3</sup> Period 886-I, experiment 221d, was to have covered 48 hours, but owing to the failure of the electric current the experiment was discontinued during the second 24 hours.

TABLE II.—Observed and computed heat production of cows

Experiment No.	Cow No.	Period No.	Dry or in milk	Gain by animal	Heat production in 24 hours		Difference	
					Computed	Observed		
				<i>Calories</i>	<i>Calories</i>	<i>Calories</i>	<i>Calories</i>	<i>Per cent</i>
221a	631	I	In milk	+221.4	11,720.5	11,463.9	+256.6	+2.2
		II	do.	+3,663.2	12,598.8	12,333.9	+264.9	+2.2
		III	Dry	+3,863.5	13,680.3	13,219.2	+461.1	+3.5
221a	615	I	In milk	+502.0	11,756.5	11,435.5	+321.0	+2.8
		II	do.	-160.7	12,286.2	12,137.3	+148.9	+1.2
		III	do.	-650.3	12,747.6	12,032.2	+715.4	+6.0
221a	579	I	do.	+384.7	13,545.9	13,235.6	+310.3	+2.3
		II	do.	+686.7	13,165.5	12,896.2	+269.3	+2.1
221c	885	I	do.	-2,883.6	11,007.1	11,221.4	-214.3	-1.9
		II	do.	-2,957.5	9,023.7	8,862.4	+161.3	+1.8
		III	do.	-4,830.4	9,032.1	8,859.0	+173.1	+2.0
221c	886	I	do.	+2,976.8	10,183.6	11,191.7	-1,008.1	-9.0
		II	do.	-1,228.2	8,228.7	8,568.6	-339.9	-4.0
		III	do.	+3,018.2	10,885.9	11,063.4	-177.5	-1.6
221d	885	I	Dry	+730.6	8,201.5	8,380.3	-178.8	-2.1
		II	do.	+4,410.1	9,099.0	9,629.1	-530.1	-5.5
		III	do.	+366.9	9,289.5	8,816.7	+472.8	+5.4
221d	886	I	do.	+897.8	8,407.9	8,036.9	+371.0	+4.6
		II	do.	+4,389.2	10,774.5	10,474.5	+300.0	+2.9
		III	do.	+895.5	8,511.0	7,892.4	+618.6	+7.8
221e	885	I	do.	+2,625.6	9,157.4	9,721.7	-564.3	-5.8
		II	do.	-155.0	8,444.7	8,716.4	-271.7	-3.1
221e	886	I	In milk	+1,795.0	11,852.0	12,047.9	-195.9	-1.6
		II	do.	+1,685.3	11,136.6	11,049.3	+87.3	+0.8
221e	874	I	do.	+537.3	11,563.9	11,417.0	+146.9	+1.3
		II	do.	+2,998.6	11,456.6	11,612.6	-156.0	-0.5
221f	874	I	Dry	+3,500.6	11,279.2	10,964.2	+315.0	+2.9
		II	do.	+159.6	9,445.2	9,076.8	+368.4	+4.1
221f	887	I	do.	+2,019.0	10,649.3	10,327.2	+322.1	+3.1
		II	do.	+240.0	8,733.5	8,395.0	+338.5	+4.0
221f	886	I	In milk	+26.5	13,187.1	13,589.4	-402.3	-3.0
		II	do.	+2,151.0	12,268.1	12,422.0	-153.9	-1.2
		III	do.	+7.0	12,701.3	12,367.7	+333.6	+2.7
221g	887	I	do.	-5,327.8	8,887.9	8,627.1	+260.8	+3.0
		II	do.	-827.5	10,429.9	9,870.3	+559.6	+5.7
		IV	do.	+2,270.1	12,010.5	11,670.4	+340.1	+2.9
Totals and averages, 36 periods.					387,349.0	383,525.2	+3,823.8	+1.0

The extreme percentage differences are +5.1 and -3.6, while the total computed heat production for the 35 trials exceeds the observed by only 0.6 per cent.

In the 57 earlier steer experiments reported by Armsby (6) the total observed heat production for the 57 trials differed from that computed by only 0.4 per cent, while the extreme percentage differences in individual cases were +7.6 and -5.1 per cent. Grouping the results of the 57 trials as above one finds: In 30 cases, or 52.6 per cent of the total number, the difference exceeds 1 per cent, of which 17 are plus (in favor of the computed heat production); in 21 cases, or 36.8 per cent of the total number, the difference exceeds 2 per cent, of which 13 are plus; in 14 cases, or 24.6 per cent of the total number, the difference exceeds 3 per cent, of which 10 are plus; in 6 cases, or 10.5 per cent of the total number, the difference exceeds 4 per cent, of which 4 are plus; in 4 cases, or

7 per cent of the total number, the difference exceeds 5 per cent, of which 3 are plus; in 2 cases, or 3.5 per cent of the total number, the difference exceeds 6 per cent, of which both are plus; and in 1 case, or 1.8 per cent of the total number, the difference exceeds 7 per cent, which is plus.

In general, the results of the steer experiments given in Table I compare favorably with those reported by Armsby in showing a close agreement between the observed and the computed heat production, though there are considerable differences in a few cases.

In the cow experiments the differences between the observed and the computed heat production are wider than with the steers. Of the 36 trials, 24 show a higher computed heat production than the observed, while in 12 cases the observed heat production is higher than that computed, the percentage differences ranging from +7.8 to -9.0. The total computed heat

production for the 36 periods is higher than that observed by 1.0 per cent. Grouping the results as before one has: In 34 cases, or 94.4 per cent of the total number, the difference exceeds 1 per cent, of which 23 are plus; in 26 cases, or 72.2 per cent of the total number, the difference exceeds 2 per cent, of which 20 are plus; in 14 cases, or 38.9 per cent of the total number, the difference exceeds 3 per cent, of which 9 are plus; in 9 cases, or 25 per cent of the total number, the difference exceeds 4 per cent, of which 6 are plus; in 7 cases, or 19.4 per cent of the total number, the difference exceeds 5 per cent, of which 4 are plus; in 2 cases, or 5.6 per cent of the total number, the difference exceeds 6 per cent, of which 1 is plus; in 2 cases, or 5.6 per cent of the total number, the difference exceeds 7 per cent, of which 1 is plus; and in 1 case, or 2.8 per cent of the total number, the difference exceeds 8 per cent which is minus.

In considering, then, the results of Tables I and II, the following questions suggest themselves: First, in comparing the heat production obtained by the direct and the indirect method which of the two possesses the greater accuracy? Second, what are the possible causes for the wider differences between the computed and the observed heat production in the cow experiments than in those made on steers? In studying these questions let us consider, briefly, the sources of error and the methods used in each.

#### SOURCES OF ERROR IN THE INDIRECT METHOD

The indirect method here considered, by which the figures for computed heat production in Tables I and II were obtained, is the second of the two indirect methods referred to above, the so-called balance method. The basic principle upon which both of these indirect methods are based is that the oxidation of a given substance in the body liberates the same amount of energy as does its oxidation outside the organism. The difference, then, between the gross energy of the feed and the energy of the total excreta, as determined by analytical methods, gives the energy derived from the feed, and is equal to the heat given off by the animal, in case the animal does not gain or lose body tissue. A loss of body tissue, of course, involves a liberation of energy exceeding that derived from the feed; therefore the energy-equivalent of the body tissue lost is added to the energy derived

from the feed in computing the total heat production. On the other hand, a gain of body tissue means that not all of the energy derived from the feed was liberated, a part of it being stored in body tissue, the energy-equivalent of which is subtracted from the energy of the feed in computing the heat production.

Representing the heat production by  $H$ , the energy of the feed by  $F$ , the energy of the total excreta (feces, urine, methane, brushings, and milk) by  $E$ , and the energy of the body gain by  $G$ , the general working formula for heat production is  $H = F - E - G$ .

The estimation of the gain or loss of body tissue, which is an essential feature of this method, is computed in accord with the conception of the schematic body, which regards the organic matter of the animal as composed essentially of protein and fat, with at most comparatively small amounts of carbohydrates (glycogen). The supply of glycogen is assumed to remain constant during the experiment, and the gain or loss of protein and fat is estimated from a balance between income and outgo of nitrogen and carbon.

The factors used in the computation are the following: protein =  $N \times 6$ ; fat =  $C \times 1.307$ ; carbon in protein = 52.54 per cent; heat of combustion of protein per gram = 5.7 Calories; heat of combustion of fat per gram = 9.5 Calories. The derivation of these factors is fully discussed by Armsby (2).

The analytical data needed for computing the heat production by the balance method are: (1) Dry matter in the feed, feces (or feces and urine mixture) and brushings; (2) nitrogen in the feed, feces, urine, milk, and brushings; (3) carbon in feed, feces, urine, milk, and brushings; (4) carbon in the gaseous excreta (respiration apparatus); (5) carbon in methane (by respiration apparatus); (6) energy in the feed, feces, urine, milk, and brushings.

The accuracy of this indirect method obviously depends on the accuracy of the factors used and on the accuracy of the analytical results. To what extent the factors used may be responsible for the differences between the computed and the observed heat production is difficult to say. Certainly any error due to the factors used should be least where the gain or loss of body tissue is least. Referring, however, to the results in Tables I and II, one notices in many cases relatively small differences between the observed and

the computed heat production where the body gains or losses are considerable, and large differences where the gains or losses are comparatively small. It will be noted that in these tables the net gain in calories is given, and that the differences are somewhat obscured in those cases in which there was a gain of one substance and a loss of another by the algebraic addition of their energy equivalents. However, the separate consideration of the gains or losses of protein and fat does not materially alter the general relationship referred to above, since in most cases in which there was a considerable gain or loss of body tissue both fat and protein were gained or lost together.

In those cases, at least, in which the gains or losses of body tissue are small and the differences between the observed and the computed heat production are large, if there is any serious error in the computed heat production it should be looked for in the analytical results. An examination of some of the analytical data recently obtained by the methods used in this institute has revealed possibilities of error which in the case of the experiments with cows deserve especial consideration. In these experiments there is, in addition to the problem of handling the milk, the necessity of collecting urine and feces together, thus giving rise to new difficulties in the handling and preparation of the samples for analysis.

With this exception, essentially the same experimental procedure and analytical methods were followed in the cow experiments as in the steer experiments (5, p. 200-222). It is important, however, to bear in mind the following points in connection with the computation of the nitrogen, carbon, and energy balances: (1) In the steer experiments the nitrogen in the feces was determined in the fresh substance, while the carbon was determined in the air-dried sample, by means of a combustion furnace. (2) In the urine the carbon was determined in the fresh material, while the nitrogen was determined both in the fresh material and in a sample dried in the vacuum desiccator in the same manner as for energy determination. From the nitrogen loss thus determined, in drying, an energy correction was computed as applying to the energy determined by the bomb, assuming that the loss of nitrogen in drying represented a loss of urea and a corresponding loss of energy.

In the cow experiments (except in 221a)<sup>4</sup> the feces were collected together with the urine. The nitrogen was determined in the composite of daily samples both in the fresh and in the air-dried materials in all cases, in order to ascertain the loss during drying. Carbon was determined in the air-dried sample by means of the bomb, in all cases, and also (in experiments 221g and 221f) in the fresh substance, by means of an electric combustion furnace. An attempt was made to determine the carbon in the fresh sample of the feces-and-urine mixture in the first of the experiments with cows, but owing to lack of laboratory help during the war and for some time thereafter, and to the initial technical difficulties with the method, no satisfactory determinations of the carbon in the fresh feces-and-urine mixture are available for the earlier cow experiments. The energy of the feces-and-urine mixture was determined in the air-dried sample.

The air-drying was conducted by spreading the material out on trays in a drying closet in which it was exposed to a current of air heated to about 60° C. by passage over a steam coil. To hasten the drying the material was carefully broken up, when partly dried, by means of a spatula. When thoroughly dry, the material (properly protected) was exposed to the air of the grinding room for several days. It was then weighed, ground as rapidly as possible, and preserved for analysis in sealed, glass-stoppered bottles. In this weighing and grinding especially dry or especially damp days were avoided.

#### LOSS OF NITROGEN AND CARBON IN AIR-DRYING FECES-AND-URINE MIXTURE

A comparison of determinations of the daily nitrogen and carbon of the feces-and-urine mixture, in the fresh samples, with determinations on air-dried samples, revealed the fact that a considerable loss of these constituents occurred during the process of drying, and that the loss of carbon exceeded the quantity required to combine with the nitrogen lost to form either urea or ammonium carbonate. Table III exhibits these losses as occurring in experiments 221f and 221g.

The figures in Table III show clearly that the carbon in the air-dried feces and urine is far from the original total, even after adding to it the carbon-equivalent of the nitrogen lost, computing this nitrogen to urea or ammonium carbonate.

<sup>4</sup> In experiment 221a the feces and urine were collected together, in the calorimeter, while during the other days of the digestion period they were collected separately.

TABLE III.—Loss of nitrogen and carbon during the drying of cow's feces-and-urine mixture

Experiment and period	Drymatter per day	Nitrogen lost per day	Carbon lost per day		
			Total	Combined with nitrogen computed from N lost)	Not combined with nitrogen
	Grams	Grams	Grams	Grams	Grams
221f:					
874 I.....	2, 225. 8	78. 3	71. 9	33. 6	38. 3
874 II.....	1, 516. 0	50. 3	58. 8	21. 6	37. 2
887 I.....	1, 887. 4	82. 0	70. 7	35. 2	35. 5
887 II.....	1, 296. 5	59. 2	48. 5	25. 4	23. 1
886 I.....	3, 178. 8	83. 9	119. 6	36. 0	83. 6
886 II.....	2, 739. 8	96. 8	124. 7	41. 5	83. 2
221g:					
887 I.....	2, 793. 1	57. 7	108. 3	32. 5	75. 8
887 II.....	1, 405. 3	58. 5	82. 2	25. 1	57. 1
887 III.....	2, 072. 2	61. 4	79. 3	26. 3	53. 0
887 IV.....	2, 924. 1	87. 3	83. 5	37. 5	46. 0

To diminish this possibility of error in the carbon balance, in those cow experiments in which the carbon was not determined in the fresh feces-and-urine mixture, the loss of carbon on drying, per gram of nitrogen lost, was computed from the average of the data available, this average being 1.15 gm. and the correction for carbon lost on drying was computed by the use of this factor. That this correction is not entirely satisfactory is realized, since the loss of carbon does not in all cases follow exactly the loss of nitrogen, but it was considered, under the conditions, as the best possible way of correcting these earlier results which were obtained from the air-dried material.

#### LOSS OF ENERGY IN AIR-DRYING FECES-AND-URINE MIXTURE

A loss of nitrogen and carbon on drying does not mean a loss of only these elements, but it also represents a certain loss of potential energy. While the original fresh feces-and-urine mixture might have contained some carbon as free CO<sub>2</sub>, the quantity so contained, in view of its solubility, could be only a very small fraction of the total quantity lost during the drying. Some of this loss no doubt came from the decomposition of urea, and this portion has been estimated in the experiments from the nitrogen lost. The remainder has been assumed, in the absence of definite knowledge, to be a result of carbohydrate fermentation, since the conditions of the drying are regarded as favorable for such a process. On this basis the energy lost on drying has been computed by using Rubner's factor, 5.45 for loss in Calories per gram of nitrogen

lost, and the factor 9.4 for computing Calories per gram of carbon lost in excess of that required to satisfy the nitrogen lost, the latter factor representing the energy per gram of carbon in starch.

Although the above computation is not without logical justification, one must admit the possibility of considerable error in this correction, in view of our imperfect knowledge as to the nature and extent of the losses. Since it is impracticable directly to determine the energy in the fresh feces-and-urine mixture, the drying process may be, therefore, a source of serious error in the computation of the heat production.

To illustrate the extent to which the above considerations, as to losses and corrections may affect the computed heat production Table IV has been compiled, the data in the three methods of computation being derived from the data of experiments 221f and 221g. In all three cases the nitrogen was determined in the fresh substance, and energy in air-dry substance. In Method I carbon was determined in the air-dry material. Corrections for carbon and energy were computed from the nitrogen lost, calculated as urea. In Method II carbon was determined in the fresh material, corrections for energy being computed as in Method I. In Method III the carbon was determined in the fresh material and the corrections for energy were computed as in Method I, with an additional correction for carbon lost in excess of that required to combine with the nitrogen to form urea (using the factors 5.45 per gram nitrogen and 9.4 per gram carbon).

TABLE IV.—Computed heat production as affected by loss of carbon during drying

Experiment No.	Cow No.	Period No.	Method I. Carbon of air-dry substance; carbon and energy corrected for loss of N		Method II. Carbon of the fresh substance; energy corrected for loss of N		Method III. Carbon of the fresh substance; energy corrected for loss of N and for C uncombined with N	
			Computed heat production	Computed ÷ observed	Computed heat production	Computed ÷ observed	Computed heat production	Computed ÷ observed
			<i>Calories</i>	<i>Per cent</i>	<i>Calories</i>	<i>Per cent</i>	<i>Calories</i>	<i>Per cent</i>
221f.....	874	I.....	11,163.7	101.8	11,639.2	106.2	11,279.2	102.9
		II.....	9,334.0	102.8	9,794.9	107.9	9,445.2	104.1
221f.....	887	I.....	10,542.4	102.1	10,983.2	106.4	10,649.5	103.1
		II.....	8,663.8	103.2	9,142.6	108.9	8,733.4	104.0
221f.....	886	I.....	12,923.8	95.9	13,972.1	102.9	13,176.0	97.0
		II.....	12,017.1	96.7	13,050.2	105.1	12,268.1	98.8
		III.....	12,472.7	100.8	13,413.8	108.5	12,701.3	102.7
221g.....	887	I.....	8,715.6	101.0	9,424.6	109.2	8,887.9	103.0
		II.....	10,270.0	104.0	10,928.1	110.7	10,429.9	105.7
		IV.....	11,871.8	101.7	12,442.9	106.6	12,010.5	102.9

In consideration of the loss of carbon on drying the feces-and-urine mixture, the results of Method I are seemingly paradoxical. Why, one may ask, by using a figure for carbon in the feces and urine, known to be much lower than the correct value, does the computed heat production agree closely with the observed, in fact, in most cases, even more closely than when computed by Method III? The answer lies in the details of the computation. Referring to the general formula for heat production,  $H = F - E - G$ , it is seen that H remains unchanged if G is increased and E is decreased simultaneously by the same amount. When, in computing the balance of carbon, the carbon lost through fermentation is ignored, the figure for carbon in the excreta is low, and the apparent gain of carbon and, therefore, of energy (G) is correspondingly high or the loss low. On the other hand, if one ignores that portion of energy of the feces and urine which was lost in drying as a result of the fermentation, the energy of the feces and urine (and therefore E) is low. These two factors, therefore, oppose each other, the net effect being the difference between the two. For example, in experiment 221f, 874-I, the carbon calculated to have been lost from the excreta by fermentation was 38.3 gm. This being ignored, the balance of carbon showed a gain which was 38.3 gm. too high. This is equivalent to 50.06 gm. of fat ( $38.3 \times 1.307$ ), or in terms of energy, 475.5 Calories ( $50.06 \times 9.5$ ). G is therefore 475.5 Calories too high. The heat lost in fermentation, computed from the carbon, was 360.0 Calories. This being

ignored, E is too low by 360.0 Calories. The net effect on the computed heat production is therefore  $360.0 - 475.5 = -115.5$  Calories, which accounts for the difference between I and III in Table IV ( $11,279.2 - 11,163.7 = 115.5$ ).

To state this somewhat differently, since the metabolizable energy equals the gross energy of the feed minus the energy of the feces, urine, and methane, when the metabolizable energy and the energy of the body gain are increased simultaneously the net effect on the computed heat production equals the increase of the former minus the increase of the latter. Thus, in the case just cited, the metabolizable energy is too great by 360 Calories, while the energy of the body gain is too great by 475.5 Calories. The net effect on the computed heat production is, therefore,  $360.0 - 475.5 = -115.5$  Calories.

Thus it is possible to obtain a figure for heat production, which may agree closely with the observed, as a result of a balance of opposing errors.

The results of Method II show unusually large differences between the computed and the observed heat production, the carbon in the feces and urine as determined in the fresh substance being used, and the energy lost in fermentation ignored.

The results of Method III are as they appear also in Table II. The basis for the computation and the possibilities of error have already been considered.

The results as set forth in Tables III and IV indicate the need of rigorous control of the conditions of drying the feces and urine, and further investigation of the nature of the material lost during drying.

Recurring now to the computation of the heat production in the steer experiments, the feces and urine were collected, dried, and analyzed separately. The carbon in the urine was determined in the fresh sample, and the correction for energy lost on drying was computed from the nitrogen lost. The carbon in the feces was, however, determined in the air-dried sample, ignoring the possibility of loss of carbon and energy during the drying. There is no data covering the losses on drying steer feces. There is, however, data which show a considerable loss of carbon in drying cow feces (not a mixture of urine and feces), which also implies a loss of energy. The results of the analysis of several samples of cow feces, which were collected by an attendant on a single day of the digestion experiments, are given in Table V.

POSSIBLE ERROR IN THE DETERMINATION OF DRY MATTER OF FECES-AND-URINE MIXTURE

The determination of the dry matter of the feces-and-urine mixture is another source of error to which attention is called.

For determinations of energy, nitrogen, carbon, etc., in the combined feces and urine, the composite sample representing several days' collection was used. In all cow experiments this composite was made up of 10 daily aliquots. The dry matter, however, was determined in this composite and also in each of the daily samples. The same routine was followed in the drying of the daily samples as in the case of the composite, except that the former were generally left in the drying closet for a longer period of time, since, as a rule, they were placed in the

TABLE V.—Losses of nitrogen and carbon occurring during the drying of cow feces

Experiment and period	Dry matter of feces	Loss of nitrogen		Loss of carbon		Loss of carbon per gram nitrogen lost	
		Grams per day	Per cent <sup>a</sup>	Grams per day	Per cent <sup>a</sup>		
221f:							
874 I.....	2,013.7		0.092	1.86	1.045	21.0	11.29
874 II.....	1,409.7				1.208	17.0	
887 I.....	1,560.2		.367	5.73	3.379	52.7	9.20
887 II.....	1,086.3		.240	2.61	1.891	20.6	7.89
886 I.....	2,762.2		.236	6.53	1.872	51.7	7.92
886 II.....	2,796.0		.208	11.41	3.114	87.1	7.63
221g:							
887 I.....	2,755.4		.165	4.54	1.672	46.1	10.15
887 II.....	1,147.2		.124	1.43	1.000	11.5	8.04
887 III.....	1,583.4		.160	2.53	1.384	21.9	8.66
887 IV.....	2,665.1		.096	2.56	3.228	86.3	33.71

<sup>a</sup> Computed to dry-matter basis.

These results show that while the loss of nitrogen is slight, the loss of carbon is several times as great.

It would, of course, be unsafe to assume that identical losses occurred in the drying of steer feces since the composition of the rations, and, therefore, presumably, of the feces differed from that applying to the experiments with cows. Furthermore, the losses on drying are shown to be too variable to be regarded as accurately applying to other conditions. However, the data suggest the probability of significant loss of carbon during the drying of steer feces also.

The bearing of the facts as to losses on drying, as disclosed by the above data, is also obviously important in connection with digestion experiments.

closet as soon as obtained, and were allowed to remain until the composite sample also was thoroughly dry.

In all cases but one the percentage of dry matter as determined on the composite was found to be higher than the true average of determinations on the daily samples. Such a uniform difference must have an explanation other than errors of sampling or weighing. Aside from these errors, such differences could be due either to an unweighed evaporation of moisture from the composite during preparation, or to a greater loss of dry matter during the drying of the daily samples. Considering the precautions taken to prevent evaporation the loss by this means could have been but slight. The loss of dry matter due to fermentation

during the drying of the daily samples appears, therefore, to be the more extensive factor of error, but since there must have been some error on both these accounts the true figure for dry matter must have been higher than the one and lower than the other estimation, and there being no means of determining this intermediate figure exactly, the average of the two was used.

Table VI gives the results of the determinations of dry matter in the composite sample, the average of determinations in the daily samples, and the average of the two, as well as the possible error, in per cent and in grams of dry matter per day.

The differences between the two percentages of dry matter are fairly uniform. The average of all differences (omitting that of experiment 221c, 886 I) is 0.869 per cent. This means that, on the average, the dry matter as determined on the composite is about 0.9 per cent higher than the true average of the daily determina-

tions or that the possible error of using the average of the two is about  $\pm 0.4$  per cent. The magnitude of the error in grams per day obviously depends on the fresh weight of the material, and ranges in these experiments from  $\pm 14.4$  gm. to  $\pm 113.2$ . It should be understood that the data presented apply only to the particular conditions and procedures which prevailed in this work.

An error in the determination of dry matter involves corresponding errors in energy, nitrogen, and carbon as determined on the dried material, all being in the same direction. These possible errors have been computed in Table VII, using the data from experiments 221f and 221g as examples. The possible errors in energy range from  $\pm 135.4$  Calories to  $\pm 451.9$  calories; the possible errors in nitrogen are small, ranging from  $\pm 0.59$  gm. to  $\pm 2.46$  gm.; the possible errors in carbon range from  $\pm 13.49$  gm. to  $\pm 44.96$  gm.

TABLE VI.—Possible error of determinations of dry matter in feces-and-urine mixture

Experiment and period	Fresh weight	Dry matter			Possible error	
		I Composite sample	II True average of daily samples	III Average of I and II	Per cent	Grams dry matter
221c:	Grams	Per cent	Per cent	Per cent	Per cent	Grams dry matter
885 I.....	20,038.4	13.182	12.312	12.747	$\pm 0.435$	$\pm 87.2$
885 II.....	14,044.4	13.170	11.946	12.558	$\pm .612$	$\pm 86.0$
885 III.....	14,785.4	15.050	13.810	14.430	$\pm .620$	$\pm 91.7$
886 I.....	17,137.8	12.595	15.097	13.846	$\mp 1.251$	$\mp 214.4$
886 II.....	12,121.0	13.044	12.242	12.643	$\pm .401$	$\pm 48.6$
886 III.....	17,364.2	15.973	14.669	15.321	$\pm .652$	$\pm 113.2$
221d:						
885 I.....	9,415.2	14.740	13.848	14.294	$\pm .446$	$\pm 42.0$
885 II.....	16,614.8	12.268	11.520	11.894	$\pm .374$	$\pm 62.1$
885 III.....	10,410.8	13.391	12.555	12.973	$\pm .418$	$\pm 43.5$
886 I.....	9,258.9	15.511	14.523	15.017	$\pm .494$	$\pm 45.7$
886 II.....	15,567.8	14.429	13.715	14.072	$\pm .357$	$\pm 55.6$
886 III.....	10,464.6	13.178	12.468	12.823	$\pm .355$	$\pm 37.1$
221e:						
885 I.....	11,784.9		14.098			
885 II.....	8,937.8	13.954	13.094	13.524	$\pm .430$	$\pm 38.4$
886 I.....	17,992.2	15.942	14.956	15.449	$\pm .493$	$\pm 88.7$
886 II.....	15,846.1	15.681	15.007	15.344	$\pm .337$	$\pm 53.4$
874 I.....	14,513.1	17.373	16.263	16.818	$\pm .555$	$\pm 80.6$
874 II.....	11,900.2	17.099	16.857	16.978	$\pm .121$	$\pm 14.4$
221f:						
874 I.....	13,370.7	17.131	16.163	16.647	$\pm .484$	$\pm 64.7$
874 II.....	9,018.0	17.362	16.206	16.784	$\pm .578$	$\pm 52.1$
887 I.....	13,424.0	14.514	13.588	14.051	$\pm .463$	$\pm 62.2$
887 II.....	8,909.0	14.992	14.110	14.551	$\pm .441$	$\pm 39.3$
886 I.....	20,344.0	16.073	15.099	15.586	$\pm .487$	$\pm 99.1$
886 II.....	18,668.0	14.830	14.510	14.670	$\pm .160$	$\pm 29.9$
221g:						
887 I.....	16,924.0	16.903	15.965	16.434	$\pm .469$	$\pm 79.4$
887 II.....	10,220.0	14.178	13.322	13.750	$\pm .428$	$\pm 43.7$
887 III.....	14,022.0	15.126	14.416	14.771	$\pm .355$	$\pm 49.8$
887 IV.....	18,531.0	16.102	15.442	15.772	$\pm .330$	$\pm 61.2$

TABLE VII.—Effect of possible error in the dry matter of feces-and-urine mixture on the energy, nitrogen, carbon, and computed heat production

Experiment and period	Possible error in daily dry matter (from Table VI)	Energy per gram dry matter	Carbon in dry matter	Nitrogen in dry matter	Possible error in energy	Possible error in carbon	Possible error in nitrogen	Energy equivalent to the carbon C×9.4	Energy equivalent to the nitrogen N×5.45	Energy equivalent to both N and C	Possible error in computed heat production
	Grams	Calories	Per cent	Per cent	Calories	Grams	Grams	Calories	Calories	Calories	Calories
221f:											
874 I....	±64.7	4.425	43.801	2.845	±286.3	±28.34	±1.84	±266.4	±10.0	±276.4	±9.9
874 II....	±52.1	4.381	43.648	2.975	±228.3	±22.74	±1.55	±213.8	±8.4	±222.2	±6.1
887 I....	±62.2	4.451	44.207	2.397	±276.9	±27.50	±1.49	±258.5	±8.1	±266.6	±10.3
887 II....	±39.3	4.394	43.890	2.590	±172.7	±17.25	±1.02	±162.2	±5.6	±167.8	±4.9
886 I....	±99.1	4.560	45.368	2.485	±451.9	±44.96	±2.46	±422.6	±13.4	±436.0	±15.9
886 II....	±29.9	4.528	45.101	1.989	±135.4	±13.49	±0.59	±127.3	±3.2	±130.5	±4.9
221g:											
887 I....	±79.4	4.555	45.469	2.557	±361.7	±36.10	±2.04	±339.3	±11.1	±350.4	±11.3
887 II....	±43.7	4.547	45.303	2.464	±198.7	±19.80	±1.08	±186.1	±5.9	±192.0	±6.7
887 III..	±49.8	4.493	45.123	2.360	±223.8	±22.47	±1.18	±211.2	±6.4	±217.6	±6.2
887 IV..	±61.2	4.545	45.323	2.258	±278.2	±27.74	±1.38	±260.8	±7.5	±268.3	±9.9

It will be noted that the possible errors in energy of the feces-and-urine mixture, as a result of the possible errors in dry matter, appear to be appreciable, and they would remain so if the nitrogen and, especially, the carbon as determined on the dried substance were not used as a basis for computing the loss of nitrogen and carbon on drying and of an energy correction corresponding to these losses.

The differences between the nitrogen and carbon as determined on the dry substance and those determined on the fresh represent the loss on drying. A positive error in the carbon and nitrogen determined on the dried substance would, therefore, mean a negative error in the computed loss on drying and, consequently, a negative error in the computed energy correction when such a correction is applied, and vice versa. As a result of this, the initial error in energy is reduced by the energy equivalent of the error in the computed loss of nitrogen and carbon during drying. These energy equivalents and the net effect on the computed heat production are shown in Table VII. The resultant error is negligible according to this method of computation. For example, if the error in dry matter in experiment No. 221f, 874I were +64.7 gm. (see Table VII), the energy of the daily feces-and-urine mixture, as computed by multiplying grams dry matter by the number of Calories per gram, would be too great by the amounts indicated in column 5, namely 286.3 Calories (64.7 times 4.425). Similarly, the figures for carbon and nitrogen as determined on the dry substance would be too high by the amounts indicated in columns 6 and 7 respectively (64.7

times  $\frac{43.801}{100} = 28.34$  gm. carbon and

64.7 times  $\frac{2.845}{100} = 1.84$  gm. nitrogen).

On the other hand, by subtracting the too large amounts of carbon and nitrogen in the dry material from those determined on the fresh the results for loss of carbon and nitrogen on drying, thus obtained, would be too small by the same amounts given in columns 6 and 7. The energy corrections based on these losses would then be too small by the amounts indicated in columns 8 and 9, totaling in the case cited 276.4 Calories (column 10), and making the final error in the energy of these excreta equal  $286.3 - 276.4 = 9.9$  Calories, or in the computed heat production,  $-9.9$  Calories.

#### DETERMINATION OF DRY MATTER IN FEED

It is obvious that an error in the determination of the dry matter of the feed would directly affect its energy equivalent, and also the carbon and nitrogen balances.

Dry matter is determined by first subjecting the sample to a preliminary determination of the air-dry substance; the sample is then ground, and the remaining hygroscopic moisture is determined in the ground sample. If there is any loss of moisture during the grinding, this is not accounted for in the determination, and would increase the figure for dry matter in the feed.

The effect of grinding on the dry matter determination is a very variable factor, the exact magnitude of which is unknown, but a preliminary investigation of this problem indicates that the

grinding of the samples may be a source of appreciable error in the dry matter determination. However, the effect of an error in the dry matter of the feed, on the computed heat production, would be relatively slight, for the reason that the metabolizable energy and the energy of the body gain would be affected in the same direction. This is illustrated by the following computation, the basis being arbitrarily assumed figures, and the effect on the nitrogen balance being disregarded:

Daily ration.....	8,000 gm. air-dry matter.
Error in dry matter..	+0.5 per cent.
Energy per gram dry matter.....	4.5 Calories.
Carbon in dry mat- ter.....	45 per cent.
Error in the daily dry matter.....	+40 gm.
Error in the metab- olizable energy....	$40 \times 4.5 = +180$ Calories.
Error in carbon bal- ance.....	+18 gm.
Error in energy of the body gain.....	$18 \times 1.307 \times 9.5 = +223$ Calories.
Error in computed heat production...	$180 - 223 = -43$ Calories.

#### OTHER SOURCES OF ERROR

**THE GLYCOGEN CONTENT OF THE BODY.**—The computation of the energy equivalent of the gain by the animal assumes that the glycogen content of the animal remains unchanged. With a submaintenance ration, or in the case of milking cows, when the ration is not sufficient fully to support the milk production, the possibility of a continuous loss of glycogen for some time may be considered. In the event of such loss the estimate for the energy equivalent of the body loss may be too large. In those cases in which there is a gain of body substance, after a considerable number of days of preliminary feeding on the ration of the experimental period to follow, the possibility that the glycogen content of the animal will come into equilibrium with the ration is greater, and the error involved in the assumption of a constant glycogen content of the body may not be appreciable.

**CARBON EQUIVALENT TO THE NITROGEN LOST BY THE BODY.**—Whether nitrogen be gained or lost by the body, it is considered to be accompanied by such an amount of carbon as is contained in an equivalent amount of protein, carbon being considered as constituting 52.54 per cent of the protein. The gain or loss in fat is computed from the carbon balance only after the carbon content of the protein gained or lost has been set aside. In case of a loss of protein, if there is a utilization of a part of the nonnitrogenous fraction of the katabol-

izing tissue protein the amount so utilized is not accounted for, and constitutes an error in the computation of the carbon equivalent to the nitrogen lost and, likewise, in the carbon balance.

**CARBON DIOXIDE OF THE WATER CONSUMED.**—The carbon dioxide content of the water consumed by the animal is usually not taken into consideration. This introduces a small error into the carbon balance, tending to reduce the apparent gain of carbon and to make the computed heat production too great.

**HEAT OF HYDRATION AND SOLUTION.**—Another small error is introduced in the determination of the energy of the visible excreta by neglecting the heat of hydration and solution. If heat is absorbed when solids come in contact with water, the reverse must be true, that is, heat is evolved when water is driven off. Conversely, if heat is evolved when solids are brought in contact with water, this heat must be absorbed on driving off the water. Thus, it is known that when urea is brought into solution an absorption of heat takes place, while when proteins are brought into contact with water an evolution of heat takes place. The feed is ordinarily given to the animal in an air-dry condition. Hydration and solution take place in the animal body, and corresponding evolution and absorption of heat must result. These processes may be considered as factors in the work of digestion. The energy of the urine, feces, and milk is determined after these substances have been dried. The heat of hydration and solution is not accounted for in the determination.

#### SUMMARY OF OBSERVATIONS ON SOURCES OF ERROR IN THE INDIRECT METHOD

Errors on the following accounts are either unavoidable or are of negligible magnitude: Possible change in the glycogen content of the body of the animal; possible utilization of the non-nitrogenous portion of the katabolized protein molecule; and the energy of hydration and solution.

It seems possible that errors due to the two factors first mentioned might be of appreciable importance in experiments in which the subjects are under submaintenance conditions.

A small determinable error is introduced by the carbon dioxide of the drinking water.

By far the most serious possibilities of error seem to lie in the methods of preparing the samples of feed, feces,

and urine for analysis. Of these the drying of the feces-and-urine mixture is the most important, since it involves the computation of corrections, the bases for which are imperfectly understood.

#### DIRECT HEAT MEASUREMENT

The direct measurement of the heat given off by the animal body is accomplished by means of the respiration calorimeter. Descriptions of such an apparatus, as used at this institute, and also of the methods employed, have appeared in several publications by Armsby and Fries (3; 4, p. 263-270; 1; 5, p. 200-222). For the present purpose the more important details may be condensed into the following:

The respiration calorimeter used at the Institute of Animal Nutrition of the Pennsylvania State College is of the Atwater-Rosa type, consisting of a Pettenkofer respiration apparatus, the chamber of which is also an animal calorimeter.

The respiration chamber is built with a double metallic wall inclosing a 3-inch dead-air space, surrounded, at a distance of 4 inches, by a wooden wall, and this in turn, at a distance of 4 inches by a second wooden wall, thus forming two air-spaces surrounding the chamber. By the heating or cooling of these air spaces the inner wall is maintained adiabatic.

The sensible heat given off by the animal, which constitutes the greater part (about three-fourths) of the total heat production, is absorbed by a current of cold water passing through copper pipes at the top of the respiration chamber, the exposure of these pipes to the air within the chamber being regulated by means of shields which can be raised or lowered by the operator. By this means the rate of removal of heat from the chamber is adjusted as required for the maintenance of an approximately constant temperature.

The temperature of the in-going air is maintained equal to that of the outcoming.

The temperature of the in-going and outcoming water is read every four minutes by means of two mercurial thermometers, graduated to 1/50° C., and carefully calibrated.

The volume of water passing through the calorimeter is measured by means of two copper meters of 100-liter capacity.

For each period of uniform water flow the product of the amount of water passing through the heat ab-

sorbers multiplied by the average temperature difference equals the amount of heat removed from the chamber in the water current.

Since a part (approximately one-fourth) of the total heat given off by the animal is in the form of latent heat of water vapor, this portion of heat, not being directly measurable by the calorimeter, is calculated from the amount of water vapor given off, using 0.587 Calorie per gram at 18° C. This heat is added to the heat removed in the water current to get the total heat emission.

#### CORRECTIONS

In order accurately to determine the amount of heat produced by the animal several corrections are made, for possible errors in the readings, for heat measured by the apparatus but not coming from the animal, and for heat withdrawn from the chamber but escaping measurement. The following is the list of corrections usually made:

(a) Corrections for difference of pressure on the bulbs of the two thermometers.

(b) Correction for friction of water in absorbers.

(c) Correction for lag in the rise or fall in temperature of the water from that at the inlet to that at the outlet of the absorber system.

(d) Correction for change in temperature of the absorber system during the experiment.

(e) Correction for heat developed by the blades of the fan used to stir the air in the calorimeter.

(f) Correction for change in temperature of the walls of the chamber during the experiment.

(g) Corrections for heat introduced into the apparatus or withdrawn from it, in feed, drink, excreta, and vessels containing these materials.

(h) Correction for the metabolism of the man entering the chamber during the experiment, to milk the cow.

(i) Correction for condensation of water on the absorber system.

(j) Correction for storage or loss of heat due to gain or loss of matter by the animal body during the experiment.

#### ACCURACY OF THE DIRECT METHOD

This list of the corrections serves to indicate the degree of accuracy which it is sought to attain. There are, however, two other sources of error which have been disregarded

because no practicable methods of measuring or estimating corrections have been found.

These errors depend on changes of body temperature of the animal during an experiment, and change in amount of moisture upon the walls of the chamber during an experiment.

Assuming, under the conditions of the experiments, the possibility of a change of  $\pm 0.6^\circ$  F. (8, p. 24-26) ( $0.3^\circ$  F. per day, in the same direction during a two-day period), the possible error in the daily heat determination, in the case of an animal weighing 400 kg., would be  $\pm 53$  Calories.

As to the moisture factor, there is the possibility of a deposit of moisture on the walls of the chamber, or of an evaporation of moisture from the same, during the experiment, thus constituting an error in the measurement of the latent heat of water vapor produced. Under ordinary conditions this error is probably not appreciable. Its magnitude and direction would depend on the initial humidity in the chamber, length of the preliminary period, rate of ventilation, and water vapor given off by the animal. It is apparent that this error would be greater in short-time experiments than in those of longer duration.

As regards the ultimate accuracy of the direct method, from a consideration of the results of the 18 alcohol check tests reported from this institute by Armsby and Fries (5, p. 200-222) the authors came to the conclusion that the results of a single experi-

ment with the respiration calorimeter may be regarded as accurate to within approximately the following percentages of the amounts determined: Heat, 1 per cent; water, 6.0 per cent.

The determination of the carbon dioxide production, which is used in the indirect method, is regarded as accurate to within approximately 0.5 per cent of the amount determined.

The results of 14 alcohol checks made subsequent to the above-mentioned report, in practically the same manner as before, and using heat values for anhydrous alcohol ranging from 7.07537 to 7.13107 Calories per gram, show also a close agreement between the computed and the observed values, the average percentages recovered being as follows: Heat 98.9 per cent; water, 108.0 per cent; carbon dioxide, 99.9 per cent. The results of these 14 individual tests are set forth in Table VIII.

It will be noted that in all these tests the observed heat is less than the computed. This rather uniform difference may be due either to the assignment of too high a heat value to the alcohol, or to the heat measurement, or to both. The range of the differences, however, is narrow. Considering the results of all the alcohol checks, and the fact that the possibility of error in short-time experiments is greater than in experiments of longer duration, the conclusion stated above, with regard to the accuracy of the direct heat measurement as well as to the carbon dioxide determination, seems to be warranted.

TABLE VIII.—Results of alcohol check tests

Date	Number of hours	Heat			Carbon dioxide			Water		
		Observed	Computed	Observed ÷ computed	Observed average of aspirator and meter	Computed	Observed ÷ computed	Observed average of aspirator and meter	Computed	Observed ÷ computed
		<i>Calories</i>	<i>Calories</i>	<i>Per cent</i>	<i>Grams</i>	<i>Grams</i>	<i>Per cent</i>	<i>Grams</i>	<i>Grams</i>	<i>Per cent</i>
Nov. 23, 1911	8	4,085.4	4,144.7	98.6	1,097.0	1,110.8	98.8	732.6	739.3	99.0
Dec. 31, 1912	8	4,252.6	4,302.0	98.9	1,170.1	1,152.9	101.4	852.2	770.0	110.7
Apr. 29, 1913	7	3,778.9	3,817.2	99.0	1,022.4	1,023.0	99.9	759.9	683.2	111.2
Dec. 4, 1913	7	3,426.8	3,491.4	98.2	928.8	935.7	99.3	679.3	631.9	107.5
Apr. 28, 1914	7	3,397.9	3,440.9	98.8	921.1	922.1	99.9	698.9	622.8	112.2
Dec. 17, 1914	7	3,481.5	3,507.7	99.3	939.5	940.1	99.9	671.8	634.9	105.8
June 23, 1915	7	3,462.8	3,478.3	99.6	931.1	932.2	99.9	689.4	629.3	109.6
May 9, 1916	7	3,507.9	3,584.5	97.9	965.1	960.6	100.5	673.0	647.4	104.0
Dec. 14, 1917	7	3,294.8	3,331.0	98.9	897.2	898.3	99.9	651.6	606.4	107.5
Jan. 3, 1918	7	3,252.1	3,310.5	98.2	884.4	893.5	99.0	637.1	602.3	105.8
Apr. 20, 1920	8	3,534.0	3,540.8	99.8	955.8	956.4	99.9	744.9	644.6	115.6
Apr. 21, 1921	8	3,383.4	3,390.7	99.8	908.4	915.9	99.2	672.1	617.2	108.9
Apr. 20, 1922	8	3,429.6	3,461.1	99.1	945.0	934.9	101.1	676.0	630.1	107.3
Nov. 24, 1922	8	3,495.2	3,542.3	98.7	949.4	949.6	100.0	685.7	637.7	107.5
Total		49,782.9	50,343.1	98.9	13,515.3	13,526.0	99.9	9,824.5	9,097.1	108.0
Average		3,555.9	3,595.9	98.9	965.4	966.1	99.9	701.8	649.9	108.0

\* By aspirator only.

## SUMMARY OF DIRECT AND INDIRECT HEAT ESTIMATION

The results of the study of the details of direct and indirect calorimetry in experiments with steers and cows at this institute may be summarized as follows:

The direct heat measurement is in general more accurate than the balance method of indirect heat computation. The direct method, therefore, serves as a valuable check on the accuracy of the analytical work involved in the indirect method.

In the measurement of the nutritive values of feeds, however, the direct and the indirect methods are about equally accurate, and the latter is much the more easily accomplished.

One source of error, not affecting direct heat measurement, but involved in the utilization of both direct and indirect heat measurements in the determination of the net energy values of feeds, is the loss of moisture of feeds and feces during grinding preparatory to analysis, but this factor affects both methods.

The most important source of error affecting direct and indirect calorimetry in different ways is loss of matter, from the urine and feces, presumably through fermentation, during drying, preliminary to the determination of energy by means of the bomb calorimeter.

This loss, which contains carbon and nitrogen, is an important factor in the computation of the heat production by the indirect method, and in the application of the results of the direct method in determining the net energy value of feeds.

This loss of matter affects the estimation of (a) metabolizable energy, as determined by subtracting from the energy of the feed the energy of the excreta, (b) heat production, as determined by subtracting from the metabolizable energy the energy of the gain (carbon and nitrogen balances multiplied by factors), (c) energy of gain, as determined by subtracting from the metabolizable energy the directly observed heat production.

The determination of the energy of the gain from the nitrogen and carbon balances does not involve the loss under discussion, since the nitrogen and the carbon can be determined on the fresh substance. When, however, the direct heat estimation is used in the final computation of net energy any such advantage as might accrue from the greater accuracy of this direct heat

estimation is lost through the fact that in order to derive the amount of the energy of the gain, which is a factor of the net energy estimation, the directly observed heat production must be subtracted from the metabolizable energy, this last datum being directly affected by the loss in drying of the excreta.

In those cases in which there is a gain of body tissues, or no appreciable loss, the error due to possible change in glycogen content of the body may be considered as negligible, and the estimation of the gain in energy, by the indirect method, may be as accurate as when determined by the direct method.

On account of the possibilities of change in the glycogen content of the bodies of cows, in connection with milk production, the chances for error in indirect calorimetry with cows seem greater than with steers.

There is need for careful control of the conditions of drying feces and urine, and of a thorough investigation into the composition and nature of the substances lost, in order to make possible the more accurate estimation of the dry matter and the energy of these excreta.

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