BETWEEN-ANIMAL VARIATION IN THE AMOUNT OF ENERGY REQUIRED FOR THE MAINTENANCE OF COWS

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PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
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CONTENTS

LIST OF SYMBOLS

dm	dry matter
om	organic matter
cp	crude protein
tr p	true protein
c fat	crude fat
c fibre	crude fibre
N-fe	nitrogen-free extractives
mm	mineral matter
dom, dcp, etc.	digestible om, digestible cp, etc.
W	body weight
M	metabolizable energy
G	gain
H	heat expenditure
c	gain correction factor
p	weight correction factor
$M_{m,W}$	kcal. metabolizable energy required for maintenance by an animal weighing W kg.
M_{m,W_o}	$M_{m,W}$ after correction to a body weight of W_o kg.
$M_{m,500}$	M_{m,W_o} in which $W_o = 500$ kg.
SD, s	standard deviation
CV	coefficient of variation

STELLINGEN

I

De individuele variatie van de voederbehoefte voor onderhoud der runderen is waarschijnlijk niet bruikbaar voor de fokkerij; deze variatie mag echter niet verwaarloosd worden bij nauwkeurige balansproeven.

Dit proefschrift

II

Het is bij de voeding van herkauwers gewenst onderscheid te maken tussen werkelijk en schijnbaar beschikbare energie.

III

In het Duitse normaalblad Din 51708 over de bepaling van de verbrandingswaarde worden de fouten, welke men kan maken bij de meting van de temperatuur van het water van de calorimeter, onderschat.

IV

Bij het kopziekte-onderzoek zijn kortdurende proeven, waarbij de veranderingen van dag tot dag in de verhoudingen van magnesium tot chromogenen in gras en mest en van magnesium tot creatinine in urine worden bepaald, van evenveel belang als conventionele magnesium-balansproeven.

V

In vergelijking tot wat momenteel in gebruik is, dient de grootte van de korting bij de betaling van de melk naar hygiënische kwaliteit slechts aanzienlijk verhoogd te worden, wanneer twee of meer malen achtereen derde klasse melk wordt geleverd; die van de toeslag kan onveranderd blijven.

VI

Het is niet waarschijnlijk, dat bij de respiratie-proeven in Kopenhagen inderdaad naast methaan ook aethaan in de uitademingslucht der runderen voorkwam.

Beretning 111 (1923) 32; 204 (1943) 44; 240 (1949) 21

VII

Bij het bestuderen van de in de voormagen van herkauwers voorkomende microbiologische omzettingen, welke van veel belang zijn voor de voeding van het dier, dienen de uitkomsten verkregen met uit deze voormagen geïsoleerde bacteriën slechts onder voorbehoud te worden aanvaard.

INTRODUCTION

In the spring of 1956 equipment for energy balance experiments with cows, which included two respiration chambers, was ready for use at the Laboratory of Animal Physiology, Agricultural University, Wageningen. In the first series of experiments it was decided to investigate the *between-animal* variation in feed requirement for maintenance of dry cows. Such an investigation is clearly of considerable importance for animal husbandry while it has the advantage that the experimental difficulties are considerably less than those of many other investigations such as the determination of the starch equivalent of roughages (see BROUWER, 1958a). During this first series the reliability of the experimental equipment could also be tested.

It could be expected that it would be possible to compose rations on which the animals would be not far from energy and from nitrogen equilibrium and thus only small amounts of carbon, nitrogen or calories would be gained or lost by the body of the animal. Afterwards the low, positive or negative energy balances might be corrected to metabolic equilibrium by applying suitable corrections to the given amounts of feed. In this way figures on the maintenance requirement of gross, digestible or metabolizable energy might be obtained. Measuring maintenance requirement expressed in net energy units is much more difficult as at least two experimental periods are needed with different amounts of feed (ARMSBY, 1912; MØLLGAARD, 1931, p. 240). From the difference in energy balance of both periods the net energy value of the feed and the requirement of net energy units during equilibrium can be computed. This computation involves extrapolation and requires therefore a very high accuracy for all data; moreover even small fluctuations of the heat expenditure of the animals from period to period will give less reliable results. It was therefore decided to do experiments with only one period with cows that were approximately in energy equilibrium, and to reduce the experimental results to zero energy balance. Meanwhile due attention was to be given to the methods used.

In the nutrition of cows feed is considered to serve two different purposes. One part, 50% and more (BROUWER, 1958a; AXELSSON and ERIKSSON, 1953), is needed for maintenance purposes, the remainder being used in the production of body gain, milk and work or in the development of the foetus.

As compared with the extensive literature dealing with the value of feeding stuffs for production few experiments have been carried out on the value of feeding stuffs for maintenance. Still less research has been done on the energy requirement for maintenance of individual cows (BROUWER, 1958a). There are a number of reasons for this rather small number of experiments. The first is that the maintenance part of the ration mainly consists of home-grown and relatively cheap bulky feeds whereas the productive part generally comprises purchased, and expensive concentrates. Furthermore it seems that the *between-animal* variation in maintenance requirement is rather high compared with that in the amount of feed required for production alone (BREIREM, 1944, p. 60; SCHIEMANN, 1958, p. 80); this variation must be considered in any measurement of the value for maintenance of feeding stuffs. The statement of RITZMAN and BENEDICT (1938, p. 127) that the basal metabolism may vary from month to month further complicates the determination of the energy requirement for maintenance and the capacity of a particular food to meet the requirement. These results of RITZMAN and BENEDICT, however, need confirmation as the investigators measured the heat expenditure of the animals after four and after one day of fasting. Another reason for the limited work in this field is that the long duration of balance experiments involving as they do an immense amount of work made this kind of research less attractive, the more especially as in the last decades experiments on vitamins and antibiotics promised quicker results.

Recent work of ARMSTRONG *et al.* (1957) on the utilization of the end products of digestion in the rumen once more indicates the importance of experiments on maintenance metabolism. These investigators have found that the heat increment of the volatile fatty acids – acetic, propionic and butyric – introduced into the rumen of sheep, was a great deal lower for maintenance than for fat production. Moreover, while the proportion of the three acids in the mixture did not influence the heat increment in maintenance experiments except when the mixture contained 97% or more acetic acid, it did considerably influence the magnitude of the heat increment in experiments above maintenance.

The main subject of this study is the *between-animal* variation in requirement of metabolizable energy for maintenance of cows. The experiments were performed with dry animals in their 7th month of pregnancy; they received the same rations which were estimated to be approximately sufficient for maintenance. It was our opinion that the energy used for reproduction in the 7th month of pregnancy would be small. Moreover, if the investigation afterwards was to be extended to farmers' cows, then only animals in this month of gestation might be obtained easily. As in the first series of experiments the energy balances of the animals turned out to be rather high, a second series was completed in which a smaller ration was fed. To get some idea as to the increment in metabolism due to pregnancy the energy balances of some of the animals were again measured two months later near the end of the gestation period.

The use of different rations in the two series, the occurrence of high and low energy balances and of differences in the body weight of the animals were reasons for some statistical work on experiments with animals near energy equilibrium found in the literature.

1. GENERAL CONSIDERATIONS

1.1. Outline of methods

For obtaining charisteristic and mutually comparable figures on the maintenance requirement of an animal, it should be maintained under circumstances which should correspond as closely as possible to those obtaining during a determination of basal metabolism, *i.e.*, the animal must be within the zone of thermoneutrality and its muscular activity should be reduced as much as possible. In ordinary circumstances the animal will certainly be above the minimum requirement because it will make certain physiologically-unnecessary movements; the maintenance requirement therefore will be somewhat higher than the theoretical minimum. In the following discussion only maintenance conditions prevailing in a cowshed with healthy, tethered animals are considered.

Only that part of the feed which is energetically important is taken into account. The rations fed should provide adequate amounts of vitamins and minerals, so that it seems justified to assume that during the few weeks of the experiments no real deficiencies invalidate the results. At the same time the ration should contain some roughage to ensure normal rumination.

The C-, N- and energy balances of the animal indicate whether there is or is not nitrogen and energy equilibrium within the body. A gain of N indicates the storage of protein in the body, a gain of C in excess of any due to a gain of protein, the formation of fat and/or glycogen. The amount of glycogen in the body is assumed to remain constant during experiments carried out with animals in the steady state. The energy balance gives the caloric value of both gains together.

The following formulae have been used:

Digestible energy = energy in feed – energy in faeces,

Metabolizable energy = energy in feed – energy in faeces – energy in urine – energy in methane,

Energy balance = metabolizable energy – heat expenditure,

N-balance = N in feed – N in faeces – N in urine,

C-balance = C in feed – C in faeces – C in urine – C in CO_2 – C in CH_4,

Energy balance = energy in gain of fat + energy in gain of protein.

Most elements of these formulae may be measured directly. For some others the following constants and the following formula are in use in this laboratory (Brouwer, 1958b; Brouwer, in prep.):

1 litre CO_2 (N.T.P.) contains 0.536 g.C,

1 litre CH_4 (N.T.P.) contains 0.536 g.C and 9.45 kcal.,

Body protein contains 52% C and yields 5.7 kcal./g.,

Body fat contains 76.73% C and yields 9.5 kcal./g.,

Heat expenditure (kcal.) = 3.869 × O_2-consumption (litres) + 1.195 × CO_2-production (litres) — 0.227 × 6.25 × N in urine (g.) — 0.516 × CH_4-production (litres).

For comparison of the maintenance requirement of different animals the results, however expressed (*i.e.* as gross, digestible or metabolizable energy) must first be converted to standard conditions viz. energy equilibrium and the same body weight. *Between-animal* variation in the maintenance requirement of gross or digestible energy will be similar to that of metabolizable energy. The principal reason is that *between-animal* variation in digestibility is small (KELLNER, 1919, p. 49; RINGEN, 1940; WATSON *et al.*, 1947, 1949; MINSON and RAYMOND, 1957; ANDERSEN *et al.*, 1959). The energy lost in urine and as methane is small compared with the energy lost in faeces and therefore, *between-animal* variation in these two losses will also have little influence on the size of the *between-animal* variation in maintenance requirement of gross or digestible energy.

In energy balance experiments the feed intake can with equal ease be expressed in terms of gross, digestible or metabolizable energy. The same holds for the correction toward energy equilibrium. The variation in maintenance requirement of metabolizable energy due to the compositions of the rations may be expected to be much smaller than variation in maintenance requirement of gross – and to a lesser extent – of digestible energy. For this reason we restrict ourselves in the following discussion to the maintenance requirement of metabolizable energy.

1.2. CORRECTION TO ENERGY EQUILIBRIUM

For correction of the metabolizable energy to energy equilibrium we must know how much metabolizable energy is required for the gain of fat and of protein and the metabolizable energy equivalent of fat or protein of the body when these are used for maintenance. On maintenance rations the nitrogen balances are nearly always small, and so the error is not great if, for simplicity, we do not make a distinction between a gain or loss of energy in fat and one in protein.

Not all the metabolizable energy in fact is truly available for the animal. A small part of it is transformed to heat by the rumen organisms and valueless for the animal which within the zone of thermoneutrality has an excess of heat. The amount of heat produced by the rumen organisms may depend on the composition of the ration. On the other hand the metabolizable energy does not include the energy of the metabolic faecal constituents. This may partly compensate the loss of energy due to the rumen organisms.

In the literature many figures are to be found on the net energy content of the metabolizable energy of single feeding stuffs for maintenance and production. All these values have been obtained from difference experiments involving two experimental periods, one experimental period with a certain ration and the second with the same ration plus the test feed. The difference in energy balance, corrected if necessary, gives the net energy 'for maintenance', perhaps better called for negative production, or for fat production of the test feed depending as to whether the experiments were done below or above energy equilibrium. With a view to minimising the experimental error large differences in energy balance are preferred.

Most investigators agree that the net energy content of metabolizable energy 'for maintenance' is higher than for fat production (MØLLGAARD and LUND, 1929, p. 29; ARMSTRONG *et al.*, 1957; NEHRING *et al.*, 1959). There is, however, no common opinion whether the relationship between net energy and metabolizable energy should be represented by an exponential graph or by two nearly straight lines going to the left and right from the point of equilibrium, each with its own slope. From an examination of the literature NEHRING *et al.* (1959) have concluded that in experiments above energy equilibrium there are very few observations which indicate a deviation from a linear relation between both kinds of energy. Nor did these authors find any deviation in linearity in their own experiments with rabbits and rats. From a theoretical point of view formation of body fat is probably a more complicated process than prevention of katabolism of body fat needed for energy purposes.

Another problem of interest for the correction to energy equilibrium is whether the net energy content of a certain feed is the same when it is used in different rations. FORBES (1933) believed that this is not always the case.

Also about the net energy content of whole rations opinions differ. AXELSSON (1939) held that there is only optimal utilization of the metabolizable energy in rations for fat production when they contain 18–23% crude fibre in the dry matter. MØLLGAARD (1939) and BREIREM (1944) have not agreed with AXELSSON's method of computation and have rejected the concept of an optimal level of crude fibre. ARMSTRONG *et al.* (1957) found in their experiments with volatile fatty acids introduced into the rumen of sheep in positive energy balance a higher utilization of the energy of the acids when the mixture was low in acetic acid. It is now generally accepted that a very large part of the carbohydrates of the feed is converted into volatile fatty acids in the rumen and that these acids are quickly absorbed from the rumen (SCHAMBYE, 1955; ANNISON and LEWIS, 1959, p. 124). There are indications that a high crude fibre content of the ration gives a higher production of acetic acid which is therefore in agreement with ARMSTRONG *et al.*'s observations.

KELLNER computed the starch equivalent of the feed from the digested true protein, digested crude fat, digested crude fibre and digested N-free extractives, but in the case of roughages applied an extra correction, viz. 0.58 times the percentage of crude fibre of the feed, in order to get values equal to those found in a few actual experiments. He ascribed the discrepancy mainly to the higher mechanical and chemical work of digestion of fibrous feed. According to ARMSTRONG *et al.* (1957) the volatile fatty acids may also be important here.

SCHIEMANN (1958) computed the requirement of metabolizable energy for fat production from pure fat, protein and carbohydrate in monogastric animals, from thermodynamic considerations of the processes involved, and found about the same figures as in actual experiments with animals. Lack of reliable information makes such a computation however very difficult. For the same reason it is nearly impossible to do it with ruminants.

MITCHELL (1942, p. 160) has said that one conclusion toward which the trend of research in energy nutrition seemed to be leading might be stated as follows: The utilization of the metabolizable energy of well-balanced rations approaches constancy for the same kind of animals in approximately the same condition of physiological functioning. This view is accepted by KRISS (1943), with reserve, and by SWIFT (1957) and by HARDISON (1959). The last-mentioned goes so far as to declare that feeding value is mainly determined by the digestible energy. He agrees that theoretically the net energy content is the better measure, but prefers the digestible energy content when rations are concerned partly because of the views of MITCHELL and partly because of the fact that only a few laboratories are able to determine net energies and then only on a small scale.

KELLNER (1900) studied the requirement of metabolizable energy for maintenance of 8 mature oxen. In the correction of the experimental results for body gain he used figures for the requirement of metabolizable energy per kcal. gain – in the following called *c* – found in actual difference experiments. In the two cases where the energy balance was negative, he added an amount of energy, equal to the energy of the negative balance, to the metabolizable energy fed. ARMSBY (1912, p. 42) did not agree with the latter correction as more metabolizable energy in the feed would have been required to get the animal in equilibrium. He proposed a value for *c* of 1.75. AXELSSON and ERIKSSON (1953) used a value of 1.61 in the correction of the balances of experiments of KELLNER and FINGERLING (optimal content of crude fibre in the dry matter of the rations), while in correcting those of ARMSBY and FORBES they used a value of 1.83 (content of crude fibre not optimal). SCHIEMANN and NEHRING (1956, p. 247) estimated *c* at 2.0 from the results of experiments of KELLNER. SCHIEMANN (1958, p. 59) used this value in the correction of 34 experiments of KELLNER and estimated the standard deviation of this figure at 0.2.

1.3. CORRECTION FOR BODY WEIGHT

In order to obtain the maintenance requirement under standard conditions (see 1.1.), we also need to know the relation between the maintenance requirement of metabolizable energy (M_m) and body weight (W). It has to be admitted that there are quite a diversity of opinions as to the nature of this relationship at the present time.

The body weight of a cow A on a ration of low dry matter intake or on a ration of concentrates will be lower than the weight of a cow B on a ration of high dry matter intake or on a ration of roughage, if their empty weights – weight of body minus weight of content of digestive tract – are equal. It is very probable that the maintenance requirement of net energy is determined by the empty weight. For this reason a relatively higher requirement per kg. total weight will be computed for cow A.

Since, however, it is impossible to determine the empty weight of living animals the actual live weights have been used in the experiments to be described. We believe that by so doing no large errors have been introduced. In the statistical investigation of results from the literature, those animals fed with concentrates only, *i.e.*, animals with relatively small weights of digestive tract contents, were excluded. The fact that animals with a positive energy balance in general have a heavier content within the digestive tract than animals with a negative energy balance, does not interfere with the results of this investigation, since these both groups of animals were treated separately. It should be pointed out that the rations used in our own experiments are not considered to have resulted in large differences in digestive tract fill.

It has been postulated that the basal metabolism of an animal is proportional to the surface area of an animal and that the surface area is proportional to body weight raised to the two-thirds power. Thus we have the relationship $B = aW^{2/3}$ in which B is basal metabolism and a is a constant. It is now considered that basal heat production is not dependent on the surface area, but on the activity of the heat producing organs; the latter are relatively bigger in smaller animals which are at the same time somewhat more active. Thus, the surface area law has a misleading name but is more or less in agreement with the experimental data.

Kleiber (1947) using relatively recent results on homeotherms found that the relation between basal metabolism and body weight was $B = aW^{3/4}$ and stated that there was no adequate reason against the application of the $^3/_4$ power rule of basal metabolism within one species of animals. Brody (1945, p. 412) found that the resting heat production (R), the heat production of lying animals just before the morning feeding, ordinarily somewhat higher than the basal heat production of ruminants, of Jersey and Holstein cattle, $^1/_2$–2 years old, was related to the body weight by $R = aW^{0.56-0.60}$. Breirem (1953) computed, from the data of 64 experiments with cattle fed hay only and having energy balances between + 2 000 and — 2 000 kcal., that the maintenance requirement of metabolizable energy (M_m) was related to body weight in a manner similar to that described by Brody: $M_m = aW^{0.61}$. Axelsson and Eriksson (1953) have concluded that in different experiments with the same animal the requirement of metabolizable energy for maintenance often appeared to be directly proportional to body weight, although great deviations from this rule occurred. These workers considered that in a population of animals, on the other hand, the power may generally be lower than 1 and they quoted values of the power computed by Axelsson (1946) of 0.93–0.89 for 34 experiments of Kellner with maintenance rations, of 0.44 for 20 experiments of Fingerling and of 0.67–0.62 for 16 experiments carried out by Møllgaard. Schiemann (1958) also showed that in the abovementioned 34 experiments of Kellner the power was very close to 1.

From the foregoing it is quite evident that there is considerable diversity of opinion as how best to correct data to energy equilibrium and to allow for differences in body weight. For this reason it seemed worth while to statistically examine the results of the experiments quoted in the literature with the object of determining the relationship between metabolizable energy of the feed, body weight of the animal, the resulting energy balance and the composition of the feed.

1.4. Causes of variation of the results of experiments on the maintenance requirement

In any series of experiments on the maintenance requirement of metabolizable energy in which there is more than one animal and more than one kind of ration the total variation in the results obtained after correction for energy gain or loss and for body weight, is composed of:

1. *analytical* variation, *i.e.* weighing and sampling errors and analytical errors,
2. *physiological* variation, *i.e.* deviations due to daily variation in production of faeces, of urine, of CO_2, of CH_4 and of heat,
3. *correction* variation, *i.e.* errors introduced by applying corrections for energy gain and for body weight,
4. *period* variation, *i.e.* errors due to changes in the maintenance requirement of the animal in the course of time which have not been accounted for during the experimental periods because these were too short,
5. *ration* variation, *i.e.* differences in the maintenance requirement of metabolizable energy due to the composition of the ration,
6. *between-animal* variation, *i.e.* differences in the true maintenance requirement of metabolizable energy of individual animals given the same rations.

The *between-animal* variation might be computed from the total variation provided that the other components of variation were known. However, part of the errors mentioned under 1., 2. and 3. may be systematic. This will not make the figure found for the *between-animal* variation less reliable as long as the systematic errors change the results of all experiments in the same manner.

The systematic errors of 1. and 2. appear to be small: in well-conducted balance experiments the difference between the energy balance computed directly, differs from that computed from carbon and nitrogen balance nearly always by less than 2% of the energy intake. Severe systematic errors may arise from 3. if wrong correction factors are used and, at the same time, the differences in gain and in body weight of the animals under experiment are considerable. In contrast to the systematic errors of 1. and 2. those of 3. will not change the results of all experiments in the same manner: results of experiments with high energy balances will have much higher systematic errors than those of experiments with low balances. Therefore, in case these correction factors are not accurately known, it is better to compute the *between-animal* variation with various factors before drawing a final conclusion. In chapter 3 we shall see that *period* and *ration* variation are small, therefore their systematic errors also will be small.

2. SURVEY OF THE LITERATURE ON BETWEEN-ANIMAL VARIATION IN MAINTENANCE REQUIREMENT

2.1. EXPERIMENTS ON BETWEEN-ANIMAL VARIATION IN MAINTENANCE REQUIREMENT

Experiments on the requirement of feed for maintenance purposes of cattle were carried out as early as the middle of the nineteenth century (HENNEBERG and STOHMANN, 1860, 1864, 1870). In 1870 (p.V), giving data of his first respiration experiments, HENNEBERG emphasized the importance of studying the conversion of the feed by individual animals. It was not until 1900 however, that reliable, comparable data on the requirement of individual animals became available (KELLNER and KÖHLER). KELLNER used the calorie as the unit in measuring metabolism and expressed the maintenance requirement in calories metabolizable energy. Thus his results were of greater general value than those of earlier investigators who expressed the requirement for maintenance in terms of kg. hay, kg. dry or organic matter or kg. digestible components of the dry matter. He compared 8 oxen in their requirement of metabolizable energy after applying corrections for energy balance and body weight. In these experiments the energy balances were less than + 1 700 kcal. The requirement of metabolizable energy of the individual feeds in the rations (hay only or hay and straw) per kcal. body gain was known from other experiments; for the hay it was 2.33 kcal., for the straw 2.63 kcal. For positive energy balances the correction to zero energy balance was made with the aid of these values. In the two cases with negative balances

TABLE 1. The experiments of KELLNER with oxen on maintenance requirement

Animal	Body weight	$M_{m,650}$ [1] (kcal.) with correction of:			
		KELLNER	KELLNER	ARMSBY	ARMSBY
	(W, kg)	$p = 2/3$	$p = 1$	$p = 2/3$	$p = 1$
V	602	12 282	12 609	12 282	12 609
A	620	13 175	13 379	13 175	13 379
IV	623	15 560	15 771	16 327	16 549
III	632	13 536	13 656	13 536	13 656
II	632	14 731	14 861	14 731	14 861
VI	644	11 842	11 877	11 842	11 877
XX	672	14 878	14 711	14 878	14 711
SD [2]		1 395	1 363	1 582	1 564
CV [2]		10.2 %	9.8 %	11.4 %	11.2 %
I	748	16 300	15 548	19 202	18 317
II	750	17 235	16 438	17 456	16 650
III	858	18 417	16 799	18 417	16 799

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[1] $M_{m,650}$ = maintenance requirement of metabolizable energy for body weight of 650 kg.

[2] SD = standard deviation; CV = coefficient of variation.

the correction was carried out by adding a number of kcal. equal to the kcal. of the balance, to the metabolizable energy according to Rubner's law of isodynamic replacement of nutrients (Rubner, 1883). The weight correction was made according to the surface area law.

One of Kellner's experimental animals (B) did not lie down in the respiration chamber during the determination of its maintenance metabolism and so the result for this animal has been excluded from the data of Kellner shown in table 1, upper 7 lines. It will be noted that the *between-animal* variation appeared to be quite large.

Similar experiments with three, heavier, animals in a more advanced state of fattening gave requirements which were 25% higher. Two of these animals did receive some concentrates; in the third case when hay alone was fed the energy balance was very low (— 4 192 kcal.). The data are given in the lower 3 lines of table 1.

Armsby (1912, p. 42) criticized the way Kellner corrected the negative energy balances. He wrote that, to hold the animal in equilibrium, more kcal. of metabolizable energy would have been necessary than an amount of kcal. equal to the energy of the balance, even perhaps about 1.75 times as much.

As already mentioned table 1 gives the results of the 10 experiments (animal B has been excluded) with and without the correction preferred by Armsby and corrected to standard body weight ($W_0 = 650$ kg.) according to the formula

$$M_{m,W} : M_{m,W_0} = W^p : W_0^p$$

with $p = {}^2/_3$ and with $p = 1$, where $M_{m,W}$ = kcal. metabolizable energy required for maintenance by an animal of W kg.

As may be computed with a formula derived in chapter 6.11., the coefficient of variation due to the sum of *analytical* and *physiological* variation and *gain correction* variation, the variation associated with the correction to zero energy gain, (see ch. 1.4.) of the results of the first seven animals will have been about 2% (the requirement of metabolizable energy for fat production was 2.5 kcal. per kcal. gain according to data of Kellner (1900); the standard deviation of this requirement was assumed by us to have been about 0.1; the standard deviations of the metabolizable energy and of the heat expenditure were about 100 kcal. and about 90 kcal. respectively, according to Schiemann (1958)). The *weight correction* variation cannot have been high as the error of weighing an animal is small (ch. 6. 12.) and furthermore the body weights of the 7 animals did not vary much. *Period* variation appears to have been absent as the CO_2-production of the animals was remarkably constant throughout the whole experimental period of 14 days. As the rations were much alike, it is not probable that the *ration* variation was high. The total variation in the results (table 1) is much higher than the sum of all variation mentioned above. This suggests the presence of *between-animal* variation in maintenance requirement of metabolizable energy. Neither the correction of negative balances according to Armsby, nor the correction of the body weight with $p = 1$ changed the total variation very much.

The results obtained with the three fat animals are much higher than those of the other animals. Nevertheless we must be careful in concluding these fat animals had a significantly higher requirement for maintenance because the number of the animals was small and two out of them received a ration which included some concentrates. Moreover, there was a difference in breed, the fat animals being a cross between the Bavarian and the Simmenthal breed while the other seven animals were of the Bavarian breed.

ARMSBY (1911, 1912, 1917) compared some steers in regard to their net energy requirements for maintenance in experiments which consisted of more than one period. In some of these periods the animal received the same kind of feed, firstly in amount only slightly below the maintenance requirement and subsequently in amount providing approximately half of this requirement. The use of net energy has the advantage that the influence of the composition of the ration on the maintenance requirement is accounted for. ARMSBY assumed that the fasting heat production could be computed by linear extrapolation of the heat expenditure to zero feed intake and called the extrapolated value the maintenance requirement of net energy. It should be understood that ARMSBY did not measure the true number of kcal. net energy used for maintenance, but only the maintenance requirement expressed in units net energy for negative production. It is not yet possible to measure this true number.

If

F_h and F_l represent amount of feed on high and low ration,
M_h and M_l represent metabolizable energy on high and low ration,
H_h and H_l represent heat expenditure on high and low ration,
G_h and G_l represent energy balance on high and low ration, and
N_m represents the net energy required for maintenance,

we have:

$$N_m = H_l - \frac{H_h - H_l}{F_h - F_l} \cdot F_l = H_l \cdot \frac{F_h}{F_h - F_l} - H_h \cdot \frac{F_l}{F_h - F_l},$$

and in the example given by ARMSBY (1917, p. 281):

$$N_m = 8\,064 - \frac{9\,812 - 8\,064}{10.21 - 6.17}\,6.17 = 5\,392 \text{ kcal.}$$

In the experiments of ARMSBY, as we shall see later (ch. 6.6.), the standard deviation of the heat expenditure as used in this formula, *i.e.*, H_h and H_l, if not corrected to a standard day of 12 hours standing and 12 hours lying of the animal, was about 1% of these values. The standard deviation of the feed intake of course was very low. Therefore, the coefficient of variation of the N_m in the example mentioned was about:

$$\frac{1}{53.92}\sqrt{\left(80.64\,\frac{10.21}{10.21 - 6.17}\right)^2 + \left(98.12\,\frac{6.17}{10.21 - 6.17}\right)^2} = 4.6\%.$$

It is to be expected that the results of other experiments will have about the same coefficient of variation since average figures were used in the above example. Of course a smaller difference in feed intake in the two periods gives a lower accuracy. ARMSBY reduced the measured heat expenditure to a standard heat expenditure based on 12 hours standing and 12 hours lying of the animal, using as appeared afterwards, rather high correction factors. This may have increased the variation of his figures of the net energy required for maintenance (1917, p. 289). The coefficient of variation in the

23 experiments with 9 animals was 13%, but since the variation in the results obtained with the same animal in different experiments was also nearly 13%, there is no evidence of *between-animal* variation. Therefore, the big difference between this percentage and that of the 4.6% found above and due to *analytical* and *physiological* variation suggests the existence of *period* variation. The *weight correction* variation was small and *gain correction* and *ration* variation were eliminated by the choice of net energy instead of metabolizable energy.

ARMSBY wrote (1917, p. 304): 'There can be little question that those differences between maintenance requirements of different animals which are ascribed somewhat vaguely to individuality are due to a large extent to varying amounts of muscular activity'. In this connection he pointed to his experiment with the steers A and B in 1905–1907. Steer A was a purebred beef steer, steer B was a scrub animal with some dairy blood and of a decidedly more nervous disposition than animal A. The maintenance requirement of net energy (kcal. per day per 1 000 lbs. body weight) of these animals was:

Animal	in 1905	in 1906	in 1907	average
A	5 873	6 272	4 723	5 623
B	6 052	6 305	6 067	6 141

While the values determined in 1907 give a significant difference between the animals, the values obtained in 1905 and 1906 do not; nor do the average results. Thus, in our opinion, the evidence for a true difference in the maintenance requirements of animals A and B is not very strong.

In 1925 (p. 1083) and 1927 (p. 167) FORBES *et al.* published revised figures on the maintenance requirement of net energy of all experiments done by ARMSBY and FORBES. They not only computed the requirement from two sub- or supermaintenance periods of one experiment, but from every combination of two periods of that experiment, assuming a linear relation between feed intake and heat expenditure. That this assumption was incorrect was shown later by FORBES *et al.* (1932). The variation of the results obtained in this way was high and showed once more the high errors inherent to the method of determination. A much smaller variation was found if the requirement was measured directly as the heat expenditure during fasting under standardized conditions. In 1931 (FORBES *et al.*, p. 1014), however, it became clear that 'the fasting heat production generally diminished continuously as the fast progressed and that no definite level of heat production was reached'. Therefore, also this direct method had to be rejected.

MØLLGAARD (1929, p. 115) refined the computation of the maintenance requirement of net energy from two periods of an experiment, using metabolizable energy and energy balance instead of gross energy and heat expenditure and using a correction for difference in body weight if this was not the same during both periods. Without the latter correction his formula of computation was, using the same notation as given above:

$$N_m = \frac{M_h\,(G_h - G_l)}{M_h - M_l} - G_h = G_h \cdot \frac{M_l}{M_h - M_l} - G_l \cdot \frac{M_h}{M_h - M_l}.$$

Since $G = M - H$, it follows that

$$N_m = H_l \cdot \frac{M_h}{M_h - M_l} - H_h \cdot \frac{M_l}{M_h - M_l}.$$

Clearly MØLLGAARD's formula differs not very much from ARMSBY's: M is used instead of F. The coefficient of variation of M is higher than that of F. The assumption, however, that the heat expenditure at zero intake of metabolizable energy can be computed by linear extrapolation is less subject to doubt than the assumption that the heat expenditure at zero intake of feed can be computed by linear extrapolation, as in general the digestibility increases with smaller rations. Therefore the coefficient of variation of an experiment similar to that of ARMSBY, but with M instead of with F will have been again about 5%.

MØLLGAARD made it clear that values of maintenance requirement of net energy computed from supermaintenance periods are not expressed in units of the same size as those computed from submaintenance periods: the quantity of metabolizable energy required per kcal. gain is namely higher than the quantity required to prevent the combustion of animal tissue with an energy content of one kcal. Using results of experiments given by FORBES *et al.* (1925, 1927) he computed that the requirement for the latter process is 0.826 times the requirement for the former. The coefficient of variation of net energy required for maintenance corrected for body weight of 6 steers with positive energy balances was 13%, of 8 steers with negative ones about 8%. Both values are higher than the coefficient of variation due to the sum of *analytical* and *physiological* variation and *weight correction* variation (somewhat less than the figure of 5% given above as some results of more than one experiment with the same animal were pooled). This suggests the presence of *period* variation and/or *between-animal* variation. The value 0.826 used above was the ratio between the average requirement of the 6 and that of the 8 animals. Its standard deviation is rather great:

$$0.826 \sqrt{\left(\frac{0.13}{\sqrt{6}}\right)^2 + \left(\frac{0.08}{\sqrt{8}}\right)^2} = 0.05.$$

MØLLGAARD (1929, p. 126) also used another method to compute the maintenance requirement expressed in units net energy for fat production. From experiments of KELLNER and of himself, 22 experiments with 12 animals, he computed the net energy of the rations used according to the starch equivalent system of KELLNER. He subtracted the energy balance if the latter was positive, in order to obtain the maintenance requirement. If the balance was negative, he added 0.826 times this negative balance. The coefficient of variation of all results was 7%; if instead of all results obtained with the same animal only the average result of this animal was used, the coefficient was 10%. The variation within animals, however, was 7%, therefore there is no significant evidence of *between-animal* variation.

There is yet a third method of estimating the maintenance requirement: Healthy, non-lactating, non-pregnant animals are given such an amount of feed that no appreciable change in body weight occurs. Then the variation in requirement is demonstrated by difference in feed intake. This method has two major difficulties, one lies in the accuracy of measuring any change in body weight, the other in the uncertainty of knowing the energy equivalent of such a change. MøLLGAARD (1929, p. 110) showed that the standard error of the average daily change in body weight of a 35 days' experiment with daily weighing was about 0.045 kg. Assuming that the gain or loss consists of 75% fat and 25% water this means a standard error of $0.045 \times 0.75 \times \times 9\,500 = 320$ kcal. net energy or about 6% of the net energy required for maintenance by a cow of 500 kg. The assumption that the gain consists of 75% fat and 25% water is not very satisfactory since the water content of body gain is not constant. The great length of such experiments and the associated high errors make this approach unsuitable for study of *between-animal* variation. It is completely unsuitable for this purpose if carried out in a way less accurate than that used by MøLLGAARD.

MøLLGAARD (1929, p. 132) ascribed the variation in maintenance requirement to breed, age and grade of fattening of the animals. As to the breed he used the already mentioned experiment (ARMSBY and FRIES, 1911) with the steers A and B, but computed the results in units of net energy for fat production for an animal of 500 kg.; he found a difference of 12–30% to the advantage of steer B. It was his opinion that no important difference in metabolism underlies the phenomenon of *between-animal* variation in maintenance requirement but that differences in temperament are the cause; therefore the same variation may be found within a breed between nervous and docile animals. As to the influence of age on maintenance requirement, MøLLGAARD considered there was as yet no convincing evidence as to its significance in cattle though there was for man. As to the grade of fattening it was his opinion that the experiments of KELLNER (1900) with the three fat animals, those of ARMSBY and FRIES (1917) with the same steer before and after fattening and perhaps those of EVVARD quoted by ARMSBY (1912) suggested a higher requirement for fat animals. Similar causes of variation and some additional ones such as oestrus, low environmental temperature, distress due to illness or insects, presence of attendants or visitors, have been suggested by COCHRANE *et al.* (1925).

2.2. ESTIMATIONS OF MAINTENANCE REQUIREMENT AND ITS BETWEEN-ANIMAL VARIATION FROM RESULTS OF EXPERIMENTS DESIGNED FOR THE EVALUATION OF FEEDS

As far as is known, after 1930 no experiments have been carried out in which the main objective has been to examine the *between-animal* variation in maintenance requirement. The experiments of FINGERLING at Möckern and those of MøLLGAARD at Copenhagen had as their main objective the evaluation of feeding stuffs, but in many of them during some periods the animals were not far from energy equilibrium. The results of such periods were used by BREIREM (1944, 1953), by AXELSSON (1946), by AXELSSON and ERIKSSON (1953) and by SCHIEMANN (1958) to calculate the requirement of metabolizable energy for maintenance. Corrections had to be applied to the results for the energy balances and for differences in body weight and, where possible, for differences in the composition of the feed and in the environment during the experiment.

The correction for *energy balance* was difficult as only in few cases was the requirement of metabolizable energy of the ration for fat production or 'for maintenance' (negative production) known accurately. The correction for differences in *body weight* was also difficult as the opinions differed on the value of p in the formula

$$M_{m,W} : M_{m,W_0} = W^p : W_0^p$$

wherein $M_{m,W}$ is the maintenance requirement of metabolizable energy of an animal of W kg. and W_0 is a body weight used for comparison (see ch. 1.3.). In most of the experiments rations of different *composition* had been used, in some either roughages or concentrates, in others a mixture of both. Very reliable corrections for differences in the composition of the rations were not available. Naturally the *environmental conditions* of the animals during the experiments differed somewhat from laboratory to laboratory, but since all investigators tried to obtain conditions not too remote from practice, the influence of this factor might be neglected.

If from results of such periods we compute the maintenance requirement of metabolizable energy at a standard body weight with corrections for energy balance and body weight only, the result will not be very accurate. If the energy balances are not too high and the gain correction is performed with the aid of an assumed instead of with a determined factor, then the variation within animals, *i.e.*, variation due to the sum of *analytical* and *physiological* variation and *correction*, *period* and *ration* variation, will be 6–8% as will be seen in chapter 3.7.2. Better results are obtained when for instance the gain correction factor is known.

Some details of the above mentioned calculations by the various workers are given in the following lines. BREIREM (1944, 1953) collected the data of 64 periods with energy balances between + 2 000 and — 2 000 kcal. He used gain correction factors as given by FORBES and KRISS and neglected the influence of the composition of the ration (hay only) on the maintenance requirement of metabolizable energy. He computed the maintenance requirement of metabolizable energy of the animals in all periods and found the lowest coefficient of total variation to be 10.4% if he used the power $p = 0.6$ in the correction for body weight. A slightly higher variation was obtained with $p = 0.5$ or $p = 0.7$. Using the results of 82 experimental periods reported by ARMSBY and FORBES in which the energy balances were of similar size to those mentioned above, and in which hay, hay and concentrates or concentrates alone were fed BREIREM found a significant correlation between the maintenance requirement of metabolizable energy and the crude fibre content (F) of the dry matter of the ration. The influence of F, however, was not very high:

$$M_m = (229.2 + 1.42\ F)\ W^{0.6}.$$

Therefore the major part of the total variation of 10.4% must have been caused by *between-animal* variation and by *analytical* and *physiological* variation and *gain* and *weight correction* and *period* variation. Taken together the four last-mentioned kinds of variation amounted to somewhat less than 6–8%, *i.e.*, the percentage given above, as the gain correction factors were known although not very accurately, and the *ration* variation was small. Therefore, it appears that the total variation of 10.5% can probably not be explained by these factors only.

AXELSSON (1946) and AXELSSON and ERIKSSON (1953) showed from results quoted in the literature and from their own experiments with rabbits that the value of the metabolizable energy of the feed 'for maintenance' was independent of the composition of the ration. They gave no figures concerning the *between-animal* variation in maintenance requirement.

SCHIEMANN (1958) assumed that in 36 experimental periods reported by KELLNER with animals of 580–760 kg. and energy balances of about + 4 000 kcal. the requirement of metabolizable energy per kcal. body gain was 2.0 kcal. He found the lowest total variation in maintenance requirement of 13.6% if the power p was 1.0. Since the heavier animals were in a more advanced state of fattening, SCHIEMANN wrote: 'Theoretically it is possible that the decrease of maintenance requirement per kg. body weight with heavier animals according to the surface area law, has been compensated by an increase in requirement because of the higher degree of fattening'. In our opinion it is not possible to explain the total variation of 13.6% only by *analytical* and *physiological* variation and *correction, ration* and *period* variation. They were very accurate experiments with rations of uniform composition and with a fairly constant daily production of CO_2 during the whole experimental period.

2.3. CONCLUSIONS

Summarizing we may conclude that:

1. the results of many experiments in the literature suggest the existence of *between-animal* variation in maintenance requirement,
2. it is difficult to measure this variation quantitatively because of the large errors associated with the results of experiments on the requirement of net energy or metabolizable energy for maintenance. These errors are due partly to *analytical* and *physiological* variation and partly to *correction, period* and *ration* variation,
3. more knowledge is needed concerning the efficiency of the utilization of the metabolizable energy of whole rations 'for maintenance' (negative gain) and for body gain and about the influences of the composition of the ration, of the degree of fattening and of the body weight on the maintenance requirement,
4. from the results of all suitable periods of experiments taken from the literature multiple regression equations of the intake of metabolizable energy on body weight with exponent p, energy balance and composition of the feed have not yet been computed.

2.4. METABOLIZABLE ENERGY REQUIRED FOR REPRODUCTIVE GAIN

As the present experiments were carried out with pregnant animals a study was first made of the literature dealing with the feed requirements of pregnancy.

A pregnant animal uses part of the metabolizable energy for reproductive gain, *i.e.*, the growth of foetus, foetal membranes, uterus and udder. Its own maintenance requirement of metabolizable energy per kg. body weight may be higher than before conception. Since it is impossible to measure the actual maintenance requirement of a pregnant ruminant, the simplest way in calculations on energy metabolism is to assume that the maintenance requirement per kg. body weight during pregnancy is equal to that before pregnancy. The maintenance requirement of the reproductive gain is thus assumed to be equal to that of the other tissues of the body. The difference between the actual maintenance requirement per kg. body weight before and after conception is seen as an extra burden on the production of reproductive gain. In a similar way with the production of body fat or of milk, the possible increase in actual maintenance requirement is usually included in the production part of the ration and not in its maintenance part.

In an energy balance experiment with a pregnant cow, in addition to reproductive gain (G_r), *i.e.*, growth of foetus, foetal membranes, uterus and udder, there is almost always non-reproductive gain (G_{nr}), *i.e.*, gain or loss of body fat and protein outside of the reproductive organs. Part of the total metabolizable energy is used to produce the reproductive gain; part of the heat expenditure is due to its production. The same holds true for the non-reproductive gain. If

M = metabolizable energy (kcal. per day),
G = gain (kcal. per day),
H = heat expenditure (kcal. per day),
m, r, nr = maintenance, reproductive and non-reproductive, we may write:

$$M_m + M_r + M_{nr} = G_r + G_{nr} + H_m + H_r + H_{nr}.$$

Only the values $M_m + M_r + M_{nr}$, $G_r + G_{nr}$ and $H_m + H_r + H_{nr}$ can be determined in a single experiment. It is very probable that the amount of G_r mainly depends on the number of days after conception, since for animals of the same breed in the same phase of gestation the weight and the chemical composition of foetus and reproductive organs are more or less constant.

Many investigators have determined the weight and the composition of foetus, foetal membranes and uterus; JAKOBSEN (1957, p. 82) also paid attention to the growth of the udder during gestation and measured the energy stored in the pregnant uterus. He used three sets of monozygotic twin heifers, only one of each pair being pregnant, and examined the within-pair differences in udder weight and composition at the end of the gestation period. On average the udders of the pregnant animals contained 200 g. N and 2 000 g. organic dry matter more than the udders of the non-pregnant animals. The difference in energy content will thus have been about 10 000 kcal. It may be assumed that it will be less for cows than for heifers. JAKOBSEN assumed that there was no appreciable growth of the udder before the 175th day of gestation. The energy stored in the pregnant uterus at the end of pregnancy amounted to about 60 000 kcal., although up to the seventh month, it was less than 20 000 kcal. The birthweight of the calves was about 40 kg.

If the G_r in an experiment is estimated with the aid of these figures, then G_{nr} can be found by subtracting G_r from total gain. It is not very difficult to carry out the experiment in such a way that G_{nr} is small, so that no great error is made in computing M_{nr} and H_{nr} with the aid of a figure for the requirement of metabolizable energy per kcal. gain that is generally found with rations as used. In a second experiment with the same, dry but non-pregnant animal, fed with a ration of about the same composition M_m ($= H_m$) may be determined provided that the body weight in both experiments does not differ; if body weights differ, a correction of 18 kcal. per kg. difference may be applied because the M_m of an animal of 500 kg. is about 12 000 kcal. ($p = 0.8$). Thus M_r and H_r may be computed. The values so computed will not be very accurate as all the figures used in the computation have their own error. Nevertheless they are more accurate than the results of most methods used for their estimation given in the literature as will be seen from what follows. Very often G_{nr} is neglected, the estimate of G_r is too low since only the energy of the newborn calf is taken in consideration and further more, to compute M_r, the requirement of metabolizable energy per kcal. reproductive gain is assumed to be equal to that per kcal. non-reproductive gain.

According to Kellner (1919, p. 614) and Kellner and Scheunert (1952, p. 271) it is only necessary to give pregnant animals a small amount of protein (70 g. of protein per day in the last 6 months of gestation) in addition to the maintenance requirement. These authors write: 'A newborn calf of 40 kg. contains about 8 kg. protein, thus the mother requires about 11–12 kg. digestible protein in the feed for its development; N-free substances are not needed as a newborn calf contains less than 1% fat.' Becker (1959, p. 167), in his recent revision of the book of Kellner and Scheunert mentioned above, gives a much higher requirement. He recommends an extra allowance in the last two months of pregnancy equal to the allowance for the production of 10 litres milk per day, *i.e.*, 500–550 g. digestible protein and 2.5–3.0 kg. starch equivalent (about 10 000 kcal. metabolizable energy).

Møllgaard (1929, p. 183) gives a figure of 700 NK_F (kcal. net energy for fat production) for the daily feed requirement for pregnancy in the last half of the gestation period. Thus the allowance of Møllgaard for pregnancy amounts to $700 \times 140 \times 1.8 \backsim 180\,000$ kcal. metabolizable energy (140 = number of days and 1.8 = requirement of kcal. metabolizable energy per kcal. gain).

Morrison (1949, p. 1147) gives as an additional allowance for the last two or three months of the gestation period about 6 lbs. total digestible nutrients, that is about $6 \times 1\,600 \times 80 \backsim 800\,000$ kcal. metabolizable energy (1 lbs. total digestible nutrients is equivalent to 1 600 kcal. metabolizable energy and 80 is the number of days). Part of this allowance is given for the improvement of the condition because it is considered that animals in a poor condition at the beginning of lactation will have a low milk yield, a view also held by Lenkeit (1953, p. 38) and by Blaxter (1957).

Axelsson and Eriksson (1953) hold that pregnant animals require about 1.05 times the metabolizable energy required for maintenance; thus for a gestation period of 280 days some $280 \times 0.05 \times 12\,000 \backsim 170\,000$ kcal. additional metabolizable energy would be necessary.

According to the feeding standards in use in the Netherlands (Centraal Veevoeder

Bureau, 1959) the allowance for pregnancy given above the maintenance feed of dry, pregnant animals in the last months of gestation, should be equal to the feed required for the production of 10 kg. milk, that is in 75 days about 75 × 10 × 0.3 ∽ 220 kg. starch equivalent or about 800 000 kcal. metabolizable energy.

MORRISON (1949, p. 212) quotes experiments of RITZMAN who found no increase in energy requirement during the first half of the gestation period but an increase of 30% at the beginning of the last month.

BRODY (1945, p. 438, 460) computed the heat increment due to gestation by comparing the heat production of pregnant, growing, non-lactating cows with estimated figures for heat production of the same animals if they had not been pregnant. The heat productions of the non-pregnant animals were computed from body weights obtained by interpolation from the actual body weight before and shortly after the pregnancy and the relationship between body weights and heat production determined prior to the cows being served. According to the author the estimation of the heat increment was rather inaccurate.

The average extra heat production per animal during the whole pregnancy of 13 Holstein cows of about 450 kg., having calves with an average birth weight of 41 kg., was 350 000 kcal. After 190 days of pregnancy it amounted to less than 1 600 kcal./day, and after 220,250 and 280 days it was respectively 2 400, 3 100 and 3 800 kcal./day. As the total reproductive gain will have been about 70 000 kcal., the total extra requirement of metabolizable energy will have been about 70 000 + 350 000 = 420 000 kcal. This requirement includes however the maintenance requirement of the reproductive gain. If, as already mentioned, it is assumed that the maintenance requirement of the reproductive gain is the same as that for the maintenance of non-reproductive body tissue, *i.e.*, 18 kcal. metabolizable energy per kg. body weight, and that the average weight of the reproductive gain during the whole pregnancy was about 30 kg., it can be seen that a total of 30 × 280 × 18 ∽ 150 000 kcal. metabolizable energy was required for the maintenance of the reproductive gain. Thus for the production of this gain 420 000 — 150 000 = 270 000 kcal. were needed.

An extra heat production of at least 2 000 kcal. per day at the end of pregnancy was found by JAKOBSEN *et al.* (1957). The value was obtained partly from measurement of the energy content of foetus, foetal membranes and uterus of pregnant animals and partly from energy balance experiments with monozygotic heifer-twins, one animal of each pair being pregnant. The pregnant animals were given an increased ration as the pregnancy advanced and the energy content of feed, faeces and urine, the nitrogen balance and the body weight of both animals determined during the experimental periods. Heat production was not measured. It was assumed that the non-reproductive gain was equal for the pregnant and the non-pregnant animal if the twins, except for the organs of reproduction, were in nutritional equilibrium. A method to estimate the non-reproductive gain failed. The non-reproductive nitrogen depositions were indeed nearly equal, but the non-reproductive body weights were not, that of the pregnant animal as compared with that of the non-pregnant heifer decreasing by 150–160 g. daily. With one exception the extra daily heat production increased as the gestation advanced. The authors subtracted from the difference in computed heat production between the two animals of a twin set the additional energy lost as methane and the additional heat resulting from the extra feed given to the pregnant animals. For comparison with the results of Brody, mentioned above, obviously this subtraction must not be made. If we omit this correction the daily heat increment after 190, 200, 232 and 260 days of pregnancy was respectively 1 600, 2 500, 3 700 and 2 600 kcal. The requirement for the production of reproductive gain will thus have been $70\,000 + 100 \times 3\,000 - 150\,000 \backsim 220\,000$ kcal. metabolizable energy ($70\,000 =$ = kcal. reproductive gain, $100 =$ number of days, $3\,000 =$ kcal. heat increment per day and $150\,000 =$ kcal. maintenance requirement of the reproductive gain). Especially during the last period the decrease in nonreproductive body weight of the pregnant animal was considerable. The authors emphasize that their figures are preliminary and that control by indirect or direct calorimetry is necessary.

The above-mentioned experiments of Brody and of Jakobsen *et al.* suggest an additional requirement for the gestation period of 250 000 kcal. metabolizable energy. Compared with this figure the feeding standards given by Kellner, by Møllgaard and by Axelsson and Eriksson appear low, while those of Becker, of Morrison and those in use in the Netherlands appear high. It must however not be forgotten that the three last-mentioned standards are meant to improve the condition of the animal; a surplus of 500 000 kcal. metabolizable energy may give a production of about 25 kg. body fat.

3. STATISTICAL TREATMENT OF THE RESULTS OF ENERGY BALANCE EXPERIMENTS NEAR ENERGY EQUILIBRIUM, GIVEN IN THE LITERATURE

3.1. INTRODUCTION

In the past many energy balance experiments were conducted with oxen, steers and cows in which the animals were almost in energy equilibrium (KELLNER, ARMSBY, FORBES, FINGERLING, MØLLGAARD, BENEDICT and RITZMAN, MITCHELL and HAMILTON). If suitable corrections could be found to reduce the data of these experiments to energy equilibrium and standard body weight, then different animals or breeds could perhaps be compared as to their requirement of metabolizable, digestible or gross energy for maintenance. Here again as in chapter 1.1. maintenance requirement refers to that of a healthy animal in energy equilibrium while tethered in the cowshed, kept within the zone of thermoneutrality and fed with a ration not deficient in protein, vitamins, macro- and microelements, and containing some crude fibre to ensure normal rumination.

Experiments involving large deviations from energy equilibrium would necessitate large corrections, a serious drawback if the accuracy of correction factors is in question. In addition the fact that seriously undernourished animals are often either very nervous or very apathetic makes the results of experiments with highly negative balances less reliable.

The efficiency of the conversion of the gross energy of the feed into body gain is *inter alia* dependent on the composition of the feed. It is well known that there exists a negative correlation between this efficiency and the crude fibre content of the feed; other properties of the feed are also important in this connection. In the case of negative energy balances energy from body reserves is drawn upon by the animal. The problem is how much feed energy is needed to save one kcal. of energy from body reserves. Here also the efficiency of the conversion is dependent on the composition of the feed and again there is a negative correlation with the crude fibre content. As already mentioned in chapter 1.2. some investigators are of the opinion that although differences in feed composition do result in differences in the efficiency of utilization of gross energy both for 'maintenance' (negative production) and for fat production nevertheless differences in feed composition do not affect the efficiency of utilization of digestible or metabolizable energy for either of these processes. Most research workers however are convinced that the efficiencies of utilization of digestible and of metabolizable energy for fat production at least are dependent on the composition of the feed.

3.2. THE MATERIAL

It was decided to compute the requirement of metabolizable energy per kcal. net energy 'for maintenance' (negative production) and for body gain and also the animal's maintenance requirement of metabolizable energy statistically using the data from experiments in which the energy balances lay between + 4 000 and — 4 000 kcal. Frequently KELLNER and FINGERLING used two experimental periods with the same animal given the same basal ration, one period before and one period after a series of periods in which the animal received larger rations. In some instances the energy balances were somewhat higher than 4 000 kcal. Nevertheless these periods were also included as they might give useful information about variation in the requirement of an animal in different periods. Animals under 280 kg. body weight were excluded. It was thought that in this case the process of growth might possibly influence the requirement for maintenance.

ARMSBY and FORBES (see COCHRANE *et al.*, 1925, p. 1069) corrected the metabolizable energy of the feed for N-equilibrium, to allow for urine energy derived from body protein instead of from feed protein (negative N-balance) or for a low urine energy in those cases (positive N-balance) that part of the feed protein was not oxidized. This correction was not used in the present instance, except in those cases where the uncorrected values were not given in the literature. Fortunately, all nitrogen balances were small, so the correction was very low. It should perhaps be pointed out that theoretically indeed the correction is valid for the feeding value of the feed. The urea in the urine that results from combustion of reserve tissue results in a lower metabolizable energy for a feed than the true value. With a positive nitrogen balance it is just the other way round. For measuring animal requirements it is the metabolizable energy as found that counts since this remains after subtracting the energy in urine, methane and faeces from the gross energy.

Nearly all experiments with positive energy balances had positive nitrogen balances; only in a few instances were positive energy balances associated with slightly negative nitrogen balances. Those with negative energy balances mostly had negative nitrogen balances. In the latter experiments not all the conditions for maintenance mentioned in 3.1. were fulfilled. For this reason these experiments will be treated separately and are considered of less value than the experiments with positive energy balances.

For convenience the kcal. of the metabolizable energy and the energy balances have been rounded to the second figure (12 683 kcal. becomes 12 680 kcal.). The average body weight in kg. determined during the experiment has been corrected to the nearest whole number. When conversion of the body weight to an exponential value followed (for instance power 0.7, 0.8 etc.), the result has been given to one decimal place. Where necessary, the crude fibre content of the whole ration was computed and has been given to one decimal place. In some of the experiments of MØLLGAARD crude fibre was not determined; as the rations in these experiments were rather simple and did not contain hay, the crude fibre content has been estimated with the aid of data of similar rations used in the years prior to and after these experiments.

Experiments with concentrates only have been excluded as they were not considered to have fulfilled the condition of normal rumination. The same holds good for experiments with hay meal.

The energy balances have not been corrected for nitrogen equilibrium for the same reasons as mentioned with the metabolizable energy. If two values for the energy balance were given, one computed from the carbon and nitrogen balances (CN-method) and one equal to the difference of metabolizable energy and heat expenditure, the latter computed from gaseous exchange and urinary nitrogen, the average of both values has been used. As ARMSBY and FORBES using direct calorimetry preferred the gain computed as difference between the metabolizable energy and the directly measured heat expenditure over the gain computed with the other methods, the former value was used (COCHRANE *et al.*, 1925, p. 1074; FORBES *et al.*, 1927).

The energy balances have not been corrected to a standard day of 12 hours standing and 12 hours lying of the animal as was done by ARMSBY and by FORBES. The standing and lying behaviour of the animals is part of their way of living and thus directly affects maintenance requirement. Of course it is important that the animal in the respiration chamber behaved in the same way as in the cowshed. For this reason experiments with animals that did not lie down in the respiration chamber were not used.

Altogether we collected the data of 64 experiments in which there were negative energy balances and data of 173 in which there were positive balances (tables 2 and 3, app.).

3.3. REGRESSION EQUATIONS WITH INTAKE OF METABOLIZABLE ENERGY AS THE DEPENDENT VARIABLE

As most research workers are convinced that the requirement of metabolizable energy for maintenance depends on the crude fibre content of the ration, we extended the well known formula $M_m = kW^p$ to read

$$M_m = (a + bF)\, W^p.$$

In these formulae and in those mentioned below the following symbols were used:

M_m = kcal. metabolizable energy for maintenance,
M_g = kcal. metabolizable energy for gain,
$M = M_m + M_g$,
G = kcal. gain,
F = crude fibre content (%) in dry matter of ration,
W = kg. body weight,
$a, b, c, d, e, f, g, h, k, p$ = constants,
$\overline{M}, \overline{G}, \overline{W}, \ldots$ = averages of $M, G, W, \ldots$

As there is no common opinion on the value of p (ch. 1.3.), it was thought best to use in the statistical computations more than one value of p, *e.g.*, 0.6, 0.7, 0.8, 0.9 and 1.0. Young, rapidly growing animals may have another value for p than full-grown animals which are being fattened. The curve $M_m = kW^p + h$ relating to the first-mentioned group is generally thought to go through the origin, and therefore the equation is written without h. If with full-grown animals the value of p is not the same as for immature animals it is unlikely that the curve $M_m = kW^p + h$ relating to full-grown animals goes through the origin. Since only experiments with full-grown or nearly full-grown animals have been used in this study we tried also the equations:

$$M_m = aW^p + h \text{ and } M_m = (a + bF)\, W^p + h.$$

For the metabolizable energy required for energy gain we may write:

$$M_g = (c + dF)\, G.$$

In the case of a negative energy balance the same formula, although perhaps with other values of the constants c and d, gives the amount of metabolizable energy needed to attain energy equilibrium.

If we call $M_m + M_g = M$, then

$$M = (a + bF)\, W^p + (c + dF)\, G \text{ and } M = (a + bF)\, W^p + (c + dF)\, G + h. \qquad \text{(1a, 1b)}$$

The mathematical calculations were done according to VAN UVEN (1946, app. 4). STUDENT's t-test was used in testing significance (SNEDECOR, 1959). The first aim was to compute from several values of p the one giving the smallest sum of squares of deviations for the regression equations

$$M = aW^p + cG \text{ and } M = aW^p + cG + h. \qquad \text{(2a, 2b)}$$

This smallest sum was divided by the number of degrees of freedom and the result called s^2. In the equation with h the value of p giving the smallest s^2 is not biased by the introduction of the condition that the plane of regression must go through the origin, *i.e.*, $h = 0$. The variate F was omitted to reduce the labour of calculation. The data from experiments with negative energy balances, those from experiments in which there were positive balances and those relating to animals of the same breed and with positive energy balances were treated separately. Afterwards the variate F was introduced using the best value of p. We also tried the equations

$$M = aW^{0.8} + cG + eF \text{ and } M = aW^{0.8} + cG + eF + h. \qquad \text{(3a, 3b)}$$

The 64 experiments with 28 animals with negative energy balances gave the following results ($\overline{W} = 435$; $\overline{W^{0.80}} = 129$; $\overline{M} = 8\,120$; $\overline{G} = -1\,430$; $\overline{F} = 28.0$, with $\overline{W}$, $\overline{M}$ etc. being the average W, M etc. of all experiments):

$$\text{2a)} \left\{ \begin{array}{lr} 108\, W^{0.75} + 1.43\, G = M, & s^2 = 1\,422\,300, \\ (79 \pm 2) W^{0.80} + (1.43 \pm 0.12) G = M, & s^2 = 1\,325\,300, \\ 58\, W^{0.85} + 1.40\, G = M, & s^2 = 1\,356\,700, \\ (42 \pm 1) W^{0.90} + (1.38 \pm 0.12)\, G = M, & s^2 = 1\,369\,100, \end{array} \right.$$

$$\text{(2b)} \left\{ \begin{array}{lr} (118 \pm 8) W^{0.75} + (1.41 \pm 0.12) G - (1\,055 \pm 803) = M, & s^2 = 1\,405\,800, \\ (81 \pm 6) W^{0.80} + (1.42 \pm 0.12) G - (287 \pm 730) = M, & s^2 = 1\,343\,600, \\ (56 \pm 4) W^{0.85} + (1.41 \pm 0.12) G + (321 \pm 700) = M, & s^2 = 1\,374\,200, \end{array} \right.$$

$$\text{(1a)} \quad \{72 \pm 4 + (0.25 \pm 0.13) F\}\, W^{0.80} + (1.42 \pm 0.11)\, G = M, \qquad s^2 = 1\,271\,900,$$

$$\text{(3a)} \quad (76 \pm 3) W^{0.80} + (1.43 \pm 0.11) G + (15 \pm 14) F = M, \qquad s^2 = 1\,321\,400,$$

$$\text{(3b)} \quad (82 \pm 6) W^{0.80} + (1.41 \pm 0.11) G + (31 \pm 18) F - (1\,293 \pm 920) = M, \qquad s^2 = 1\,309\,900.$$

The power 0.8 gives the lowest s^2 in both the equations (2a) and (2b) with and without h, but the difference in the values of s^2 for the powers 0.75 or 0.85 is small; obviously, within certain limits, the choice of p has little influence on s^2. The value of h does not differ significantly from zero. The experiments have been carried out with animals of 300–500 kg., thus it is not surprising that the computation of h, involving extrapolation toward $W = 0$, cannot be done with great accuracy. The fact that h is not far from zero explains why the same value of p gives the smallest s^2 in the equations with and without h. The values of b in (1a) and e in (3a) and (3b), both coefficients of F, are not significantly different from zero at the 5% level of probability. The value of c, the coefficient of G, in all cases differs significantly from 1.00. This suggests that to obtain equilibrium more kcal. metabolizable energy must be given than an amount equal to the kcal. of negative energy balance the surplus appearing as heat. Indeed, it is difficult to understand that an added amount of metabolizable energy would not give additional heat expenditure.

The 173 experiments with 72 animals with a positive energy balance gave the following results ($\overline{W} = 569$; $\overline{W^{0.80}} = 160$; $\overline{M} = 16\,350$; $\overline{G} = 2\,120$; $\overline{F} = 22.9$):

$$\text{(2a)}\;\left\{\begin{array}{ll} (154 \pm 3)W^{0.70} + (1.63 \pm 0.12)G = M, & s^2 = 3\,739\,200, \\ (\;82 \pm 2)W^{0.80} + (1.61 \pm 0.10)G = M, & s^2 = 3\,301\,900, \\ (\;43 \pm 1)W^{0.90} + (1.61 \pm 0.10)G = M, & s^2 = 3\,087\,700, \\ (\;22 \pm 0.4)W^{1.00} + 1.64G \qquad = M, & s^2 = 3\,086\,300, \end{array}\right.$$

$$\text{(2b)}\;\left\{\begin{array}{ll} (95 \pm 4)W^{0.80} + (1.61 \pm 0.10)G - (2\,238 \pm 640) = M, & s^2 = 3\,099\,100, \\ (45 \pm 2)W^{0.90} + (1.62 \pm 0.10)G - (\;624 \pm 575) = M, & s^2 = 3\,084\,400, \end{array}\right.$$

$$\text{(3a)}\quad (166 \pm 6)W^{0.70} + (1.59 \pm 0.12)G - (43 \pm 20)F = M, \qquad s^2 = 3\,658\,800,$$

$$\text{(3b)}\;\left\{\begin{array}{l} (205 \pm 9)W^{0.70} + (1.65 \pm 0.11)G + (46 \pm 24)F - (5\,530 \pm 950) = M, \\ \hfill s^2 = 3\,065\,200, \\ (\;95 \pm 4)W^{0.80} + (1.66 \pm 0.11)G + (48 \pm 24)F - (3\,480 \pm 880) = M, \\ \hfill s^2 = 3\,043\,200, \end{array}\right.$$

$$\text{(1a)}\quad \{81 \pm 6 + (0.02 \pm 0.28)F\}\, W^{0.80} + \{1.38 \pm 0.50 + (0.01 \pm 0.02)F\}G = M, \qquad s^2 = 3\,321\,500,$$

$$\text{(1b)}\quad \{92 \pm 7 + (0.15 \pm 0.27)F\}W^{0.80} + \{1.50 \pm 0.50 + (0.01 \pm 0.02)F\}G - (2\,355 \pm 650) = M,\; s^2 = 3\,098\,300.$$

With these a higher value for p gives a lower residual variance but in the equations (2b) in which h is included the differences are small. These data thus throw little light on the question of which value for p is to be preferred within the range 0.8–1.0. In the equation (2b) with $p = 0.8$ h differs highly significantly from zero. The values of b and d in (1a) and (1b), both coefficients of F, differ not significantly from zero, that of e in (3b), also a coefficient of F, differs significantly from zero at the 5% level of probability. The negative sign of the term 43 F in equation (3a) is caused by the condition $h = 0$; here this term serves more or less as h; in the equation (3b) with h the value of h is highly negative.

The value of c, the coefficient of G, differs from that of c in the experiments with negative balances, but the difference is not significant. The values of c in the equations (2a) and (2b) without F of both series of experiments, *i.e.*, 1.4 and 1.6, would mean that the efficiency of the utilization of the metabolizable energy 'for maintenance' (negative production) was about 70% and that for gain about 60%. Both efficiencies are rather high, figures of 65 and 50 respectively would have been more in accordance with figures derived from difference experiments (see also ch. 1.2.). The values of c obviously are not biased by the condition $h = 0$ since the introduction of h changed c very little.

The data relating to experiments in which there were positive balances were divided according to the breed and the regression equations of each breed were computed.

Bavarian oxen (KELLNER, FINGERLING; 69 experiments with 34 animals; $\overline{W} = 695$; $W^{0.80} = 188$; $\overline{M} = 20\,930$; $\overline{G} = 2\,960$; $\overline{F} = 22.3$):

$$\text{(2a)}\quad \left\{\begin{array}{ll} (175 \pm 5)W^{0.7} + (1.28 \pm 0.16)G = M, & s^2 = 3\,667\,300, \\ (\ 90 \pm 3)W^{0.8} + (1.34 \pm 0.16)G = M, & s^2 = 3\,759\,500, \\ (\ 46 \pm 1.4)\ W^{0.9} + (1.41 \pm 0.16)\ G = M, & s^2 = 3\,936\,800, \\ (\ 23.6 \pm 0.8)W^{1.0} + (1.48 \pm 0.17)G = M, & s^2 = 4\,194\,900, \end{array}\right.$$

$$\text{(2b)}\quad \left\{\begin{array}{ll} (163 \pm 28)W^{0.7} + (1.26 \pm 0.17)G + (1\,278 \pm 2\,940) = M, & s^2 = 3\,712\,200, \\ (\ 73 \pm 13)W^{0.8} + (1.26 \pm 0.17)G + (3\,400 \pm 2\,580) = M, & s^2 = 3\,718\,400, \\ (\ 33.6 \pm 5.9)W^{0.9} + (1.26 \pm 0.17)G + (5\,051 \pm 2\,340) = M, & s^2 = 3\,725\,200, \\ (\ 15.5 \pm 2.7)W^{1.0} + (1.26 \pm 0.17)G + (6\,367 \pm 2\,085) = M, & s^2 = 3\,731\,200, \end{array}\right.$$

$$\text{(3a)}\quad (182 \pm 12)W^{0.7} + (1.27 \pm 0.16)G - (29 \pm 45)F = M, \qquad s^2 = 3\,699\,100,$$

$$\text{(3b)}\quad \left\{\begin{array}{l} (144 \pm 31)W^{0.7} + (1.14 \pm 0.19)G - (89 \pm 64)F + (5\,470 \pm 4\,200) = M, \\ \hfill s^2 = 3\,660\,200, \\ (\ 65 \pm 14)W^{0.8} + (1.14 \pm 0.19)G - (90 \pm 64)F + (7\,370 \pm 3\,800) = M, \\ \hfill s^2 = 3\,664\,300. \end{array}\right.$$

Red Danish cows (MØLLGAARD *et al.*; 27 experiments with 10 animals; $\overline{W} = 508$; $\overline{W^{0.80}} = 146$; $\overline{M} = 13\,520$; $\overline{G} = 1\,840$; $\overline{F} = 20.7$):

$$\text{(2a)}\quad \left\{\begin{array}{ll} (127 \pm 4)W^{0.7} + (1.94 \pm 0.17)G = M, & s^2 = \quad 566\,100, \\ (\ 68 \pm 2.5)W^{0.8} + (1.97 \pm 0.18)G = M, & s^2 = \quad 633\,200, \\ (35.9 \pm 1.4)W^{0.9} + 2.01G = M, & s^2 = \quad 743\,600, \end{array}\right.$$

$$\text{(2b)}\quad \left\{\begin{array}{ll} 112\ W^{0.7} + 1.93\,G + 1\,269 = M, & s^2 = \quad 569\,000, \\ (52 \pm 8)W^{0.8} + (1.93 \pm 0.17)G + 2\,341 = M, & s^2 = \quad 567\,800, \\ 25\ W^{0.9} + 1.93\,G + 3\,179 = M, & s^2 = \quad 564\,300. \end{array}\right.$$

Shorthorn steers (ARMSBY, FORBES; 19 experiments with 8 animals; $\overline{W} = 420$; $\overline{W^{0.80}} = 126$; $\overline{M} = 12\,060$; $\overline{G} = 1\,410$; $\overline{F} = 22.4$):

$$\text{(2a)}\quad \left\{\begin{array}{ll} 143\ W^{0.7} + 1.65\,G = M, & s^2 = 1\,190\,000, \\ (77 \pm 3)W^{0.8} + (1.71 \pm 0.20)G = M, & s^2 = 1\,146\,700, \\ 42\ W^{0.9} + 1.77\,G = M, & s^2 = 1\,187\,100, \end{array}\right.$$

$$(2b)\quad (78 \pm 12)\, W^{0.8} + (1.71 \pm 0.22)G - 177 = M, \qquad s^2 = 1\,217\,400.$$

Jersey cows (Benedict, Forbes; 17 experiments with 6 animals; $\overline{W} = 410$; $\overline{W^{0.80}} = 123$; $\overline{M} = 12\,220$; $\overline{G} = 1\,760$; $\overline{F} = 24.3$):

$$(2a)\quad \left\{\begin{array}{ll} (146 \pm 12) W^{0.7} + (1.34 \pm 0.40)G = M, & s^2 = 3\,202\,600, \\ (\ 80 \pm \ \ 7) W^{0.8} \pm (1.35 \pm 0.41)G = M, & s^2 = 3\,230\,200. \end{array}\right.$$

Holstein cows (Benedict; 13 experiments with 4 animals; $\overline{W} = 592$; $\overline{W^{0.80}} = 165$; $\overline{M} = 15\,180$; $\overline{G} = 1\,370$; $\overline{F} = 30.4$):

$$(2a)\quad \left\{\begin{array}{ll} (152 \pm 8) W^{0.7} + (1.39 \pm 0.43)G = M, & s^2 = 1\,775\,800, \\ (\ 80 \pm 4) W^{0.8} + (1.40 \pm 0.44)G = M, & s^2 = 1\,796\,800. \end{array}\right.$$

Aberdeen Angus steers (Armsby, Forbes; 14 experiments with 4 animals; $\overline{W} = 424$; $\overline{W^{0.80}} = 126$; $\overline{M} = 10\,740$; $\overline{G} = 1\,180$; $\overline{F} = 22.6$):

$$(2a)\quad \left\{\begin{array}{ll} (131 \pm 4) W^{0.7} + (1.42 \pm 0.18)G = M, & s^2 = \ \ 444\,900, \\ (\ 72 \pm 3) W^{0.8} + (1.38 \pm 0.21)G = M, & s^2 = \ \ 569\,100. \end{array}\right.$$

All animals with positive energy balances except those of Bavarian, Red Danish or Shorthorn breed (58 experiments with 16 animals):

$$(2a)\quad (80 \pm 2) W^{0.8} + (1.40 \pm 0.21)G = M, \qquad s^2 = 2\,652\,700,$$

$$(2b)\quad (85 \pm 6) W^{0.8} + (1.43 \pm 0.21)G - 734 = M, \qquad s^2 = 2\,673\,500.$$

In the equations (2a) and (2b) of the Bavarian animals without and with h a higher value of p gives a higher residual variance, although in the equation including h this effect is very small. With the Red Danish cows s^2 increases with higher values of p in the equations (2a) without h, but it decreases, slightly, in those (2b) with h. Again the main part of the residual variance is not caused by the choice of p.

High values of h biased the values of c, the coefficient of G, in equations (2a) where h had been put equal to zero. There is a considerable difference between the values of c in the various breeds, the Bavarian breed having a very low and the Red Danish breed a very high value.

With the Bavarian animals the influence of the crude fibre content differs not significantly from zero.

3.4. REGRESSION EQUATIONS WITH ENERGY GAIN AS THE DEPENDENT VARIABLE

The independent variables of these kind of regression equations should be accurately known as in the computations they are considered to be true values. The accuracy of the gain (G) is not very high, the coefficient of variation being about 10%, the accuracy of the body weight (W) and of the dependent variable of 3.3., the metabolizable energy (M), is higher (chapter 6.12. and 6.10.). As the low accuracy of the values of G might have caused the rather low values of c in the regression equations of 3.3.,

$$M = aW^p + cG,$$

we also computed other regression equations with gain as the dependent variable:

$$G = fM + gW^p.$$

The following results have been obtained using the same groups of experiments as in 3.3.

All experiments with a negative energy balance:

$$(0.49 \pm 0.04)M - (57 \pm 4)W^{0.75} = G, \qquad s^2 = 486\,800,$$
$$(0.50 \pm 0.04)M - (43 \pm 3)W^{0.80} = G, \qquad s^2 = 466\,000,$$
$$(0.50 \pm 0.04)M - (32 \pm 2)W^{0.85} = G, \qquad s^2 = 486\,800.$$

All experiments with a positive energy balance:

$$(0.33 \pm 0.02)M - (40 \pm 5)W^{0.7} = G, \qquad s^2 = 759\,800,$$
$$(0.35 \pm 0.02)M - (23 \pm 2)W^{0.8} = G, \qquad s^2 = 719\,700,$$
$$(0.36 \pm 0.02)M - (13 \pm 1)W^{0.9} = G, \qquad s^2 = 693\,000,$$
$$(0.36 \pm 0.02)M - (6.6 \pm 0.7)W^{1.0} = G, \qquad s^2 = 683\,500.$$

Bavarian oxen (positive energy balance):

$$(0.38 \pm 0.05)M - (52 \pm 10)W^{0.7} = G, \qquad s^2 = 1\,091\,900,$$
$$(0.38 \pm 0.05)M - (27 \pm 5)W^{0.8} = G, \qquad s^2 = 1\,067\,000,$$
$$(0.38 \pm 0.04)M - (14 \pm 3)W^{0.9} = G, \qquad s^2 = 1\,050\,900.$$

Red Danish cows (positive energy balance):

$$(0.43 \pm 0.04)M - (51 \pm 7)W^{0.7} = G, \qquad s^2 = 125\,100,$$
$$(0.42 \pm 0.04)M - (26 \pm 4)W^{0.8} = G, \qquad s^2 = 134\,200,$$
$$(0.40 \pm 0.04)M - (13 \pm 2)W^{0.9} = G, \qquad s^2 = 148\,200.$$

Shorthorns (positive energy balance):

$$(0.48 \pm 0.06)M - (64 \pm 11)W^{0.7} = G, \qquad s^2 = 343\,500,$$
$$(0.47 \pm 0.06)M - (34 \pm 5)W^{0.8} = G, \qquad s^2 = 316\,800,$$
$$(0.46 \pm 0.05)M - (18 \pm 3)W^{0.9} = G, \qquad s^2 = 307\,600.$$

Table 4 gives a survey of the results obtained with M and with G respectively as the dependent variable; in the latter case the inverse of the coefficient of M, $1/f = c'$, is given.

TABLE 4. The values of p and c in the regression equations with M and with G respectively as the dependent variable

Animals Energy balance	all neg.	all pos.	Red Danish pos.	Bavarian pos.	Shorthorn pos.
p (M = dep. var.)	0.8	$\backsim$ 1.0	$\leqq$ 0.7	$\leqq$ 0.7	0.8
c (M = dep. var.)	1.4	1.6	$\leqq$ 1.9	$\leqq$ 1.3	1.7
p (G = dep. var.)	0.8	$\geqq$ 1.0	$\leqq$ 0.7	$\geqq$ 0.9	$\geqq$ 0.9
c' (G = dep. var.)	2.0	$\leqq$ 2.8	$\leqq$ 2.3	$\backsim$ 2.7	$\geqq$ 2.2

The values of p giving the smallest s^2 are in most of the new equations higher than those in the equations of 3.3. In both sets of equations the main part of s^2 is, within limits, not due to the choice of p.
c' is in all cases considerably higher than c, the coefficient of G in 3.3. Again c' calculated from the experiments in which there were positive energy balances is higher than c' calculated from those in which there were negative energy balances.

From these results it appears correct to use a power between 0.8 and 1.0 in the term aW^p, *i.e.*, for the correction for body weight. However, no definite value of c can be deduced in the term cG, *i.e.*, the correction for caloric gain.
In the following we shall call c the *gain correction factor* and p the *weight correction factor*. Of course, p is not a factor in the mathematical sense.

3.5. THE REQUIREMENT OF METABOLIZABLE ENERGY PER KCAL. ENERGY GAIN ACCORDING TO THE STARCH EQUIVALENT SYSTEM

Because no definite gain correction factor had yet been found, it was decided to computed the requirement of metabolizable energy per kcal. gain according to the starch equivalent system (KELLNER and SCHEUNERT, 1952, Anhang).
The amounts (g.) of digestible true or, if these were not available, of digestible crude protein, digestible crude fat, digestible crude fibre and digestible N-free extractives of all experiments of 3.2. were collected. These figures were multiplied by 0.94 (or 0.80 if digestible crude protein was used, assuming that 85% of the crude protein consisted of true protein), 2.00, 1.00 and 1.00 respectively. The fat content of the rations was so low that it was thought not necessary to differentiate between fat from hay and fat from concentrates. Two corrections were applied to the sum (S) of these products. For each gramme of crude fibre in hay or straw we subtracted 0.58 units, for each gramme in silage 0.40 units (KELLNER and SCHEUNERT give values between 0.58 and 0.29 according to the crude fibre content of the fresh material, for green fodder). For concentrates and beets a correction was computed with the aid of the digestibility of the organic matter and the value number, given by WERNER and FRANKE (1953), SCHIEMANN (1956) and KELLNER and SCHEUNERT (1952). From the digestibility of the organic matter of one concentrate-component of the ration and the total quantity digested organic matter of the whole ration we computed which share (r%) the component approximately had in the above-mentioned sum S; if the value number of the component was W, then the correction to be applied to S obviously was:

$$- (1 - W/100) \,.\, r/100 \quad . S.$$

The corrected sum, the approximate starch equivalent of the ration, was multiplied by 2.356 to give the (computed)net energy content of the ration (the net energy of 1 g. digested starch is about 2.356 kcal.). The ratio between the (determined) metabolizable energy of the ration and the (computed) net energy gave a third figure,

c_s,

for the requirement of metabolizable energy per kcal. gain (table 2 (app.) and 3 (app.)). It is true that the crude fibre correction is a rather weak point in the starch equivalent system (SCHIEMANN, 1958, p. 84). Also the value number of dried beet pulp, often used by KELLNER and FINGERLING in basal rations, is not accurately known. On the other hand the alternative is to use only one gain correction factor c computed with regression in experiments with widely divergent rations, and this may be no more satisfactory.

c_s at the same time is useful while comparing the make-up and composition of the experimental rations (low value numbers and high crude fibre corrections giving high values of c_s). For this reason also the coefficients of digestibility of the organic matter of all experiments have been collected (table 2 (app.), 3 (app.) and 5).

TABLE 5. Make-up of the rations

			Crude fibre in dm (%)		Digestibility of om (%)		c_s	
Animals	Energy balance	Ration [1]	Average	Stand. dev.	Average	Stand-dev.	Average	Stand. dev.
All	—	sb+, h, h+, h++, d	28	8	65	7	2.12	0.30
All	+		23	6	70	6	2.02	0.26
Red Danish	+	sb++, sb+, sh++	21	3	73	4	1.93	0.22
Bavarian	+	h++; a few h, h+ or hs+	22	4	71	4	1.98	0.12
Shorthorn	+	h, h+	22	9	70	9	2.07	0.33

[1] h = hay; s = straw; b = beets; d = other roughage, mostly corn silage; + = one kind of concentrate; ++ = concentrate mixture; sh++ = ration of straw, hay and a concentrate mixture.

All the average values of c_s are close to 2.0, a value intermediate between the values of c in the first and of c' in the second set of regression equations (see table 4). The rations of the Bavarian animals were much more uniform than those of the Shorthorns. The intakes of metabolizable energy in all experiments in which there were positive energy balances were corrected for gain using the factor c_s calculated for each experiment. The following equations were computed for the regression of this corrected intake (M_m) on body weight (W) with power p 0.8 and 1.0:

All animals $(76 \pm 1)W^{0.8} = M_m,$ $s^2 = 3\,419\,300,$
$(21 \pm 0.3)W^{1.0} = M_m,$ $s^2 = 3\,065\,100.$
Red Danish cows $(67 \pm 1)W^{0.8} = M_m,$ $s^2 = 539\,500,$
$(19 \pm 1)W^{1.0} = M_m,$ $s^2 = 617\,800.$
Bavarian oxen $(79 \pm 1)W^{0.8} = M_m,$ $s^2 = 4\,126\,200,$
$(21 \pm 0.4)W^{1.0} = M_m,$ $s^2 = 4\,228\,000.$
Shorthorns $(74 \pm 2)W^{0.8} = M_m,$ $s^2 = 775\,800,$
$(22 \pm 0.5)W^{1.0} = M_m,$ $s^2 = 733\,200.$

Compared with the first set of regression equations ($aW^p + cG = M$; 3.3.) in all cases except that of the Shorthorns the smallest s^2 is found with the same value of p and in general, the values of s^2 are about the same in both sets, obviously, within limits, the value of the gain correction factor has little influence on s^2, except perhaps in those instances where the rations are not uniform (Shorthorns). It is clear that with a positive energy balance a higher value of the gain correction factor c will give a lower value of the coefficient a in the regression equations $aW^p + cG = M$ for constant values of W, p and M. Therefore the a's of the above-mentioned equations are lower than the a's of the equations derived in 3.3.

It was decided in future calculations to use the correction factors $p = 0.8$ and $c = c_s$ (or $c = 0.83\ c_s$ in experiments with negative energy balances). The regression method to compute c gives less accurate results because most of the metabolizable energy in these experiments was used for maintenance and also because the figures of body weight, metabolizable energy and especially energy gain were not free from errors. Furthermore the value c_s has been derived according to a system which has been shown to be reasonably accurate. Moreover, the average c_s was intermediate between the values of c and c' of the first and the second set of regression equations.

For additional check the value $p = 1.0$ and other gain correction factors, namely those of the first set of regression equations ($c = 1.43$ and 1.61 for negative and positive energy balances respectively) and values nearly intermediate between those of the first and the second set of equations ($c = 1.67$ and 2.00 for negative and positive energy balances respectively) have occasionally been used.

In the experiments with negative energy balances the correction factors of the regression equations were lower than those derived from the experiments with positive energy balances. MØLLGAARD has also computed that the requirement of metabolizable energy was smaller 'for maintenance' than for fattening (ch. 2.1.). Therefore in those experiments $0.83\ c_s$ was used instead of c_s, the figure 0.83 only being an approximate estimate.

The tables 2 and 3 (app.) give the requirement ($M_{m,500}$) of the metabolizable energy of the animals after correction of the intake of metabolizable energy for energy gain and for body weight to that for a weight of 500 kg. Various values of c and p have been used.

3.6. THE VARIATION IN MAINTENANCE REQUIREMENT OF METABOLIZABLE ENERGY DUE TO DIFFERENCES IN RATION

Ration variation, the variation in maintenance requirement of metabolizable energy due to the composition of the ration (ch. 1.4.), was studied by computing coefficients of correlation between $M_{m,500}$ (correction factors: $p = 0.8$ and $c = c_s$) on one hand, and (i) the crude fibre content of the dry matter of the ration, (ii) the digestibility of the organic matter or (iii) c_s, *i.e.*, the gain correction factor computed by means of the starch equivalent system, on the other hand (table 6).

The only significant correlations were found in experiments where the rations varied much in composition, especially in the ratio between concentrate and roughage.

TABLE 6. Correlation between $M_{m,500}$ [1]) and a) the percentage of crude fibre in the dry matter, b) the percentage of the digestibility of the organic matter or c) c_s

Experiments	Energy balance	Number	$M_{m,500}$ × %c fibre	$M_{m,500}$ × %dom	$M_{m,500}$ × c_s
All	neg.	54	+ 0.39** [2])	— 0.35**	+ 0.33*
Shorthorns	,,	20	+ 0.71**	— 0.47**	+ 0.52*
All	pos.	165	+ 0.04	— 0.02	+ 0.02
Red Danish cows	,,	21	— 0.21	+ 0.06	— 0.20
Bavarian oxen	,,	68	— 0.03	+ 0.05	— 0.11
Shorthorns	,,	19	+ 0.47*	— 0.52*	+ 0.42
Ration of only hay		63	+ 0.07	+ 0.01	

[1]) $M_{m,500}$ = maintenance requirement of kcal. metabolizable energy of an animal of 500 kg.
[2]) * and ** = significant at 5 and 1 % levels of probability respectively.

The regression equation of $M_{m,500}$ on c_s in the experiments with negative energy balances was:

$$(1\,600 \pm 600)c_s + (8\,600 \pm 1\,300) = M_{m,500}.$$

Obviously a 10% change of c_s gives only a change of less than 2% in $M_{m,500}$.
BREIREM (1944, p. 15) found a highly significant correlation (r = + 0.39) between the crude fibre content (F, % in dry matter) of the ration and the maintenance requirement of metabolizable energy corrected for body weight, in 82 experiments of ARMSBY and FORBES in which there were small energy balances; the regression equation was:

$$229.6\,W^{0.6} + 1.4\,F\,W^{0.6} = M_m.$$

Here also the influence of the term with F on M_m is small compared with that of the term without F. The same is found in the first set of regression equations (3.3.) in the cases where the coefficient of F is not far from the 5% level of significance.
We may conclude, taking all the rations examined into account, that differences in ration affect but little the metabolizable energy requirement of cattle for maintenance. This is especially true for the data from experiments in which similar types of rations have been used. Where widely divergent rations have been fed the differences in requirement may be of some magnitude.

3.7. WITHIN-ANIMAL VARIATION AND BETWEEN-ANIMAL VARIATION IN MAINTENANCE REQUIREMENT OF METABOLIZABLE ENERGY

3.7.1. Introduction

The magnitude of *between-animal* and within-animal variation in maintenance requirement of metabolizable energy might be estimated by analysis of variance of the values M_{m,W_o}, *i.e.*, the amounts of metabolizable energy reduced to zero energy balance and body weight W_o. This is possible because a considerable number of the animals has been used in two or more experiments (tables 2 and 3, app.).

With this intention a correction for the energy balance was first applied to M, the intake of metabolizable energy, to obtain M_m using the correction factors of 3.5.

Next with the formula

$$M_{m,W_o} = \left(\frac{W_o}{W}\right)^p . M_m,$$

in which W is the actual body weight and W_o the weight of comparison, all values were made comparable. W_o was taken as 500 kg. for all the experiments together and as 350, 475, 650 or 800 kg. for experiments in which W lay in the ranges 280–400, 401–550, 551–750 and higher than 750 kg. respectively. The small weight ranges were used in order to reduce additional variation due to a possible error in the choice of a value for p.

Within a breed the range of W was not very great, but between breeds it was considerable. Within a breed the range of body weight within an animal, *i.e.*, from experiment to experiment, was only slightly smaller than that between animals (many animals were measured only once). Therefore, the variation within an animal of a breed includes nearly all additional variation due to the use of a possibly incorrect value of p; with all animals of more than one breed together the computed within-animal variation included only part of the additional variation due to this correction.

The variation due to the use of the above-mentioned values of c instead of the unknown true values is for the greater part also included in the within-animal variation of each breed since the range of the energy balances obtained and the variety of the rations fed in the experiments to the same animal of a breed were fairly similar to those obtaining in the experiments between the animals of that breed. The range of energy balances and the type of food fed, however, differed considerably between the breeds thus tending to give an incorrect, low within-animal variation if animals of two or more breeds are taken together.

For the reasons mentioned in the last two sections the true within-animal variation for different breeds will be higher than the variation computed from the data of the experiments dealt with here.

3.7.2. The within-animal variation

First we computed the within-animal variation of M_{m,W_o} for animals weighing less than 400 kg., for those weighing 401–550 kg., for those weighing 551–750 kg. and for those weighing more than 750 kg. (table 7). Nearly all Bavarian and Holstein animals fell in the third class, some Bavarian and most Simmenthal animals fell in the heaviest class while almost all animals of the other breeds fell in the lighter classes.

The within-animal variation was about 7% of the average maintenance requirement. It slightly increased when the correction factors for gain (c) were 1.67 and 2.00 instead of 1.43 and 1.61.

Table 7. Between-animal variation (V_I) and within-animal variation (V_W) of the maintenance requirement of metabolizable energy at body weight W_o, expressed as a percentage of the average maintenance requirement ($\overline{M_{m,W_o}}$)

Body weight		Energy balance	Number of		p=0.8; c=1.43 (neg. bal.) or 1.61 (pos. bal.)			p=0.8; c=1.67 (neg. bal.) or 2.0 (pos. bal.)		
W (kg)	W_o (kg)		animals	exp.	$\overline{M_{m,W_o}}$ (therms)	V_I (%)	V_W (%)	$\overline{M_{m,W_o}}$ (therms)	V_I (%)	V_W (%)
300–400	350	neg.	15	29	8.95	0.7	6.8	9.31	0	6.8
		pos.	15	26	8.51	11.8	8.8	8.07	10.3	12.4
401–550	475	neg.	12	27	10.27	6.6	6.8	10.58	7.5	5.5
		pos.	21	46	10.20	12.3	7.6	9.57	12.9	9.6
551–750	650	neg.	6							
		pos.	41	80	14.87	8.7	7.1	13.88	10.3	7.4
> 750	800	neg.	1							
		pos.	9	21	18.55	16.4	3.7	17.70	17.6	4.4

Next we computed the within-animal variation of M_{m,W_o} for all animals and for all the animals of some breeds, using the value for W_o = 500 kg. instead of the four values of W_o (table 8). Again taking all experiments the within-animal variation was not far from 7%. It changed but little for the experiments in which there were only negative energy balances, and the same applied to those in which there were positive balances.

The within-animal variation increased slightly if an exponent 1.0 instead of 0.8 was used. The value of the correction factor c had little influence on the within-animal

Table 8. Between-animal variation (V_I) and within-animal variation (V_W) of the maintenance requirement of metabolizable energy at 500 kg body weight, expressed as a percentage of the average maintenance requirement ($\overline{M_{m,500}}$)

		Number of		p	c = 1.43 (neg. balance) or 1.61 (pos. balance)			c = 1.67 (neg. balance) or 2.0 (pos. balance)			c = 0.83 c_s (neg. balance or c_s (pos. balance)		
Animals	Energy balance	animals	exp.		$\overline{Mm,500}$ (therms)	V_I (%)	V_W (%)	$\overline{Mm,500}$ (therms)	V_I (%)	V_W (%)	$\overline{Mm,500}$ (therms)	V_I (%)	V_W (%)
Red Danish	neg.	4	9	0.8	10.70	0	7.1	10.87	3.6	6.0	10.97	7.7	5.4
	,,			1.0				11.17	4.7	5.5			
	pos.	10	27	0.8	10.38	8.2	5.3	9.70	9.9	4.7	9.57	8.4	4.1
	,,			1.0	10.45	10.1	5.5	9.76	11.9	3.4	9.61	8.8	5.7
Bavarian	pos.	34	68	0.8	12.30	9.2	6.6	11.43	11.1	7.2	11.41	10.2	8.3
	,,			1.0	11.58	8.6	7.6	10.76	10.7	8.1	10.75	9.6	9.0
Shorthorns	neg.	9	20	0.8	11.44	8.7	6.1	11.89	8.0	6.1	11.72	8.2	7.9
	,,			1.0				12.38	10.1	7.9	12.21	9.6	10.3
	pos.	8	19	0.8	10.91	8.8	8.1	10.32	7.5	9.8	10.37	6.5	7.6
	,,			1.0	11.31	9.3	8.9	10.69	6.4	11.1	10.74	5.3	7.9
All animals	neg.	28	64	0.8	11.44	8.5	7.8	11.79	9.1	7.1	11.77	9.0	9.5
	,,			1.0				12.08	8.7	8.7	12.00	7.4	11.3
	pos.	72	173	0.8	11.67	12.1	7.1	10.93	12.8	8.8	10.87	12.0	8.7
	,,			1.0	11.43	11.5	7.8	10.71	12.1	9.6	10.62	10.7	9.6

variation. Evidently neither the correction for gain nor the correction for body weight affected the within-animal variation much. The within-animal variation of the Red Danish breed was lower than that of the Bavarian or Shorthorn breed. There was no difference in within-animal variation between the Bavarian oxen of Kellner and those of Fingerling.

The within-animal variation is caused by *analytical* and *physiological* variation and by *gain correction*, *weight correction*, *period* and *ration* variation (ch. 1.4.). The size of the sum of the first three kinds of variation may approximately be computed with the aid of $s'_{M_{m,W}}$, the standard deviation of $M_{m,W}$ if there were no *weight correction*, *period* and *ration* variation nor *between-animal* variation. This sum obviously is $\frac{s'_{M_{m,W}}}{M_{m,W}} \cdot 100\%$.
$s'_{M_{m,W}}$ may be computed by means of a formula that will be derived in chapter 6.11.:

$$s'^{2}_{M_{m,W}} = (c-1)^2 s_M^2 + \frac{1}{n} s_c^2 \sum_{i=1}^{n} G^2 + c^2 s_H^2, \text{ with} \qquad (4)$$

G = gain (kcal.); c = average gain correction factor; n = number of experiments; s_M, s_H and s_c = standard deviations of the average daily intake of metabolizable energy, of the average daily heat expenditure, both due to errors and to physiological deviations only, and of c respectively.
For lack of sufficient information we will only compute $s'_{M_{m,W}}$ in the experiments with positive energy balances with the Red Danish, Bavarian and Shorthorn breeds. First we consider which values for the various factors are to be used in (4):
c: In all three cases c may be estimated at 2.0 (table 5).
$\frac{1}{n}\sum_{i=1}^{n} G^2$: 4 080 422, 10 669 446 and 3 288 536 respectively.
s_M: The coefficient of variation (CV) of the figure found for the average daily intake of metabolizable energy in an experimental period differed for the three breeds mainly because of the length of this period. A CV of 0.5% (s_M = 100) in the experiments with the Bavarian breed (experimental period of 14 days) has been derived from errors and physiological deviations of energy in feed, faeces, urine and methane computed by Schiemann (1958, p. 38). Our own experiments (experimental period also 14 days) had a CV of about 0.7% (ch. 6.10.). The average of both values is 0.6%,therefore according to the length of the experimental period we roughly estimated the CV in the experiments with the Red Danish breed (experimental period of 28 days) at 0.4% (s_M = 50) and that in those with the Shorthorns (experimental period of 12 days) at 0.7% (s_M = 85).
s_H: In chapter 6.6. the CV of the average daily heat expenditure in a period due to errors and physiological deviations will be found to be about 1.2% (s_H = 140) for the Red Danish breed, about 0.5% (s_H = 90) for the Bavarian oxen and 1.0% (s_H = 110) for the Shorthorns (number of respiration days per experimental period 6–8, 3–5 and 2–3 respectively).

s_c: It is difficult to estimate s_c because the true c in every experiment depends on the ration (ration effect) and perhaps also on the animal (animal effect) since even animals fed with the same ration may have different values for c. The latter effect is generally thought to be small although it has not been studied intensively (BREIREM, 1944, p. 33). The ration effect on s_c very roughly has been estimated from the variation of c_s, *i.e.*, the requirement of metabolizable energy per kcal. gain computed according to the starch equivalent system (table 5). Assuming the animal effect to be small we will use values s_c of 0.25, 0.20 and 0.35, *i.e.*, figures a little higher than the standard deviations of c_s of table 5, for the Red Danish, Bavarian and Shorthorn animals respectively. It must be realized that since the values of s_c are not very accurate the values of $s'_{M_m,W}$ will not be very accurate especially as in the right member of equation (4) the term with s_c often predominates.

Using the above-mentioned values $s'_{M_m,W}$ becomes:

Red Danish breed	580 kcal. (CV = 5.9%),
Bavarian breed	685 kcal. (CV = 4.6%),
Shorthorn breed	680 kcal. (CV = 7.3%).

As the body weights of most of the animals within each of the three breeds were not very far from the average weight, the *weight correction* variation will have been small compared with the sum of *analytical* and *physiological* variation and *gain correction* variation. We have already noted (3.6.) that *ration* variation is of minor importance, especially in experiments with rations of more or less the same composition. Therefore, if *period* variation is neglected, the sum of the six kinds of variation will be about 6, 5 and 7.5% respectively for the three breeds. The first and the last of these figures are higher than the within-animal variation of table 8, 11th column. Therefore, if the values 6 and 7.5 are not too far from the truth, in those experiments *ration* and *period* variation were correctly neglected. The value for the Bavarian breed is much lower than the variation mentioned in table 8. Here, *period* variation may have been of importance.

3.7.3. The between-animal variation

We shall now assume that the within-animal variation noted for animals for which M was measured only once to be the same as that computed for individuals of the same breed for which M was measured more than once and shall compute the *between-animal* variation.

By abbreviation we call M_{ij} the maintenance requirement of metabolizable energy at 500 kg. body weight of animal i in experiment j. Then

$$M_{ij} = \mu + \alpha_i + \varepsilon_{ij}, \text{ with}$$

μ = population mean, α_i = deviation from population mean due to individual properties, and ε_{ij} = deviation from $\mu + \alpha_i$ due to errors and accidental deviations in experiment j.

Let the number of animals be m and let animal i be used in n_i experiments. For values of m and n_i equal to infinity

$$\frac{1}{m} \cdot \sum_{i=1}^{m} \alpha_i^2 \text{ and } \frac{1}{n_i} \sum_{j=1}^{n_i} \varepsilon_{ij}^2$$

become σ_I^2 and $\sigma_{W_i}^2$, the squares of the standard deviations due to *between-animal* variation and due to errors and accidental deviations respectively. It may be assumed that $\sigma_{W_I} = \sigma_{W_2} = \ldots \sigma_{W_i} \ldots \sigma_{W_n} = \sigma_W$. In case m and n_i are not infinite only best estimates (s_I^2 and s_W^2) of σ_I^2 and σ_W^2 have been computed.

For the average $M_{i.}$ of n_i experiments with animal i we have

$$M_{i.} = \mu + \alpha_i + \varepsilon_{i.}, \text{with } \varepsilon_{i.} = \frac{1}{n_i} \cdot \sum_{j=1}^{n_i} \varepsilon_{ij},$$

and for the average $M_{..}$ of all averages $M_{i.}$

$$M_{..} = \mu + \alpha_{.} + \varepsilon_{..}, \text{with } \alpha_{.} = \frac{1}{m} \cdot \sum_{i=1}^{m} \alpha_i \text{ and } \varepsilon_{..} = \frac{1}{m} \cdot \sum_{i=1}^{m} \varepsilon_{i.} \,.$$

Therefore:

$$\sum_{i=1}^{m} (M_{i.} - M_{..})^2 = \sum_{i=1}^{m} (\mu + \alpha_i + \varepsilon_{i.} - \mu - \alpha_{.} - \varepsilon_{..})^2$$

$$= \sum_{i=1}^{m} (\alpha_i^2 - 2\alpha_i \alpha_{.} + \alpha_{.}^2 + \varepsilon_{i.}^2 - 2\, \varepsilon_{i.} \varepsilon_{..} + \varepsilon_{..}^2 + \ldots)$$

$$\backsim m s_I^2 - 2 s_I^2 + s_I^2 + \sum_{i=1}^{m} \frac{s_W^2}{n_i} - \frac{2}{m} \sum_{i=1}^{m} \varepsilon_{i.}^2 + \frac{1}{m} \sum_{i=1}^{m} \varepsilon_{i.}^2$$

$$\backsim (m - 1)\, s_I^2 + \sum_{i=1}^{m} \frac{s_W^2}{n_i} - \frac{1}{m} \sum_{i=1}^{m} \frac{s_W^2}{n_i}$$

$$= (m - 1)\left(s_I^2 + s_W^2 \cdot \frac{1}{m} \sum_{i=1}^{m} \frac{1}{n_i}\right).$$

SNEDECOR (1959, p. 268) computed s_I^2 with another formula, derived from

$$\sum_{i=1}^{m} n_i \left(M_{i.} - \frac{\sum_{i=1}^{m} \sum_{j=1}^{n_i} M_{ij}}{\sum_{i=1}^{m} n_i}\right)^2 \text{ instead of from } \sum_{i=1}^{m} (M_{i.} - M_{..})^2.$$

In case n_i varies much from animal to animal, clearly our formula is to be preferred, if s_I is high compared with s_W, SNEDECOR's formula, if s_I is low compared with s_W. We compared some values s_I (expressed as a percentage V_I of $\overline{M_{m,W_0}}$) computed with

SNEDECOR's formula with those computed with our formula (table 9). As with both formulae s_W generally was lower than s_I, the results obtained with our formula may be slightly better than those obtained with that proposed by SNEDECOR. Table 9 also gives the results of tests for significance of s_I. The F-tests used were

$$F = \frac{s_I^2 \dfrac{\sum\limits_{i=1}^{m} n_i - \left(\sum\limits_{i=1}^{m} n_i^2 \Big/ \sum\limits_{i=1}^{m} n_i\right)}{m-1} + s_W^2}{s_W^2} \quad \text{and} \quad F = \frac{s_I^2 + s_W^2 \cdot \dfrac{1}{m} \sum\limits_{i=1}^{m} \dfrac{1}{n_i}}{s_W^2 \cdot \dfrac{1}{m} \sum\limits_{i=1}^{m} \dfrac{1}{n_i}}$$

for SNEDECOR's formula and our formula respectively.

TABLE 9. Between-animal variation (V_I) of M_{m,W_0} computed with two formulae

	Formula	Energy balance	M_{m,W_0} ($p = 0.8$; $c = 1.43$ or 1.61) of all animals with body weight:				$M_{m,500}$ ($p = 0.8$; $c = 1.43$ or 1.61) of			
			300–400 kg	401–550 kg	551–750 kg	> 750 kg	all animals	Red Danish animals	Bavarian animals	Shorthorn animals
V_I	SNEDECOR	neg.		7.0* 1)			6.7***	3.1		5.4
,,	,,	pos.	10.5*	12.6***	10.3***	14.3***	13.1***	6.9***	9.9***	8.2*
,,	Own	neg.		6.6*			8.5***	0		8.7**
,,	,,	pos.	11.8*	12.3***	8.7***	16.4***	12.1***	8.2***	9.2***	8.8*

1) *, ** and *** = significant at 5, 1 and 0.5% levels of probability respectively

With our formula s_I was computed for the same groups of experiments as those used in the computation of s_W. In tables 7 and 8 the result is given as a percentage (V_I) of $\overline{M_{m,W_0}}$.

Approximately the same values for V_I were found for all the gain and the weight correction factors which had been used. Therefore the significance tests were only applied to the values M_{m,W_0} computed with the correction factors $p = 0.8$ and $c = 1.43$ or 1.61 (table 9).

Both the variation between animals of the same breed and the variation between all animals regardless of breed was high. The latter was higher than the former, but it must be kept in mind that the true variation within all animals will have been higher than the value computed by us (3.7.1.). Doubtless the difference also has been caused by the variation in maintenance requirement between the breeds. The average maintenance requirement of the Red Danish cows is clearly lower than the average requirement of all animals (table 8).

It is questionable whether in practice similar variations between animals and between breeds exist at least to day. Nearly all the animals used in the experiments recorded in the literature have been selected for docility (ch. 6.6.). For this reason the computed variation between animals may have been too low. On the other hand, it may be that in the many years that have elapsed since the experiments were completed, breeding may have lowered such variation.

3.8. CONCLUSIONS

The best value for the weight correction factor p, *i.e.*, the power in the equation $M_{m,W_0} = M_{m,W} (W_0/W)^p$, appeared to lie between 0.8 and 1.0. Within certain limits the choice of p did not considerably decrease or increase the residual variance in the regression of M on W^p and G. The same was true with the choice of the gain correction factor c, *i.e.*, the requirement of metabolizable energy per kcal. gain.

The gain correction factor computed according to the starch equivalent system was intermediate between that computed by regression of M on W^p and G and that computed by regression of G on M and W^p.

The influence of the composition of the ration on the maintenance requirement of metabolizable energy was small.

The variation of $M_{m,500}$ within animals was about 7%. For the Red Danish and the Shorthorn breed this variation might be accounted for by *analytical* and *physiological* variation and *correction* variation. In the Bavarian breed there is evidence that *period* variation may also play a part.

The variation in $M_{m,500}$ between animals was high (5–10%).

4. DESIGN OF THE EXPERIMENTS CONDUCTED AT WAGENINGEN AND A GENERAL DESCRIPTION OF THE TECHNIQUES USED

Feed is used for maintenance purposes only when the carbon, nitrogen and energy balances are zero and when no milk, work etc. is produced. However, for obtaining the minimum maintenance requirement of energy some further conditions must be fulfilled: the maintenance requirements of protein, minerals and vitamins must be met and in addition there must be some crude fibre in the ration to ensure normal rumination. In this connection a ration of hay and mixed concentrates with the addition of a little salt (NaCl) appears satisfactory. For comparison of the requirements of different animals the same feed must be used for all animals.

Under practical conditions, *i.e.*, in the cowshed, the theoretical minimum requirement will not be reached. As the variation in this practical maintenance requirement is the subject of this study (see ch. 1.1.), it would be necessary to do the experiments in such a way that the environmental conditions during the experiments were as equal as possible to those in the cowshed.

In the Netherlands cows are economically far more important than male animals and since the maintenance requirements of the latter perhaps differ from those of females, it is important to do the experiments with cows even though they present more technical difficulties.

Animals for the experiments were available on the laboratory farm, although it was not possible to get a sufficient number of dry, non-pregnant cows. Since the weight of the foetus of cows in their 7th month of pregnancy is small, it seemed feasible to use such animals for the experiments. To obtain information about the increasing metabolism during the last months of pregnancy experiments with the same animals in the 9th month of pregnancy might be done.

In the first experiment two cows were still giving some milk and difficulties arose in correcting results for milk production in addition to the inevitable, small body gain. Therefore, the cows of the other experiments were dried off some weeks before the preliminary period of the 7th month pregnancy experiment, that is a few weeks earlier than is done in practice. Altogether experiments with 15 dry animals in about their 7th month of pregnancy were done. With 8 of these cows an experiment was also carried out in the 9th month of pregnancy (table 10).

In the 9th month pregnancy experiments the preliminary and experimental periods during which the same quantity of feed was given every day, had to be curtailed since the requirements of the animals were changing rapidly with the growth of the foetus. Short periods, however, give less accurate results. Therefore preliminary periods of 16 days and experimental periods of 14 days were used in normal experiments and preliminary periods of 12 days and experimental periods of 10 days in the experiments during the 9th month of pregnancy. The experimental periods of 14 days were divided in two subperiods, in order to compare the first week's results with those of the second

TABLE 10. Various data on the animals

Exp.	Name of animal	Birth date	Experimental period	Age during exp. period		Days before parturation	% of 24 h. spent standing in:	
				years	months		resp. ch.	stall
FIRST SERIES								
R 1	Witschoft	6– 4–'53	15/7–30/7 '56	3	3	225	48	not determ.
R 1	Zwartschoft	18– 6–'50	15/7–30/7 '56	6	1	92	51	,, ,,
R 2	Annie 7	13– 1–'52	2/9–17/9 '56	4	8	55	66	,, ,,
R 2	Alie 1	24– 2–'52	2/9–17/9 '56	4	6	39	57	,, ,,
R 4	Clara	29– 4–'53	18/1– 2/2 '57	3	9	58	56	60
R 4	Klaske	spring '52	18/1– 2/2 '57	∽5		52	50	55
R 5	Zwartkop 2	29– 3–'51	15/3–30/3 '57	6	0	65	58	51
R 5	Coba 6	12– 8–'50	15/3–30/3 '57	6	7	69	60	52
SECOND SERIES								
R 7	Eke 42	23– 2–'54	9/ 8–24/ 8 '57	3	6	69	43	40
R 7	Jansje	19– 4–'54	9/ 8–24/ 8 '57	3	4	75	42	45
R 7 III	Eke 42	23– 2–'54	25/ 8–31/ 8 '57	3	6	57	39	43
R 7 III	Jansje	19– 4–'54	25/ 8–31/ 8 '57	3	4	63	44	45
R 10	Alie 1	24– 2–'52	28/11–13/12 '57	5	9	77	67	56
R 10	Klaasje 3	31– 3–'50	28/11–13/12 '57	7	8	95	52	41
R 12	Kee 2	10–11–'50	16/ 1–31/ 1 '58	7	3	62	60	52
R 12	Alke	5– 5–'52	16/ 1–31/ 1 '58	5	8	57	61	66
R 13	Betsy	1– 5–'53	20/ 2– 7/ 3 '58	4	10	90	77	63
R 13	R. Willy 12	20– 3–'51	20/ 2– 7/ 3 '58	7	0	81	59	61
LATE PREGNANCY EXPERIMENTS								
R 3	Annie 7	13– 1–'52	28/ 9– 6/10 '56	4	9	34	48	not determ.
R 3	Alie 1	24– 2–'52	28/ 9– 6/10 '56	4	7	18	67	,, ,,
R 6	Zwartkop 2	29– 3–'51	6/ 5–17/ 5 '57	6	2	14	49	43
R 6	Coba 6	12– 8–'50	6/ 5–17/ 5 '57	6	9	18	47	59
R 9	Eke 42	23– 2–'54	7/10–18/10 '57	3	8	11	39	40
R 9	Jansje	19– 4–'54	7/10–18/10 '57	3	6	17	54	56
R 14	Betsy	1– 5–'53	14/ 4–25/ 4 '58	4	11	39	73	52
R 14	R. Willy 12	20– 3–'51	14/ 4–25/ 4 '58	7	1	30	52	51
EXPERIMENT WITH GRASS								
R 8	Klaske	spring '52	16/ 9–27/ 9 '57	∽5		—	44	53
R 8	Lamkje	9– 5–'54	16/ 9–27/ 9 '57	3	4	44	47	42
R 8	Roosje	8– 1–'54	16/ 9–27/ 9 '57	3	8	78	49	54

week. In the middle of each week there were two respiration experiments of 24 h. each on successive days. In the 9th month pregnancy experiments three respiration experiments were carried out. During the whole experimental period faeces and urine were collected dayly, weighed and sampled.

The animals were Friesian cows of 3–8 years' age from the laboratory farm (table 10). The herd of this farm had just been bought and was rather heterogeneous. We did not select the animals for docility. Few of the animals had been used in any previous experiment. On arrival at the laboratory they were in a moderate condition. In the preliminary period the cows were familiarized with their new surroundings, especially with the respiration chambers. Their feed was the same as in the experimental period, although sometimes the quantity offered was slightly changed in the first week when the body weight had become more accurately known.

The experimental rations of the cows (table 11) were computed according to the feeding standards of FREDERIKSEN (1931) for maintenance and for milk production with the exception of the standard for digestible crude protein for milk production which was

Table 11. Rations

Exp.	Name of animal	Body weight (kg)	*f*	Hay (kg)	Sugar beet pulp (kg)	Concentr. mixture (kg)	Salt (g)	Milk (kg *FCM*)
R 1	Witschoft	511	0.900	5.4 (A)	1.5	2.80 (I)	30	7.58
R 1	Zwartschoft	600	1.000	6.0 (A)	1.7	2.70 (I)	34	6.37
R 2	Annie 7	653	1.060	6.4 (A)	1.8	0.80 (I)	36	
R 2	Alie 1	613	1.000	6.0 (A)	1.7	0.80 (I)	34	
R 3	Annie 7	650	1.060	6.4 (A)	1.8	1.85 (I)	36	
R 3	Alie 1	610	1.000	6.0 (A)	1.7	1.85 (I)	34	
R 4	Clara	573	0.970	5.80 (A)	1.65	0.80 (I)	33	
R 4	Klaske	573	0.970	5.80 (A)	1.65	0.80 (I)	33	
R 5	Zwartkop 2	538	0.930	5.58 (A)	1.58	0.80 (I)	32	
R 5	Coba 6	497	0.887	5.32 (A)	1.51	0.80 (I)	30	
R 6	Zwartkop 2	590	0.990	5.94 (A)	1.68	1.85 (I)	34	
R 6	Coba 6	540	0.933	5.60 (A)	1.59	1.85 (I)	32	
R 7	Eke 42	460	0.843	5.08 (B)		0.73 (II)	30	
R 7	Jansje	498	0.887	5.32 (B)		0,76 (II)	30	
R 9	Eke 42	464	0.864	5.18 (B)		1.11 (III)	30	
R 9	Jansje	500	0.898	5.39 (B)		1.15 (III)	30	
R 10	Alie 1	570	0.967	5.80 (B)		0.83 (II)	30	
R 10	Klaasje 3	538	0.931	5.59 (B)		0.80 (II)	30	
R 12	Kee 2	599	1.000	6.00 (B)		0.86 (II)	30	
R 12	Alke	519	0.910	5.46 (B)		0.78 (II)	30	
R 13	Betsy	519	0.910	5.46 (B)		0.78 (II)	30	
R 13	R. Willy 12	619	1.020	6.12 (B)		0.88 (II)	30	
R 14	Betsy	573	0.973	5.84 (B)		1.25 (III)	30	
R 14	R. Willy 12	631	1.030	6.18 (B)		1.32 (III)	30	

reduced by 10%, a reduction advised by the Centraal Veevoeder Bureau (1956):

(*W* = kg. body weight; *SV* = starch equivalent; dcp = digestible crude protein; *FCM* = fat corrected milk)

Maintenance: $\left(\frac{W}{300} + 1\right)$ kg. *SV* and 0.7 *W* g. dcp,

Milk production: 0.278 kg. *SV* and 63 g. dcp per kg. *FCM*.

The extra allowance for gestation given by the Centraal Veevoeder Bureau was reduced to prevent large, positive energy balances:

For pregnancy (7th month)
in experiment R 1: requirement for 1 kg. *FCM*,
in experiments R 2, 4, 5: requirement for 2 kg. *FCM*,
in experiments R 7, 10, 12, 13: 0 kg. *SV* and 70 g. dcp,
For pregnancy (9th month)
in experiments R 3, 6: requirement for 5 kg. *FCM*,
in experiments R 9, 14: 0.3 kg. *SV* and 250 g. dcp.

The composition of the preliminary samples of hay A, used in the experiments R 1–6, and of hay B, used in the experiments R 7, 9–10, 12–14, and of the various concentrate mixtures and the dried sugar beet pulp were:

	dry matter (%)	crude protein (% in dm)	crude fibre (% in dm)		ash (% in dm)	*SV* 1)	dcp 1) (g/kg)
hay A	83.9	12.5	30.7		9.3	33.6	58
hay B	85.4	10.4	27.6		8.7	39.0	45
	corn-meal, %	barley-meal, %	linseed-meal, %	cocos-meal, %	groundnut-meal, %	*SV* 2)	dcp 2) (g/kg)
mixture I	22.2	22.2	22.2	22.2	11.1	75.3	182
mixture II	17.05	17.05	17.05	17.05	31.8	75.2	238
mixture III	10.1	10.1	10.1	10.1	59.7	75.2	314
sugar beet pulp						58.8	37

1) computed by the method of BROUWER and DIJKSTRA (see DIJKSTRA, 1954).
2) given bij Centraal Veevoeder Bureau, 1956.

The five kinds of meal and the sugar beet pulp were bought some weeks before an experiment. The concentrates were mechanically mixed at the laboratory.
A cow of 600 kg. requires for maintenance 3.00 kg. *SV* and 420 g. dcp, an amount nearly contained in 6 kg. of hay A plus 1.7 kg. of the dried sugar beet pulp. This ration was used as a standard ration during the experiments R 1–6. Cows having a body weight W in the first week of the preliminary period received $\frac{(W/300) + 1}{(600/300) + 1} = f$ times this ration. In addition some of mixture I, 0.369 kg. was needed for each kg. *FCM*, was given to make up for the requirement due to milk production and to pregnancy. The pregnancy supplement in these experiments did not change with the weight of the animal. The weights of the animals during the experimental period of course were slightly different from those in the first week of the preliminary period.
After the results of the first six experiments had been computed, it was clear that the rations had been rather high; they gave fairly large, positive energy balances. Therefore the allowance for pregnancy was reduced to zero kg. *SV* and 70 g. dcp in normal experiments and to 0.3 kg. *SV* and 250 g. dcp in the 9th month pregnancy experiments; the amounts of dcp were in accordance with the feeding standards given by JAKOBSEN (1956). Since by this time no hay A was left and the second batch of hay (B) contained less protein and more *SV* it was necessary to enrich 6 kg. of hay B with concentrates: in normal experiments 6 kg. of hay B plus 0.86 kg. of mixture II was given for maintenance and for pregnancy; in the 9th month pregnancy experiments 6 kg. of hay B plus 1.28 kg. of mixture III was given. These standard rations were used in the experiments R 7, 9, 10, 12–14; the weight correction f was computed in the usual way except in the 9th month pregnancy experiments R 9 and R 14 where f was $\frac{(W/300) + 1 + 0.3}{(600/300) + 1 + 0.3}$. Therefore in this series of experiments the pregnancy supplement changed slightly with the body weight of the animal. This change was, however, negligible as the weight variations were not large and as the pregnancy supplement was small.

The animals ate their rations almost completely; any feed residues were collected immediately after the last day of each (sub)-period.

Drinking water was given as soon as the feed had been eaten. After experiment R 3 no more than 25 kg. daily was allowed.

The cows used in experiment R 2 were not completely dry at the beginning of the experimental period although they were no longer being milked. As result some milk was pressed out of the udders when the animals lay down. About one year later a second experiment (R 10) was carried out with one of these animals and on this occasion the animal was completely dry.

One cow of experiment R 4, Klaske, had a digestive disturbance during the second subperiod; its temperature rose to 40.2° C. and for some days feed was refused. Only the results of the first subperiod were used.

Experiment R 7 was prolonged by one additional subperiod to test whether it made any difference when unchopped hay was eaten.

In experiment R 8 fresh autumn grass was used as the only feed. Originally this experiment was set up as a digestion trial. Since it was not very difficult to have the animals for some days in the respiration chambers and to do slightly more analytical work, the experiment was carried out as a complete balance trial. Fresh grass was mowed in the morning and chopped. The *SV* of the grass, amounting to 56 in the dry matter, was computed by the method of BROUWER and DIJKSTRA (see DIJKSTRA, 1954), we predicted the crude fibre and ash content of the grass in the middle of the experimental period from those of samples taken in the preliminary period. The standard ration was $\frac{(W/300) + 1}{0.56}$ kg. dry matter of the grass. About half a day's ration was weighed and given to the animals at 10.00 h. At the same time a sample of the grass was taken and its dry matter content was immediately determined. The loss of water during the day of the remainder was also determined and hence it was possible to calculate the amount of grass that had to be given at 16.00 h., to obtain the correct daily intake of dry matter. The experimental period lasted 10 days. The animals had some feed residues, partly because the very wet grass contained appreciable quantities of soil.

5. METHODS

5.1. GENERAL OUTLINE

Since nitrogen, carbon and energy balances were to be computed, and at the same time the apparent digestibility of the major feedcomponents had to be determined, the following information was needed:

The quantity and composition (dry matter, crude protein = 6.25 N, crude fat, crude fibre, true protein, carbon, calorific value) of the feeds (hay, concentrate mixture and dried sugar beet pulp separately), of the feed residues and of the faeces; the quantity and composition (dry matter, fat, nitrogen, carbon, calorific value) of the milk; the quantity and composition (nitrogen, carbon, calorific value, the sum of free and bound CO_2) of the urine and the quantities of CO_2 and of CH_4 produced and the amount of O_2 consumed by the animal.

To be sure that from day to day the same and equivalent amounts of hay were given and that a representative sample of the hay which was fed should be obtained, the hay was chopped to pieces of length 1–4 cm. and sieved in three fractions. For a day's ration aliquote portions of the three fractions were weighed out.

For the separate collection of urine and faeces urinals described by MØLLGAARD (1929, p. 60) were used and until experiment R 5 these were fastened to the animals with a net. Afterwards a leather harness (VAN ES and VOGT, 1959) was used. The urine flowed through the urinal and bicycle tubes (diameter 4 cm.) into a 10 litre flask while the faeces fell into big galvanized containers. The faeces were transferred to galvanized washing boilers fitted with cover and of 40 litres about four times aday. Any urine which escaped collection in the urinal fell into the big container and was collected as free from faeces as possible. The amount of this spilled urine was seldom more than 5% of the total amount. The respiration experiments were carried out in the two respiration chambers of the laboratory (BROUWER, in prep.).

5.2. WEIGHING AND SAMPLING

The *rations* (in paper bags) were weighed by an ordinary balance with an error less than 0.2%. A decimal balance was used for the weighing of the milk, the urine and the faeces. The weighing error was again less than 0.2%.

All the day's rations of one experimental period were weighed out on the same day. Immediately before the weighing of each day's ration a spoonful of concentrate mixture or of dried sugar beet pulp was put into a bottle with a tight fitting stopper to get a composite sample. During the filling of every single paper bag with the day's rations of hay a handful of the material was twice put into each of two washing boilers to obtain two composite samples of each fraction of the hay.

Small quantities of the *feed left* by the animals at the end of the (sub)-periods, mainly hay, were collected, dried at 60–70° C. and weighed after cooling. Then they were assumed to have a dry matter content of 95%. This material was not analysed since the amount was so small. In the computations we supposed it had the same composition of the dry matter as the hay. Large feed residues were weighed, sampled and analysed.

The *milk* was weighed within an hour after milking. After mixing a constant percentage of the total amount (usually 6%) was put into a sampling bottle having a tight fitting stopper and containing 0.5 g. $K_2Cr_2O_7$. The total subperiod sample weighed about 2–4 kg. The bottle was stored at + 5° C.

The *urine* was weighed within two hours after the end of the day of collection (9.00 h.). It was sampled in the same way as the milk, but in duplicate. The urine of one of the composite sampling bottles was acidified with HCl (s.g. 1.1) to prevent losses of N. At the beginning of the period 100 ml. of the acid was put into the bottle, an amount that was enough in most cases for the total subperiod sample of about 4 litres. If the reaction after addition of a fresh portion of urine became neutral or alkaline more HCl was added. Into the other sampling bottle 15 ml. formalin was put as a preservative; the sample of this bottle (in total again about 4 litres) was used for the determination of carbon and the sum of free and bound CO_2. In experiments R 1 and R 2 formalin was also added to the acidified urine. As it appeared to be unnecessary from the point of view of conservation, and as it necessitated an additional correction to the energy content of the urine it was omitted in later experiments. The sampling bottles were stored at + 5° C.

The *faeces* were weighed within two hours after the end of the day of collection (9.00 h.) and then intensively kneaded and mixed by hand. A weighed amount, equal to a constant percentage of the total weight (usually 6%), was taken by random sampling and put into a composite sampling bottle. This bottle contained 15 ml. formalin and was stored at about — 25° C. In the experiments R 4–6 no formalin was added. It was found that the loss of dry matter and N by drying at 60–70° C. during about two days was somewhat higher than the loss by drying of faeces with formalin added. Therefore, from experiment R 7 onwards again formalin was added.

If the *concentrate sample* was dry enough, it was immediately ground by means of a small hammer mill through a 1.25 mm. sieve. Samples that were not dry enough for grinding directly, were treated as follows: 200.0 g. were weighed, dried at 60–70° C. and weighed again after 3–4 hours' cooling in a room with normal humidity before being ground.

From each of the two *samples* of each of the three *hay* fractions subsamples of 200.0 g. were taken. The 6 samples thus obtained were dried for one day at 60–70° C., weighed after 3–4 hours' cooling and ground through the hammer mill. The 6 ground samples were mixed three to three in aliquot parts, so that two mixed samples of equal value were obtained, both corresponding in composition with the original hay if dried at 60–70° C.

At the end of the subperiod the composite *samples* of the *milk* were heated to 40° C., mixed and immediately analysed.

The composite *samples* of the *urine* were mixed and immediately analysed.

The composite *samples* of the *faeces* after being defrosted were intensively kneaded and mixed. Random samples were taken and immediately analysed for dry matter, crude protein, true protein and sometimes volatile fatty acids. Afterwards 1000.0 g. of the sample was dried at 60–70° C. for two days, weighed after cooling and ground. The determinations not listed above and in addition those of dry matter and crude protein were done on the ground, air-dry material.

5.3. ANALYTICAL METHODS

The *dry matter* content of all samples was determined by drying at 101–102° C.; that of the milk after preliminary drying of the sample mixed with sand at 100° C. Two values of the dry matter content of the faeces were obtained, one from the determination of the wet faeces and one computed from that in the air-dry material and the content of air-dry matter in the wet faeces. The first value was used in the computation, the other served as a check on the loss by drying at 60–70° C.

Nitrogen was determined by the KJELDAHL-method with selenium as a catalyst. The digestion was continued for half an hour after the fluid became clear. Here again there were two values for the N-content of the faeces; that determined on the air-dry material served as a check on losses by drying at 60-70° C.

True protein was determined in the feed and in the wet faeces. NaOH, $CuSO_4$ and $KAl(SO_4)_2$ were used to precipitate the protein. The N-content of the precipitate was determined by the KJELDAHL-method.

Crude fat was determined by ether extraction.

For the determination of *crude fibre* 1.25% H_2SO_4, 1.25% KOH and acetone were used as reagents, asbestos fibres as a filter and pear-shaped crude fibre vessels as the reaction flasks.

Mineral matter was determined by combustion at 500–550° C.

All the above-mentioned determinations were carried out in duplicate by each of two technicians, independently of each other. Duplicates were done on different days. Feed and faeces of the same period were always analysed in the same series of determinations. When duplicates differed more than usual one or more additional determinations were done.

The content of *volatile fatty acids* of the faeces was estimated by titration of the destillates of four successive destillations of acidified aqueous extracts of the samples. Each of the four destillations lasted until half of the original volume of the fluid remained; before the next destillation the fluid was brought back to its original volume by addition of water. Only acetic acid and butyric acid were assumed to be present.

The determinations of the *calorific value* was performed with a bomb calorimeter similar to that described by KLEIN and STEUBER (1926). The water equivalent of the calorimeter was determined by combustion of pure benzoic acid. It appeared that the BECKMAN-thermometer when compared with an improvised gas thermometer, gave errors in some parts of the scale as high as 0.015° C. Therefore a correction table was used to overcome the errors of the temperature reading.

The quantity of material used for a combustion was chosen so that the increase in temperature due to the combustion was about 1.5–2.0° C. The usual corrections for combustion of the ignition wire, for unburnt carbon and for the HNO_3 produced were applied. Very dry samples often burned only partly; a moisture content of the sample of about 12% was found to give the best results and therefore the moisture content of some samples had to be increased before combustion in the bomb.

The (acidified) urine and the milk were dried before combustion; urine (10 g.) on a cellulose block in a vacuumdesiccator over silicagel, milk (5 g.) in a tube of thin glass in a similar desiccator.

The determination of the calorific value and carbon content of the faeces after drying at 60–70° C. did not satisfy us. Several authors mention losses of carbon in drying faeces (GHONEIM, 1929, p. 133; KLEIBER *et al.*, 1936). COLOVOS *et al.* (1957) compared the calorific values of faeces dried at 65° C. with those found after combustion of the wet faeces with alcohol as a primer and found rather high losses of energy by the former method. The calorific value of the alcohol given by him, however, is rather low. The amount of heat due to the combustion of the alcohol was great compared with the heat due to the combustion of the faeces. Moreover it is very difficult to introduce an accurately measured amount of alcohol into the bomb without loss. Prof. COOPS in Amsterdam (1958) stated that a high water content of the sample considerably diminishes the accuracy of determination of calorific value and that the combustion of volatile substances is very often incomplete. FLATT (1957) found in faeces dried at 89° C. a calorific value that was about 3% lower than the one obtained with the wet combustion method of COLOVOS. We also tried to estimate the loss in drying (NIJKAMP, in prep.). One half of each of a few samples was freeze-dried, a very accurate method of drying, and the other half was dried at 60–70° C. Only slightly (less than 0.5%) lower values were obtained by the latter method. In earlier investigations quick drying at 100° C. with collection of all carbon gave a carbon content that was only slightly higher than the one found after drying at 60–70° C. Very recently BRATZLER and SWIFT (1959) have shown that the wet combustion method with alcohol is incorrect. They burned wet faeces with an accurately known amount of benzoic acid instead of with alcohol and obtained the same calorific values as those found when the faeces were dried for 22 hours at 65° C. in a forced air oven.

The loss of volatile fatty acids during drying of faeces at 60–70° C. in experiment R 7 was about 20%. Fortunately, the content of acetic acid and butyric acid in the fresh faeces was low, about 0.14 and 0.03% respectively. This was another reason for considering the calorific values obtained on dried faeces as being very close to the true values for the faeces as produced. The loss of volatile acids during the determinations of dry matter at 101–102° C. was about 15%, equivalent to a loss of only 0.03% dry matter. Therefore no corrections for losses of volatile fatty acids were applied.

The *carbon determination* was done in combination with that of the calorific value. The gases in the bomb were led through an absorption train consisting of two GOMBERG vessels containing a 35% KOH-solution and U-shaped CO_2-absorption tubes. The carbon of the urine was determined by wet combustion with a potassium dichromate-sulphuric acid mixture. The gases liberated in this combustion were first led through a tube containing pumice stones saturated with concentrated sulphuric acid in order

to absorb most of the SO_2 and SO_3, then through a tube filled with lead chromate and copper turnings heated to about 525° C. to reduce N-oxides, to trap halogens and the remainder of SO_2 and SO_3 and to oxidize any partly oxidized components. Finally the gases passed through a CO_2-absorption train.
The content of *free and chemically bound CO_2* was found by acidifying the (not acidified) urine sample and absorbing the resulting CO_2 in an absorption train.
The determinations of carbon and calorific value were effected by one technician in duplicate (both on different days), or if these duplicates differed more than 0.3%, in triplicate or quadruplicate. Again the feed and the faeces of the same (sub)-period were analysed at the same time.

5.4. CORRECTION FOR ADDED PRESERVATIVES

The addition of HCl and/or formalin resulted in the composition of the composite samples differing somewhat from that of the original urine or faeces. Correction formulae were established to transform the data of the analyses of the samples to data of the original urine or faeces. The calorific value of formaldehyde is about 4 kcal./g. and its carbon content about 40 %, thus for formalin, a 40 % solution of formaldehyde in water, these figures are 1.6 and 16 respectively. It was assumed that no loss of formaldehyde occurred during drying of the faeces. No correction was applied for the addition of $K_2Cr_2O_7$ to the milk.

Now we put:
U = quantity of original urine (g.) in composite sample,
F = quantity of original faeces (g.) in composite sample,
p = quantity of HCl (g.), added to the composite sample,
q = quantity of formalin (g.), added to the composite sample,
r = quantity of CO_2 (g.), lost by acidifying of 1 g. original urine,
n = % of nitrogen in original urine,
c = % of carbon in original urine,
w = calorific value (kcal.) per g. original urine,
a = % of dry matter in original faeces,
b = % of nitrogen, crude or true protein in original faeces,
c = % of nitrogen, crude protein, crude fat, crude fibre or mineral matter in dry matter of original faeces,
d = % of N-free extractives in dry matter of original faeces,
e = % of carbon in dry matter of original faeces,
f = calorific value (kcal.) per g. dry matter of original faeces,
$n' \ldots f'$ = % or value in composite sample instead of in original material.

Suppose U g. original urine had been diluted with p g. HCl and q g. formalin. The addition of the acid gave a loss of r g. CO_2 per g. urine. Then:

$$nU = n' \, (U + p + q - rU) \text{ and } n = n' \left(1 + \frac{p+q}{U-rU}\right)(1-r).$$

For the C-content holds good (addition of formalin only):

$$cU + 16q = c'(U + q) \text{ and } c = c'\left(1 + \frac{q}{U}\right) - 16\frac{q}{U}.$$

For the calorific value we get (addition of HCl and formalin):

$$wU + 1.6q = w'(U + p + q - rU) \text{ and } w = \left\{w'\left(1 + \frac{p + q}{U - rU}\right) - \frac{1.6q}{U - rU}\right\}(1 - r).$$

In a similar way the correction formulae for use with faeces were derived. All the correction formulae of urine and faeces are given below:

Urine

nitrogen $n = n'\left(1 + \frac{p + q}{U - rU}\right)(1 - r)$,

carbon $c = c'\left(1 + \frac{q}{U}\right) - 16\frac{q}{U}$,

calorific value $w = \left\{w'\left(1 + \frac{p + q}{U - rU}\right) - \frac{1.6q}{U - rU}\right\}(1 - r)$.

Faeces

dry matter $a = a'\left(1 + \frac{p}{F}\right) - 40\frac{p}{F}$,

nitrogen, crude and true protein $b = b'\left(1 + \frac{p}{F}\right)$,

nitrogen, crude protein, crude fat, crude fibre and ash, all in dry matter $c = c'\left(1 + \frac{40p}{aF}\right)$,

N-free extractives in dry matter $d = d'\left(1 + \frac{40p}{aF}\right) - \frac{4\,000p}{aF}$,

carbon in dry matter $e = e'\left(1 + \frac{40p}{aF}\right) - \frac{1\,600\,p}{aF}$,

calorific value of dry matter $f = f'\left(1 + \frac{40p}{aF}\right) - \frac{160p}{aF}$.

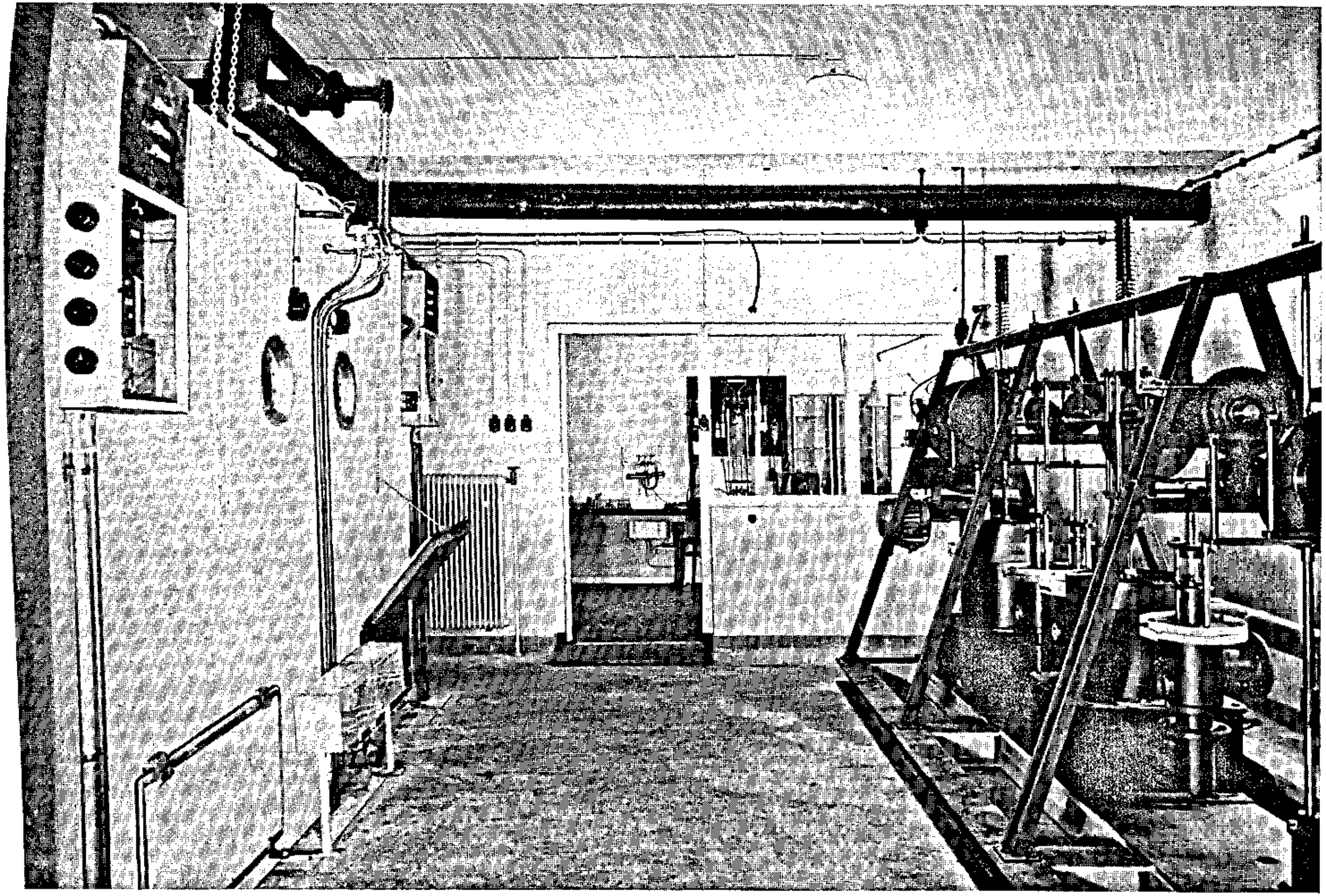
As U and F, being about 4 000, were high compared with q, being about 16, and as U was rather high compared with p, being about 110, all corrections were small:

Urine

N, C and kcal.: about 5% of total % or value.

Faeces

dm: about 0.8% of total %,
N, cp, c fat, c fibre, N-fe, all in dm: about 1% of total %,
C and kcal. in dm: about 0.2% of total % or value.


Front of the respiration chambers, part of the gas analysis room and mercury pump

As mentioned above the dry matter and crude protein contents of the fresh faeces were determined directly, but they might also be computed from the analysis of the dried faeces. Drying apparently gave losses. The average loss of dry matter and crude protein was 0 and 5% of total % respectively with the faeces plus formalin (32 samples) and 2 and 9% respectively with faeces without formalin (9 samples). If the separation of urine and faeces had not been complete, *i.e.*, when there had been some spilled urine, then the losses seemed to be higher.

The content of dry matter and of crude protein of the fresh faeces determined directly were used in all calculations and therefore the contents of the components of the dry matter determined in the air-dry material, had to be changed. They were in fact lowered in proportion to the change in crude protein that resulted from drying. Here it was assumed that indeed protein as a whole had been lost by drying and not simpler N-compounds. It does not matter so very much whether this is really the case since the loss by drying is not high. The carbon content and the calorific value were also determined in the dried faeces and therefore these values were increased in proportion to the change in organic matter due to the correction for the loss of protein by drying. It gave a very small correction.

5.5. THE MEASUREMENT OF RESPIRATORY EXCHANGE

Total CO_2- and CH_4-production and O_2-consumption of an animal in an open-circuit system respiration chamber per 24 h. can be computed from the volumes (standard conditions) of the gases entering, leaving and remaining in the respiration chamber. In the following a description is given of the manner in which these data were collected and used.

5.5.1. The equipment and its use

In each of the two respiration chambers used (chamber 3 and chamber 4) a negative pressure of 5–10 mm. H_2O was maintained in order to draw in fresh outdoor air through tube A (fig. 1). While a very small volume, less than 20 litres/h., of indoor air leaked into the chamber through small holes, no chamber air leaked out. A continuous sample of the ingoing air was drawn into S_i, a pyrex tube which, prior to the beginning of the experiment, had been filled with 800 ml. mercury. During the experiment the mercury flowed out of this tube through a gradually lowered overflow and, thus, a sample of the gas was sucked in. Occasionally a sample of the indoor air surrounding the chamber was taken. These and all other gas samples were analysed with a modified SONDEN volumetric gas analysis apparatus (VAN ES, 1958); 10% KOH was used to absorb CO_2, a hot platinum wire to combust CH_4 if any was present, and pyrogallol (15 g. in a saturated solution of KOH) and later a $CrCl_2$-solution (Ruhrchemie-O_2-Absorbens) to absorb the O_2. If there was a difference for O_2, CO_2 or CH_4 of more than 0.003 vol.% between duplicate analyses, then a third analysis was

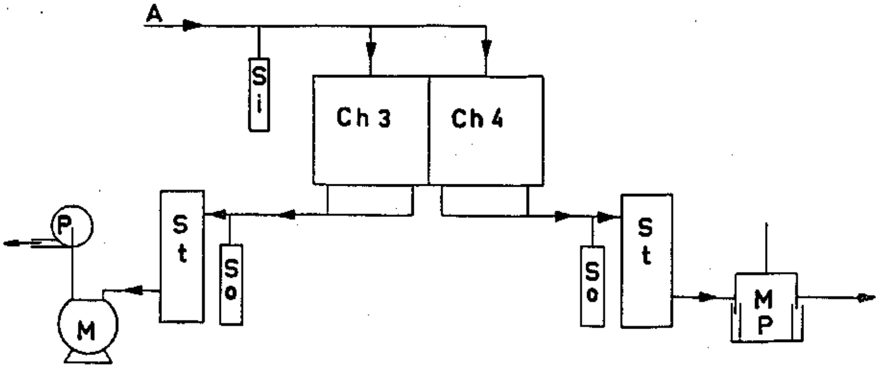


FIG. 1. The respiration equipment. A = inlet outdoor air; Ch = respiration chamber; S_i and S_o = sampling devices of in- and outgoing air; St = saturation tower; M = wet gasmeter; P = centrifugal pump; Mp = mercury pump

made. Indoor air was found to contain 0.01–0.03 vol. % more CO_2 and proportionately less O_2 than outdoor air. As so little air leaked into the chamber its composition was assumed to be that found for the air outside.

The standard volume (0°C., 760 mm. Hg) of the ingoing air (V_i, litres) was computed with the aid of its N_2-content (n_i, vol. %), the standard volumes of N_2 that went into and out of the chamber (N_i and N_o, litres) and the increase of the standard volume of the N_2 in the chamber during the experiment (ΔN_{Ch}, litres), in the following way:

$$N_i = N_o + \Delta N_{Ch};\ V_i = \frac{100}{n_i}(N_o + \Delta N_{Ch}).$$

At the start and at the end of each respiration experiment samples of the chamber air were taken for analysis. The volume of the chambers was computed from measurements of their length, height and breadth. These values agreed within 1% of those found by another method: A known amount of CO_2 was introduced into the chamber, the resulting increase in CO_2-content of the chamber air was measured and from these figures the volume was computed. The volume of the animal, body weight (kg.) × 1 litres, was subtracted from the total volume in experiments with cows.

The volume of the outgoing air of chamber 3 was measured with a large wet gasmeter, that of chamber 4 with a mercury pump.

Samples of the outgoing gas were taken in duplicate (each in sampling tubes of 1 500 ml. capacity) in the same way as with the ingoing air. The overflow vessels of all the sampling tubes were lowered by the mercury pump. For chamber 4 there was therefore a constant ratio between the volume of the sample and the total volume of the outgoing air. The flow of the outgoing air of both chambers was very constant during the experiment, fluctuations in flow being less than 0.5% of total flow. Thus also, there was no danger of getting an inaccurate sample from chamber 3. The gas for the continuous samples was taken from the tube connecting the chamber with the saturation tower in order to avoid condensation of water vapour in the narrow copper sampling capillary (inner width 1 mm., length 8 m.). The temperature of the pyrex sampling tubes was held constant (water mantle, nearly constant temperature in room).

All air volumes were corrected to standard volumes from the average temperature, relative humidity and barometric pressure using the tables of CARPENTER (1948). The data required were computed partly from values given by an automatic resistance wire temperature recorder with dry and wet measuring units (once each hour) and a barograph (once per two hours) and partly by reading every two hours the dry and wet mercury thermometers and a mercury barometer. The relative humidity of the air in the wet gasmeter was taken as 100%, while that of the air in the mercury pump was computed from the temperature of the water in the saturation tower and the temperature of the pump, assuming 100% relative humidity at the temperature of the water inside the tower.

5.5.2. The calibration of the mercury pump

In order to determine the actual volume of air that passed the mercury pump or the gasmeter with each rotation, both meters were calibrated before and after every 2- or 3-days' experiment. The mercury pump is so constructed that the volume delivered per revolution is independent of the speed at which the pump is working. Before the inlet valve of the pump closes, there is a short period during which the piston remains stationary, thus allowing time for the air in the piston and in other parts of the pump between the outlet and the inlet valves to assume the pressure of the gas in the inlet tube. The same happens with the outlet valve just before closing and therefore the pressure of the remaining gas equilibrates with that of the gas in the outlet tube.

From this it is clear that the volume of the transferred air is not equal to the volume (P, about 30 litres) computed from the surface and the stroke of the piston. The volume P is about one and a half times as great as the (dead) volume (D, about 20 litres) which exists between the inlet and outlet valves and the piston when the latter is in the lower position. If, just before closing of the inlet valve, the pressure is $-a$ mm. H_2O and, just before closing of the outlet valve, $+b$ mm. H_2O, then the volume of the transferred air is:

$$(P + D)\left(1 + \frac{-a}{10\,000}\right) - D\left(1 + \frac{+b}{10\,000}\right) \backsim P\left(1 - \frac{5a + 2b}{30\,000}\right). \qquad (1)$$

P was determined by connecting the outlet tube of the mercury pump to an accurate, small, wet gasmeter (fig. 2).

The volume given by the gasmeter after every complete stroke of the very slowly operating pump was read. The values of a and b just before closing of the valves were very constant: $a = 0$ and $b = +2$ mm. H_2O. The air used for this calibration had been saturated with water vapour in the saturation tower and, after having passed the mercury pump, went through the small gasmeter at a pressure of about 12 mm. H_2O;

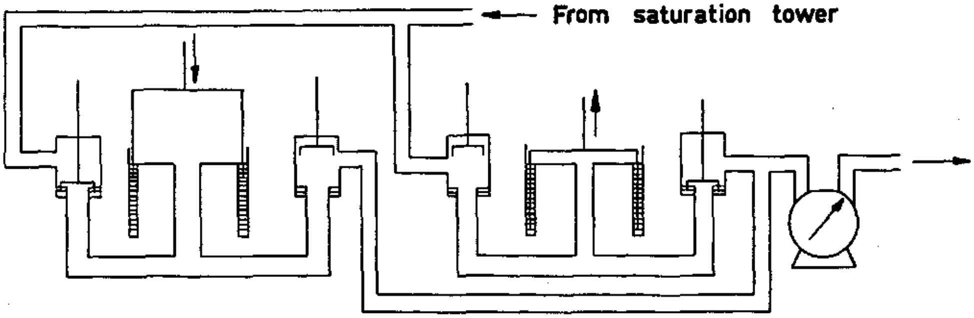


FIG. 2. Calibration of mercury pump with small wet gasmeter

only at the start and at the end of each turn the pressure was lower; therefore the volume delivered by the pump,

$$P\left(1 - \frac{5a + 2b}{30\,000}\right) = 0.999\,9\,P,$$

was compressed to

$$0.998\,8 \times 0.999\,9\,P = 0.998\,7\,P.$$

Appropiate corrections were applied when the temperature of the small gasmeter differed from that of the mercury pump or/and that of the saturation tower.

The small wet gasmeter was calibrated at the same velocity as was used for it during the calibration of the mercury pump: in 6 minutes about 30 litres of wet air were passed through the wet gasmeter by transferring air from a 50 litre carboy by displacement with a weighed volume of water (V_w), fig. 3. The temperature of the water in the carboy had been made equal to that of the gasmeter. The pressure of the air at the start and at the end was + 2 mm. H_2O. Therefore each time

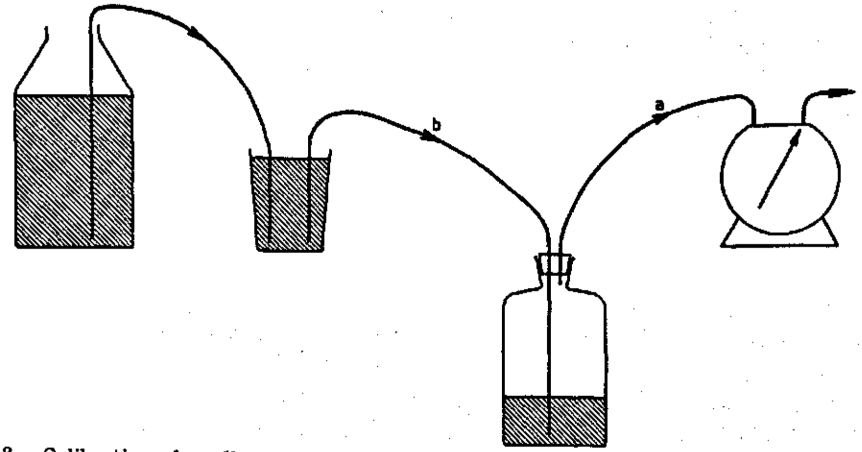


FIG. 3. Calibration of small wet gasmeter

$$\left(1+\frac{2}{10\,000}\right) V_w \text{ litres of air}$$

passed out of the carboy. However, during the passage of the air through the gasmeter the pressure was increased up to 12 mm. H_2O. Thus, the gas was measured in a compressed state, as V_g gasmeter units, it was, while entering the gasmeter, only:

$$\left(1-\frac{12}{10\,000}\right)\left(1+\frac{2}{10\,000}\right) V_w = 0.999\,0\ V_w.$$

Thus, one unit of the gasmeter was actually

$$\frac{0.999\,0\ V_w}{V_g} = f_H \text{ litres.}$$

When therefore during the calibration of one piston of the mercury pump with this gasmeter M units were measured, then actually f_HM litres of compressed saturated air passed through the meter. Thus, after applying suitable corrections for temperature differences and incomplete saturation (M became M_c), f_HM_c litres were transferred by the piston, a volume equal to 0.998 7 P, thus

$$0.998\,7\ P = f_HM_c \text{ and therefore } P = 1.001\,3\ f_HM_c \text{ litres.}$$

During a respiration trial in equation (1) $b = 0$, therefore the volume that went through the mercury pump was per rotation:

$$P\left(1-\frac{5a}{30\,000}\right) = 1.001\,3\left(1-\frac{5a}{30\,000}\right) f_HM_c \text{ litres.}$$

The small wet gasmeter was also checked in a different manner. After one calibration as described above had been finished, tube a was connected to the outlet of the meter and tube b was put into another weighed pail about 1 m. below the carboy (fig. 4).

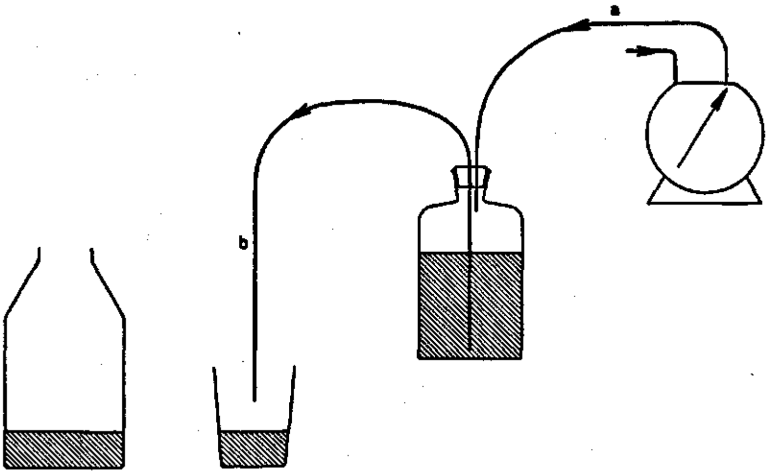


FIG. 4. Calibration of small wet gasmeter

When the plug was taken out of b the water flowed out of the carboy and, only partially saturated, air entered the gasmeter. It was assumed that the air was fully saturated by the water in the gasmeter before the measuring compartments of the meter were emptied. The pressure of the air in the carboy at the start and at the end of the calibration was -2 mm. H_2O ,that of the gas at the moment of measuring by the meter 0 mm. H_2O. If the volume of the weighed water was V_w, then

$$\left(1 - \frac{2}{10\,000}\right) V_w \text{ litres of air}$$

passed into the carboy and the same volume without compression through the gasmeter which measured V_g units. Therefore one unit of the meter was actually

$$\left(1 - \frac{2}{10\,000}\right) \frac{V_w}{V_g} = f_T \text{ litres.}$$

The factors f_H and f_T never differed by more than 0.2%.
Similar values for P were found when the mercury pump instead of forcing air through the gasmeter sucked it through.
The average of two determinations of f_H and two of f_T, determined in the order f_H-f_T-f_H-f_T, and taking 30 min. to complete, and the average of six determinations of M for each piston, taking some 50 min., were used in the calculation of P_{1+2}, *i.e.*, the volume of one stroke of piston 1 plus that of one stroke of piston 2.
SCHNEIDER (1957) calibrated his mercury pump in another way. He closed the inlet and outlet valves and made a connection between the piston and a 50 litre carboy, the latter filled with water at a temperature equal to that of the pump. Then he moved the piston from the higher to the lower position and maintained atmosferic pressure inside the piston by emptying the carboy. The volume of the water that passed out of the carboy was equal to P, provided only that there was the same pressure in the piston at the end as at the start and that the air in the piston was saturated.
Recognizing the simplicity of SCHNEIDER's technique we tried this method of calibrating the mercury pump. Two modifications were introduced. The valves were not closed thus establishing conditions more comparable to those existing when the pump is in actual use. In addition by the use of a nearly empty carboy it was possible to calibrate an upward moving piston (fig. 5). The results of all measurements differed less than 0.2% from those of our calibration method with the small wet gasmeter. For routine purposes we prefer the gasmeter method which takes 80 min. and is fairly safe. It requires two men, one for 30 min. and the other for 80 min. Our modification of SCHNEIDER's method took 80 min., was a little risky as the mercury pump might easily get in disorder by a wrong act. It required two men for the whole period.

5.5.3. The calibration of the large gasmeter

The calibration of the large wet gasmeter of chamber 3 was performed with the aid of the mercury pump. A wet gasmeter should always be calibrated at the velocity at

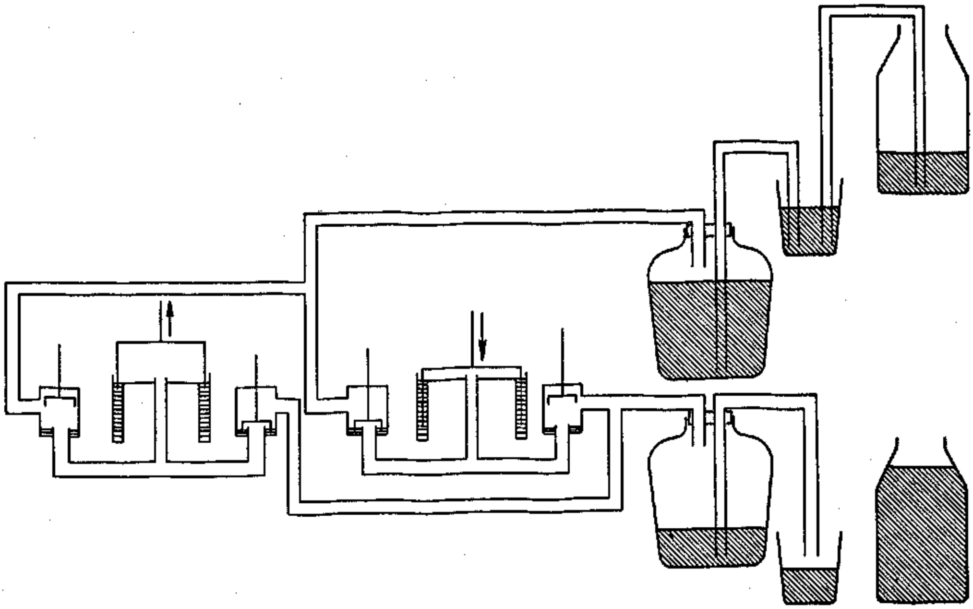


FIG. 5. Calibration of mercury pump according to SCHNEIDER, with modifications

which it is used, otherwise the water level in the measuring drum is not at the same height in both cases and this results in a different volume being indicated. It was possible to connect the outlet of the gasmeter with the inlet of the mercury pump by filling or emptying the U-shaped bends of the connecting pipes (fig. 6). During a calibration test of the large gasmeter the mercury pump was used to suck air through it. To avoid the undesirable pulsating suction of the mercury pump the pump was left connected to chamber 4 which acted as a buffer.

During one calibration the mercury pump made 200 rotations and transferred, since $b = 0$,

$$200\,P_{1+2}\left(1 - \frac{5a + 2b}{30\,000}\right) \text{ or } 200\,P_{1+2}\left(1 - \frac{5a}{30\,000}\right) \text{ litres.}$$

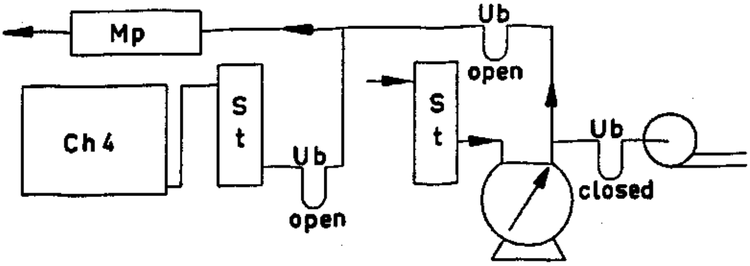


FIG. 6. Calibration of the big wet gasmeter. Mp = mercury pump; St = saturation tower; Ch 4 = chamber 4; Ub = U-shaped bend

While a small part of the measured gas volume possibly arose from leakage, rise in temperature and/or humidity, or fall in barometric pressure, the principal part understandably was drawn from the wet gasmeter. Admittedly it was measured at a lower temperature than existed at the moment the gas passed through the pump.
To obtain an estimation of the leakage, in a separate test, the chamber was totally closed and some air was sucked out of it resulting in a negative pressure within. The increase in pressure after 30 min. corrected for changes in temperature, humidity and/or barometric pressure, was a measure of the volume of air that leaked in, a corrected increase of 10 mm. H_2O indicating a gain of, if V_{Ch} = volume of the chamber,

$$\frac{10}{10\,000} V_{Ch} = \frac{10}{10\,000} 10\,800 \backsim 11 \text{ litres.}$$

The amount of gas that left the chamber because of rise in temperature and/or humidity or fall in barometric pressure could easily be computed from the changes in wet- and dry-bulb temperature and in barometric pressure that occurred from the beginning to the end of the calibration.
Until experiment R 6 we did not make allowance for the humidity of the air in the chamber during calibration of the wet gasmeter and during the leakage test. The error introduced by this omission was small as the humidity changed little during these tests.

5.5.4. Computation of respiratory exchange and heat expenditure

We now put:

V_i = standard volume of air entering the chamber, litres,
V_o = standard volume of air leaving the chamber, litres,
c_i, m_i, o_i = vol. % of CO_2, CH_4 and O_2 in V_i,
c_o, m_o, o_o = vol. % of CO_2, CH_4 and O_2 in V_o,
$\Delta C_{Ch}, \Delta M_{Ch}, \Delta O_{Ch}$ = increase of standard volume of CO_2, CH_4 and O_2 in the chamber during one experiment, litres.

When no use had been made of the airlocks of the chamber, the production of CO_2 was computed as:

$$\frac{V_o c_o}{100} - \frac{V_i c_i}{100} + \Delta C_{Ch},$$

that of CH_4 as:

$$\frac{V_o m_o}{100} - \frac{V_i m_i}{100} + \Delta M_{Ch} \text{ (with } m_i = 0)$$

and the consumption of O_2 as:

$$-\left(\frac{V_o o_o}{100} - \frac{V_i o_i}{100} + \Delta O_{Ch}\right).$$

ΔC_{Ch}, ΔM_{Ch} and ΔO_{Ch} were computed from the standard volume and the composition of the air in the chamber at the end and at the start of a respiration experiment. The CH_4-content of the chamber air was not determined with the aid of gas analysis; it was computed from the CO_2-content at the mentioned moments and from the ratio CH_4/CO_2 of the 24-hours-sample.

No allowance was made for using the large airlock (500 litres). The composition of the air in the airlock after one visit to the chamber increased by 0.2–0.5% CO_2 and therefore a volume of about 2 litres CO_2 was not recorded. Since however a man expired CO_2 at the rate of about 0.3 litres per minute while inside the chamber the loss of CO_2 to the airlock was compensated for. The chamber was entered very seldom more than three times per 24 h. The milkhand was inside for about 10 minutes and the other visits lasted on an average 3 minutes. Nevertheless, the number and lengths of the visits and the time at which they occurred were noted.

Feed was given via the small airlock twice a day, if possible the whole half-day's ration at once. If this were not possible the concentrates were given first and then the hay. Each time the airlock was used all the chamber air it held was replaced with room air. As the air in this airlock measured about 210 litres (standard conditions), the loss of CO_2 and the gain of O_2 was about 2 litres each time. A correction was applied for this loss and gain.

Finally the CO_2-production measured in the respiration experiment was augmented with the free and bound CO_2 of the urine to obtain total CO_2-production.

From the O_2-consumption (litres), the (total) CO_2- and the CH_4-production (litres) and the amount of nitrogen in the urine ($E = 6.25 \times$ g.N) the heat expenditure (H, kcal.) was computed with the formula of BROUWER (1958 b):

$$H = 3.869\, O_2 + 1.195\, CO_2 - 0.227\, E - 0.516\, CH_4.$$

5.5.5. Test experiments

Many test experiments were done to check for sources of errors. Known volumes of CO_2, O_2, N_2 and/or CH_4 were produced in or led into the chamber and it was tried to recover these gases in the same way as was done with the respiratory gases of animals. In the first instance known weights of ethyl alcohol were passed into the chamber at a constant rate of 3 g. per min. and burned on a dish of 10 cm. diameter. Prior to the commencement of the test and in order to raise the CO_2-content of the chamber air, alcohol from another bottle was passed into the chamber at the same rate for half an hour and allowed to burn. Ventilation of the chamber started 10 min. before the beginning of the test. The alcohol burned with a flame of about 40 cm. and produced about as much CO_2 as a non-lactating cow. The only difference between this experiment and an animal experiment was the length of the trial (6 instead of 24 h.), the absence of CH_4 and a respiratory quotient of 0.67 instead of about 1.0. The short duration of the test necessitated a larger sampling percentage as the sampling tubes had to be threequarters filled to obtain sufficient gas sample for analysis. As the composition of the outgoing air during an animal experiment was rather constant, it

never varied more than 10% in 15 min. as shown by physical gas analysis, this higher sampling rate will not have biased the result very much.
The water content of the alcohol was computed from its specific gravity. The oxidation of 1 g. of waterfree alcohol when complete requires 1.460 litres O_2 and produces 0.973 litres CO_2.

The results of all tests were:

Chamber 3			Chamber 4		
Date	CO_2 recovered	O_2 recovered	Date	CO_2 recovered	O_2 recovered
28-2-'56	99.6%	99.7%	2-3-'56	96.7%	97.6%
6-3-'56	100.2%	101.6%	6-3-'56	98.7%	99.2%
3-7-'56	97.6%	99.1%	3-7-'56	96.5%	99.0%
22-8-'56	96.1%	98.0%	23-8-'56	96.2%	97.5%
25-9-'56	98.0%	98.4%	25-9-'56	97.0%	97.7%
12-3-'57	96.8%	98.3%	12-3-'57	96.6%	98.4%
17-4-'57	98.3%	98.6%	12-4-'57	97.7%	98.7%
3-5-'57	98.2%	98.9%	17-4-'57	97.6%	97.8%
			3-5-'57	97.5%	98.7%
			29-5-'57	98.6%	99.7%
			4-6-'57	98.7%	99.5%

The recoveries were too low, though fairly constant. Some of the tests were done with different types of combustion-dishes or with twice destilled alcohol, without giving better results. No combustible gases could be found in the outgoing air.
Another test method was tried. CO_2 from a high-pressure cylinder was first saturated with water, then measured with a calibrated wet gasmeter and passed into the chamber. A continuous sample of the gas passing this gasmeter was collected and analysed for CO_2. The temperature of the meter and the barometric pressure were recorded, thus it was possible to correct the volume of the introduced CO_2 to standard conditions. The results of all tests were:

Date	10/11-1-'57	4-6-'57	18-7-'57
CO_2 recovered, chamber 3	99.3%	99.7%	
CO_2 recovered, chamber 4	98.8%		99.9%

These results further strengthened our doubts about the reliability of the alcohol method. SCHIEMANN (1958) experienced the same difficulties with alcohol, in the respiration chambers for large animals and also, though to a lesser degree, in those for rabbits. SCHNEIDER (1957, p. 52) rejected alcohol tests as unreliable and used methods based on the introduction of CO_2 and O_2. MØLLGAARD and ANDERSEN (1917, p. 42) introduced CO_2 or burned H_2. BENEDICT *et al.* (1934, p. 674) used only CO_2. ARMSBY and FORBES did use alcohol tests and claimed good recoveries (BRAMAN, 1933, p. 35) as did KLEIBER (1945, p. 56).

In the computation of the heat expenditure the O_2-volume is multiplied by 3.869 and the CO_2-volume by only 1.195. It was therefore of great importance to have a reliable O_2-test. Introducing O_2 out of an O_2-cylinder was possible, but it gave a high instead of the low O_2-content in the outgoing air found in animal experiments. A few experiments were carried out. Since our gas analysis apparatus was not devised for analysing samples with less than 78.5% N_2 taken from the outgoing air in such tests, some accurately measured N_2, stored in the combustion vessel, had to be added to the sample in the apparatus after most of the O_2 had been absorbed (VAN ES, 1958). The O_2 introduced into the chamber was saturated with water and measured with a 20 litre wet gasmeter. At the same time CO_2 was led in. A sample of both gases was taken continuously; the samples were analysed for CO_2 and O_2. The results of these tests were:

Date	21-2-'58	11-3-'58
CO_2 recovered, chamber 3	99.4%	
O_2 recovered, chamber 3	103.4%	
CO_2 recovered, chamber 4		99.1%
O_2 recovered, chamber 4		100.1%

The gas analysis was rather difficult. Theoretically it was incorrect in a test trial to use the gas analysis apparatus, for analysing gases with a low N_2-content, in a manner that differed from the manner used in experiments with animals. Therefore we chose another method: N_2 and CO_2 were led into the chamber in proportion 4:1 at a rate of 12 and 3 litres per min. respectively, the ventilation rate being as usual 300 litres per min. There thus flowed into the chamber per min. 12 litres N_2 plus 3 litres CO_2 out of the cylinders and (300–15) 0.79 $\backsim$ 225 litres N_2, (300–15) 0.21 $\backsim$ 60 litres O_2 and (300–15) 0.000 4 $\backsim$ 0.1 litre CO_2 with the ingoing air. Thus, the outgoing air had a composition of:

$$\frac{225+12}{300}\,100 = 79\%\ N_2;\ \frac{60}{300}\,100 = 20\%\ O_2 \text{ and } \frac{3+0.1}{300}\,100 \backsim 1\%\ CO_2.$$

Except for the CH_4, the composition was equal to that of the outgoing air in an experiment with an animal.

If C_a, N_a = the standard volumes of the CO_2 and N_2 introduced into the chamber from the gas cylinders, litres,
$V_a = C_a + N_a$,
V_i, O_i, N_i = the standard volumes of outdoor air, outdoor O_2 and outdoor N_2 entering the chamber, litres,
V_o, O_o, N_o = the standard volumes of air, O_2 and N_2 leaving the chamber, litres,
$\Delta C_{Ch}, \Delta O_{Ch}, \Delta N_{Ch}$ = the increases in the standard volumes of CO_2, O_2 and N_2 in the chamber during an experiment, litres,
c_i, o_i, n_i = vol. % of CO_2, O_2 and N_2 in V_i,
c_o, o_o, n_o = vol. % of CO_2, O_2 and N_2 in V_o,

then $\frac{V_o c_o}{100} + \Delta\, C_{Ch} - \frac{V_i c_i}{100}$ must be equal to C_a and the recovery percentage is given by the ratio $\frac{V_o c_o + 100\, \Delta\, C_{Ch} - V_i c_i}{100\, C_a}$ multiplied by 100.

For the computation of the O_2-recovery we assume N_a to be outdoor air from which all the O_2 (and CO_2), *i.e.*, $\frac{o_i}{n_i} N_a$ (and $\frac{c_i}{n_i} N_a$), has been absorbed. This 'absorbed' amount of O_2 (equal to $\frac{o_i}{n_i} N_a$) can be computed with the same accuracy as that of N_a (error about $3^0/_{00}$) since the accuracy of values for o_i and n_i is extremely high (error about $0.2^0/_{00}$). Next we compute the O_2-consumption in the same manner as in an experiment with an animal:

O_2-consumption $= O_i - O_o - \Delta\, O_{Ch} = \frac{o_i}{n_i}\,(N_o + \Delta\, N_{Ch}) - O_o - \Delta\, O_{Ch}$. For a recovery of 100% this figure must be equal to $\frac{o_i}{n_i} N_a$.

Small corrections had to be made as the cylinders did not contain pure N_2 or CO_2. The results of the tests were:

Date	6-2-'58	25-3-'58	26-3-'58	20-3-'59
CO_2 recovered, chamber 3			100.4%	
O_2 recovered, chamber 3			101.1%	
CO_2 recovered, chamber 4	99.2%	100.0%		100.9%
O_2 recovered, chamber 4	100.2%	99.7%		101.2%

In the method described above for testing the chamber recoveries, the difficulty of achieving complete saturation of the introduced gases, and of calibrating the wet gasmeter used to measure their volumes have to be remembered. Undoubtedly a further improvement would have been the weighing of the gas introduced into the chamber with a balance sensitive to 2 g. and towards the conclusion of the experiments such a balance became available. Nevertheless using a less sensitive balance (accurate to 10 g.) the difference between the weighed and the measured amount of gas was never more than 1% of the total amount.

A few tests were done introducing CH_4 from a high pressure cylinder. In experiments with ruminants in an open system respiration chamber the error of the CH_4-analysis is fairly considerable, because the CH_4-content of the outgoing air is low (0.05–0.10%). This content was also low in the CH_4-tests. They were mainly tests of the gas analysis, the error of the measurement of the volume of the outgoing air and that of the sampling of in- and outgoing air being much smaller.

The results of the two experiments where CH_4 of the cylinder was introduced into the chamber were satisfactory: on 10-9-'58 with chamber 3 98.8% was recovered and on 3-12-'58 with chamber 4 103.3% was recovered.

In the analysis of the gas the decrease of the volume due to the combustion of combustible gases is first measured and afterwards the resulting CO_2 is absorbed, giving a further decrease in volume. Combustions of outdoor air or already 'burned' air also gave slight decreases in volume. This blank value was therefore determined when analysing gas samples and subtracted from the total decrease. Even with this correction the ratio between the first and the second decrease was often slightly higher than that for CH_4 (theoretically 2.0), perhaps indicating the presence of H_2. Samples of rumen gas of a fistulated cow were analysed: the ratio was distinctly higher than 2 only within an hour after feeding beets or dried sugar beet pulp. The same was found after feeding dried pulp to cows in the respiration chambers if the chambers were closed for one hour immediately after the feed had been given, to allow accumulation of combustible gas. Moreover, physical gas analysis of the outgoing air during the first hour after feeding dried sugar beet pulp indicated the presence of a gas with very high thermal conductivity, perhaps H_2. As all the measurements except the last-mentioned depended on the accuracy of the volumetric analysis of the combustible gases, it seemed desirable to test the gas analysis apparatus by analysing gas of known composition. The gas in the cylinder mentioned above held 96% CH_4; this content was determined with the gas analysis apparatus in samples containing about 5 times more CH_4 than a sample of normal air from a respiration experiment with a cow. In these samples and also in samples with a lower content of CH_4 the ratio between the first and the second decrease was close to 2.0, obviously the gas analysis apparatus functioned fairly well. Therefore, in the cases mentioned above with ratios clearly above 2.0 another combustible gas may have been present.

When one is introducing a gas or even alcohol from the outside into the respiration chamber care has to be taken to ensure complete entry of the test material into the chambers. Leaks in the entry leads have to be guarded against while several pressure cylinders had to be discarded because of leaking when connected with the needle valve. These difficulties were eventually overcome by putting the cylinders and the accurate balance *inside* the chamber. CO_2 and N_2 were first released in the nearly closed chamber in such quantities that the composition of the air in the chamber, after a ventilation of 20 min., became about 1% CO_2 and 20% O_2. During this ventilation of 20 min. the (closed) cylinders were weighed and connected with a capillary sampling tube and with a small dry gasmeter. At the end of the 20 min. the experiment was started in the normal way and the cylinders were opened from the outside by means of the rubber gloves inset in the large door of the chamber. The velocity of the gas given by the dry gasmeter could be adjusted with the needle valve in the same way. The test experiment with CO_2 and N_2 of 20-3-'59 mentioned above was performed in this manner.

5.5.6. Execution of experiments with animals

In the preliminary period the animals were familiarized with the respiration chambers. Most animals were inside from 9.00 till 16.00 h. for 4-6 days. The animals quickly became quiet because they could see each other through the window in the partitionwall. As already mentioned, respiration experiments were nearly always carried out on two successive days. The heat expenditure of the first respiration day of the experimental period was never significantly different from that determined on the second day. Furthermore, the time spent standing in the respiration chambers never differed much from that in the digestion stall (table 10).

About 2 h. before the start of the first respiration day the animals were led into the chambers. During experiment R 1 the cows were milked at 7.00 h. in the stall after the concentrates had been given, and moved to the chambers after milking. The hay was then given. The respiration experiment started at 9.00 h. As physical gas analysis showed an increase of rather variable magnitude in CO_2- and CH_4-production and in O_2-consumption in the first hours after feeding, it was thought better to delay the start of the experiment by a further hour. Therefore, from experiment R 3 onwards the animals were led into the chamber at about 8.00 h. after they had eaten the concentrates (given at 7.00 h.) and the hay (given after they had eaten the concentrates), and the experiment started at 11.00 or 11.30 h.

The system of feeding was changed after experiment R 6. We no longer gave the cows the first part of the day's ration (approximately half of it) at 7.00 h. but at 16.45 h., to make sure that they did get during the respiration day (11.00-11.00 h.) one full ration instead of two estimated halves of two rations.

The day before an experiment the gasmeter and the mercury pump were calibrated. The ventilation of the chambers was started about 30 min. after the animals had entered the chambers. In the half hour without ventilation the CO_2-content rose to a value maintained during the remainder of the experiment. During the hours previous to the start of the experiment (hour *U*, *e.g.* 11.00 h.) all the necessary equipment was put into operation (mercury pump, centrifugal pump, water pumps of the saturation towers; air conditioners of the chambers, recorders and physical gas analysis apparatus). At *U*-30 (*i.e.*, 30 min. prior to hour *U*) the gas sampling system was started, use was made, however, of the sampling tubes of the second day with the object of filling all the sampling capillaries with gas entering resp. leaving the respiration chamber.

At hour *U* the counter of the gasmeter was read, a sample of the air inside chamber 3 was taken, the dry and wet thermometers of chamber 3 were read and the drainpipe for condensed water from the air conditioner was connected to an empty bottle. At $U+1$ the same was done with the chamber 4 with the exception that the counter of the mercury pump was read instead of that of the gasmeter. Two minutes later the samples of the air leaving the respiration chambers taken at hour *U* and $U+1$, flowing through the sampling capillaries, reached the sampling tubes. At that moment the sampling tubes of the first day were opened and those of the second day closed. From *U* until $U+4$ records were taken of the temperatures of the pump, of the gasmeter and of the ingoing air, of the barometric pressure and of the negative pressure of the outgoing air. During an experiment all readings were taken on the hour at two-hourly (at night three-hourly) intervals.

At the end of the first respiration day which frequently coincided with the time of the start of the second day, the same actions were repeated as at the start; the sampling tubes used for the first day were closed and those for the second opened.

6. RESULTS OF THE EXPERIMENTS

In this study on the maintenance metabolism of individual cows much time was devoted to the testing of equipment. The experience gained during these trials and the accuracy of the various data obtained will be mentioned first. Evidence obtained as to *between-animal* variation in maintenance requirement for metabolizable energy will then be referred to.
The main results of all experiments are given in the tables 10, 11, 12 (appendix), 21, 22, 24. All experimental data are given in a separate paper (BROUWER *et al.*, in prep.).

6.1. SOME REMARKS ON THE DIGESTION EXPERIMENTS

No difficulties were experienced with either the weighing or the sampling of the feeds. With hay the method of sampling sieved fractions as practised in this laboratory for many years worked very well. The differences in composition between the two separately treated samples A and B were small (table 13, a, b).
The standard deviations of the results computed from the differences between sample A and sample B are nearly all due to variation of mere chemical analysis. The differences between the samples of the whole hay (R 7, subperiod III) are larger; here samples A and B were taken separately during the weighing of the rations; the hay of these larger samples was cut to pieces of 1–2 cm. length before two subsamples, A_1,

TABLE 13a. Difference (Δ) between amounts of individual components in hay samples A and B

Experiment	Δ dm (%)	on a dry matter basis						Δ C (%)	Δ cal (cal)
		Δ cp (%)	Δ c fat (%)	Δ c fibre (%)	Δ N-fe (%)	Δ ash (%)	Δ tr p (%)		
R 1 coarse fraction	−0.34	0.40	−0.02	0.33	−0.58	−0.13	0.06	0.08	0
middle fraction	−0.26	0.07	0.05	0.14	−0.27	0.01	−0.13		
fine fraction	0.17	0.06	0.29	0.89	−0.70	−0.54	−0.06		
R 2	−0.03	−0.14	−0.16	0.20	−0.13	0.23	0.06	−0.06	−17
R 3	0.02	−0.07	0.02	0.36	−0.32	0.01	0.03	0.04	4
R 4	0.41	0.07	−0.05	−0.22	0.10	0.10	0.08	−0.01	−7
R 5	0.12	−0.07	−0.04	0.49	−0.23	−0.15	−0.15	0.07	−3
R 6	−0.11	0.14	−0.04	0.12	−0.25	0.03	0.07	−0.17	−2
Standard deviation	0.16	0.12	0.09	0.30	0.27	0.16	0.06	0.06	6
Average composition	89	12	2.6	30	46	9	10	45	4300
R 7	0.45	−0.08	0.04	−0.13	−0.03	0.20	−0.03	−0.19	−13
R 9	−0.05	−0.04	0.02	0.23	−0.06	−0.15	−0.02	0.04	−4
R 10	−0.03	0.01	0.07	−0.19	0.10	0.01	−0.01	−0.13	−12
R 12	0.01	−0.11	−0.39	−0.01	−0.18	0.69	−0.05	−0.34	−40
R 13	−0.03	−0.03	0.17	0.00	−0.22	0.08	0.09	0.06	−5
R 14	0.11	−0.05	0.21	−0.23	0.06	0.01	−0.09	0.01	−3
Standard deviation	0.14	0.04	0.14	0.12	0.09	0.21	0.04	0.12	13
Average composition	90	10	2.7	29	50	9	8	45	4300

Table 13b. Composition of the unchopped hay used in R 7 III and the grass used in R 8

		on a dry matter basis							
	dm (%)	cp (%)	c fat (%)	c fibre (%)	N-fe (%)	ash (%)	tr p (%)	C (%)	cal/g
Hay									
sample A 1	85.35	10.27	2.32	28.28	50.98	8.15	7.56	44.80	4344
,, A 2	85.70	9.79	2.19	27.22	48.96	11.84	7.27	43.43	4192
,, B 1	85.35	10.26	2.49	27.73	51.60	7.92	7.71	44.94	4342
,, B 2	85.61	10.03	2.32	28.00	51.43	8.22	7.23	45.01	4330
$\frac{A\,1 + A\,2}{2} - \frac{B\,1 + B\,2}{2}$	0.04	−0.11	−0.14	−0.11	−1.55	1.93	−0.05	−0.86	−68
Grass									
sample A (fr.)	12.03	18.84				16.42		42.47	4208
,, B (fr.)	11.97	19.04				14.56		42.99	4269
,, C (dr.)	11.93	18.87				13.75		43.43	4318
,, D (dr.)	11.98	18.88				13.87		43.24	4311
$\frac{A + B}{2} - \frac{C + D}{2}$	0.04	0.06				1.68		−0.61	−76

A_2 and B_1, B_2 respectively, were taken from each. The differences in composition of samples A_1, B_1 and B_2 are not so very much higher than those found for the fractionated hay already mentioned; differences between sample A_2 and the other three are large as a result of the exceedingly high ash content in sample A_2.

The sampling of the fresh grass of experiment R 8 was a difficult matter. During the weighing of the rations two samples (A and B) were taken separately and each put into one of two composite sample bottles; two more samples were weighed, dried at 80° C., weighed after cooling and put into two other sampling bottles (dried, C and D). At the end of the experimental period the composite samples A and B were dried at 60–70° C. and ground after cooling and the samples C and D were ground without additional drying. Here again three samples are much alike in their chemical composition, the fourth has a high ash content. Even with the method of fractionating the hay very high differences in the ash content of the samples A and B occurred occasionally (table 13a). An unequal distribution of soil particles in the material and sedimentation during sampling may have been important in this respect.

The collecting, weighing and sampling of milk, of urine and of faeces gave no great trouble.

Although the digestion stall was not air conditioned its average temperature in summer could be kept below 22° C. and in winter by central heating above 12° C. The animals were therefore always within the zone of thermoneutrality.

6.2. SOME REMARKS ON THE RESPIRATION EXPERIMENTS

The respiration chambers answered their purpose. Standing and lying behaviour of cows in the chambers and in the digestion stall showed little difference (table 10). As mentioned the cows were first accustomed to the chamber. A cow entering the chamber for the first time, normally did not lie down during the first six hours, even when there was a companion in the other chamber. Most cows did lie down during their second stay of 6 hours, all did so during the third stay. The result was that afterwards in the 48-hours-respiration trial, the animals were standing during the first day only about 10% longer than during the second day. Thus, the difference was very small.

The number of calibrations of the mercury pump and of the gasmeter was rather high. Each calibration lasted 3 hours and required 2 persons. From the point of view of accuracy less frequent calibrating, *e.g.* once before or after instead of before and after every two respiration days, seems possible.

The calibration values found with the mercury pump did not vary much, all values with the exception of a few where oil had covered the in- and outlet valves, lying between 60.90 and 61.30 litres.

The calibration value of the wet gasmeter became gradually higher in the course of every respiration day, *e.g.*, it rose from 0.994 to 0.996 or from 1.000 to 1.002. It was evident that the level of water in the gasmeter fell as the experiment proceeded, the loss being induced by the slight temperature rise that occurred in the air after leaving the saturation towers and before leaving the wet gasmeter. All values were between 0.994 and 1.012; after filling up to the mark we always had a value between 0.995 and 1.000; water was added as soon as the value rose above 1.010. Although the scheme of calibrations practised was rather laborious it had the advantage that the whole equipment was tested, thus leaving little risk of error during the experiment.

The gas sample of the outgoing air was taken from the tube connecting the chamber with the saturation tower (fig. 1). Theoretically it is better to take the sample as near to the gasmeter or mercury pump as possible. However the passage of saturated air through the capillary sampling tubes may lead to obstruction of the gasflow by waterdroplets when the temperature of the sampling capillary falls below that of the meter or the pump. Since the saturation towers with adjacent pipes did not leak, this was controlled during every calibration, there was no objection against taking the sample as it was done.

6.3. POSSIBLE SIMPLIFICATION OF GAS ANALYSIS

The analysing of the in- and outgoing air and of the samples of air from the chamber at the start and at the end of each respiration day was timeconsuming. One technician needed 8 hours to analyse all samples of one respiration day with two animals. The analysis of the CH_4-content took as much time as that of the O_2- and CO_2-content. In the computation of heat expenditure the importance of the production of CH_4 is far less than that of the consumption of O_2 and of the production of CO_2 (BROUWER, 1958 b). In the energy balance the energy of the CH_4 produced is only about 8% of the gross energy of the feed. Measurement of the content of CH_4 of the outgoing air with an instrument based on thermal conductivity or absorption of infrared light would very much simplify the gas analysis. The analysis with these instruments is rapid and for CH_4 sufficiently accurate.

6.4. POSSIBLE SIMPLIFICATION OF THE COMPUTATION OF O_2-CONSUMPTION AND CO_2-PRODUCTION

The computation of the O_2-consumption and CO_2- and CH_4-production from the various data was rather lengthy, especially due to the need to correct for differences in composition, temperature and pressure of the air in the chamber at the start and at the end of each respiration day.

We put:

$W(O_2)$, $W(CO_2)$, $W(N_2)$ = volumes of O_2, CO_2, N_2 pumped out of the chamber (litres, N.T.P.: 760 mm. Hg and 0° C.),

$V(O_2)$, $V(CO_2)$, $V(N_2)$ = volumes of O_2, CO_2, N_2 in chamber at the start (litres, N.T.P.),

V = volume of the respiration chamber (litres),

o, c, n, o_i, c_i, n_i = vol. % of O_2, CO_2, N_2 in dry chamber air and in dry ingoing air respectively,

t, B, p = temperature (°C.), barometric pressure (mm. Hg) and pressure of water vapour (mm. Hg) respectively of chamber at the start,

$\Delta V(O_2)$, Δo, Δt etc. = the increase of $V(O_2)$, o, t etc. during the experiment.

Thus:

$$V(O_2) = V \cdot \frac{273}{273+t} \cdot \frac{B-p}{760} \cdot \frac{o}{100}\text{; let } V \cdot \frac{273}{273+t} \cdot \frac{B-p}{760} \cdot \frac{1}{100} \text{ be } F,$$

then $V(O_2) = Fo$ and also $V(CO_2) = Fc$, $V(N_2) = Fn$.

If at the end of the experiment the chamber contained the same amounts of O_2, CO_2 and N_2 as at the start, then the O_2-consumption and the CO_2-production of the animal in the chamber could be computed - only the volume of the air leaving the chamber was measured, not the volume of the ingoing air- from:

$$\frac{o_i}{n_i} \cdot W(N_2) - W(O_2) \text{ and} \qquad (1)$$

$$-\left\{\frac{c_i}{n_i} \cdot W(N_2) - W(CO_2)\right\} \qquad (2)$$

respectively.

Since the amounts of O_2, CO_2 and N_2 in the chamber at the end are not the same as at the start, a correction has to be applied to (1) and (2). This correction may be calculated in the following way. We assume that the volume of N_2 in the chamber increased by $\Delta V(N_2) = \Delta(Fn)$. The volume of O_2 which entered the chamber together with $\Delta(Fn)$ was $\frac{o_i}{n_i}\Delta(Fn)$. However, the actual increase of the volume of O_2 in the chamber was $\Delta V(O_2) = \Delta(Fo)$.

The difference

$$\frac{o_i}{n_i}\Delta(Fn) - \Delta(Fo) \qquad (3)$$

has evidently been used by the animal in addition to volume (1) and, therefore, the correction ($\Delta\, O_2$) mentioned above is equal to (3).

Now

$$\Delta\, O_2 = \frac{o_i}{n_i}\,\Delta\,(Fn) - \Delta\,(Fo) = \frac{o_i}{n_i}\,F\,\Delta\,n + \frac{o_i}{n_i}\,n\,\Delta\,F - F\,\Delta\,o - o\,\Delta\,F$$

or

$$\Delta\, O_2 = \left(\frac{o_i}{n_i}\,n - o\right)\Delta\,F + F\left(\frac{o_i}{n_i}\,\Delta\,n - \Delta\,o\right). \qquad (4)$$

Differentiation of F gives:

$$\Delta\,F = -\,V\,\frac{273\,(B-p)}{(273+t)^2\,76\,000}\,\Delta\,t + V\,\frac{273}{(273+t)\,76\,000}\,\Delta\,(B-p)$$

or

$$\Delta\,F = -\,F\,\frac{\Delta\,t}{273+t} + F\,\frac{\Delta\,(B-p)}{B-p}. \qquad (5)$$

Substitution of (5) in (4) gives:

$$\Delta\,O_2 = F\left(\frac{o_i}{n_i}\,\Delta\,n - \Delta\,o\right) + F\left(\frac{o_i}{n_i}\,n - o\right)\left(-\,\frac{\Delta\,t}{273+t} + \frac{\Delta\,B - \Delta\,p}{B-p}\right). \qquad (6)$$

During the experiments the averages of $B, t, p, c, o, n, \frac{c_i}{n_i}$ and $\frac{o_i}{n_i}$, V and the maximal absolute values of $\Delta\,B$, $\Delta\,t$, $\Delta\,p$, $\Delta\,c$, $\Delta\,o$ and $\Delta\,n$ were:

B 760, t 15, p 10, c 1.00, o 20.00, n 79.00,
$\Delta\,B$ 10, $\Delta\,t$ 1, $\Delta\,p$ 1, $\Delta\,c$ 0.3, $\Delta\,o$ 0.3, $\Delta\,n$ 0.04 [1]),
c_i/n_i 0.000 443, o_i/n_i 0.264 8, V 10 600.

Using these values in the second term on the right-hand side of (6) this term reaches a maximum for values of $\Delta\,B = +10$, $\Delta\,t = -1$, $\Delta\,p = -1$ equal to

$$+\,1.8\,(20.92 - o) \text{ litres}$$

which, for the minimum value $o = 19.7$, is $+\,2.2$ litres. Its minimum (for $\Delta\,B = -10$, $\Delta\,t = +1$, $\Delta\,p = +1$) is -2.2 litres. Of course, other values of t and p also occurred, but the difference from the above-mentioned averages was only small. With the chambers being air conditioned t only varied between 14.5 and 15.5° C. and p between 9.5 and 10.5 mm. Hg from one balance experiment to another. Naturally the atmosferic pressure (B) fluctuated appreciably over the range 730–780 mm. Hg although within anyone day the range was quite small.

The use of these other values of t, p and B in the second term on the right-hand side of (6) does not change the figure 2.2 computed above. It has, however, some influence on the first term on the right-hand side of (6). F was, using these values of t, p and B, always between 102 and 95 or about between $99 + 4$ and $99 - 4$. From the values of o_i, n etc., given above, it may be derived that $\frac{o_i}{n_i}\,\Delta\,n - \Delta\,o$ always was between $+\,0.3$

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[1]) Only in rare cases was the value of $\Delta\,n$ higher and then always below 0.065.

and − 0.3. Therefore it is clear that using a value of 99 for F gave only a maximal error of $4 \times 0.3 = 1.2$ litre. Neglect of $F \frac{o_i}{n_i} \Delta n$ also gave only a maximal error of 1 litre as $\frac{o_i}{n_i} \Delta n$ was always below 0.01. Therefore the error made by using the formula

$$\Delta O_2 = -99 \Delta o$$

is less than $2.2 + 1.2 + 1 = 4.4$ litres, *i.e.*, less than about 0.2% of the O_2-consumption of a cow in 24 h.

ΔCO_2, the correction to be *subtracted* from (2) to compute the CO_2-production of the animal, may be found in a similar way as ΔO_2:

$$\Delta CO_2 = F\left(\frac{c_i}{n_i}\Delta n - \Delta c\right) + F\left(\frac{c_i}{n_i} n - c\right)\left(-\frac{\Delta t}{273 + t} + \frac{\Delta B - \Delta p}{B - p}\right). \quad (7)$$

In (7) the maximal ,absolute value of the second term on the right-hand side becomes

$$1.8\,(0.035 - c) \text{ litres}$$

which, for the maximum value $c = 1.3$, is 2.3 litres.

In the first term $\frac{c_i}{n_i} \Delta n - \Delta c$ was always between + 0.3 and −0.3 and $\frac{c_i}{n_i} \Delta n$ very small. Therefore the error made by using the formula

$$\Delta CO_2 = -99 \Delta c$$

is less than $2.3 + 1.2 = 3.5$ litres, *i.e.*, again less than 0.2% of the CO_2-production of a cow in 24 h.

From these formulae it is clear that there is no great need for very accurate analysis of the samples of the gas from the chamber taken at the start and at the end of each respiration day. If Δc or Δo are wrong by 0.01, then the error in the CO_2-production and O_2-consumption during 24 h. is only greater by 0.03%. Here again rapid, but somewhat less accurate gas analysis with instruments based on thermal conductivity or absorption of infrared light would lessen the analytical work without damage to the accuracy of the whole experiment.

6.5. POSSIBLE SIMPLIFICATION IN THE CARRYING OUT OF A RESPIRATION EXPERIMENT

To be able to correct the volume of the outgoing air to standard conditions the average temperature and pressure of the air in the mercury pump and the gasmeter are required. The agreement between the average values computed from the automatically-recorded data, and from those read every two hours by the technician supervising the respiration equipment, was so close that the former data were considered sufficient to rely on. From experiment R 12 onwards the barograph was used to record all the information on atmospheric pressures that was needed. The mercury barometer was read once every 6 or 8 h. as a check. Thus from a point of view of accuracy, constant supervision and the two-hourly recordings of temperature, pressure etc. is not necessary. Of course there must be somebody in the vicinity who can hear the alarm that is rung automatically when the electricity fails or a fault develops in the ventilation or air conditioning systems of the chambers.

6.6. VARIATION IN THE DAILY O_2-CONSUMPTION AND CO_2-PRODUCTION OF THE ANIMALS

The results of the test experiments mentioned in chapter 5.5.5. where alcohol was burned or CO_2, O_2 and/or N_2 were introduced into the chamber, made it clear that the accuracy of the respiration equipment was high. The coefficient of variation in the measurement of CO_2-production and O_2-consumption may be estimated to be about 1%.

From the point of view of labour it is important to know how many days during an experimental period the respiratory exchange of an animal must be measured in order to obtain accurate average values for the 24 h. period. Constancy of the animal in its daily respiratory exchange combined with an accurate respiration equipment would give the most satisfactory results.

The standard deviation (SD) and the coefficient of variation (CV) of the CO_2-production and O_2-consumption during one respiration day was computed from the differences between these data on two successive respiration days. All experiments R 1 – 14 including 41 × 2 respiration days were used:

	SD chamber 3	SD chamber 4	Average SD	Average CV	Deviation correlation
O_2	54 litres	63 litres	59 litres	1.9%	r = +0.88
CO_2	40 litres	50 litres	45 litres	1.4%	

The same was done with the results of successive respiration days of the Danish experiments already mentioned in chapter 3.2. in which the animals were dry but non-pregnant cows receiving a maintenance ration:

Experiments in which there were negative energy balances (29 x 2 resp. days)

	SD	CV	Deviation correlation
O_2	61 litres	3.2%	r = + 0.16
CO_2	31 litres	1.7%	

Experiments in which there were positive energy balances (81 × 2 resp. days)

	SD	CV	Deviation correlation
O_2	54 litres	2.4%	r = + 0.41
CO_2	43 litres	1.7%	

Clearly the daily variation in the respiratory exchange of an animal is fairly large since the CV's are higher than those due to errors of the respiration equipment itself. Compared with the Danish data our results appear very satisfactory. Perhaps the smaller variation in our experiments is related to the fact that the animal in the respiration chamber can see a companion; this was not the case in the Danish experiments. Theoretically, the results of the first day might be higher than those of the second: during the first day the animal might be unduly excited by the transfer to the respiration chamber. Nevertheless neither our results nor the Danish ones showed any such effect.

In the same way variations in respiratory exchange over a longer period can be estimated from the data obtained in respiration experiments at the beginning and end of the period. Again the same two series of experiments were used in the computation of differences in respiratory exchange after intervals of 7, 14 or 28 days. The mean values for each two-day respiration experiment were used. The resulting standard deviation of the average of two days was multiplied by $\sqrt{2}$ to reduce it to a standard deviation of one day. The Danish experiments lasted about 28 days and included 3 or 4 two-day respiration experiments. Our experiments lasted 14 days and included 2 two-day respiration experiments. Over the whole period of every experiment the ration was not changed.

	The present experiments (interval 7 days)	SD	CV	Deviation correlation
O_2	(R1,2,4,5,7,10,12,13), 15 intervals	116 litres	3.8%	
CO_2	(R1,2,4,5,7,10,12,13), 15 intervals	78 litres	2.4%	
O_2	(R4,5,7,10,12,13), 11 intervals	91 litres	3.0%	$r = +0.95$
CO_2	(R4,5,7,10,12,13), 11 intervals	68 litres	2.0%	
	Danish experiments (interval 14 days)			
O_2	(neg. energy balance), 20 intervals	68 litres	3.6%	$r = +0.55$
CO_2	(neg. energy balance), 20 intervals	42 litres	2.2%	
O_2	(pos. energy balance), 54 intervals	86 litres	3.7%	$r = +0.48$
CO_2	(pos. energy balance), 54 intervals	59 litres	2.3%	
	Danish experiments (interval 28 days)			
O_2	(neg. energy balance), 10 intervals	98 litres	5.2%	
CO_2	(neg. energy balance), 10 intervals	70 litres	3.6%	
O_2	(pos. energy balance), 26 intervals	104 litres	4.5%	
CO_2	(pos. energy balance), 26 intervals	76 litres	3.0%	

The CV's for O_2-consumption and CO_2-production computed from the data obtained just before and just after intervals of 7 or 14 days are higher, especially in our experiments, than those computed from data for successive days. The interval of 28 days gave again higher CV's. Now it might be thought that a negative energy balance might lower and a positive one might increase the body weight and in consequence alter the maintenance metabolism of the animal. Such a trend was not found: in the Danish experiments the differences in O_2-consumption and in CO_2-production between the respiration data recorded at the end of the period and those recorded at the beginning were almost as often positive as negative.

In our experiments the correlations between the deviations of the O_2-consumption and the deviations of the CO_2-production in the same experiment are high compared with those in the Danish experiments. In Møllgaard's laboratory the CO_2-content of the in- and outgoing air of the respiration chamber was not only measured volumetrically but also gravimetrically, *i.e.*, with two different methods. This may explain part of the difference.

It is thus clear that variation in respiratory exchange obviously increases with greater intervals between determinations. This suggests the presence of slow changes in metabolism. The Danish experiments show that these changes are much smaller than those mentioned by Ritzman and Benedict (1938, p. 127), but more information is needed, especially about the length of the period during which there is an increase or decrease and about the causes of these changes (rumen organisms, hormones?).

For accurate results it is necessary to reduce in each experiment the standard errors of the average figures on gaseous exchange as far as possible. This necessitates many respiration days, distributed over a long period in such a way that there are respiration experiments during the first, the middle and the last days of the period. In addition the preliminary period must be of sufficient duration to allow the animal to attain equilibrium with its diet. A length of the preliminary period of 14–20 days is thought necessary (Nehring and Schiemann, 1954, p. 206). In the Danish experiments there was a preliminary period of 4 weeks.

From the figures given by Schiemann (1958, p. 19) concerning the accuracy of the experiments of Kellner with oxen it can be seen that the CV of the amount of carbon in the CO_2 and CH_4 produced by the animal during one respiration day was about 1%. So the CV of CO_2-production will not differ much from this figure. In Kellner's experiments the experimental period lasted about two weeks and there were 3–5 one-day respiration experiments, therefore the CV of the average daily heat expenditure in the whole experimental period was about $1/\sqrt{4} = 0.5\%$.

In the experiments of Armsby and Forbes with steers, mentioned in chapter 3.2., we computed a CV for the directly measured heat expenditure of one day of about 1.5%. These investigators used an experimental period of about 12 days followed by 2 or 3 calorimeter days. Thus, the CV of the average daily heat expenditure of the whole experimental period was about $1.5/\sqrt{3} \backsim 1\%$. Perhaps the variation would have been higher if the calorimeter days had been distributed over the whole experimental period.

In the Danish experiments already mentioned (ch. 3.2.) the CV of one determination of the heat expenditure was about 3.2%, therefore the CV of the average daily heat expenditure in the experimental period was $3.2/\sqrt{7} \backsim 1.2\%$.

If Kellner and if Armsby and Forbes had used longer periods the CV's in their experiments might have been higher. However, even compared with the CV's computed from the present and from the Danish respiration data for short intervals, the CV's obtained by Kellner and by Armsby and Forbes are rather low. Like the Danish investigators and Fingerling they only used animals which had been selected for use in balance experiments.

Nevertheless it may well be that the difference in accuracy of the determination of the heat expenditure of one day between Kellner's and perhaps also Armsby's and

FORBES' experiments on the one hand, and those of MØLLGAARD and of ourselves on the other hand, may lie in the fact that the firstmentioned investigators used castrated male animals while in those carried out in Denmark and at Wageningen cows have been used.

6.7. ACCURACY OF THE DETERMINATION OF THE AVERAGE DAILY AMOUNT OF FEED EATEN BY THE ANIMALS

The accuracy of measuring the quantities of the various components of the feed eaten by the animals depends on the errors made in weighing the ration, in sampling the feed and in analysing the sample. As the hay, being the feed given in the largest amount, was sampled in duplicate, the standard deviation (SD) of the results, due to sampling and analysis, for a single sample, could be computed from the differences in composition between the two samples: $(\mathrm{SD})^2 = \frac{\Sigma (A - B)^2}{2n}$, where A and B are the results of the analysis of the two samples and n is the number of duplicates. Values for $(A - B)$ have been given in table 13a. The results were:

	dm	cp	c fat	c fibre	N-fe	ash	tr p	C	cal
SD	0.16%	0.09%	0.11%	0.24%	0.21%	0.18%	0.05%	0.10%	10 cal
SD/$\sqrt{2}$	0.11%	0.06%	0.08%	0.17%	0.15%	0.13%	0.04%	0.07%	7 cal

Since the average of the analytical results of the duplicate samples was used in further calculations, the SD had to be divided by $\sqrt{2}$. It was assumed that the SD's obtained for the components of the hay could also be applied to those supplied by the dried sugar beet pulp and by the concentrate mixture. Although with these materials only one sample was taken it is considered that less error is involved in sampling such material as compared with hay.

The SD of weighing a 2 kg. quantity with the balance used for weighing the rations was about 2 g. As there were three fractions of the hay (coarse, intermediate and fine), 3 weighings were needed for a one day's ration of hay. Therefore the SD of weighing the hay, about 6 kg., was $2\sqrt{3}$ g.; that of the pulp (about 1.7 kg.), and of the concentrate mixture (about 0.8 kg.), was 2 g. if it is assumed that the weighing error is purely accidental. This gives a SD of the weighing of the total ration of $\sqrt{12 + 4 + 4} = 4.5$ g. and, since during the experiments R 1, 2, 4, 5, 7, 10, 12 and 13 the ration averaged about 7.6 kg., the CV was 0.06%. This figure must be divided by $\sqrt{7}$ to get the error of the average amount of feed given daily in one subperiod. However as not all weighing errors were purely accidental and as some feed was lost during and after feeding we did not divide by $\sqrt{7}$.

The SD of the quantities fed of each component of the ration may approximately be computed from the average weight and the average composition of the ration and the SD's of weighing and of analysis and sampling. Let a be the weight of the ration, b and c the contents of dry matter and of crude protein in dry matter, S_a, S_b and S_c the various SD's and C_a, C_b and C_c the various CV's then the SD of the amount of protein fed is approximately:

$$a \frac{b}{100} \frac{c}{100} \sqrt{\left(\frac{S_a}{a}\right)^2 + \left(\frac{S_b}{b}\right)^2 + \left(\frac{S_c}{c}\right)^2}$$

and its CV:

$$\sqrt{C_a^2 + C_b^2 + C_c^2},$$

equal to $\sqrt{(0.06)^2 + (0.126)^2 + (0.46)^2} \backsim 0.5\%$, according to table 14.

TABLE 14. Coefficients of variation of the amounts of the components of the ration fed daily to the animals in a subperiod

			dm	cp	c fat	c fibre	N-fe	ash	tr p	C	cal
a) Weight of the ration	(g)	7 600									
b) Dry matter content of the ration	(%)		87								
c) Composition of dry matter of ration	(%)			13	2.7	25	51	8.5	11	44.6	4330 [1]
Standard deviation of a)	(g)	4.5									
,, ,, ,, b)	(%)		0.11								
,, ,, ,, c)	(%)			0.06	0.08	0.17	0.15	0.13	0.04	0.07	7
Coeff. of variation of a)	(%)	0.06									
,, ,, ,, ,, b)	(%)		0.126								
,, ,, ,, ,, c)	(%)			0.46	2.96	0.68	0.29	1.53	0.36	0.157	0.162
Coeff. of variation of amount of component fed	(%)		0.14	0.5	3.0	0.7	0.3	1.5	0.4	0.2	0.2

[1] per g. dm

Table 14 shows that the magnitude of the errors due to sampling and to analysis largely determined the accuracy of the measurements of the amounts of the components of the ration fed. In the following we shall denote variation due to errors of weighing, of sampling and of analysis as *analytical* variation (see ch. 1.4.).

SCHIEMANN (1958, p. 38) estimated the CV of the intake of carbon at 0.25 in the experiments of KELLNER a value which is in accordance with the value determined in the present experiments (0.2%).

6.8. ACCURACY OF THE DETERMINATION OF THE AVERAGE DAILY AMOUNT OF THE COMPONENTS OF THE FAECES

The coefficient of variation (CV), due to *analytical* and *physiological* variation, of the average daily quantities of the components of the faeces, *i.e.*, faecal crude protein, faecal C, etc., may be computed from the differences between the data of the two subperiods of the experiments R 1, 2, 4, 5, 7, 10, 12 and 13 in the same manner as was done with the data on composition of the two hay samples (ch. 6.7.).

However, the deviations of the average daily quantities of the first subperiod from the (unknown) true average daily quantity may be correlated with those of the daily quantities of the second subperiod immediately following the first subperiod. In this case neglecting the correlation gives a SD of the average daily quantity in a subperiod which is too low. Too high a value for the SD might be computed from the differences between the data of the subperiods of one animal of each pair of animals used in the same experiment, and those of the subperiods of the other animal, provided that both animals of each pair got the same quantity of the same ration. In this case it includes possible *between-animal* variation in digestibility.

For the present we shall neglect this correlation, although in 6.15. when dealing with differences in digestibility between animals, some comment shall be made as to the correctness or otherwise of so-doing. The various data of the digestion experiments are given in a paper of BROUWER *et al.* (in prep.). The results of the computations are given in table 15. Obviously, even if the errors made during weighing, sampling and analysing of the faeces had been somewhat higher than those made during weighing, sampling and analysing of the feed, even then these alone could not have been the cause of the high CV found. It is clear that there was *physiological* variation of considerable size.

TABLE 15. Standard deviations and coefficients of variation of the average daily amounts of faecal components (subperiod of 7 days)

	dm	cp	c fat	c fibre	N-fe	ash	tr p	C	cal
Standard deviation, all exp. (g)	35	10	4	14	13	9	6	16	166 cal
Standard deviation, all exp. except R 1, 2 (g)	34	10						15	155 cal
Coefficient of variation (%)	2	3	6	3	2	3	3	2	2

6.9. ACCURACY OF THE DETERMINATION OF THE APPARENT DIGESTIBILITY

Only in the experiments R 1–4 did the animals not eat all the feed that was given to them. The feed residues were however very small (on one occasion 1% of the daily ration, in all other cases less than 0.2%). Therefore, the SD's of the coefficients of apparent digestibility might be estimated from those of the average daily amount of feed and of the average daily amount of faeces, the latter computed in 6.7. and 6.8. respectively, (method A). Already in 6.8. it has been noted that the SD of the faeces may be too low, but here again we assume that the obtained values were correct.

If:

x_i = the average daily amount (g.) of a component of the feed in experiment i,
y_i = the average daily amount (g.) of a component of the faeces in experiment i,
d_i = the apparent digestibility (%) of a component in experiment i,
x, y, d = the average x_i, y_i and d_i in all n experiments,

$\Delta x_i, \Delta y_i, \Delta d_i$ = deviations due to *analytical* and *physiological* variation from the unknown true x_i, y_i and d_i in experiment i,

c_x, c_y, s_d = CV of x_i, CV of y_i and SD of d_i, due to *analytical* and *physiological* variation, we have:

$$d_i = 100\left(1 - \frac{y_i}{x_i}\right);\ \Delta d_i = \Delta\left\{100\left(1 - \frac{y_i}{x_i}\right)\right\} = -100\left(\frac{x_i\,\Delta y_i - y_i\,\Delta x_i}{x_i^2}\right)$$

$$\frac{1}{n}\sum_{i=1}^{n}(\Delta d_i)^2 = \frac{100^2}{n}\left\{\sum_{i=1}^{n}\frac{y_i^2}{x_i^2}\left(\frac{\Delta x_i}{x_i}\right)^2 + \sum_{i=1}^{n}\frac{y_i^2}{x_i^2}\left(\frac{\Delta y_i}{y_i}\right)^2 - 2\sum_{i=1}^{n}\frac{y_i^2}{x_i^2}\,\frac{\Delta x_i\,\Delta y_i}{x_i\,y_i}\right\}. \qquad (8)$$

We neglect in (8) the term with $\Delta x_i\,\Delta y_i$ because the correlation between Δx_i and Δy_i may be assumed to be small and because Δx_i is small in comparison with Δy_i. As in all experiments y_i/x_i was nearly the same we replace it by y/x. If n is not too small, $\frac{1}{n}\sum_{i=1}^{n}(\Delta d_i)^2$ becomes approximately s_d^2, $\frac{1}{n}\sum_{i=1}^{n}\left(\frac{\Delta x_i}{x_i}\right)^2$ approximately c_x^2 and $\frac{1}{n}\sum_{i=1}^{n}\left(\frac{\Delta y_i}{y_i}\right)^2$ approximately c_y^2 since Δx_i resp. Δy_i were small compared with x_i resp. y_i and neither x_i nor y_i differed very much from x and y, therefore

$$s_d^2 \backsim 100^2\frac{y^2}{x^2}(c_x^2 + c_y^2) \text{ and } s_d \backsim 100\frac{y}{x}\sqrt{(c_x^2 + c_y^2)}.$$

The value of c_x and c_y can be got from the tables 14 and 15, that of y/x from the paper of BROUWER *et al.* (in prep.).

The s_d was also computed from the differences in digestibility in the first and in the second subperiod (method B). Data have been supplied in the above-mentioned paper. There is a close agreement between the results of both methods A and B (table 16).

TABLE 16. Standard deviations (s_d) of the coefficient of apparent digestibility of feed components (subperiod)

	dm	cp	c fat	c fibre	N-fe	ash	tr p	C	kcal
s_d (method A)	0.53	1.17	2.48	0.87	0.39	1.73	0.83	0.54	0.61
s_d (method B)	0.55	1.13	2.02	0.84	0.41	1.54	0.83	0.56	0.59

6.10. ACCURACY OF THE FIGURES FOR AVERAGE DAILY DIGESTIBLE AND METABOLIZABLE ENERGY, HEAT EXPENDITURE, ENERGY BALANCE AND NITROGEN BALANCE

The SD's of the average daily intakes of digestible and of metabolizable energy and those of the energy and nitrogen balances were estimated in the same way as those for digestibility, *i.e.*, firstly from the differences between the results of the two subperiods (method B) and secondly from the SD's of the separate components of the equations: digestible energy = feed energy – faecal energy, metabolizable energy = feed energy – faecal energy – energy in urine – energy in methane, etc., and the correlations of the deviations of these terms from their unknown true value (method A). Firstly the SD's for urine energy, for gaseous exchange and for heat expenditure were computed from the differences between the results of the two subperiods, in the same manner as used in 6.8. with faecal energy.

As in 6.8. a possible correlation between the deviation of an item in the first subperiod from its unknown true average value and the deviation of the same in the second subperiod was neglected. Thus, some of the SD's obtained may be too low. Whether this is the case will be discussed later.

Only the data of experiments R 4, 5, 7, 10, 12 and 13, each with two subperiods, were used (table 12, app.). Experiment R 1 was excluded since the animals were lactating; the same was done with experiment R 2 during which the animals had an abnormally high energy requirement (6.18.). Table 17 gives the SD's computed from the subperiods (method B).

TABLE 17. Standard deviations of various items (subperiod of 7 days, method B)

	kcal (CV)	C (g)	N × 6.25 (g)	litres
SD feed	61 (0.2%)	6.2	4.1	
,, faeces	155 (2%)	15.2	10	
,, urine	27 (2%)	3.1	12.3	
,, CH_4-production	63 (2.8%)	3.6		6.7
,, CO_2-production		25.7		48.7
,, O_2-consumption				65
,, heat expenditure	307 (2.1%)			
,, digestible energy	155			
,, metabolizable energy	125			
,, energy balance	323			
,, energy balance computed from C- and N-balance	355			
,, nitrogen balance			9.4	

It was possible to compute the correlation between the deviations of the metabolizable energy and those of the heat expenditure in the same way as the SD's were computed, *i.e.*, from the results of the two subperiods; it was negligible ($r = +0.07$). The correlations between the deviations of the energy of the feed and those of the energies contained in faeces, urine and methane may be neglected as equal rations had been weighed out in both subperiods with a very small weighing error and as the feed residues were small or absent.

Table 18 shows the SD's of some items computed with the aid of the SD's and the correlation of the deviations of the separate terms of the equations (method A). In view of the small number of experiments the agreement with the figures of table 17 is not unsatisfactory. *In the following we use values of 150, 160* and *310 kcal. as the SD's of the average daily amount of digestible and metabolizable energy and heat expenditure in a subperiod, 100, 110 (CV = 0.7%) and 220 kcal. as those in a period.*

TABLE 18. Standard deviations of various items (subperiod of 7 days, method A)

SD digestible energy	162 kcal
,, metabolizable energy	176 ,,
,, energy balance	356 ,,
,, nitrogen balance (× 6.25)	16 g

The correlation between the deviations of the energy balances computed directly and computed from the C- and N-balances was + 0.97, so that the SD's of the average of both balances differs little from that of each balance alone. The high correlation is caused by the relatively small SD's of the carbon and energy in the feed and by the high correlations between the deviations of the other corresponding terms of the two equations used in the computation of the balances: the correlation between the deviations of energy and carbon in the faeces was very high ($r = +0.996$), the same was the case with the deviations of heat expenditure and CO_2-production ($r = +0.95$). The SD of the difference between both balances is obviously low. The fact that this difference is often about 1% of the energy of the feed instead of 0% is caused by systematic errors.

The SD of the faecal energy was the major determinant of the SD's of digestible and metabolizable energy. With reference to the value for energy balance the major determinant was the SD of heat expenditure with that of faecal energy of next importance. Therefore it is clear that, in order to obtain higher accuracy, the standard error of the day-average of heat expenditure must be reduced. This can only be done, as was seen in 6.6., by increasing the number of respiration days, as the error is mainly due to *physiological* and not to *analytical* variation. More respiration days involve a great deal more work (gas analysis, supervision, computations of results) and therefore means must be found to reduce the work involved in respiration chamber experiments to a minimum (less readings during the respiration day; physical analysis of CO_2 and O_2 in samples of the chamber-air at the start and at the end of one respiration day and of CH_4 in the composite sample of the outgoing air; respiration experiments of 48 h. instead of 24 h.). When there are 6 (= 3 × 2) respiration days during the subperiod of one week, then the standard error of the heat expenditure about equals the SD of the faecal energy ($310/\sqrt{3} = 180$) but is three times the SD's of the energy in feed and in methane.

6.11. CORRECTION OF THE AVERAGE DAILY INTAKE OF METABOLIZABLE ENERGY TO ENERGY EQUILIBRIUM

To be able to compare the maintenance requirement of metabolizable energy of animals with different body weights, on different feed intakes and with varying energy balances, it is first necessary to apply certain corrections. As these corrections are made with factors that are not free from errors and deviations, it is important to learn what will be the SD of the resulting figures. According to chapter 1.2., the correction of the energy balance can be made by subtracting from the metabolizable energy eaten a value of c times the energy balance. We have already called c the gain correction factor (see 3.4.).

We now shall compute the SD ($s'_{M_{m,W}}$) of the average daily amount of metabolizable energy required for maintenance ($M_{m,W}$) in a subperiod or period due only to *analytical* and *physiological* variation and *gain correction* variation. Therefore we assume that in n experiments of similar design as the present experiments, there was only variation in the resulting figures $M_{m,W}$ due to the above-mentioned kinds of variation and not due to *ration* and *period* variation and *between-animal* variation (see ch. 1.4.).

In experiment i of n experiments we have:

$$M_{m,W} = M - cG = M - c\,(M - H) = (1 - c)\,M + cH,$$

wherein

M = average daily intake of metabolizable energy, kcal.,
H = average daily heat expenditure, kcal.,
G = average daily energy balance, kcal.,
$G = M - H$,
W = body weight of the animal, kg.,
$M_{m,W}$ = average daily intake of metabol. energy corrected to energy equilibrium,
c = the average gain correction factor in all experiments.

We denote the deviations of the various items M, H etc. from the true value in experiment i and their approximate SD's, due to *analytical* and *physiological* variation, with ΔM, ΔH etc. and s_M, s_H etc. respectively and the deviation of c in experiment i from the average c in all experiments and its approximate SD with Δc and s_c, then:

$$\Delta M_{m,W} = \Delta M - c\Delta M - M\Delta c + c\Delta H + H\Delta c = (1 - c)\,\Delta M - G\Delta c + c\Delta H.$$

Squaring and averaging over all n experiments with neglect of all terms with $\frac{1}{n}\sum_{i=1}^{n} \Delta H\Delta M$, $\frac{1}{n}\sum_{i=1}^{n} \Delta M\Delta c$ and $\frac{1}{n}\sum_{i=1}^{n} \Delta H\Delta c$ gives, if n is not too small:

$$s'^{2}_{M_{m,W}} \backsim (c - 1)^2\, s_M^2 + \frac{1}{n}\, s_c^2 \sum_{i=1}^{n} G^2 + c^2 s_H^2. \qquad (9)$$

We may ask whether it is permissable to neglect the above-mentioned terms. We already found no correlation between ΔM and ΔH (6.10.). It is not probable that there exists correlation between ΔM and Δc; moreover ΔM is small. ΔH may be thought to include 1) analytical deviations, 2) daily variation in the maintenance requirement of net energy and 3) daily variation in the efficiency of the utilization of the metabolizable energy for maintenance and production. Δc is 1) partly due to the fact that the ration in experiment i differed from the average ration in all experiments so that the true c in experiment i differed from the average c in all experiments, and 2) partly due to the fact that c, even in experiments with the same ration, for physiological reasons will not be absolutely constant. Only the third part of ΔH and the second of Δc may be correlated, therefore the correlation between ΔH and Δc will be low.

In our experiments c was not known and neither was s_c. Both figures had to be estimated. In computing $s'_{M_{m,W}}$ with (9) using estimated figures of c and s_c, we must realize that the resulting value due to *analytical* and *physiological* variation and *gain correction* variation is not very accurate since the equation is meant for the average c and its SD s_c and not for other values of c and s_c. If only estimated values are available it is probably better to use more than one of the possible, estimated values before drawing a final conclusion.

In the 11 experiments with 2 subperiods used in the computation of s_M and s_H (6.10.) the average of M was 15 228 kcal., that of G + 780 kcal.; $\frac{1}{n}\sum_{i=1}^{n} G^2$ was 2 754 000 for all 22 subperiods and 2 697 000 for all 11 periods. According to the regression equations of 6.18. ($M = 93\ W^{0.8} + 1.42\ G$ and $G = \frac{1}{1.73}\ M - 53\ W^{0.8}$) c will not have been far from 1.6. The composition of the rations in the various experiments differed little; for this reason s_c is estimated at 0.2, a value which is in agreement with the considerations on s_c given in chapter 3.7.2. Then we get from (9) using the values of s_M and s_H given in 6.10. (*i.e.* in a subperiod: 160 and 310 kcal. respectively; in a period: 110 and 220 kcal. respectively):

Average $M_{m,W}$ 13 980 kcal.,
$s'_{M_{m,W}}$ (subperiod) = 600 kcal. or 4.3% of the average $M_{m,W}$,
$s'_{M_{m,W}}$ (period) = 485 kcal. or 3.5% of the average $M_{m,W}$.

6.12. CORRECTION TO AVERAGE BODY WEIGHT

The results of the average daily intake of metabolizable energy corrected to energy equilibrium ($M_{m,W}$) can only be compared if they apply to animals of the same body weight .The correction of $M_{m,W}$ to a value $M_{m,500}$ for an average weight W_o, *e.g.* 500 kg., involves a second source of *correction* variation, the *weight correction* variation, since the power in the equation

$$M_{m,W} : M_{m,500} = W^p : 500^p$$

is not accurately known and may even vary slightly from animal to animal and from experiment to experiment.

Also there is a considerable error attached to the value of W, again mainly for physiological reasons, especially variable fill of the digestive tract and of the bladder. The weight of the body minus digestive tract and bladder will not have changed sensibly during the experimental periods as the energy balances were small. The SD of W was about 6.4 kg. for a subperiod (weighing was done at the beginning and the end of each period).

In chapter 3.4., it was found that a value of 0.8 for p was perhaps slightly better than values of 0.6 or 1.0. An idea of the possible errors made in using $p = 0.8$ can be obtained by computing the factor f needed to reduce $M_{m,W}$ to $M_{m,500}$ with $p = 0.6$, 0.7, 0.8, 0.9 or 1.0, f being $\left(\frac{500}{W}\right)^p$ (table 19). If we use $p = 0.8$ and assume that the true value of p is somewhere between 0.6 and 1.0, then it follows from table 19 that the maximal error made with cows of 400–600 kg. is ± 5%, with cows of 300–400 kg. it is ± 10% and with cows of 600–700 kg. ± 8%. The additional error of f due to the inaccuracy of W is not large as the coefficient of variation of W is about 1.3%. Nevertheless more frequent weighing is to be preferred; this can be done with very little labour after each 48-hours-respiration experiment when the cows return to the digestion stall. The easiest way is to add the effect of this weighing error to the standard deviation $s'_{M_{m,W}}$ of ch. 6.11. Since the latter changes very little by the addition, we may even neglect the error made in the determination of the body weight W. In the experiments of KELLNER and FINGERLING large animals were used (W about 700 kg.), in those of ARMSBY and FORBES small ones (W about 400 kg.). The *weight correction* variation introduced in using the reduction factor $f = \left(\frac{500}{W}\right)^{0.8}$ for the values $M_{m,W}$ of large and small cows may be very high, even higher than the sum of *analytical* and *physiological* variation and *gain correction* variation. It is thus better to correct animals of 300–400, of 400–550 and of 550–750 kg. to a weight W_o of 350, 475 and 650 kg. respectively; this is shown in table 20. The *weight correction* variation therefore lies between 0 and 3% of M_{m,W_o}.

TABLE 19. Values of $f = \left(\frac{500}{W}\right)^p$ with various values of p and W

	$p = 0.6$	$p = 0.7$	$p = 0.8$	$p = 0.9$	$p = 1.0$
$W = 300$	1.36	1.43	1.51	1.58	1.67
$W = 400$	1.14	1.17	1.20	1.22	1.25
$W = 500$	1.00	1.00	1.00	1.00	1.00
$W = 600$	0.90	0.88	0.86	0.85	0.83
$W = 700$	0.82	0.79	0.76	0.74	0.71

6.13. ACCURACY OF $M_{m,W}$ AND M_{m,W_o} IF THE HEAT EXPENDITURE HAD BEEN MEASURED MORE ACCURATELY

It is interesting to compute what would have been the value for s' of $M_{m,500}$ in a 14-days' experimental period if it had been possible to use a coefficient of variation for average daily heat expenditure in a period of 0.5% (as in KELLNER's experiments, 6.6.) or 1% (as in the experiments of ARMSBY and FORBES, 6.6.), instead of the value

TABLE 20. Values of $f = \left(\frac{W_0}{W}\right)^p$ with various values of p, W_0 and W

$W_0 = 350$	$p = 0.6$	$p = 0.8$	$p = 1.0$
$W = 300$	1.10	1.13	1.17
$W = 350$	1.00	1.00	1.00
$W = 400$	0.92	0.90	0.88
$W_0 = 475$	$p = 0.6$	$p = 0.8$	$p = 1.0$
$W = 400$	1.11	1.15	1.19
$W = 475$	1.00	1.00	1.00
$W = 550$	0.92	0.89	0.86
$W_0 = 650$	$p = 0.6$	$p = 0.8$	$p = 1.0$
$W = 550$	1.11	1.14	1.18
$W = 650$	1.00	1.00	1.00
$W = 750$	0.92	0.89	0.87

of $2.1/\sqrt{2} = 1.5\%$ (as in the present experiments, 6.10.). In the computation of s' of $M_{m,W}$ of the periods (6.11.) instead of 220 kcal. the SD of the heat expenditure would have been about 1/3 or 2/3 as much, thus about 70 and 150 kcal. respectively. This gives:

$s'_{M_{m,W}}$ (period, CV of H 0.5%) 360 kcal. or 2.6% of average $M_{m,W}$,
„ (period, CV of H 1.0%) 380 kcal. or 2.7% of average $M_{m,W}$,
„ (period, CV of H 1.5%) 485 kcal. or 3.5% of average $M_{m,W}$.

In all the three cases $(c-1)^2 s_M^2$ of the equation (9) was small compared with $s'^2_{M_{m,W}}$; $\frac{1}{n} s_c^2 \sum_{i=1}^{n} G^2$ was about equal to $c^2 s_H^2$ where the CV of H was 1.5%, while where the CV of H was 0.5 or 1.0%, $c^2 s_H^2$ was much smaller, and $\frac{1}{n} s_c^2 \sum_{i=1}^{n} G^2$ mainly determined $s'^2_{M_{m,W}}$.

$s'_{M_{m,500}}$ will not be much higher than $s'_{M_{m,W}}$ since in our experiments the standard deviation of W was small (ch. 6.12.) and the weight correction not very high as the body weights did not differ very much.

6.14. SIMILARITY IN COMPOSITION OF THE RATIONS

It has already been seen from the results shown in table 11 that the composition of the rations was not always the same in the present experiments. Even the components of the rations (hay A and B, concentrate mixture I, II and III, sugar beet pulp) had not exactly the same composition in all experiments (table 21).

TABLE 21. Composition of the components of the rations

			on a dry matter basis							
Exp.	Component	dm (%)	cp (%)	c fat (%)	c fibre (%)	N-fe (%)	ash (%)	tr p (%)	C (%)	cal/g
R 1	Concentrate mixture I	89.25	23.92	3.85	10.85	56.42	4.96	22.58	45.56	4536.2
R 2	,, ,, I	88.91	24.08	3.85	10.72	56.32	5.03	22.77	45.60	4538.8
R 3	,, ,, I	88.78	23.62	5.40	10.53	55.75	4.70	22.46	46.15	4632.6
R 4	,, ,, I	88.39	25.82	5.36	9.47	54.80	4.55	24.02	46.34	4630.6
R 5	,, ,, I	88.90	26.05	5.34	9.96	54.08	4.57	24.37	46.27	4629.0
R 6	,, ,, I	89.57	25.67	5.17	9.23	55.42	4.51	23.98	46.30	4631.0
R 7 I, II	,, ,, II	90.49	27.43	6.28	14.70	47.00	4.59	26.08	47.55	4761.0
R 7 III	,, ,, II	90.26	28.50	6.05	13.80	47.04	4.61	26.93	47.42	4768.0
R 9	,, ,, III	90.56	35.71	6.33	16.52	36.78	4.66	33.49	48.21	4887.0
R 10	,, ,, II	90.31	27.58	5.47	15.25	46.98	4.72	25.72	47.18	4714.9
R 12	,, ,, II	90.54	27.71	5.54	14.72	47.25	4.78	26.13	47.14	4724.7
R 13	,, ,, II	89.06	29.42	4.65	14.23	47.30	4.40	28.01	47.01	4711.4
R 14	,, ,, III	90.56	34.52	5.61	18.94	36.62	4.31	32.77	48.01	4858.7
R 1	Beet pulp	87.86	7.19	0.37	13.60	72.98	5.86	5.74	42.62	4023.8
R 2	,, ,,	89.22	7.17	0.39	13.39	72.79	6.26	5.76	42.34	4006.7
R 3	,, ,,	89.30	7.14	0.38	13.31	73.46	5.71	5.70	43.00	4056.9
R 4	,, ,,	88.68	7.30	0.38	14.12	72.73	5.47	6.01	42.77	4038.3
R 5	,, ,,	94.46	7.52	0.34	13.83	73.25	5.06	6.12	43.18	4078.0
R 6	,, ,,	90.93	7.09	0.27	13.39	73.96	5.29	5.83	43.19	4072.0
R 1	Hay A	85.99	12.91	2.68	30.23	44.96	9.22	10.73	44.76	4352.6
R 2	,, ,,	84.86	12.90	2.95	30.42	44.49	9.24	10.50	44.91	4359.4
R 3	,, ,,	84.99	12.62	2.65	29.91	45.64	9.18	10.42	44.76	4347.0
R 4	,, ,,	88.76	12.08	2.68	29.68	46.47	9.09	10.07	44.56	4320.4
R 5	,, ,,	90.84	12.04	2.80	29.86	46.04	9.26	10.00	44.64	4324.5
R 6	,, ,,	91.24	12.05	2.49	30.79	45.33	9.34	9.98	44.64	4321.0
R 7 I, II	,, B	87.56	10.21	2.40	27.90	50.98	8.51	7.72	44.96	4352.5
R 7 III	,, ,,	85.50	10.09	2.33	27.81	50.74	9.03	7.44	44.54	4302.0
R 9	,, ,,	85.80	10.27	2.44	27.18	51.51	8.60	7.69	44.99	4348.0
R 10	,, ,,	85.82	10.46	2.64	27.12	50.88	8.90	7.76	44.64	4342.2
R 12	,, ,,	87.10	10.46	2.48	26.94	51.14	8.98	7.92	44.58	4316.8
R 13	,, ,,	88.08	10.24	2.68	29.14	49.46	8.48	7.82	44.90	4343.0
R 14	,, ,,	89.54	10.22	2.72	28.64	49.90	8.52	7.76	44.84	4344.8
R 8	Grass	11.98	18.91	3.90	26.85	35.69	14.65	14.38	43.03	4276.5

The sugar beet pulp and the components of the concentrate mixtures were bought a few weeks before each experiment from a local dealer. Thus, they did not all come from the same stock during all experiments and therefore it is not perhaps surprising that their composition varied.

Hay A all came from one stack, hay B all from another, but neither batch of hay was intensively mixed when it arrived at the laboratory. Although care was taken that the hay used in one experiment came from the upper, the middle and the lower part of the stack, the figures for the composition of the hay from experiment to experiment nevertheless vary slightly more than can be explained by errors due to sampling and analysis (see table 13). It should however be mentioned that the rations for both cows for a given experiment were always made up at the same time and from previously well-mixed quantities of hay, concentrate mixture and sugar beet pulp.

In looking for *between-animal* variation in for example digestibility it is clear that it is not justifiable, without corrections, to compare data from animals which received rations of different composition, *e.g.*, of experiments R 1 and R 2, of R 2 and R 3, of R 2 and R 7, of R 3 and R 9 (table 11). Following this line of thought we must recognize that even the rations of the 6 cows in experiments R 2, R 4 and R 5 and those of the 8 cows in experiments R 6, R 10, R 12 and R 13 were not completely equal, because

1. during the first series of experiments (R 1–6) the pregnancy allowance was the same for large and small cows; in the second series (R 7–14) this allowance was very low, but it was given in proportion to the body weight;
2. the composition of the hay, the concentrate mixture and the sugar beet pulp varied slightly from experiment to experiment;
3. the chemical and/or the physical properties of the hay may have slowly changed in the 11 months between R 1 and R 6 and in the 10 months between R 7 and R 14. The hay was stored on a very dry loft. POIJARVI (1950) found a decrease of 3.2, 2.2 and 4.8 units in the coefficients of digestibility of dry matter, crude protein and N-free extractives respectively in hay after storing for one year.

Except for the rations of the cows in experiment R 1 those of both cows in each experiment may be considered to have been of equal composition. The difference in body weight between the two cows in each of the experiments R 2, R 3, R 4, R 5 and R 6 were fortunately small thereby resulting in only very slight differences in the ratio between maintenance feed and pregnancy feed.

6.15. BETWEEN-ANIMAL VARIATION IN APPARENT DIGESTIBILITY

The coefficients of digestibility found in all experiments are given in table 22. We compare, in all experiments except R 1, the data of one animal (called cow 3 in every experiment except R 8) with those of its companion (called cow 4 in every experiment except R 8) since the two animals received the same feed in fairly equal quantities in any one experiment.

If

z_3 and z_4 = digestion coefficient of dry matter, crude protein, etc., in the experiment with cow 3 and cow 4 respectively, and

s_{W3} and s_{W4} = standard deviation of z_3 and z_4, due to *analytical* and *physiological* variation (variation within animals),

then s_{W3} is obviously equal to s_{W4}; therefore we call s_{W3} and s_{W4} s_W. This s_W was estimated in ch. 6.9. from the differences in the digestibility obtained with the same animal in the first and in the second half of the experimental period, *i.e.*, in each of the two subperiods. However, a possible correlation between the deviations of the data of each of the two subperiods from the unknown true daily average had to be neglected for lack of information on its size. From the calculated values for s_W we may compute the standard deviation of the difference z_3-z_4, due to *analytical* and *physiological* variation only. It is equal to $\sqrt{s_W^2 + s_W^2} = s_W\sqrt{2}$, a value which because of the unknown, neglected correlation mentioned above, also may be too low. If z_3-z_4 were

TABLE 22. Coefficients of digestibility of all experiments R 1–14

Exp.	Animal											
	nr.	name	dm	cp	c fat	c fibre	N-fe	ash	tr p	C	kcal	Number of subperiods
R 1	3	Witschoft	75.4	68.8	64.4	71.5	83.4	48.8	70.1	74.8	74.1	2
R 1	4	Zwartschoft	74.0	65.6	63.5	69.7	82.6	47.6	67.2	73.4	72.6	2
R 2	3	Annie	70.6	58.4	59.6	70.6	78.4	46.6	55.4	69.7	68.8	2
R 2	4	Alie	71.8	59.4	56.4	72.1	79.2	48.6	57.7	70.6	69.6	2
R 3	3	Annie	73.1	63.4	67.8	72.0	80.3	47.4	62.3	72.3	71.5	1
R 3	4	Alie	72.4	63.8	68.3	70.5	79.4	48.1	64.0	71.6	70.8	1
R 4	3	Clara	75.3	59.8	65.3	79.0	82.4	46.0	62.2	75.0	74.1	2
R 4	4	Klaske	73.7	58.8	61.3	77.1	80.8	45.2	58.8	73.1	72.1	1
R 5	3	Zwartkop	74.0	60.3	66.8	74.5	81.1	50.6	60.4	73.3	72.4	2
R 5	4	Coba	75.0	64.0	65.8	76.7	81.6	47.4	63.1	74.5	73.6	2
R 6	3	Zwartkop	72.0	63.3	67.4	70.4	79.2	46.9	64.8	71.5	70.7	1
R 6	4	Coba	73.0	63.7	66.5	72.3	80.4	45.8	65.0	72.5	71.7	1
R 7 I, II	3	Eke	73.5	67.9	63.4	72.8	79.9	49.1	63.7	72.8	71.9	2
R 7 III		Eke	74.1	68.2	62.0	73.9	80.4	52.2	64.1	73.2	72.3	1
R 7 I, II	4	Jansje	73.2	66.9	62.6	73.0	79.1	51.4	64.2	72.2	71.3	2
R 7 III		Jansje	71.3	64.6	58.0	70.6	77.9	49.8	61.4	70.0	69.0	1
R 8	R	Roosje	72.2	76.3	49.3	79.7	80.4	34.3	72.3	75.1	74.0	1
R 8	K	Klaske	72.1	70.4	50.6	79.9	83.3	32.2	71.6	75.0	73.9	1
R 8	L	Lamkje	73.5	78.2	50.5	80.7	80.2	39.2	74.2	76.0	75.0	1
R 9	3	Eke	70.1	68.8	65.6	67.1	76.5	46.2	66.6	69.3	68.4	1
R 9	4	Jansje	70.8	70.2	65.2	68.6	76.7	46.6	68.2	70.1	69.2	1
R 10	3	Alie	72.6	67.6	66.3	72.3	78.4	50.0	63.4	71.6	70.9	2
R 10	4	Klaasje	73.8	68.6	65.2	74.0	79.9	48.6	64.9	73.0	72.1	2
R 12	3	Kee	71.3	65.7	60.5	70.4	77.6	49.2	62.4	70.3	69.4	2
R 12	4	Alke	71.4	63.8	60.7	70.6	77.8	52.0	60.5	70.2	69.1	2
R 13	3	Betsy	70.5	63.3	63.6	70.4	76.8	47.5	60.7	69.6	68.6	2
R 13	4	R. Willy	70.1	59.9	64.0	70.4	77.5	43.5	59.9	69.4	68.4	2
R 14	3	Betsy	68.3	66.1	69.0	64.0	75.8	42.9	65.7	67.7	67.0	1
R 14	4	R. Willy	67.3	62.5	68.4	63.5	75.4	41.3	65.0	66.7	65.9	1

higher than three times $s_W \sqrt{2}$, provided that s_W were the true standard deviation, then there would be highly significant evidence of *between-animal* variation in digestibility. If s_W were too low, then the evidence would be less conclusive.

A study was made to see if any difference in digestibility of dry matter, crude protein etc., between cow 3 and cow 4 in the same experiment was higher than $3 \sqrt{2}$ times s_W. The values of s_W used were those given in table 16 (method B) for the experiments R 3, 6, 8, 9 and 14, all having only one subperiod although of 10 days' length, and for the experiment R 4 in which one animal had only one subperiod of 7 days' length. In the other experiments, all with two subperiods of 7 days each, we used $1/\sqrt{2}$ times the value given in table 16. The difference exceeded $3 \sqrt{2}$ times s_W in the following experiments:

R 2: true protein only,
R 4: N-free extractives, true protein, C and kcal. only,
R 5: crude protein, crude fibre and true protein only,
R 8: crude protein, N-free extractives and mineral matter only,
R10: N-free extractives and carbon only,
R12: true protein only,
R13: crude protein and mineral matter only,
R14: crude protein only.

The difference in R 4 may have been due to other causes since one of the animals had a digestive upset in the second subperiod. The experiment R 8 (fresh grass) with its lower accuracy will be discussed later. Thus, even if we use these values of s_W which may be too low, the evidence of *between-animal* variation in digestibility is not very great.

Analysis of variance gives us another way of estimating *between-animal* variation since part of the total variation in the results obtained with different animals, all on the same ration, is caused by *analytical* and *physiological* variation (variation within animals) and the remainder by variation between animals. We have already computed the standard deviation s_W, due to variation within animals. The values of s_W to be used now are slightly higher than $1/\sqrt{2}$ times those of table 16 (method B) as some experiments (R 3, 6, 9, 14) had only one subperiod of 10 days instead of one period consisting of two subperiods of 7 days each. The standard deviation s_T, due to total variation, may be estimated from all differences $z_3 - z_4$. The differences of the variances s_T^2 and s_W^2 gives the variance s_I^2, due to variation between animals.

In this computation we used the data from all experiments R 2–14 with the exception of those from experiment R 8. Table 23 gives the results; the accuracy is of course limited by the fairly small number of data. There is one chance in 10 that the obtained s_T (11 degrees of freedom) for the digestibility of the dry matter, of C and of kcal. could have arisen from s_W (15 degrees of freedom) alone (F-test; $P = 0.10$, $F = 2.0$). This suggests *between-animal* variation in digestibility although of a very small size. However, s_W may have been too low. In that case the evidence for *between-animal* variation would become even weaker and the size of s_I even smaller.

TABLE 23. Standard deviations of the coefficients of digestibility due to total variation (s_T), to variation within animals (s_W) and to variation between animals (s_I), (whole experimental period)

	dm	cp	c fat	c fibre	N-fe	ash	tr p	C	kcal
s_T	0.7	1.5	1.2	1.0	0.7	1.5	1.3	0.7	0.7
s_W	0.5	0.9	1.6	0.7	0.4	1.3	0.7	0.5	0.5
s_I	0.5	1.2	—	0.7	0.5	0.8	1.1	0.5	0.5

The digestibility in the third subperiod of R 7 during which the hay was not chopped, differed little from that of the subperiods 1 and 2.

In the experiment R 8 with fresh grass the technical errors were higher than those in the other experiments. Only the digestibility of the crude protein of cow K when compared with cows R and L, differs somewhat more than could be expected from the higher variation within animals. The dry matter content of the faeces of this cow K was very low (average 9.3%), that of the other animals averaging 15.3 and 13.6% respectively.

Between-animal variation in digestibility, although of small size, has also been noted by KELLNER (1919, p. 49), RINGEN (1940), WATSON *et al.* (1947, 1949), MINSON and RAYMOND (1957) and ANDERSEN *et al.* (1959). Therefore, it seems correct to assume that in the present experiments also there was *between-animal* variation of small size. Thus, the computed values for s_W will have been close to the true values, *i.e.*, there was little correlation between a deviation of the digestibility in the first subperiod from the unknown true average and that in the second.

6.16. BETWEEN-ANIMAL VARIATION IN ENERGY LOST IN URINE AND AS METHANE AND BETWEEN-ANIMAL VARIATION IN INTAKE OF METABOLIZABLE ENERGY

Between-animal variation in energy lost in the urine and as methane each expressed as a percentage of the total energy intake can be estimated in the same way as *between-animal* variation in digestibility. Table 24 shows the energy of the urine and of the methane and in addition the metabolizable energy intake all expressed as a

TABLE 24. Energy lost in urine and as methane, and the metabolizable energy intake of experiments R 1–14 expressed as a percentage of total energy intake

Exp.	Animal		Energy in urine			Energy in methane			Metaboliz. energy		
	Nr.	Name	Subperiod		Average	Subperiod		Average	Subperiod		Average
			I	II		I	II		I	II	
R 1	3	Witschoft	4.5	4.5	4.5	8.2	8.4	8.3	60.8	61.7	61.2
R 1	4	Zwartschoft	4.7	4.3	4.5	7.5	7.4	7.4	60.1	61.2	60.6
R 2	3	Annie	4.7	4.7	4.7	8.1	7.9	8.0	56.4	55.8	56.1
R 2	4	Alie	4.7	4.8	4.8	8.1	8.4	8.2	56.5	56.7	56.6
R 3	3	Annie	4.4			8.4			58.7		
R 3	4	Alie	4.4			8.0			58.4		
R 4	3	Clara	3.8	4.0	3.9	8.8	9.0	8.9	61.5	61.2	61.4
R 4	4	Klaske	4.0			8.5			59.6		
R 5	3	Zwartkop	4.4	4.2	4.3	8.3	8.2	8.2	60.3	59.2	59.8
R 5	4	Coba	4.5	4.4	4.4	8.7	8.8	8.8	60.4	60.6	60.5
R 6	3	Zwartkop	4.1			7.8			58.7		
R 6	4	Coba	4.2			8.0			59.5		
R 7	3	Eke	5.6	5.4	5.5	8.8	9.2	9.0	57.8	57.0	57.4
R 7	4	Jansje	5.5	5.3	5.4	8.6	8.7	8.6	57.9	56.6	57.2
R 8	R	Roosje	8.0			7.2			58.8		
R 8	K	Klaske	8.3			7.8			57.8		
R 8	L	Lamkje	7.9			8.2			58.9		
R 9	3	Eke	5.5			8.3			54.6		
R 9	4	Jansje	5.7			8.0			55.5		
R 10	3	Alie	5.2	5.2	5.2	8.9	8.6	8.8	56.8	57.0	56.9
R 10	4	Klaasje	5.4	5.3	5.4	8.8	8.4	8.6	58.7	57.7	58.2
R 12	3	Kee	5.2	5.0	5.1	8.3	8.9	8.6	55.7	55.7	55.7
R 12	4	Alke	4.9	5.0	5.0	7.9	8.4	8.2	55.8	56.4	56.1
R 13	3	Betsy	5.0	5.2	5.1	8.2	7.8	8.0	55.5	55.5	55.5
R 13	4	R. Willy	4.5	4.5	4.5	8.1	7.9	8.0	55.9	56.0	56.0
R 14	3	Betsy	5.5			7.4			54.1		
R 14	4	R. Willy	4.8			7.5			53.6		

percentage of total energy intake. s_W was estimated from the results of the two subperiods of the experiments R 1, 2, 4, 5, 7, 10, 12 and 13 (15 degrees of freedom). s_W might again be too low as subperiod correlation was neglected. s_T was estimated from the differences in the results of the animals 3 and 4 of all experiments except R 1 and R 8 (11 degrees of freedom). Table 25 gives s_T, s_W and s_I (for comparison the values for faecal energy have been included). There was 1 chance in 100, 1 in 5 and 1 in 10 respectively that the total variation found was caused solely by variation within animals for the urine, the methane and the metabolizable energy respectively (F-test). If s_W were indeed too low, then the evidence for *between-animal* variation would be weaker and its size even lower. The existence of some *between-animal* variation in the metabolizable energy percentage is probable in view of the probable *between-animal* variation in digestibility. Therefore, the computed value of the s_W of this percentage will have been close to the true value and subperiod correlation was rightly neglected. We may conclude that a large *between-animal* variation in energy requirement for maintenance if it exists, must be due to other causes than *between-animal* variation in the percentage of energy of the feed lost in faeces, in urine and as methane since the latter variation is very small.

TABLE 25. Standard deviations of faecal, urine, methane and metabolizable energy expressed as a percentage of energy intake, due to total variation (s_T), to variation within animals (s_W) and to variation between animals (s_I), (whole experimental period)

	Energy in			Metabolizable energy
	faeces	urine	methane	
s_T	0.7	0.21	0.23	0.6
s_W	0.5	0.10	0.18	0.4
s_I	0.5	0.18	0.14	0.4

6.17. THE NITROGEN, CARBON AND ENERGY BALANCES

All nitrogen balances were positive except those of the animals in experiment R 8 and of one animal in experiments R 1 and R 7. According to JAKOBSEN (1956) at the 200th day of pregnancy there is a protein retention for reproductive purposes of 40 g. per day, rising by the 250th day to one of 110 g. Total protein or nitrogen retention is the sum of reproductive and non-reproductive retention and therefore with the aid of the figures of JAKOBSEN the non-reproductive retention may roughly be estimated.

In our experiments, a positive non-reproductive protein retention was in nearly all cases accompanied by a positive carbon balance, the latter corrected for the carbon of the N-balance (table 26), a negative non-reproductive protein retention by a negative carbon balance.

TABLE 26. Nitrogen and carbon balances

Exp.	Animal Nr.	Animal Name	N-balance × 6.25 (g)	Reproductive protein retention (g)	Non-reproductive protein retention (g)	C-balance corrected for C of N-balance (g)
R 1	3	Witschoft	— 58	0	— 58	+ 147
R 1	4	Zwartschoft	+ 32	30	+ 2	+ 185
R 2	3	Annie	+ 29	60	— 31	— 166
R 2	4	Alie	+ 18	80	— 62	— 173
R 4	3	Clara	+ 127	60	+ 67	+ 131
R 4	4	Klaske	+ 112	60	+ 52	+ 142
R 5	3	Zwartkop	+ 106	50	+ 56	+ 215
R 5	4	Coba	+ 55	50	+ 5	+ 257
R 7	3	Eke	— 26	50	— 76	+ 104
R 7	4	Jansje	+ 22	50	— 28	+ 56
R 10	3	Alie	+ 21	40	— 19	+ 11
R 10	4	Klaasje	+ 25	30	— 5	+ 116
R 12	3	Kee	+ 19	60	— 41	— 46
R 12	4	Alke	+ 38	50	— 12	— 87
R 13	3	Betsy	+ 11	30	— 19	— 68
R 13	4	R. Willy	+ 67	40	+ 27	— 83
R 3	3	Annie	+ 150	80	+ 70	+ 142
R 3	4	Alie	+ 211	130	+ 81	— 87
R 6	3	Zwartkop	+ 189	130	+ 59	+ 209
R 6	4	Coba	+ 162	130	+ 32	+ 266
R 9	3	Eke	+ 45	130	— 85	— 85
R 9	4	Jansje	+ 73	130	— 57	— 60
R 14	3	Betsy	+ 45	80	— 35	— 182
R 14	4	R. Willy	+ 119	100	+ 19	— 122
R 8	R	Roosje	— 56	40	— 96	— 45
R 8	K	Klaske	— 141	0	— 141	— 24
R 8	L	Lamkje	— 85	70	— 155	— 129

In general the energy of the non-reproductive gain in protein was small compared with the energy gain as fat, but in the experiment R 8 (fresh grass) it was almost as high.

The energy balance computed directly was nearly always lower than when computed from the C- and N-balances; the average difference was 430 kcal. or 1.6% of the energy intake. In ch. 6.10. we have seen that this difference must have been caused by systematic errors. In the following calculations the average of both values has been used.

6.18. BETWEEN-ANIMAL VARIATION IN MAINTENANCE REQUIREMENT OF METABOLIZABLE ENERGY

6.18.1. Method 1

In order to study the *between-animal* variation in maintenance requirement of metabolizable energy it is in first necessary to reduce the observed intake of metabolizable energy (M) to energy equilibrium ($M_{m,W}$) and afterwards to the same body weight (M_{m,W_0}).

Therefore M taken in by each animal was corrected for the energy balance, the values for requirement of M per kcal. net energy 'for maintenance', in the case of negative energy balances, and for gain, in the case of positive energy balances, being those given in chapter 3.3., viz. 1.43 and 1.61 kcal. respectively. The energy balances ranged from — 2 500 kcal. to + 3 500 kcal.

For reduction to constant body weight all values of $M_{m,W}$ were converted to values of $M_{m,500}$ with the use of the formula

$$M_{m,W} : M_{m,500} = W^{0.8} : 500^{0.8}.$$

The body weights of the animals were not far from 500 kg., and thus, a different value of the power in this formula would have made little difference to the results.

In experiment R 1 we have assumed that the requirement of metabolizable energy for one kcal. milk energy was about 1.50 kcal., that is between the requirement 'for maintenance' and that for production (ARMSBY, 1917, p. 497; MØLLGAARD, 1929, p. 180; SCHIEMANN and NEHRING, 1956, p. 253). Experiment R 2 was excluded for the possibility that the cows of this experiment were not completely dry as already was mentioned (ch. 4.); compared with the other cows the requirement of the animals in this experiment was indeed very high. One of the animals (Alie) was measured about one year later when it was also in the 7th month of pregnancy but this time completely dry, then the requirement was considerably less. The experiments in the 9th month of pregnancy were also not included since we were interested in maintenance only. It was assumed that until the seventh month of gestation the requirement for pregnancy was negligible (see ch. 4.).

The *between-animal* variation in maintenance requirement of metabolizable energy may be found in the same way as that of *between-animal* variation in digestibility, *i.e.*, by subtracting the square of the standard deviation due to variation within animals from that of the standard deviation due to total variation. The variation within animals consists of *analytical* and *physiological* variation and *gain* and *weight correction* variation and *period* and *ration* variation.

Ration variation might be excluded by computing the total variation of results obtained with animals fed exactly the same ration, *i.e.*, by comparing the results obtained with cow 3 with those obtained with cow 4 in every experiment (see 6.14.). *Ration* variation will have had only slight influence on the total variation of the results of all animals in experiments R 1, 4 and 5 and of those in experiments R 7, 10, 12 and 13 respectively since in these experiments the rations of all cows were nearly equal. Of course total variation in the results of all cows of all experiments together might include a higher *ration* variation as the rations were rather variable (hay A, hay B, grass).

The contribution of *period* variation to the total variation might be smaller if total variation were computed from the results of both cows in the same experiment than if total variation were computed from all results together since in the former case more circumstances during the experiments were equal.

The *weight correction* variation was small as the body weights of the animals did not vary much.

In 6.11. we saw that the calculated standard deviation of combined *analytical* and *physiological* variation and *gain correction* variation was not very accurate because the true values for the requirement of metabolizable energy per kcal. gain and its standard deviation were not available and estimated values had to be used. Moreover, in computing the standard deviation of the average daily intake of metabolizable energy and of heat expenditure in 6.10. from the results of the two subperiods with the same animal, correlation between the deviations of the results of the subperiods from the unknown true average was neglected. We have seen in 6.16. that this could be permitted for the metabolizable energy. In 6.6. we saw that the calculated variation in average daily respiratory exchange increased when computed from two sets of respiration days with increasing number of days between the execution of these sets. Therefore it is very probable that our estimation of the standard deviation of the heat expenditure is too low and thus also that of the standard deviation $s'_{M_{m},W}$ of $M_{m,W}$, due to *analytical* and *physiological* variation and *gain correction* variation only. Better values would have been obtained if the experimental periods had been longer. Therefore, the difference between $s'_{M_{m},W}$ in an infinitely long period and $s'_{M_{m},W}$ in a period as used by us may be attributed to *period* variation since in chapter 1.4., this kind of variation was defined as follows: Errors due to changes in the maintenance requirement of the animal in the course of time which have not been accounted for during the experimental periods as these were too short.

The within-animal variation in the experiments of ARMSBY and FORBES and of MØLLGAARD, computed by us (3.7.2.), was mainly caused by *analytical* and *physiological* variation and *gain* and *weight correction* variation. Neither *ration*, nor *period* variation appeared to have had a big influence. In the experiments of KELLNER and FINGERLING with Bavarian oxen, however, probably also *period* variation played a part. For the present we assume that in our experiments neither *ration* nor *period* variation had a big influence on the variation within animals.

From the differences in $M_{m,500}$ of the animal in the first subperiod and that in the second subperiod we computed a within-animal variation of 2.5%; this figure, however, does only include part of the *correction*, *period* and *ration* variation because energy balance, body weight and ration were equal or nearly equal in both subperiods and because there was no interval between the first and the second subperiod. The within-animal variation was about 3.5% according to the computation of 6.11. when it was assumed to consist mainly of *analytical*, *physiological* and *gain correction* variation. In that computation the standard deviation of the requirement of metab-

olizable energy per kcal. gain (s_c) was assumed to be 0.2 in all experiments with hay. s_c may have been higher if the experiment R 8 (grass) is included, in which case the within-animal variation would have been 4%. For the pairs of animals within every experiment a lower value than 0.2 may be used since both animals received exactly the same ration, and also for all cows in experiments R 4 and R 5 and for those in experiments R 7, 10, 12 and 13 since the rations in any of both sets of experiments were nearly of constant composition. As even the value $s_c = 0$ gives a variation of 2.6%, here a within-animal variation of 3% seems to be a better estimate.
Table 27 gives the total variation computed from the results of the whole experimental period for pairs of animals and for groups of animals. Moreover the *between-animal* variation computed from this total variation and the within-animal variation is also given.

TABLE 27. Average $M_{m,500}$ and total, within-animal and between-animal variation of $M_{m,500}$ (whole experimental period)

Animals	Average $M_{m,500}$ (therms)	Variation Total (df) [1] (%)	Within (df) (%)	Between (%)	F	F (P = 0.10)
Pairs of:						
R 4, 5, 7, 8, 10, 12, 13	13.26	4.8 (8)	3 (11)	3.7	2.56	2.30
R 4, 5, 7, 10, 12, 13	13.13	4.7 (6)	3 (11)	3.6	2.45	2.39
All animals of:						
R 1, 4, 5	13.00	7.5 (5)	3 (11)	6.9	6.25	2.45
R 7, 10, 12, 13	12.95	7.9 (7)	3 (11)	7.3	6.93	2.34
R 4, 5, 7, 10, 12, 13	13.16	7.1 (11)	3.5 (11)	6.2	4.11	2.23
R 1, 4, 5, 7, 8, 10, 12, 13	13.10	7.6 (15)	4 (11)	6.5	3.61	2.17

[1] df = degrees of freedom

Since assumed values for the requirement of metabolizable energy per kcal. net energy 'for maintenance' or gain (c) had been used, it was important to know whether it was probable that the true value of c differed from the assumed ones and to what extent other values would influence the results. In the same way as with the results of the experiments of the literature (3.3. and 3.4.) regression equations

$$(M = aW^{0.8} + cG \text{ and } G = hW^{0.8} + kM)$$

were computed using the experiments R 4, 5, 7, 10, 12 and 13. In all these experiments the rations of hay and concentrates gave a crude fibre content of 24.4–27.1% in the dry matter and all 12 animals were dry. The equations were:

$$(92.6 \pm 2.2)W^{0.8} + (1.42 \pm 0.20)\,G = M, \qquad s^2 = 1\,047\,000,$$
$$(0.58 \pm 0.09)M - (53 \pm 9)W^{0.8} = G, \qquad s^2 = 497\,300.$$

It was not thought necessary to use different values of the exponent 0.8 as the range of body weight (626 — 446 kg.) was small.

The requirement of metabolizable energy per kcal. net energy 'for maintenance'or gain used above (1.43 and 1.61 respectively) differs but little from the requirement computed by regression (1.42; 1/0.58 = 1.73).

6.18.2. Method 2

In the same way as in chapter 3.5., we have computed the starch equivalent of the rations from the quantities of digested true protein, crude fat, N-free extractives and crude fibre, from the crude fibre content of the hay and from the value numbers of the constituents of the concentrate mixtures and of the sugar beet pulp. This figure was multiplied with 2 356 and the number of kcal. metabolizable energy of the ration was divided by the resulting product. The result, the requirement of metabolizable energy per kcal. gain, varied only from 1.91 to 2.04 in all experiments except R 8. Therefore we computed from the total and within-animal variations the *between-animal* variation using a value of 2.00 in all experiments with positive energy balances. As in chapter 3.5., in all experiments with negative balances a lower value, $0.83 \times 2.00 \backsim 1.67$, was used (table 28). We assumed that the within-animal variation was slightly higher than that of method 1 as the coefficient of variation of $M_{m,W}$, $100\, s'_{M_{m,W}}/\overline{M}_{m,W}$, with the old correction factor was 3.5% and with the new factor 3.9%. Obviously the computed *between-animal* variation is higher than that of method 1.

TABLE 28. Average $M_{m,500}$ and total, within-animal and between-animal variation of $M_{m5,00}$ (whole experimental period)

	Average	Variation				
Animals	$M_{m,500}$	Total (df) [1]	Within (df)	Between	F	F
	(therms)	(%)	(%)	(%)		(P = 0.10)
Pairs of:						
R 4, 5, 7, 10, 12, 13	12.76	6.0 (6)	3.4 (11)	5.0	3.11	2.39
All animals of:						
R 4, 5, 7, 10, 12, 13	12.76	9.0 (11)	3.9 (11)	8.1	5.32	2.23

[1] df = degrees of freedom

We may conclude that the *between-animal* variation in our experiments was probably between 4 and 8%. The accuracy of these figures is not very high mainly because of the small number of experiments and also because of the fact that the within-animal variation has not been determined with the aid of experiments one or two years later with the same animals while again in their 7th month of pregnancy. If it is not permitted to neglect *period* and *ration* variation, then the within-animal variation becomes higher and the *between-animal* variation yet lower. *Ration* variation may explain the difference in *between-animal* variation found with the pairs of animals and with all animals together.

6.19. THE REQUIREMENT OF METABOLIZABLE ENERGY PER KCAL. REPRODUCTIVE GAIN ESTIMATED FROM EXPERIMENTS WITH COWS IN THE 7TH AND 9TH MONTH OF PREGNANCY

In chapter 2.4., we established the following equation:

$$M_m + M_r + M_{nr} = G_r + G_{nr} + H_m + H_r + H_{nr}, \tag{10}$$

in which M = metabolizable energy, G = gain, H = heat expenditure, m = for maintenance, r = reproductive and nr = non-reproductive. We may use this equation to estimate M_r, the metabolizable energy required for reproductive gain during pregnancy, from the results of the pairs of experiments R 5 and 6, R 7 and 9 and R 13 and 14. In each pair of experiments the energy metabolism of every animal was measured twice, first (R 5, 7 and 13) after 190–210 days of pregnancy (7th month) and secondly (R 6, 9 and 14) after 240–265 days (9th month). Because of the fact that the results of experiment R 2 and perhaps those of R 3 are not reliable, these experiments have not been taken into consideration.

We assume that the requirements of metabolizable energy per kcal. gain due to production of reproductive and non-reproductive gain respectively for the rations used in the 7th month pregnancy experiments (R 5, 7, 13) were equal to those for the rations used in the 9th month experiments (R 6, 9, 14). The amount of hay of both rations was about the same, the quantities of the concentrates differed slightly in R 5, 6, but were nearly equal in R 7, 9 and R 13, 14; the concentrate mixture of the ration of the 9th month pregnancy experiment contained more protein (ch. 4.). The correction of the intake of metabolizable energy for non-reproductive gain was made with the same correction factors as in 6.18.1. and 6.18.2. ($c = 1.43$ or 1.61 and $c = 1.67$ or 2.00).

The daily reproductive gain may be estimated from data on the energy deposition in the uterus during pregnancy given by JAKOBSEN *et al.* (1957). These authors in their experiments used animals of about the same body weight as our animals. They computed from the data the following function between the energy content of the pregnant uterus (E_u) and the number of days after conception (t):

$$E_u = 416.2\ \mathrm{e}^{0.0174\,t}\ \text{kcal.}$$

According to this function at the end of pregnancy ($t = 280$) E_u is 54 000 kcal. and the daily gain at the 200th, 240th and 265th day 240, 470 and 730 kcal. According to us the actual data of energy in the uterus given by JAKOBSEN *et al.* (1957) suggest a slightly lower daily gain at the 200th day and a much higher one in the last month of pregnancy. The foetus of 280 days' age, in the uterus with a total energy content of 78 000 kcal., however weighed 45.4 kg. (JAKOBSEN, 1957, p. 78). The birthweight of a Netherland, Friesian calf is about 40 kg. If the foetus mentioned above had weighed 40 kg., the energy content of the uterus might have been about 70 000 kcal., thus much more than 54 000 kcal. Therefore 60 000 kcal. appears to be a better estimate of E_u than 54 000 kcal.

From the figures of JAKOBSEN (1957, p. 82) on the composition of udders of 3 twins, one member of each twin being pregnant, we computed a total energy deposition in the udder due to pregnancy at the end of the gestation period of 5 000, 5 000 and 30 000 kcal. The above-mentioned work by JAKOBSEN was done with heifers. Since it might be supposed that the additional energy required in the growth of the udder in subsequent lactations might be less than that required for the first lactation we have assumed a value of 10 000 kcal. for the energy associated with udder growth in all experimental animals.

Therefore in the present experiments we estimated the energy deposited in the uterus and in the udder at 280 days after conception at 70 000 kcal. and the daily reproductive gain (G_r) at the 200th, 240th and 265th day at 200, 650 and 1250 kcal. respectively. In every experiment (tables 29 and 30) we computed G_{nr} from $G_{nr+r} - G_r$, M_{nr} from $c_{nr} \times G_{nr}$ (as in 6.18. c_{nr} was estimated to be 1.43 (negative energy balance) and 1.61 (positive energy balance) or 1.67 and 2.00) and H_{nr} from $M_{nr} - G_{nr}$. The resulting figures have been subtracted from the corresponding terms of equation (10); this gave:

$$M_m + M_r = G_r + H_m + H_r.$$

In this equation the sums $M_m + M_r$ and $H_m + H_r$ are known and a value for G_r can be estimated as mentioned above. As M_m and H_m were not measured with non-pregnant animals, we assumed that the requirement (c_r) of metabolizable energy per kcal. reproductive gain was constant during the whole pregnancy, thus, it was possible to compute M_r and M_m from the data of the 7th and 9th month pregnancy experiments with the same animal. Table 29 shows the main steps of the computations

TABLE 29. Computation of the requirement (c_r) of metabolizable energy per kcal. reproductive gain (c_{nr} = 1.43 (neg. balance) or 1.61 (pos. balance))

Exp.	Animal	Body weight W (kg)	Days after conception	Metab. energy M_{m+r+nr} (kcal)	Gain G_{r+nr} (kcal)	[1] G_r (kcal)	[2] G_{nr} (kcal)	[3] M_{nr} (kcal)	[4] Corr. for ΔW (18 kcal per kg)	[5] M_{m+r} (kcal)	$\frac{\Delta G_r}{\Delta M_r}$	c_r
R 5	Zwartkop	544	211	18 700	2 940	200	2 740	4 410		14 290	1 050	3.7
R 6	Zwartkop	590	262	21 910	3 040	1 250	1 790	2 880	830	18 200	3 910	
R 5	Coba	511	210	18 140	3 180	200	2 980	4 800		13 340	1 050	3.4
R 6	Coba	551	261	21 210	3 450	1 250	2 200	3 540	720	16 950	3 610	
R 7	Eke	447	206	12 920	960	200	760	1 220		11 700	1 050	3.7
R 9	Eke	486	264	13 250	—890	1 250	—2 140	—3 060	700	15 610	3 910	
R 7	Jansje	492	206	13 500	610	200	410	660		12 840	1 050	3.3
R 9	Jansje	505	264	13 990	—490	1 250	—1 740	—2 490	230	16 250	3 410	
R 13	Betsy	523	189	13 420	—860	200	—1 060	—1 520		14 940	450	8.0
R 14	Betsy	556	240	15 150	—2 140	650	—2 790	—3 990	590	18 550	3 610	
R 13	R. Willy	625	200	15 160	—780	200	—980	—1 400		16 560	650	3.2
R 14	R. Willy	651	251	16 420	—1 010	850	—1 860	—2 660	470	18 610	2 050	
St. dev. R 5, 7, 13		7		110	250	200	320	560		570	280	1.4
St. dev. R 6, 9, 14		7		140	300	200	360	630	190	670	880	

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[1] calculated from data of JAKOBSEN *et al.*

[2] $G_{nr} = G_{r+nr} - G_r$

[3] $M_{nr} = G_{nr} \times c_{nr}$

[4] ΔW is an attempt to equate for body weight change in the animal between the 7th and 9th months of pregnancy. Allowance of 18 kcal. per kg. calculated from maintenance requirement of 12 000 kcal. metabolizable energy for 500 kg., ($p = 0.8$)

[5] $M_{m+r} = M_{m+r+nr} - M_{nr}$

TABLE 30. The requirement (c_r) of metabolizable energy per kcal. reproductive gain, computed with various figures for the requirement (c_{nr}) of metabolizable energy per kcal. non-reproductive gain

Animal	Zwartkop	Coba	Eke	Jansje	Betsy	R. Willy	Average
c_r, if c_{nr} = 1.43 or 1.61	3.7	3.4	3.7	3.3	8.0	3.2	3.9
c_r, if c_{nr} = 1.67 or 2.00	4.1	3.7	4.5	3.8	8.9	3.4	4.4

with the lower gain correction factors (c_{nr}); in table 30 the results with lower and higher factors are given. Since the maintenance requirement of metabolizable energy of an animal of 500 kg. is about 12 000 kcal., a correction of 18 kcal. per kg. difference in body weight has been applied to every pair of experiments. *Period* variation has been neglected.

The individual values obtained for c_r vary less than was expected from the, indirectly computed, high standard deviation (table 29). If we had used a lower value of G_r in the experiments during the 9th month of pregnancy, *e.g.*, 1 000 or 750 kcal., then c_r would have become even higher. It is evident that the requirement of metabolizable energy per kcal. reproductive gain is high and this is reflected in the high additional heat expenditure, a fact also found by BRODY and JAKOBSEN *et al.* (see ch. 2.4.). According to our figures in the last two months of pregnancy the average daily reproductive gain of 1 000 kcal. necessitates an additional daily intake of about 4 000 kcal. metabolizable energy above that needed for maintenance. For the whole pregnancy the additional requirement is about (total G_r) × (average c_r) = 70 000 × 4.2 ∽ 300 000 kcal. metabolizable energy. This requirement is 5 times that given by KELLNER, 1.7 times that given by MØLLGAARD and about 1/3 of the standards given by MORRISON and those in use in the Netherlands (table 31; see also ch. 2.4.). If c_r is about 4, then in the 7th month of pregnancy (with G_r = 200 kcal.) M_r is about 800 kcal. This figure was more or less in agreement with later experiments in which 4 of the animals were measured again under comparable circumstances, this time, however, when they were not pregnant (fig. 7, exp. R 16 and 21).

6.20. THE INFLUENCE OF PREGNANCY ON THE BETWEEN-ANIMAL VARIATION IN MAINTENANCE REQUIREMENT OF METABOLIZABLE ENERGY

In 6.18. while computing the *between-animal* variation of the animals in the 7th month of gestation it was tacitly assumed that the pregnancy did not influence the variation. It may be doubted whether this assumption was correct since it is possible that the daily amount of reproductive gain and the requirement of metabolizable energy for this gain has varied from animal to animal. Furthermore the number of days of gestation was not exactly equal for all animals in the 7th month pregnancy experiments (table 10). Thus, part of the *between-animal* variation found in 6.18. may have been due to pregnancy. If the requirement of metabolizable energy for reproductive

TABLE 31. Requirement of metabolizable energy for the whole pregnancy (M_r), estimated from various data

	M_r
Exp. of BRODY	270 000 kcal
Exp. of JAKOBSEN *et al.*	220 000 ,,
Own experiments	300 000 ,,
Standard of KELLNER	60 000 ,,
,, of MØLLGAARD	180 000 ,,
,, of MORRISON	800 000 ,,
,, of AXELSSON and ERIKSSON	170 000 ,,
,, in use in the Netherlands	800 000 ,,

gain in the 7th month were low, a view held when initiating the 7th month pregnancy experiments, the part of the variation associated with foetal requirements would also be low and could be neglected. However the size of this requirement, about 800 kcal. metabolizable energy if $c_r = 4$ and $G_r = 200$, makes it questionable whether it is justifiable to neglect the effect of pregnancy.

Pregnancy may also have lowered the activity of the animals making them more uniform.

6.21. THE MAINTENANCE REQUIREMENT OF A NON-PREGNANT COW

Emphasis has been laid so far on the variability in average maintenance requirement of a non-pregnant dry cow of 500 kg. under the conditions of our experiments and not on the actual requirement. Until now no correction was applied for the metabolizable energy required for reproductive gain in the 7th month (about 800 kcal. if c_r is 4). In ch. 6.18. we found an average requirement of 13 000 kcal. metabolizable energy for a pregnant cow of 500 kg., but there the correction factor for non-reproductive gain was used for both the reproductive and the non-reproductive gain. Since the correction factor for reproductive gain is higher than the factor for non-reproductive gain, $M_m + M_r$ actually was about 13 400 kcal. Therefore in these experiments the non-pregnant animal required for maintenance about 12 600 kcal. metabolizable energy. This figure is above those given in chapter 3.7., for the maintenance requirement computed from the results of experiments with non-pregnant animals recorded in the literature.

6.22. THE RESULTS OF THE EXPERIMENTS R 1 (WITH LACTATING COWS) AND R 8 (RATION OF FRESH GRASS FED TO DRY COWS)

Table 12 (App.) shows that the values $M_{m,500}$ of 4 cows used in the experiments R 1

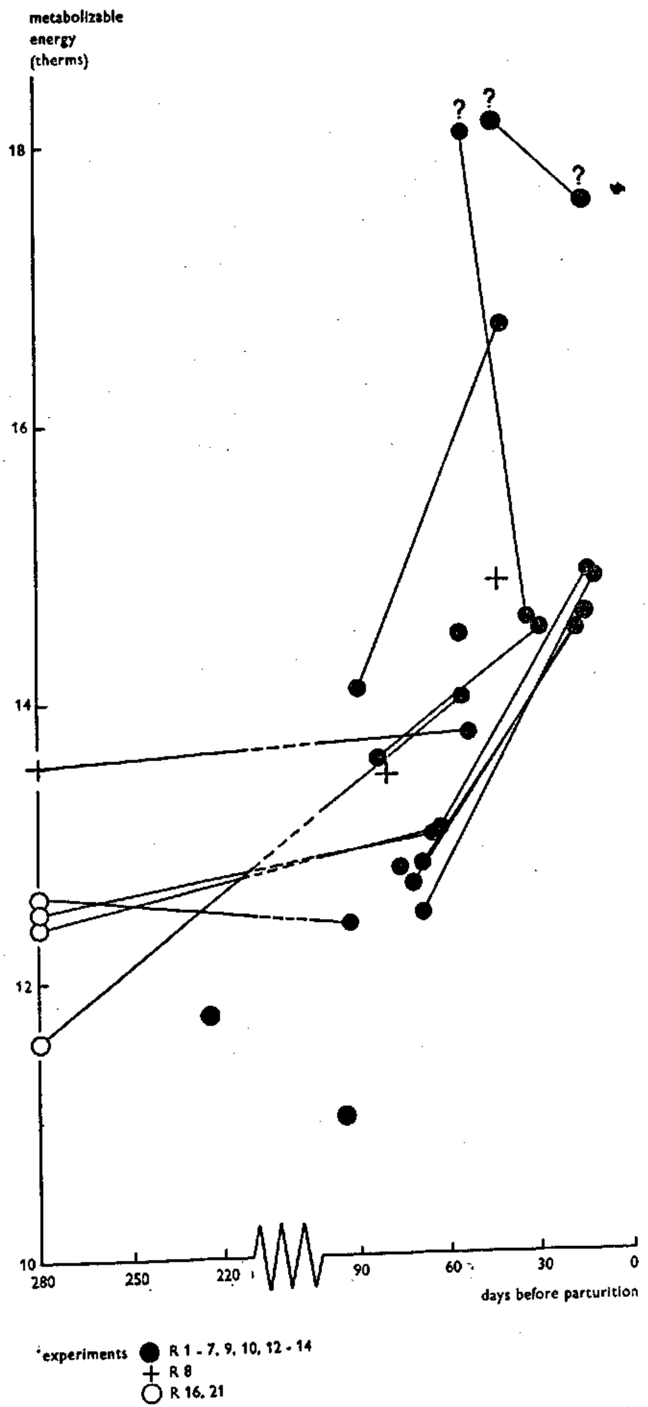

FIG. 7. Requirement of metabolizable energy for maintenance at 500 kg. body weight ($M_{m,500}$) of all animals; correction factors: p = 0.8; c (for all gain) = 1.43 (neg. balance) or 1.61 (pos. balance); the results obtained for the same animals in different experiments have been connected with each other

(lactating cows) and in R 8 (ration of fresh autumn grass) do not differ from the values $M_{m,500}$ of the cows used in their 7th month of pregnancy in the other experiments, even though in R 1 the large correction of the metabolizable energy used for milk production and in R 8 the short length of the experimental period, the changing composition of the grass and the difficulties of sampling grass introduced additional sources of errors. The 5th animal (Lamkje in experiment R 8) was in the 8th month of gestation instead of in the 7th and its higher requirement was to be expected.

In figure 7 the results of all experiments are plotted against the number of days of pregnancy; here in correcting to zero energy balance all gain has been assumed to have been non-reproductive gain; all corrections were carried out as in ch. 6.18.1. The figure clearly demonstrates the increasing requirement at the end of pregnancy.

7. DISCUSSION

THE PERFORMANCE OF ENERGY BALANCE EXPERIMENTS

Daily variation in the measured amounts of faecal energy and heat expenditure is much higher than the variation caused by the methodical errors of the separate analytical and other determinations involved. Mainly physiological causes underly this phenomenon, a conclusion already drawn by MØLLGAARD (1929, p. 83). Therefore to obtain better results it is more effective to increase the number of determinations of faecal energy and of heat expenditure than to increase the accuracy of the various analytical determinations themselves.

All standard deviations of the separate terms of the energy balance, expressed as a percentage of energy intake, should be more or less of the same size. In the present experiments the standard deviations (6.7.–6.10.) of energy intake and of energy of the urine are very low, that of the heat expenditure very high. Obviously without adversely affecting the overall accuracy the former two standard deviations may be allowed to increase slightly to economize in work (*e.g.*, by analysis of the hay in triplicate instead of in quadruplate; by estimation of the energy content of the urine from its nitrogen content). A higher total accuracy may only be obtained by increasing the number of respiration days. This demands simplification of the work connected with every respiration experiment. To do this does not necessarily imply diminishing the accuracy of a single determination of heat expenditure. The simple correction for difference in composition of the gas in the chamber at the start and at the end of each experiment given in chapter 6.4., the gas analysis by physical methods of CH_4 in the 24 hour-sample and of CO_2 and O_2 in the sample of the gas in the chamber at the start and at the end, and allowing each determination to extend to 48 hours' instead of 24 hours' respiratory exchange will not lower the overall accuracy, but it will considerably reduce the amount of work.

THE CORRECTIONS FOR BODY WEIGHT AND ENERGY BALANCE

In the statistical study of the results of experiments of the literature neither a definite value for p in the formula

$$M_{m,W} : M_{m,W_o} = W^p : W_o^p$$

nor one for the requirement of metabolizable energy for maintenance or gain was found. BREIREM's value $p = 0.6$ was not in agreement with our results which indicated a higher value. Within certain limits the choice of factors for both corrections had little influence on the high residual variance of the regression equations.

PERIOD VARIATION IN MAINTENANCE REQUIREMENT OF METABOLIZABLE ENERGY

Some evidence for *period* variation was found in the experiments of KELLNER and

FINGERLING in which the variation within animals was very high. Furthermore the increasing variation in heat expenditure in MØLLGAARD's and in the present experiments if variation is computed from two separate determinations made at greater time intervals, indicates *period* variation. This important subject requires further study.

COMPOSITION OF THE RATION AND THE MAINTENANCE REQUIREMENT OF METABOLIZABLE ENERGY

It is an important problem whether the requirement of metabolizable energy for maintenance is dependent in part on the composition of the ration. In the experiments in the literature which we have treated statistically there appeared only to be a correlation between this requirement and the crude fibre content of the dry matter of the ration, between this requirement and the digestibility of the organic matter and between this requirement and the gain correction factor itself, computed according to the starch equivalent system, and then only when the rations differed considerably, especially in the ratio between concentrates and roughage. The correlation coefficients even then were low and the influence of fibre content on maintenance requirement of metabolizable energy small. No correlation was found between maintenance requirement and crude fibre content of rations consisting only of hay. The material was, however, too small and too heterogeneous to draw any final conclusion. According to the experiments of ARMSTRONG *et al.* (1957) on volatile fatty acids the influence of the ration on maintenance requirement of metabolizable energy is small. Other investigators found no (AXELSSON and ERIKSSON, 1953) or only a small influence (BREIREM, 1944).

If indeed the composition of the ration has no or only limited influence on the feeding value of its metabolizable energy for maintenance, then the computation of the requirement of growing, fattening or lactating animals with the starch equivalent system is not correct since the value number and crude fibre corrections should only be applied to that part of the feed which the animal uses for production.

COMPARISON OF THE BETWEEN-ANIMAL VARIATION IN MAINTENANCE REQUIREMENT OF METABOLIZABLE ENERGY IN THE PRESENT EXPERIMENTS WITH THOSE RECORDED IN THE LITERATURE

The *between-animal* variation in maintenance requirement of metabolizable energy found in the experiments performed on animals of one breed and recorded in the literature was higher than that in the present experiments. For this several explanations may be given:

1. selection in the course of time has made our breed more uniform,
2. in our experiments pregnancy may have lowered the activity of the animals making them more uniform,
3. the difference is due to chance.

CONSEQUENCES OF BETWEEN-ANIMAL VARIATION IN MAINTENANCE REQUIREMENT OF METABOLIZABLE ENERGY

BROUWER (1958 a) has said that the existence of a large *between-animal* variation in maintenance requirement might be an attractive point for selective breeding and that the opposite, *i.e.*, a small variation, would considerably simplify the determination of the feeding value for maintenance of roughages. A variation of 5% in the maintenance requirement of metabolizable energy in combination with a high hereditability of maintenance requirement is adequate enough to allow for selective breeding provided that it would be possible to measure the requirement easily and accurately. As the determination is rather complicated it seems for technical reasons to be nearly impossible to make use of the *between-animal* variation in maintenance requirement in breeding.

If we feed equal amounts of metabolizable energy of various kinds of hay to different animals and compare the maintenance requirements of the animals after correction for body weight and energy balance, then it is not permitted to neglect an *between-animal* variation of 5%, if we wish to detect differences in feeding value of 10% or less. Obviously to obtain absolute feeding values first a number of animals with average requirements should be collected. To obtain relative values, various hays should be fed in succession to the same animals. As the influence of the composition of the ration on the maintenance requirement of metabolizable energy appears to be small results of such experiments will mainly be of value for maintenance and less for production.

ADDITIONAL REQUIREMENT OF METABOLIZABLE ENERGY FOR PREGNANCY

The requirement of metabolizable energy for pregnancy found in the present experiments was in agreement with the few results of earlier experiments. Undoubtedly the much higher requirement that is accepted in the feeding standards at present in use, is partially a reflection of the desire on the part of the farmer to 'steam-up' his cows prior to parturition. It is generally accepted that to meet the requirements of high milk yields the better cows may need to draw upon their body reserves both for energy and for minerals in the early part of the lactation. The process of 'steaming-up' provides for this contingency although it is our opinion that cows of lowyielding capacity can well consume an energy intake adequate to meet their demands.

SUMMARY

In a lecture in 1958 **Brouwer** called attention to the fact that a great part of the feed of a lactating cow is used for maintenance, that the most important maintenance feed is roughage and that only a few complete balance experiments have been carried out on such feeding stuffs. **Brouwer** has also expressed the view that while the existence of non or only limited *between-animal* variation in the maintenance requirement of the animals would considerably facilitate the determination of the feeding value of roughage for maintenance, on the other hand a high *between-animal* variation might be an attractive point for selection by breeding. Therefore, when the equipment for the determination of carbon, nitrogen and energy balances at the Laboratory of Animal Physiology at Wageningen was ready for use, an investigation into *between-animal* variation in maintenance requirement of cattle was commenced. During this investigation attention was at the same time paid to the accuracy of the measurements. During the determinations of maintenance energy requirement the animals were housed under conditions as near as possible to those pertaining in practice. For this reason also no experiments were conducted either with fasted animals or animals on low and high planes of nutrition. The animals were fed according to their estimated maintenance requirements. Corrections had to be applied for the inevitable, small, positive or negative energy balances to obtain the true requirement of gross, digestible or metabolizable energy for maintenance. A second correction was needed to make comparable the results of animals with unequal body weights. Since in the literature opinions on both corrections differ, it was decided to compute these corrections statistically from the results of experiments by **Kellner**, **Armsby**, **Møllgaard**, **Fingerling**, **Forbes** and **Benedict** in which the animals gave small, positive or negative energy balances.

Variation in the requirement of metabolizable energy for maintenance per 500 kg. body weight found in this manner was assumed to be caused by:

1. *analytical* variation (differences as a result of weighing and sampling errors and of analytical errors),
2. *physiological* variation (differences in true daily production of faeces, of urine, of CO_2, of CH_4 and of heat),
3. *correction* variation (differences due to applying corrections for energy gain and for body weight),
4. *period* variation (fluctuations of the maintenance requirement in the course of time which have not been accounted for in the experimental period as this was too short),
5. *ration* variation (differences due to the composition of the ration) and
6. *between-animal* variation.

In the investigation of the literature (ch. 2.) it was found that the results of many experiments suggested the existence of *between-animal* variation in maintenance

requirement, but that figures on the size of this variation were lacking. Little was known about the influence of the composition of the ration and the state of fattening and the age of the animals on the requirement for maintenance. As nearly all of the own experiments described herein were carried out with non-lactating animals in their 7th month of pregnancy, some attention was paid to the requirement of feed for pregnancy. The amount of dry matter and energy deposited in uterus and udder during pregnancy, in total about 70 000 kcal., was fairly well known, but less was known about the energy required for this reproductive gain. Most of the feeding standards for pregnant animals are rather high particularly during the latter part of pregnancy since an improvement of the condition of the animal is thought desirable to prepare for the subsequent lactation.

In the treatment of the data of 237 experiments from the literature in which there were small energy balances (ch. 3.) regression coefficients were calculated with M as the dependent variable for such equations as

$$M = aW^p + cG + eF + k \text{ or } M = (a + bF)\, W^p + (c + dF)\, G + k,$$

wherein M = metabolizable energy of feed; F = % crude fibre in dry matter of feed; W = body weight; G = energy balance; a, b, c, d, e and k = constants. We found no significant influence of crude fibre on the maintenance requirement of metabolizable energy. These and simpler regression equations in which b, d, e and k were put equal to zero, made it probable that the best value of p lay between 0.8 and 1.0. Further, they gave an improbably low requirement of metabolizable energy per kcal. gain. Other regression equations with energy gain as the dependent variable ($G = fM + gW^p$) gave improbably high requirements (table 4). This requirement per kcal. gain (c_s) computed according to the starch equivalent system was intermediate between those calculated from both kinds of regression equations. The residual variance of the regression equation of maintenance requirement of metabolizable energy, corrected to zero energy balance with c_s, on body weight with power p differed only slightly from the residual variance of the first kind of regression equations. Obviously the choice of the gain correction factor had, within limits, little influence on the residual variance. The same was the case with the choice of p in the weight correction.

Only in certain American experiments there was correlation between the maintenance requirements of metabolizable energy per 500 kg. body weight and crude fibre content of the dry matter of the ration. In these experiments correlations were also found between metabolizable energy requirement and digestibility of organic matter and between metabolizable energy requirement and the requirement per kcal. gain c_s. In these experiments widely differing rations particularly in their concentrate to roughage ratio were fed. Furthermore an increase in content of crude fibre raised the maintenance requirement only slightly.

It was decided to use more than one figure both in the correction for gain (factor c) and in the correction for body weight (power p), viz.: c = 1.43 (negative energy balance) or 1.61 (positive energy balance), 1.67 or 2.00 and 0.83 c_s or c_s (c_s is the gain correction factor according to the starch equivalent system, varying between 1.6 and 2.4); p = 0.8 and 1.0.

Many animals were used in more than one experiment. It was therefore possible by analysis of variance to get information on the variation in maintenance requirement of metabolizable energy within animals and between animals. The within-animal variation was about 7% (tables 7 and 8) both for $p = 1.0$ and for $p = 0.8$ and also with the three pairs of figures (c and c_s) of requirement 'for maintenance' (negative production) and for gain. While in the experiments with Red Danish cows and in those with Shorthorn steers this variation was mainly due to *analytical* and *physiological* variation and *correction* variation, in those with Bavarian oxen there also appeared to be *period* variation. The *between-animal* variation was rather high (about 11%, tables 7 and 8), although it is probable that breed differences in requirement account for some of this. Within some breeds this variation was 8-10%.

Most of the experiments described herein were carried out with 13 fullgrown, non-lactating Friesian animals in their 7th or 9th month of pregnancy (ch. 4.). The animals belonged to the recently acquired and not very uniform herd of the experimental farm of the laboratory. In selecting the animals account was taken of age, non-lactation, month of pregnancy and health; disposition and temperament of the animals were not considered. The animals were in moderate condition when used in the experiments. In each experiment there were two animals both receiving a ration of the same make-up and chemical composition. The animals were given a ration of good-quality hay with some concentrate at a level sufficient for maintenance and pregnancy. One kind of hay was used in the first series of experiments (R 1–6), another in the second series (R 7, 9, 10, 12, 13, 14). It was assumed that the requirement for pregnancy in the 7th month of gestation was small. To get some information concerning the requirement for pregnancy in the last stages of the gestation period some experiments (R 3, 6, 9 and 14) were also carried out with animals when 9 months pregnant. In another experiment (R 8) three animals were given fresh grass as their feed.

The experimental methods used have been described in chapter 5: preparation of the rations, sampling, methods of analysis, feeding, separate collection of urine and faeces, weighing, sampling and analysis of urine and faeces and the measurement of respiratory gas exchange have all been referred to.

Corrections for addition of formalin and/or acid to faeces and urine have been discussed. A general outline of the respiration equipment has been given and the chapter includes a detailed survey of the method of carrying out respiration chamber experiments and of the calibrations and test experiments carried out before and after the equipment is used. The results of the test experiments suggest that the O_2-consumption and the CO_2-production of a cow in 24 hours was measured with an accuracy of about 1%. Test experiments involving the combustion of alcohol did not appear satisfactory; we preferred test experiments in which CO_2 and N_2 in proportion 1:4 were released from accurately weighed cylinders standing in the respiration chambers. On the whole the apparatus worked correctly although it did entail a lot of work, especially with reference to the determination of the respiratory gas exchange (ch. 6.). Some simplifications in technique have been discussed and a simple formula derived for the computation of the correction for difference in composition of the gas in the respiration chamber at the start and at the end of an experiment. To obtain information on the size of the daily variation in respiratory exchange or in heat expenditure the extent of this variation in the experiments at Möckern, at Pennsylvania and at Copenhagen has been examined: the coefficient of variation was 1, 1.5 and 3.2% respectively. During the relatively long-term experiments at Copenhagen slow changes in O_2-consumption were found with time: the variation computed from the results in the first and those in the second week was smaller than that computed from the results in the first and those in the fourth week. The variation found in the present experiments in which the respiratory exchange was measured on two consecutive days in each of two consecutive weeks is not far from that found in the Danish experiments for an interval of two weeks between the determination of respiratory exchange. The difference in variation between the Danish and the present experiments on the one hand and the German and the American experiments on the other might have been caused by the fact that in the first-mentioned female animals were used while in the last-mentioned the experimental animals were oxen or steers.

From the results of the first and second weeks of each experimental period it was possible to compute standard deviations for the average daily amounts of faeces and of urine and of their components, of CO_2, of CH_4 and of heat (ch. 6. 7. - 6.10.). The standard deviation of the heat production was especially high, that of the energy of the urine and of the methane low. The standard deviation of the quantities of the feed components consumed was small. For a complete experimental period of 14 days each with 4 respiration chamber days we computed standard deviations for the average daily intake of digestible and of metabolizable energy and for the average daily heat expenditure to be about 100, 110 and 220 kcal. respectively. After correction to energy equilibrium (energy balance = 0) the coefficient of variation of the computed requirement of metabolizable energy for maintenance due to *analytical* and *physiological* variation and *gain correction* variation, was about 3.5%. This variation was not appreciably affected by the correction to constant body weight as the weight of the experimental animals was not very far from 500 kg.

Since the rations of the two animals (3 and 4) of every experiment were of equal composition, although the composition of the rations differed slightly from experiment to experiment, the total variation was computed from the differences in the results between pairs of cows. The total variation was compared with the variation which could be expected as a result of *analytical* and *physiological* variation and *correction* variation; a difference between these variations could be attributed to *between-animal* and to *period* variation. It appeared that the *between-animal* variations in the digestion of the various components of the feed were less than 0.5%. Also the *between-animal* variation of energy lost in urine and as methane both losses expressed as a percentage of the energy intake was very small. Thus, as was expected, there was little difference in *between-animal* variation in maintenance requirement of metabolizable energy and *between-animal* variation of maintenance requirement of digestible or of gross energy.

For the computation of the *between-animal* variation in maintenance requirement of metabolizable energy the results of the experiments with the animals which were in the 7th month of gestation were corrected for energy balance and body weight. It was assumed that the variation within animals was mainly caused by *analytical* and *physiological* variation and *correction* variation. *Between-animal* variation was computed as the total variation minus within-animal variation, this was done 1) by comparison of the results between pairs of animals in each experiment, 2) by comparison of the results in which all animals were fed the same kind of hay and finally 3) by comparison of all results together. The *between-animal* variation obtained by the first comparison was fairly low (4-5%), but in the other cases higher (6-8%) perhaps because of *ration* variation. *Period* variation in maintenance requirement could not be measured since none of the animals was used in two or more experiments of equal design. If this variation is considerable, then the figure found for *between-animal* variation becomes lower.

By comparison of the results of the experiments with the same animals in the 7th and in the 9th month of pregnancy (ch. 6.19.) we found that the requirement per kcal. reproductive gain, in comparison with the requirement per kcal. non-reproductive gain was high, about 4 kcal. metabolizable energy, a value which approximates to that calculated from the experiments of BRODY and JAKOBSEN. The total requirement during the whole pregnancy for growth of foetus, uterus and udder was about 300 000 kcal. metabolizable energy, this is 1/2–1/3 of the quantity which should be given according to the present feeding standards. These standards have been set rather high as an improvement of the condition of the animal at the end of the gestation period is thought desirable.

In the discussion (ch. 7.) it has been pointed out that the standard deviations of heat production and of energy in faeces and in urine were mainly of physiological origin and that only a small part was due to analytical errors. Therefore it is of little value to reduce the latter errors. It is possible to make the physiological deviations less perceptible by using longer experimental periods or more respiration experiments. In that case the experiments entail even more work and consideration is given to possible means of reducing amount of work. More research is needed about *period* variation. Although it has not yet been proven conclusively that the composition of the ration has little influence on the requirement of metabolizable energy for maintenance there were many indications that this is so. The experimental material which was examined statistically proved too heterogeneous to allow a definite conclusion to be drawn. The difference in *between-animal* variation in the experiments of the literature and in the present experiments might have been caused 1) by a higher grade of uniformity through selection by breeding in the course of time or 2) by the influence of pregnancy in the latter experiments or 3) by chance. The consequences of an *between-animal* variation of 5% in maintenance requirement of metabolizable energy has been considered from the point of view of breeding and of setting up experiments to measure the feeding value for maintenance of roughages. For breeding such a variation was thought, for technical reasons, nearly useless. For the determination of feeding value it was thought to be too large to be completely neglected.

SAMENVATTING

Brouwer vestigde er in een voordracht in 1958 de aandacht op, dat het onderhoudsvoeder een groot deel van het totale rantsoen van melkvee uitmaakt en dat voor onderhoud vooral ruwvoeder wordt gebruikt, een complex van voedermiddelen, waarvan de waarde voor onderhoud met volledige energiebalansproeven nog weinig onderzocht is. Geen of slechts een geringe *individuele* variatie van de onderhoudsbehoefte der dieren zou volgens hem de bepaling van de waarde van ruwvoer voor onderhoud sterk vergemakkelijken, overigens zou een grote variatie wellicht aantrekkelijk kunnen zijn voor de fokkerij. Na het gereedkomen van de apparatuur voor het meten van stikstof-, koolstof- en energiebalansen in het Laboratorium voor Fysiologie der dieren van de Landbouwhogeschool te Wageningen werd dan ook een onderzoek begonnen naar de *individuele* variatie van de onderhoudsbehoefte van runderen. Tijdens dit onderzoek zou tevens ruime aandacht geschonken worden aan de nauwkeurigheid der metingen.

Getracht werd tijdens de meting van de onderhoudsbehoefte zo veel mogelijk de levensvoorwaarden van een rund in een gewone stal te benaderen. Daarom werden ook geen hongerproeven of proeven met hoog en laag voederniveau uitgevoerd ter bepaling van de behoefte aan netto energie voor onderhoud, maar werden de dieren naar geschatte onderhoudsbehoefte gevoerd. Voor de onvermijdelijke, kleine, positieve of negatieve energiebalansen diende derhalve gecorrigeerd te worden ten einde de juiste behoefte aan bruto, verteerbare of beschikbare energie voor onderhoud te leren kennen. Een tweede correctie, omrekening tot behoefte bij een bepaald lichaamsgewicht, was nodig ter vergelijking van de onderhoudsbehoeften van dieren met verschillend gewicht. Daar er in de literatuur nogal wat verschil van mening is over beide correcties, werd besloten deze tevens statistisch uit de uitkomsten van proeven met kleine energiebalansen van Kellner, Armsby, Møllgaard, Fingerling, Forbes en Benedict te berekenen.

De variatie van de gevonden behoeften aan beschikbare energie voor onderhoud per 500 kg. lichaamsgewicht werd verondersteld te zijn veroorzaakt door:

1. *analytische* variatie (verschillen door fouten bij het wegen, het bemonsteren en het analyseren),
2. *fysiologische* variatie (verschillen in werkelijke, dagelijkse produktie van mest, van urine, van CO_2, van CH_4 en van warmte),
3. *correctie* variatie (verschillen ten gevolge van het gebruik van gemiddelde correctiefactoren voor aanzet en voor gewicht),
4. *periode* variatie (dat deel van de fluctuatie van de onderhoudsbehoefte over tijdvakken langer dan de proefperiode, dat niet uit de uitkomsten van de eerste en tweede helft der proefperiode te berekenen valt),
5. *rantsoen* variatie (verschillen in behoefte ten gevolge van de samenstelling van het rantsoen) en
6. *individuele* variatie.

Bij het literatuuronderzoek (hoofdstuk 2) bleek, dat de uitkomsten van vele proeven weliswaar het bestaan van individuele verschillen in onderhoudsbehoefte aannemelijk maakten, maar dat cijfers over de grootte van deze verschillen ontbraken. Er was weinig bekend over de invloed van de samenstelling van het rantsoen en de mesttoestand en de leeftijd van de dieren op de behoefte. Daar bijna al de eigen proeven werden uitgevoerd met droogstaande dieren in de 7e maand van de dracht, werd ook enige aandacht geschonken aan de voederbehoefte voor drachtigheid. Over de tijdens de dracht afgezette hoeveelheid droge stof en energie in uterus en uier, in totaal ongeveer 70 000 kcal., was wel het een en ander bekend, over de energiebehoefte echter veel minder. De voedernormen voor hoogdrachtig vee zijn veelal vrij hoog, daar met het oog op de komende melkproduktie tijdens het laatste deel van de dracht een verbetering van de conditie van het dier gewenst wordt geacht.

Bij de verwerking van de gegevens van 237 proeven met kleine energiebalansen uit de literatuur (hoofdstuk 3) met behulp van regressievergelijkingen met M als afhankelijk veranderlijke, zoals

$$M = aW^p + cG + eF + k \text{ of } M = (a + bF)\, W^p + (c + dF)\, G + k,$$

waarin M = opgenomen hoeveelheid beschikbare energie, F = het percentage ruwe celstof in de droge stof van het rantsoen, W = het lichaamsgewicht, G = de energiebalans en a, b, c, d, e en k=constanten, werd geen significante invloed van de ruwe celstof op de onderhoudsbehoefte aan beschikbare energie gevonden. Deze en eenvoudiger regressievergelijkingen, waarbij b, d, e en k gelijk aan nul gesteld werden, maakten het waarschijnlijk, dat de beste waarde van p tussen 0,8 en 1,0 lag. Voorts gaven zij een onwaarschijnlijk lage behoefte aan beschikbare energie per kcal. aanzet. Regressievergelijkingen met de aanzet als afhankelijk variabele ($G = fM + gW^p$) gaven onwaarschijnlijk hoge behoeften (tabel 4). De behoefte voor aanzet, berekend volgens het zetmeelwaarde systeem, lag tussen die van beide soorten regressievergelijkingen in. De restvariantie van de regressievergelijking van de op deze laatste wijze vóor aanzet gecorrigeerde onderhoudsbehoefte aan beschikbare energie en het gewicht tot de macht p (onafhankelijk variabele) verschilde slechts weinig van die van de eerste soort regressievergelijkingen. Klaarblijkelijk had de keuze van de aanzetcorrectiefactor binnen zekere grenzen weinig invloed op de restvariantie. Hetzelfde was het geval met de keuze van p bij de gewichtscorrectie.

Correlatie tussen de onderhoudsbehoefte aan beschikbare energie, berekend met laatstgenoemde aanzetcorrectiefactor, bij een lichaamsgewicht van 500 kg. enerzijds en het gehalte aan ruwe celstof in de droge stof van het rantsoen, de verteerbaarheid van de organische stof respectievelijk genoemde correctiefactor zelf anderzijds, was er slechts in een aantal Amerikaanse proeven, waarin vrij uiteenlopende rantsoenen, vooral ten aanzien van de verhouding ruwvoer tot krachtvoer, gebruikt werden. Een hoger gehalte aan ruwe celstof verhoogde de onderhoudsbehoefte evenwel slechts weinig.

Er werd besloten meer dan één waarde te gebruiken zowel voor de aanzetcorrectie (factor c) als voor de gewichtscorrectie (macht p), en wel: c = 1,43 (negatieve energiebalans) of 1,61 (positieve energiebalans), 1,67 of 2,00 en 0,83 c_s of c_s (c_s is de factor, berekend met het zetmeelwaarde systeem, variërend tussen 1,6 en 2,4); $p = 0{,}8$ en 1,0. Vele dieren waren meer dan eens in een proef betrokken. Het was dus mogelijk met variantieanalyse een indruk te verkrijgen van de variatie van de onderhoudsbehoefte aan beschikbare energie binnen de dieren en die tussen de dieren (= *individuele* variatie). De variatie binnen de dieren was ongeveer 7% (tabel 7 en 8) zowel voor $p = 0{,}8$ als voor $p = 1{,}0$ en ook bij de drie paren waarden (c en c_1) voor behoefte aan geschikbare energie 'voor onderhoud' (negatieve productie) en voor aanzet. Deze variatie was bij de proeven met Rode Deense koeien en met Shorthorn ossen grotendeels het gevolg van *analytische* en *fysiologische* variatie en *correctie* variatie, bij die met de Beierse ossen echter wellicht tevens van *periode* variatie. De variatie tussen de dieren was vrij hoog (ongeveer 11%, tabel 7 en 8), deels omdat er vermoedelijk verschillen in behoefte per ras bestaan. Binnen enige rassen (het Rode Deense, het Beierse en het Shorthorn vee) was de variatie 8-10%.

Het merendeel van de eigen proeven werd uitgevoerd met 13 volwassen, droogstaande, drachtige (7e of 9e maand) dieren van het Fries-Hollandse veeslag (hoofdstuk 4). De dieren behoorden tot de vrij recent aangekochte, derhalve niet uniforme veestapel van de proefboerderij van het laboratorium. Bij de keuze der dieren werd alleen rekening gehouden met leeftijd, droogstand, maand van afkalven en gezondheid, niet met temperament of nervositeit. De dieren waren in middelmatige conditie, wanneer zij voor de proeven gebruikt werden. Per proef werd gewerkt met twee dieren, die een rantsoen van dezelfde samenstelling ontvingen. De dieren werden gevoerd met een rantsoen van goed hooi met wat krachtvoer, voldoende voor onderhoud en dracht. Eén soort hooi werd gebruikt in een eerste serie proeven (R I-6), een andere soort in een tweede serie (R 7, 9, 10, 12, 13, 14). Er werd van uitgegaan, dat de extra voederbehoefte voor drachtigheid in de 7e maand gering was. Teneinde enige indruk te verkrijgen over deze extra behoefte werd met enige koeien ook een proef uitgevoerd in de maand voor het afkalven (R 3, 6, 9, 14). Ook was er een proef met drie dieren, die naar onderhoudsbehoefte gevoederd werden met vers gras (R 8).
De gebruikte methoden werden beschreven in hoofdstuk 5. Dit hoofdstuk omvat het bereiden der rantsoenen, het bemonsteren, de analysemethoden, het voederen, het gescheiden opvangen van mest en urine, het wegen, bemonsteren en analyseren van mest en urine en de meting van de respiratorische gaswisseling. Enige correcties voor toevoeging van formaline en/of zuur aan mest en urine werden besproken. Voorts werd een globaal overzicht van de respiratieapparatuur gegeven, alsmede een gedetailleerd overzicht van de uitvoering van respiratieproeven, van ijkingen en van controleproeven. De uitkomsten van de laatste maakten het waarschijnlijk, dat de koolzuurproductie en de zuurstofconsumptie van een koe per 24 uur tot op 1% nauwkeurig werden gemeten. Controleproeven met verbranding van alcohol voldeden niet; er werd de voorkeur gegeven aan proeven, waarbij CO_2 en N_2 in de verhouding 1:4 uit nauwkeurig gewogen cilinders in de respiratiekamers werden vrijgelaten.
De gehele proefopstelling werkte in het algemeen naar wens, maar was zeer arbeidsintensief, vooral wat betreft de bepaling van zuurstofverbruik en koolzuur- en methaanproductie (hoofdstuk 6). Enige vereenvoudigingen werden besproken. Tevens werd

een eenvoudige formule ter berekening van de correctie voor de samenstelling van het gas in de respiratiekamer bij begin en einde van een etmaal ontwikkeld. Teneinde een indruk te verkrijgen van de grootte van de variatie van warmteafgifte, zuurstofverbruik of koolzuurproductie van dag tot dag werd berekend hoe groot deze was bij de proeven in Möckern, Pennsylvanië en Kopenhagen: de variatiecoefficient bij deze proeven was respectievelijk ongeveer 1, 1,5 en 3,2%. Daarbij bleek, dat er tijdens de – vrij lange – proeven in Kopenhagen langzame veranderingen in zuurstofverbruik optraden, de variatie berekend uit de uitkomsten in een eerste week en die in een tweede week was kleiner dan die berekend uit de uitkomsten in de eerste en die in de vierde week. De variatie in de eigen proeven met twee respiratie-etmalen in de eerste week en twee in de tweede week van de proefperiode kwam ongeveer overeen met die van de Deense proeven bij een interval van twee weken. Wellicht werd het verschil in variatie tussen de Deense en de eigen proeven enerzijds en de Duitse en de Amerikaanse anderzijds veroorzaakt door het feit, dat in de eerste vrouwelijke dieren en in de andere ossen gebruikt werden.

Uit de uitkomsten van de twee helften van de proefperioden was het mogelijk de standaardafwijkingen van de gevonden gemiddelde dagproductie aan mest- en urinebestanddelen, aan koolzuur, aan methaan en aan warmte te berekenen (hoofdstuk 6.7.–6.10.). Vooral de standaardafwijking van de warmteproductie was hoog, die van de energie van de urine en van het methaan zeer laag. De standaardafwijking van de hoeveelheid opgenomen voederbestanddelen was gering. Voor een totale proefperiode van 14 dagen met vier respiratie-etmalen werden standaardafwijkingen van de verteerbare en de beschikbare energie en van de warmteafgifte, alle drie gemiddeld per 24 uur, berekend van ongeveer 100, 110 respectievelijk 220 kcal. Na correctie tot stofwisselingsevenwicht (energiebalans = 0) was de variatiecoefficient van het getal, berekend voor de onderhoudsbehoefte aan beschikbare energie, ten gevolge van *analytische* en *fysiologische* variatie en *aanzetcorrectie* variatie, ongeveer 3,5%. De *gewichtscorrectie* variatie verhoogde deze coefficient niet noemenswaard, daar het gewicht van de dieren niet zeer veel van 500 kg. verschilde.

Daar de rantsoenen van de beide dieren (3 en 4 genoemd in elke proef, alhoewel het van proef tot proef bijna nooit dezelfde dieren betrof) van elke proef gelijke samenstelling hadden, doch de rantsoenen van proef tot proef niet volkomen van dezelfde samenstelling waren, werd uit de verschillen in de uitkomsten van koe 3 en koe 4 de totale variatie berekend. Deze werd vergeleken met de variatie, die te verwachten was ten gevolge van *analytische* en *fysiologische* variatie en *correctie* variatie; indien de laatste lager was dan de eerste kon dit derhalve worden toegeschreven aan *individuele* variatie en *periode* variatie. Het bleek, dat de *individuele* variaties van de vertering van de diverse bestanddelen van het voer kleiner dan 0,5% waren. Ook de *individuele* variatie van het energieverlies in de vorm van urine en methaan als percentage van de energie van het voer was zeer gering. De *individuele* variatie van de onderhoudsbehoefte aan beschikbare energie zou dus, zoals ook werd verwacht, weinig of niet verschillen van de *individuele* variatie van de onderhoudsbehoefte aan verteerbare of bruto energie.

Ter berekening van de *individuele* variatie van de onderhoudsbehoefte aan beschikbare energie werden de uitkomsten van de proeven met de dieren in de 7e maand van de dracht gecorrigeerd tot energetisch evenwicht en tot die van dieren met een lichaamsgewicht van 500 kg. Er werd aangenomen, dat de variatie binnen de dieren grotendeels bestond uit *analytische* en *fysiologische* variatie en *correctie* variatie. Met behulp hiervan werd uit de totale variatie de *individuele* berekend en wel door vergelijking van de uitkomsten van de dieren 3 en 4 van elke proef, voorts van alle dieren voorzover gevoerd met dezelfde soort hooi en tenslotte van alle dieren tezamen. De *individuele* variatie in het eerste geval was vrij laag (4-5%), die in de andere gevallen hoger (6-8%), wellicht door *rantsoen* variatie. De *periode* variatie van de onderhoudsbehoefte kon niet worden bepaald, daar geen der dieren twee of meermalen in proeven met gelijke omstandigheden werd betrokken. Indien deze aanzienlijk is, dan wordt het getal, gevonden voor de *individuele* variatie, nog lager.

Door vergelijking van de uitkomsten van de proeven met dezelfde dieren in de 7e en de 9e maand van de dracht (hoofdstuk 6.19.) kon berekend worden, dat in vergelijking tot de produktie van lichaamsvet veel kcal. beschikbare energie, wellicht ongeveer 4, nodig waren per kcal. energie afgezet in uterus en uier tijdens de dracht, een waarde, die ook ten naaste bij uit proeven van BRODY en JAKOBSEN was af te leiden. De totale extra behoefte tijdens de gehele dracht voor groei van foetus, uterus en uier was ongeveer 300 000 kcal. beschikbare energie, dit is 1/2 à 1/3 van de hoeveelheid, die volgens de huidige voedernormen verstrekt dient te worden. Deze normen zijn vrij hoog gesteld, daar men een verbetering van de conditie van het dier tijdens de droogstand gewenst acht.

In de discussie werd gezegd (hoofdstuk 7), dat de gevonden variatie van de warmteproductie en de energie in mest en urine grotendeels van fysiologische aard was en slechts voor een klein deel veroorzaakt werd door meetfouten. Het heeft derhalve weinig zin om de meetfouten te verkleinen; wel kan men de variatie van fysiologische aard door langere proefperioden of door een groter aantal respiratie-etmalen minder voelbaar maken. De proeven worden dan evenwel zeer arbeidsintensief; het werd evenwel mogelijk geacht op de hoeveelheid werk aanzienlijk te bezuinigen. Voorts werd opgemerkt, dat meer onderzoek nodig is over de *periode* variatie en dat nog niet afdoende bewezen is, dat de samenstelling van het rantsoen weinig invloed heeft op de behoefte aan beschikbare energie voor onderhoud ,ook al waren er daarvoor aanwijzingen. Het statistisch verwerkte materiaal was te heterogeen voor een definitieve conclusie. Het verschil in *individuele* variatie van de onderhoudsbehoefte in de literatuurproeven en in de eigen proeven werd wellicht veroorzaakt door hogere graad van uniformiteit door selectie bij het fokken in de loop der jaren en door de invloed van de dracht bij de eigen proeven en door toeval. Ook werden de gevolgen van een *individuele* variatie van de onderhoudsbehoefte aan beschikbare energie van 5% bezien uit een oogpunt van fokkerij en van proeftechniek voor het meten van de voederwaarde voor onderhoud van ruwvoeders. Voor de fokkerij werd een dergelijke variatie om technische redenen zo goed als onbruikbaar geacht en voor het meten van de voederwaarde als te groot om verwaarloosd te kunnen worden.

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TABLE 2. Requirement of metabolizable energy for maintenance at W_0 or 500 kg. body weight (M_{m,W_0} or $M_{m,500}$) computed from data of literature experiments with various values of the gain and weight correction factors c and p

Reference (see list at the end of table 3)	Exp. or period number	Breed	Name	Body weight (kg)	Metabol. energy (kcal)	Gain (kcal)	Crude fibre in feed (% in dm)	Digestib. of om (%)	c_s	Ration	M_{m,W_0}, therms $p = 0.8$ $W_0 = 350, 475, 650$		$M_{m,500}$, therms				
													$p = 0.8$			$p = 1.0$	
											$c = 1.43$	$c = 1.67$	$c = 1.43$	$c = 1.67$	$c = 0.83\, c_s$	$c = 1.67$	$c = 0.83\, c_s$
MØLLGAARD '23	10	Red Danish	St.	417	8 240	— 160	34.2**	—	—	sb+	9.40	9.44	9.79	9.83	—	10.20	—
" '23	14	" "	"	404	7 220	— 670	29.9	61	2.14	s++	9.32	9.50	9.71	9.90	9.98	10.32	10.41
" '23	20	" "	"	431	8 650	— 0	29.3	64	2.42	sb+	9.35	9.35	9.74	9.74	9.74	10.03	10.03
" '23	31	" "	"	484	9 620	— 290	22.2**	—	—	sb+	9.88	9.95	10.30	10.37	—	10.44	—
" '23	30	" "	K	426	8 660	— 570	30.4**	—	—	sb+	10.34	10.48	10.77	10.93	—	11.28	—
" '29	71	" "	X	402	8 560	— 640	41.0	61	2.31	h	10.83	11.00	11.28	11.47	11.66	11.98	12.18
" '29	58	" "	Y	494	10 810	— 760	25.6	64	1.79	h	11.53	11.70	12.01	12.20	12.06	12.22	12.08
" '29	62	" "	"	501	10 180	— 340	36.6	58	2.29	h	10.22	10.30	10.65	10.74	10.82	10.73	10.81
" '29	64	" "	"	468	7 190	— 1 610	36.8	57	2.62	h	9.61	9.99	10.01	10.42	11.27	10.54	11.41
KELLNER '00	1b	Bavarian	IV	623	14 140	— 980	37.6	58	2.43	hs	16.09	16.33	13.04	13.23	13.52	12.67	12.94
" '00		Bav. × Simm.	I	748	13 700	— 4 190	29.8	59	2.11	h	17.61	18.49	14.28	15.00	15.25	13.82	14.05
" '00		" " "	II	750	18 640	— 320	21.6	68	2.15	h+	17.03	17.10	13.81	13.86	13.89	12.79	12.81
FINGERLING '33	XVI	Simmenthal	10	841	17 190	— 40	23.1	73	2.00	h++	16.57	16.58	11.38	11.39	11.39	10.27	10.27
ARMSBY '03	174 A	Shorthorn	I	387	6 590	— 2 620	35.3	59	2.28	h+	9.55	10.11	12.69	13.46	14.17	14.16	14.91
" '03	174 B	"	"	403	9 480	— 810	36.1	61	2.38	h+	12.14	12.36	12.65	12.88	13.17	13.44	13.75
" '05	179–1	"	"	544	8 310	— 3 220	33.9	60	2.18	h	11.60	12.28	12.08	12.79	13.22	12.57	12.99
" '05	179–2	"	"	520	6 020	— 4 100	33.7	61	2.20	h	11.05	11.95	11.51	12.45	13.10	12.36	13.01
" '05	179–3	"	"	514	8 220	— 2 320	26.0	66	1.87	h+	10.83	11.35	11.28	11.82	11.56	11.76	11.50
" '08	186–2a	"	"	562	10 680	— 750	28.0	63	2.13	h	13.21	13.41	10.71	10.87	10.94	10.62	10.69
" '08	186–3a	"	"	566	8 570	— 2 160	28.8	62	2.10	h	13.02	13.59	10.56	11.03	11.17	10.75	10.89
FORBES '25	212–5	"	H	337	6 570	— 730	30.8	64	2.41	h	7.85	8.03	10.44	10.68	11.02	11.56	11.92
ARMSBY '18	216–2	"	J	366	8 850	— 700	22.5	72	1.88	h+	9.51	9.67	12.65	12.86	12.76	13.68	13.58
" '18	216–4	"	"	356	5 570	— 2 450	20.9	72	1.76	h+	8.95	9.53	11.91	12.67	12.01	13.55	12.85
" '18	216–7	"	"	377	6 850	— 1 550	30.0	62	2.20	h	8.56	8.90	11.37	11.83	12.15	12.51	12.85
" '16	220–1	"	K	514	11 360*	— 760*	34.3	58	2.37	h	11.69	11.86	12.17	12.35	12.58	12.29	12.51
" '16	220–2	"	"	497	7 970*	— 2 320*	34.3	61	2.29	h	10.88	11.41	11.34	11.90	12.44	11.91	12.45
" '16	220–5	"	"	491	7 380*	— 2 670*	11.8	81	1.57	h+	10.91	11.52	11.37	12.01	11.01	12.04	11.05
FORBES '31	1	"	17	364	9 130	— 20	35.1	60	2.47	h	8.88	8.89	11.81	11.82	11.83	12.59	12.60
" '31	4	"	85	332	8 840	— 540	36.3	62	2.52	h	10.03	10.16	13.34	13.52	13.84	14.67	15.01
" '30	240–10	"	57	398	4 270	— 3 670	18.6	76	1.72	h+	8.60	9.38	11.42	12.46	11.42	13.05	11.96
" '30	240–11	"	60	381	4 140	— 3 340	18.7	75	1.72	h+	8.34	9.08	11.08	12.07	11.09	12.74	11.70
" '30	240–13	"	60	412	9 680	— 110	34.5	59	2.62	h	11.03	11.06	11.48	11.51	11.58	11.97	12.04
MITCHELL '32	3	"	X	597	7 140	— 2 610	9.6	—	1.62	h++	11.64	12.29	9.44	9.97	9.24	9.63	8.92
FORBES '25	209–2	Hereford	F	293	7 330*	— 70*	14.2	—	—	h++	8.57	8.59	11.39	11.42	—	12.70	—
" '25	209–3	"	"	283	4 440*	— 1 900*	14.2	—	—	h++	8.49	9.02	11.29	12.00	—	13.44	—

Table 2, continued

Reference (see list at the end of table 3)		Exp. or period number	Breed	Name	Body weight (kg)	Metabol. energy (kcal)	Gain (kcal)	Crude fibre in feed (% in dm)	Digestib. of om (%)	c_s	Ration	M_{m,W_0}, therms $p = 0.8$ $W_0 = 350, 475, 650$		$M_{m,500}$, therms				
														$p = 0.8$			$p = 1.0$	
												$c = 1.43$	$c = 1.67$	$c = 1.43$	$c = 1.67$	$c = 0.83\, c_s$	$c = 1.67$	$c = 0.83\, c_s$
Forbes	'25	209–5	Hereford	F	308	6 620*	— 1 110*	31.7	—	—	h	9.09	9.38	12.10	12.48	—	13.75	—
,,	'25	209–6	,,	,,	292	3 840*	— 2 880*	31.7	—	—	h	9.21	10.00	12.24	13.29	—	14.79	—
,,	'25	210–1	,,	D	345	8 520*	— 940*	35.2	—	—	d	9.98	10.21	13.28	13.58	—	14.62	—
,,	'25	210–2	,,	,,	331	6 990*	— 1 200*	35.2	—	—	d	9.10	9.40	12.12	12.51	—	13.58	—
,,	'25	210–3	,,	,,	316	4 920*	— 2 190*	35.2	—	—	d	8.74	9.31	11.63	12.38	—	13.56	—
Armsby	'17	211–1	,,	,,	460	11 350*	— 200*	33.8	57	2.33	h	11.94	11.99	12.44	12.49	12.55	12.70	12.76
,,	'17	211–2	,,	,,	432	9 440*	— 160*	19.4	75	1.76	h+	10.44	10.48	10.88	10.92	10.88	11.23	11.19
,,	'17	211–4	,,	,,	455	6 960*	— 2 240*	33.8	62	2.23	h	10.52	11.07	10.96	11.53	11.97	11.75	12.20
,,	'17	211–5	,,	,,	428	3 280*	— 4 680*	33.8	60	2.18	h	10.84	12.04	11.30	12.55	13.31	12.94	13.72
,,	'17	211–2	,,	G	358	6 890*	— 1 310*	13.5	83	1.67	h+	8.61	8.92	11.45	11.86	11.38	12.68	12.17
,,	'17	211–4	,,	,,	387	6 220*	— 2 720*	33.8	62	2.25	h	9.34	9.93	12.41	13.20	13.89	13.89	14.61
,,	'17	211–5	,,	,,	364	3 020*	— 3 860*	33.8	61	2.16	h	8.29	9.17	11.02	12.19	12.81	12.99	13.64
,,	'11	200–4	Ab. Angus	A	407	7 930	— 280	35.6	54	2.46	h	9.43	9.51	9.82	9.90	10.02	10.32	10.45
,,	'11	207–3	,, ,,	,,	507	6 100	— 1 670	31.1	64	2.04	h	8.06	8.43	8.39	8.79	8.82	8.76	8.80
Forbes	'27	237–3	,, ,,	254	345	5 180	— 1 930	22.3	74	1.76	d+	8.04	8.50	10.69	11.30	10.77	12.17	11.59
,,	'21	237–5	,, ,,	254	347	7 030	— 1 330	31.2	65	2.33	dh	8.99	9.31	11.97	12.39	12.86	13.32	13.83
,,	'27	237–10	,, ,,	36	330	7 560	— 870	33.2	58	2.52	h	9.23	9.44	12.27	12.56	13.08	13.65	14.21
,,	'28	238–6	,, ,,	36	471	4 790	— 3 360	17.8	76	1.74	h+	9.66	10.46	10.06	10.90	10.10	11.03	10.23
,,	'28	238–10	,, ,,	36	500	11 610	— 20	33.5	60	2.64	h	11.17	11.18	11.64	11.64	11.65	11.64	11.65
,,	'27	237–11	,, ,,	47	355	8 370	— 720	32.9	58	2.52	h	9.30	9.46	12.36	12.58	12.98	13.48	13.90
,,	'28	238–5	,, ,,	47	475	4 790	— 2 960	17.8	77	1.75	h+	9.02	9.72	9.40	10.13	9.46	10.24	9.56
Armsby	'11	200–1	Scrub	B	298	8 480	— 450	25.1	67	1.97	h++	10.37	10.49	13.80	13.96	13.95	15.49	15.47
,,	'11	200–4	,,	,,	309	7 030	— 350	34.1	56	2.39	h	8.32	8.41	11.06	11.18	11.34	12.32	12.49
,,	'11	207–1	,,	,,	373	9 440	— 100	22.6	68	1.82	h++	9.11	9.14	12.12	12.15	12.13	12.87	12.85
,,	'11	207–3	,,	,,	374	5 580	— 2 310	31.1	63	2.01	h	8.42	8.94	11.20	11.89	11.90	12.61	12.62
,,	'11	207–4	,,	,,	385	9 430	— 0	30.0	62	2.05	h	8.74	8.74	11.63	11.63	11.63	12.25	12.25
Cochrane	'25	221E2	Jersey	885	434	8 290	— 450	18.9	74	1.69	h++	9.60	9.72	10.01	10.12	9.99	10.41	10.28
Ritzman	'38		,,	J-II	368	8 710	— 660	9.0**	77	1.80	d+	9.28	9.43	12.34	12.54	12.38	13.33	13.17
,,	'38		Holstein	H-IV	623	11 190	— 510	9.0**	77	1.88	d+	12.34	12.46	10.00	10.10	10.06	9.67	9.63

* = corrected for N-equilibrium
** = estimated

TABLE 3. Requirement of metabolizable energy for maintenance at W_o or 500 kg. body weight (M_{m,W_o} or $M_{m,500}$) computed from data of literature experiments with various values of the gain and weigh correction factors c and p

											M_{m,W_o}, therms $p = 0.8$ W_o=350, 475, 650, 800		$M_{m,500}$, therms $p = 0.8$			$M_{m,500}$, therms $p = 1.0$		
Reference (see list at the end of this table)	Exp. or period number	Breed	Name	Body weight (kg)	Metabol. energy (kcal)	Gain (kcal)	Crude fibre in feed (% in dm)	Digestib. of om (%)	c_s	Ration	c=1.61	c=2.00	c=1.61	c=2.00	$c = c_s$	c=1.61	c=2.00	$c = c_s$
MØLLGAARD '23	15	Red Danish	St.	400	8 420	90	27.1	64	2.07	s++	7.44	7.41	9.89	9.86	9.84	10.34	10.30	10.29
,, '23	11	,, ,,	,,	432	12 110	1 750	26.7**	—	—	sb+	10.04	9.30	10.45	9.69	—	10.76	9.96	—
,, '23	16	,, ,,	,,	412	12 230	2 250	21.6	69	1.88	s++	9.65	8.67	10.05	9.02	9.34	10.45	9.38	9.71
,, '23	22	,, ,,	,,	455	12 220	1 920	24.3	69	2.46	sb+	9.45	8.67	9.84	9.03	8.08	10.03	9.21	8.24
,, '23	33	,, ,,	,,	507	14 080	2 270	18.0**	—	—	sb+	9.89	9.05	10.31	9.44	—	10.28	9.41	—
,, '23	12	,, ,,	,,	453	15 600	2 830	23.1**	—	—	sb+	11.47	10.33	11.96	10.77	—	12.19	10.97	—
,, '23	24	,, ,,	,,	480	15 380	2 910	29.2	71	2.40	sb+	10.61	9.48	11.05	9.89	8.69	11.14	9.96	8.75
,, '23	35	,, ,,	,,	532	17 330	3 280	16.1**	—	—	sb+	11.01	9.84	11.47	10.25	—	11.32	10.12	—
,, '23	21	,, ,,	X	393	10 770	540	20.3	73	2.13	sb+	9.03	8.84	12.01	11.75	11.67	12.60	12.33	12.24
,, '23	23	,, ,,	,,	400	13 630	1 930	17.9	76	2.18	sb+	9.46	8.78	12.58	11.68	11.27	13.15	12.21	11.78
,, '23	32	,, ,,	K	432	11 570	910	25.5**	—	—	sb+	10.91	10.53	11.37	10.97	—	11.70	11.28	—
,, '23	34	,, ,,	,,	445	13 880	1 700	22.8**	—	—	sb+	11.74	11.05	12.24	11.51	—	12.52	11.78	—
THORBEK '36	105	,, ,,	A 38	589	12 390	990	18.7	75	1.62	hs++	11.68	11.26	9.47	9.13	9.46	9.16	8.84	9.16
MØLLGAARD '41	109	,, ,,	,,	614	13 680	1 370	19.2	74	1.74	s++	12.01	11.45	9.74	9.29	9.59	9.34	8.90	9.20
THORBEK '36	101	,, ,,	,,	515	12 920	1 630	19.4	74	1.75	hs++	9.66	9.06	10.06	9.44	9.84	10.00	9.38	9.78
,, '36	107	,, ,,	,,	625	16 050	2 330	22.2	69	1.87	hs++	12.69	11.75	10.29	9.53	9.78	9.84	9.11	9.35
,, '36	103	,, ,,	,,	553	17 100	3 370	20.9	71	1.87	hs++	13.29	11.79	10.77	9.56	9.97	10.56	9.36	9.76
,, '36	106	,, ,,	B 4	608	12 810	780	19.3	72	1.74	hs++	12.19	11.87	9.88	9.62	9.79	9.50	9.25	9.41
,, '36	102	,, ,,	,,	577	13 310	1 410	18.6	73	1.75	hs++	12.14	11.54	9.85	9.36	9.67	9.57	9.10	9.40
MØLLGAARD '41	110	,, ,,	,,	624	13 820	780	17.4	75	1.74	s++	12.99	12.68	10.53	10.27	10.44	10.07	9.82	9.98
THORBEK '36	108	,, ,,	,,	627	16 300	2 520	23.1	69	1.90	hs++	12.60	11.59	10.22	9.40	9.61	9.76	8.97	9.17
,, '36	104	,, ,,	,,	600	17 480	3 160	20.0	70	1.90	hs++	13.22	11.91	10.71	9.65	9.93	10.33	9.30	9.56
MØLLGAARD '41	116	,, ,,	A 8	500	11 720	1 100	16.7	75	1.93	sb++	9.55	9.14	9.95	9.52	9.60	9.95	9.52	9.60
,, '41	114	,, ,,	C 11	516	14 100	2 200	19.5	74	1.79	sd++	9.88	9.08	10.29	9.46	9.91	10.23	9.40	9.85
,, '41	122	,, ,,	50	550	13 030	1 720	16.7	80	1.92	sb++	9.13	8.54	9.51	8.89	9.02	9.33	8.72	8.84
HANSEN '43	124	,, ,,	B 89	435	11 690	1 860	16.4	76	1.96	sb++	9.33	8.55	9.72	8.91	8.99	9.99	9.16	9.24
,, '43	125	,, ,,	B 90	435	11 530	1 980	17.0	75	1.97	sb++	8.95	8.12	9.32	8.46	8.53	9.59	8.70	8.77
KELLNER '00		Bav. × Simm.	III	858	24 530	1 300	24.5	71	2.09	h++	21.23	20.75	14.57	14.23	14.15	13.08	12.78	12.72
,, '00	I	Bavarian	II	632	16 390	830	33.0	62	2.11	h	15.40	15.07	12.48	12.21	12.12	11.91	11.65	11.56
,, '00	I	,,	III	632	14 860	630	37.4	59	2.41	hs	14.16	13.91	11.48	11.27	11.08	10.95	10.76	10.57
,, '00	II	,,	III	649	19 670	3 010	30.8	63	2.14	hs+	14.85	13.68	12.04	11.08	10.78	11.42	10.51	10.22
,, '00	II	,,	IV	631	18 790	1 760	30.9	61	2.13	hs+	16.34	15.64	13.25	12.67	12.49	12.64	12.09	11.92
,, '00	I	,,	V	602	15 140	1 490	30.8	61	2.19	hs+	13.54	12.93	10.98	10.48	10.26	10.58	10.10	9.89
,, '00	I	,,	VI	644	15 920	1 680	30.8	64	2.15	h	13.32	12.66	10.80	10.26	10.09	10.26	9.75	9.58
,, '00	I	,,	XX	672	17 440	960	27.5	67	1.90	h	15.48	15.12	12.55	12.25	12.33	11.83	11.55	11.63
,, '00		,,	A	620	16 370	1 550	27.2	67	2.04	h	14.42	13.79	11.68	11.17	11.12	11.19	10.70	10.65
,, '00	IV	,,	E	768	26 700	3 150	20.6	72	2.10	h++	22.34	21.07	15.34	14.46	14.24	14.08	13.28	13.07
,, '00	III	,,	F	596	17 680	2 060	21.7	73	2.08	h++	15.40	14.54	12.49	11.78	11.64	12.05	11.38	11.24

Table 3, continued

Reference (see list at the end of this table)	Exp. or period number	Breed	Name	Body weight (kg)	Metabol. energy (kcal)	Gain (kcal)	Crude fibre in feed (% in dm)	Digestib. of om (%)	c_s	Ration	M_{m,W_o}, therms $p = 0.8$ W_o=350, 475, 650, 800		$M_{m,500}$, therms $p = 0.8$			$p = 1.0$		
											$c = 1.61$	$c = 2.00$	$c=1.61$	$c=2.00$	$c=c_s$	$c=1.61$	$c=2.00$	$c = c_s$
KELLNER '00	III	Bavarian	G	622	17 380	1 780	23.6	73	2.03	h++	15.04	14.32	12.19	11.61	11.57	11.67	11.11	11.07
,, '00	I	,,	,,	617	22 710	3 680	28.5	64	2.23	hs+	17.51	16.01	14.19	12.97	12.25	13.60	12.43	11.74
,, '00	IV	,,	H	669	17 440	2 060	23.3	76	1.93	h++	13.80	13.01	11.19	10.55	10.66	10.56	9.95	10.05
,, '00	I	,,	,,	646	21 980	2 740	31.0	64	2.18	hs++	17.66	16.58	14.31	13.45	13.05	13.60	12.77	12.39
,, '00	IV	,,	J	635	17 870	2 300	22.5	77	1.94	h++	14.44	13.52	11.70	10.96	11.08	11.16	10.44	10.55
,, '00	I	,,	,,	615	22 630	3 570	30.5	67	2.15	hs++	17.64	16.19	14.30	13.12	12.66	13.73	12.59	12.15
FINGERLING '38	IV	,,	K	678	17 670	2 220	21.0	77	1.92	h++	13.63	12.79	11.05	10.37	10.51	10.40	9.75	9.88
NEHRING '53	V	,,	M	707	20 990	3 420	21.6	75	1.95	h++	14.48	13.23	11.74	10.73	10.85	10.95	10.00	10.12
,, '53	IV	,,	N	661	20 920	2 300	21.6	74	1.98	h++	16.99	16.11	13.77	13.06	13.10	13.02	12.34	12.38
,, '56	VI	,,	,,	681	20 770	1 890	21.6	74	1.97	h++	17.09	16.38	13.85	13.27	13.32	13.02	12.47	12.51
,, '56	XI	,,	O	753	24 660	1 520	23.2	72	2.05	h++	23.30	22.68	16.01	15.59	15.53	14.75	14.36	14.30
,, '56	II	,,	,,	697	26 300	3 600	27.4	65	2.15	h++	19.40	18.07	15.72	14.65	14.24	14.71	13.70	13.31
,, '56	I	,,	,,	651	22 560	3 150	20.3	76	1.92	h++	17.47	16.24	14.16	13.17	13.37	13.43	12.49	12.68
,, '53	VI	,,	P	720	22 370	3 120	20.3	74	1.94	h+	15.99	14.87	12.96	12.05	12.19	12.05	11.19	11.33
,, '53	I	,,	,,	636	22 330	3 880	20.3	75	1.91	h++	16.37	14.83	13.27	12.02	12.31	12.64	11.45	11.73
FINGERLING '34	VIII	,,	Q	740	21 610	3 660	19.9	71	1.87	h++	14.18	12.89	11.49	10.45	10.80	10.62	9.66	9.98
,, '34	I	,,	,,	636	21 420	5 130	19.7	71	1.86	h++	13.40	11.36	10.86	9.21	9.80	10.35	8.77	9.34
NEHRING '53	III	,,	R	713	21 340	4 000	23.7	72	2.04	h++	13.84	12.39	11.22	10.05	9.92	10.45	9.35	9.24
,, '56	V	,,	T	734	20 010	3 670	19.3	77	1.93	h++	12.80	11.50	10.38	9.33	9.52	9.61	8.63	8.81
,, '56	II	,,	,,	670	20 010	4 820	19.2	77	1.93	h++	11.96	10.12	9.70	8.21	8.48	9.14	7.74	7.99
FINGERLING '33	VII	,,	U	758	21 850	3 630	22.4	71	2.03	h++	16.71	15.23	11.47	10.53	10.38	10.56	9.70	9.56
,, '33	IV	,,	,,	700	21 580	4 250	22.4	71	2.03	h++	13.90	12.33	11.26	9.99	9.89	10.53	9.34	9.25
,, '33	I	,,	,,	648	21 510	4 920	22.3	71	2.00	h++	13.63	11.71	11.05	9.49	9.49	10.49	9.01	9.01
,, '33	VII	,,	V	719	21 760	4 010	22.4	72	1.99	h++	14.13	12.68	11.45	10.28	10.31	10.64	9.55	9.58
,, '33	IV	,,	,,	665	21 820	4 810	22.4	71	2.03	h++	13.82	11.98	11.21	9.71	9.60	10.58	9.17	9.07
,, '33	I	,,	,,	596	22 150	5 910	22.4	72	2.01	h++	13.54	11.07	10.98	8.98	8.92	10.60	8.67	8.62
,, '34	IV	,,	W	708	22 020	3 800	19.1	72	1.89	h++	14.85	13.47	12.04	10.92	11.23	11.23	10.18	10.48
,, '34	I	,,	,,	641	21 900	4 280	19.1	72	1.88	h++	15.17	13.49	12.31	10.94	11.36	11.71	10.40	10.80
,, '34	IV	,,	X	706	21 940	3 960	19.1	70	1.92	h++	14.57	13.12	11.81	10.64	10.88	11.02	9.93	10.15
,, '34	I	,,	,,	638	21 530	4 570	19.0	71	1.87	h++	14.38	12.58	11.67	10.20	10.68	11.11	9.71	10.18
NEHRING '56	VII	,,	Y	742	23 060	3 580	19.6	70	2.06	h++	15.57	14.31	12.62	11.61	11.45	11.66	10.72	10.58
FINGERLING '37	IV	,,	,,	682	23 530	4 500	19.5	71	2.09	h++	15.68	13.99	12.71	11.33	11.02	11.94	10.65	10.36
,, '37	I	,,	,,	596	23 730	5 370	19.4	71	2.10	h++	16.17	13.93	13.11	11.29	10.82	12.65	10.90	10.45
,, '37	IV	,,	Z	668	23 670	5 650	19.6	72	2.07	h++	14.27	12.11	11.56	9.81	9.49	10.91	9.26	8.97
,, '37	I	,,	,,	588	23 410	6 480	19.4	71	2.08	h++	14.07	11.33	11.40	9.19	8.73	11.04	8.88	8.44
,, '34	II	,,	16	685	19 670	310	20.4	75	1.89	h++	18.38	18.27	14.90	14.80	10.73	13.99	13.91	10.08
,, '34	IV	,,	16	733	24 660	3 240	16.6	77	1.83	h++	17.67	16.53	14.32	13.40	13.80	13.26	12.40	12.77
,, '34	III	,,	16	731	25 360	3 320	16.6	77	1.86	h++	18.21	17.04	14.77	13.82	14.15	13.69	12.80	13.12
,, '37	I	,,	16	671	25 840	3 470	16.6	77	1.80	h++	19.75	18.43	16.01	14.95	15.50	15.09	14.08	14.59
NEHRING '53	XI	,,	17	826	18 620	1 020	22.8	69	1.95	h++	16.54	16.15	11.36	11.09	11.13	10.28	10.03	10.06

Table 3, continued

Reference (see list at the end of this table)	Exp. or period number	Breed	Name	Body weight (kg)	Metabol. energy (kcal)	Gain (kcal)	Crude fibre in feed (% in dm)	Digestib. of om (%)	c_s	Ration	M_{m,W_o}, therms $p = 0.8$ W_o=350, 475, 650, 800		$M_{m,500}$, therms $p = 0.8$			$M_{m,500}$, therms $p = 1.0$		
											c=1.61	c=2.00	c=1.61	c=2.00	$c = c_s$	c=1.61	c=2.00	$c = c_s$
FINGERLING '34	II	Bavarian	17	675	19 250	1 630	20.4	73	1.93	h++	16.14	15.53	13.08	12.58	12.67	12.32	11.85	11.93
NEHRING '53	XX	,,	17	978	23 720	2 950	18.7	75	1.91	h++	16.14	15.16	11.09	10.42	10.58	9.70	9.11	9.24
,, '53	XVI	,,	17	904	23 590	4 120	18.7	75	1.87	h++	15.38	13.92	10.56	9.56	9.90	9.38	8.49	8.79
FINGERLING '37	I	,,	18	629	19 920	2 010	18.5	73	1.80	h++	17.13	16.33	13.89	13.24	13.58	13.26	12.64	12.96
NEHRING '53	XV	,,	19	1006	23 400	780	18.7	75	1.86	h++	18.45	18.19	12.66	12.49	12.56	11.01	10.85	10.91
,, '53	V	,,	19	802	19 910	850	18.4	73	1.78	h++	18.50	18.17	12.71	12.49	12.62	11.56	11.34	11.46
FINGERLING '37	I	,,	19	705	20 730	1 620	18.6	74	1.82	h++	16.98	16.39	13.77	13.29	13.51	12.85	12.40	12.61
NEHRING '53	XI	,,	19	890	23 410	2 740	18.7	75	1.85	h++	17.44	16.46	11.98	11.31	11.57	10.67	10.08	10.31
FINGERLING '44	XIII	,,	22	646	16 130	1 770	22.1	71	1.93	h++	13.35	12.65	10.82	10.26	10.36	10.28	9.74	9.84
,, '44	X	,,	24	795	18 910	2 120	22.9	71	1.95	h++	15.57	14.74	10.69	10.12	10.20	9.75	9.23	9.30
NEHRING '53	I	,,	24	613	18 770	2 580	20.4	74	1.89	h++	15.32	14.26	12.42	11.57	11.81	11.92	11.11	11.33
FINGERLING '44	XI	,,	24	839	22 850	3 510	20.8	72	1.93	h++	16.55	15.23	11.37	10.46	10.63	10.25	9.44	9.58
,, '44	VII	,,	24	792	19 030	2 610	22.5	72	1.94	h++	14.95	13.92	10.27	9.56	9.67	9.36	8.71	8.82
,, '44	X	,,	25	692	18 320	2 290	22.9	70	1.92	h++	13.92	13.07	11.29	10.59	10.73	10.57	9.93	10.06
NEHRING '53	VI	,,	25	697	19 320	2 910	20.4	74	1.93	h++	13.84	12.77	11.22	10.35	10.51	10.50	9.68	9.82
,, '53	I	,,	25	614	19 410	3 300	20.4	74	1.95	h++	14.76	13.41	11.97	10.88	11.01	11.48	10.43	10.56
,, '53	I	,,	26	688	18 520	1 280	20.4	74	1.87	h++	15.72	15.24	12.76	12.37	12.50	11.96	11.60	11.73
,, '53	III	,,	26	713	23 150	3 380	18.6	73	1.90	h++	16.45	15.23	13.34	12.34	12.60	12.42	11.49	11.73
ARMSBY '03	174C	Shorthorn	I	416	11 200	450	36.6	58	2.47	h+	11.65	11.45	12.14	11.94	11.69	12.59	12.38	12.13
,, '03	174D	,,	,,	424	12 190	700	37.2	55	2.50	h+	12.13	11.83	12.63	12.32	11.92	13.05	12.72	12.31
,, '05	179–4	,,	,,	532	18 050	3 400	16.8	78	1.71	h+	11.49	10.28	11.97	10.71	11.65	11.82	10.58	11.51
FORBES '25	212–3	,,	H	354	10 710	990	30.1	62	2.40	h	9.04	8.66	12.02	11.51	10.99	12.88	12.33	11.76
,, '25	212–1	,,	,,	349	13 450	2 360	29.4	62	2.35	h	9.68	8.76	12.87	11.65	10.54	13.83	12.51	11.32
ARMSBY '17	217–4	,,	J	642	14 280	50	13.2	78	1.71	h++	14.34	14.32	11.63	11.61	11.62	11.06	11.05	11.05
,, '18	216–6	,,	,,	403	12 020	1 190	30.0	61	2.26	h	11.53	11.00	12.01	11.46	11.09	12.54	11.96	11.58
,, '18	216–3	,,	,,	387	12 070	1 210	22.6	69	1.90	h+	9.34	8.91	12.43	11.85	12.00	13.08	12.47	12.62
,, '17	217–1	,,	,,	490	12 290	1 390	13.4	78	1.72	h++	9.80	9.27	10.21	9.66	10.06	10.26	9.70	10.10
,, '17	216–5	,,	,,	404	15 180	2 350	29.5	60	2.25	h	12.98	11.94	13.52	12.44	11.74	14.10	12.97	12.24
,, '16	220–3	,,	K	490	11 290*	540*	12.0	80	1.66	h+	10.16	9.95	10.59	10.37	10.56	10.63	10.41	10.60
FORBES '31	5	,,	17	338	7 780	700	18.8	75	1.81	h+	6.84	6.56	9.10	8.73	8.91	9.84	9.44	9.63
,, '30	240–12	,,	57	426	10 200	240	34.5	60	2.57	h	10.71	10.60	11.16	11.05	10.89	11.52	11.41	11.25
,, '30	240–2	,,	57	360	8 210	300	18.3	77	1.81	h+	7.56	7.44	10.05	9.90	9.98	10.73	10.57	10.65
,, '30	240–4	,,	57	384	12 580	3 090	18.4	77	1.85	h+	7.07	5.95	9.40	7.90	8.47	9.90	8.33	8.93
,, '30	240–1	,,	60	311	7 530	270	18.4	76	1.81	h+	7.80	7.68	10.37	10.22	10.29	11.41	11.24	11.32
,, '30	240–3	,,	60	333	11 340	2 520	18.4	74	1.88	h+	7.58	6.56	10.09	8.73	9.14	10.94	9.46	9.91
,, '30	240–5	,,	60	358	15 060	3 900	18.4	74	1.87	h+	8.63	7.14	11.48	9.49	10.16	12.26	10.14	10.85
MITCHELL '32	2	,,	X	613	13 630	1 110	9.6	—	1.60	h++	12.41	11.96	10.06	9.70	10.07	9.66	9.31	9.67
RITZMAN '38		Jersey	J–I	415	12 680	1 060	32.8	62	2.55	h	12.22	11.76	12.74	12.26	11.59	13.22	12.72	12.03
,, '38		,,	,,	448	14 850	1 710	30.2	60	2.45	h	12.68	11.98	13.21	12.48	11.64	13.50	12.76	11.90
,, '38		,,	,,	445	14 700	1 840	26.5	62	2.40	h	12.37	11.62	12.89	12.10	11.29	13.19	12.39	11.55

Table 3, continued

Reference (see list at the end of this table)		Exp. or period number	Breed	Name	Body weight (kg)	Metabol. energy (kcal)	Gain (kcal)	Crude fibre in feed (% in dm)	Digestib. of om (%)	c_s	Ration	M_{m,W_o}, therms $p = 0.8$ W_o=350, 475, 650, 800		$M_{m,500}$, therms $p = 0.8$			$p = 1.0$		
												$c = 1.61$	$c = 2.00$	$c=1.61$	$c=2.00$	$c=c_s$	$c=1.61$	$c=2.00$	$c = c_s$
Ritzman	'38		Jersey	J–I	402	14 530	2 790	30.9	62	2.75	h	11.47	10.23	11.95	10.66	8.17	12.49	11.13	8.53
,,	'38		,,	J–II	410	12 940	740	32.8	63	2.58	h	13.22	12.89	13.77	13.43	12.93	14.33	13.98	13.46
,,	'38		,,	,,	411	14 730	1 680	30.2	61	2.46	h	13.50	12.77	14.07	13.30	12.40	14.63	13.84	12.90
,,	'38		,,	,,	404	13 600	1 680	26.5	59	2.40	h	12.41	11.66	12.93	12.15	11.36	13.48	12.68	11.85
Cochrane	'25	221F–2	,,	874	416	9 600	530	20.5	72	1.79	h++	9.73	9.50	10.14	9.90	10.03	10.51	10.26	10.40
,,	'25	221F–1	,,	874	429	14 780	3 820	20.5	70	1.87	h++	9.36	7.75	9.76	8.08	8.64	10.06	8.32	8.91
,,	'25	221D–1	,,	885	415	8 930	550	21.2	72	1.83	h++	9.01	8.72	9.34	9.09	9.20	9.69	9.44	9.54
,,	'25	221D–3	,,	885	426	9 660	840	20.2	75	1.75	h++	9.06	8.71	9.45	9.07	9.31	9.75	9.37	9.62
,,	'25	221E–1	,,	885	443	11 780	2 060	20.2	74	1.74	h++	8.95	8.10	9.32	8.44	9.04	9.55	8.65	9.26
,,	'25	221D–2	,,	885	430	13 510	3 900	21.4	72	1.80	h++	7.83	6.18	8.16	6.44	7.32	8.41	6.64	7.55
,,	'25	221D–1	,,	886	400	9 310	1 250	20.8	74	1.79	h++	6.56	6.12	8.72	8.14	8.46	9.12	8.51	8.84
,,	'25	221D–3	,,	886	420	9 410	1 490	19.8	73	1.80	h++	7.74	7.10	8.06	7.39	7.74	8.35	7.65	8.01
,,	'25	221F–2	,,	887	320	8 970	580	19.5	75	1.71	h++	8.64	8.40	11.48	11.17	11.41	12.56	12.20	12.46
,,	'25	221F–1	,,	887	335	13 670	3 340	19.5	69	1.90	h++	8.59	7.24	11.43	9.63	10.09	12.38	10.44	10.93
Ritzman	'38		Holstein	H-I	555	14 940	1 390	34.1	55	2.40	h	14.42	13.80	11.69	11.19	10.67	11.44	10.96	10.45
,,	'38		,,	,,	594	15 050	1 590	34.9	50	2.79	h	13.43	12.76	10.88	10.34	9.24	10.51	10.00	8.93
,,	'38		,,	H–IV	670	15 170	720	26.5	62	2.31	h	13.67	13.40	11.09	10.87	10.70	10.46	10.24	10.08
,,	'38		,,	,,	629	16 920	2 930	34.1	58	2.45	h	12.53	11.36	10.16	9.21	8.11	9.70	8.79	7.74
,,	'38		,,	H-V	621	14 180	200	26.5	59	2.33	h	14.37	14.29	11.65	11.59	11.53	11.16	11.09	11.04
,,	'38		,,	,,	562	11 740	240	9.0**	75	1.78	d+	12.76	12.66	10.34	10.26	10.30	10.10	10.02	10.07
,,	'38		,,	,,	577	13 600	530	34.9	56	2.56	h	14.02	13.79	11.37	11.19	10.92	11.05	10.87	10.61
,,	'38		,,	,,	621	16 550	1 090	30.2	61	2.46	h	15.34	14.90	12.44	12.09	11.66	11.91	11.57	11.17
,,	'38		,,	,,	632	17 440	1 300	30.2	60	2.40	h	15.70	15.18	12.73	12.30	11.87	12.14	11.74	11.33
,,	'38		,,	,,	599	15 390	1 300	32.8	60	2.57	h	14.20	13.66	11.51	11.08	10.44	11.10	10.68	10.06
,,	'38		,,	,,	564	12 400	1 720	34.1	56	2.59	h	10.79	10.04	8.75	8.14	7.22	8.54	7.95	7.05
,,	'38		,,	,,	622	18 480	3 060	34.7	66	2.51	h	14.04	12.80	11.38	10.38	9.07	10.90	9.94	8.68
,,	'38		,,	H-VI	473	15 480	1 700	32.8	59	2.65	h	12.79	12.13	13.32	12.64	11.49	13.47	12.77	11.61
Armsby	'11	207–4	Ab. Angus	A	514	10 150	650	30.0	63	2.06	h	8.55	8.31	8.91	8.66	8.62	8.86	8.61	8.57
,,	'11	200–1	,, ,,	,,	404	10 740	1 410	22.9	68	1.94	h++	9.65	9.02	10.05	9.40	9.50	10.48	9.80	9.90
,,	'11	207–1	,, ,,	,,	499	12 070	1 900	21.0	70	1.83	h++	8.67	7.96	9.03	8.29	8.61	9.03	8.29	8.61
Forbes	'27	237–12	,, ,,	36	318	8 170	370	22.8	65	1.90	h+	8.19	8.03	10.89	10.68	10.73	11.91	11.68	11.74
,,	'28	238–8	,, ,,	36	481	10 310	470	18.0	77	1.85	h+	9.46	9.28	9.85	9.66	9.73	9.93	9.74	9.82
,,	'28	238–4	,, ,,	36	490	14 680	2 820	18.0	76	1.89	h+	9.89	8.81	10.30	9.18	9.50	10.35	9.22	9.54
,,	'27	237–6	,, ,,	47	357	8 730	170	31.2	64	2.45	d	8.33	8.26	11.07	10.98	10.88	11.84	11.75	11.64
,,	'28	238–9	,, ,,	47	499	11 680	420	33.5	60	2.66	h	10.59	10.43	11.03	10.86	10.58	11.03	10.86	10.58
,,	'27	237–4	,, ,,	47	362	8 750	460	23.1	72	1.84	d	7.80	7.63	10.37	10.14	10.23	11.06	10.81	10.91
,,	'27	237–13	,, ,,	47	347	9 080	720	23.0	68	1.89	h+	7.98	7.69	10.61	10.24	10.34	11.41	11.01	11.42
,,	'28	238–7	,, ,,	47	485	10 570	1 180	17.9	77	1.87	h+	8.53	8.08	8.89	8.42	8.57	8.94	8.46	8.62
,,	'27	237–2	,, ,,	47	360	10 800	1 550	21.6	73	1.60	d	8.12	7.53	10.81	10.02	10.82	11.53	10.70	11.56

Table 3, continued

Reference (see list at the end of this table)		Exp. or period number	Breed	Name	Body weight (kg)	Metabol. energy (kcal)	Gain (kcal)	Crude fibre in feed (% in dm)	Digestib. of om (%)	c_s	Ration	M_{m,W_o}, therms $p = 0.8$ W_o=350, 475, 650, 800		$M_{m,500}$, therms $p = 0.8$			$M_{m,500}$, therms $p = 1.0$		
												$c = 1.61$	$c = 2.00$	$c=1.61$	$c=2.00$	$c=c_s$	$c=1.61$	$c=2.00$	$c = c_s$
Forbes	'28	238–3	Ab. Angus	47	495	15 560	3 870	18.0	76	1.91	h+	9.03	7.57	9.41	7.88	8.24	9.42	7.90	8.25
,,	'27	237–8	,, ,,	254	340	9 040	460	15.8	70	1.72	d	8.50	8.31	11.30	11.05	11.23	12.20	11.94	12.14
Fingerling	'32	V	Simmenthal	10	756	17 940	360	18.3	75	1.77	h++	18.16	18.01	12.48	12.38	12.44	11.48	11.38	11.44
,,	'32	IX	,,	10	786	18 670	380	20.6	74	1.83	h++	18.31	18.16	12.58	12.47	12.51	11.49	11.39	11.43
,,	'32	I	,,	10	669	19 120	1 700	16.9	76	1.82	h++	16.01	15.36	12.98	12.45	12.70	12.24	11.74	11.97
,,	'33	XV	,,	10	849	22 060	1 880	23.0	72	1.88	h++	18.14	17.44	12.46	11.99	12.14	11.21	10.78	10.91
,,	'32	IV	,,	10	753	23 400	2 790	15.4	77	1.78	h++	19.83	18.69	13.63	12.85	13.29	12.56	11.83	12.24
,,	'32	VI	,,	10	777	23 350	2 870	21.4	74	1.84	h++	19.16	18.02	13.16	12.38	12.70	12.05	11.34	11.64
,,	'31	I	,,	25	762	15 440	1 010	29.2	67	2.07	h	14.37	13.96	9.86	9.58	9.53	9.06	8.80	8.76
,,	'31	III	,,	25	758	15 850	1 070	29.2	68	2.09	h	14.75	14.31	10.13	9.83	9.76	9.32	9.05	8.98
,,	'31	II	,,	25	768	19 570	2 900	28.6	69	2.06	h+	15.39	14.22	10.57	9.76	9.64	9.70	8.96	8.85
Forbes	'25	209–4	Hereford	F	321	11 400*	330*	31.7	—	—	h	11.65	11.51	15.50	15.32	—	16.93	16.73	—
,,	'25	209–1	,,	,,	301	11 360*	2 370*	14.2	—	—	h++	8.52	7.47	11.33	9.94	—	12.53	11.00	—
Armsby	'17	211–1	,,	G	389	12 050	340	33.8	61	2.27	h	10.57	10.45	14.07	13.91	13.80	14.78	14.61	14.49
,,	'11	200–2	Scrub	B	310	10 910	1 180	20.7	66	1.90	h++	9.94	9.43	13.21	12.53	12.71	14.53	13.79	13.98
,,	'11	207–2	,,	,,	386	14 090	2 570	18.9	73	1.80	h++	9.21	8.28	12.24	11.01	11.64	12.89	11.59	12.25

* = corrected for N-equilibrium
** = estimated

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TABLE 12. Data from the experiments and the requirements of metabolizable energy for maintenance at 500 kg. body weight ($M_{m,500}$) of the animals

																	$M_{m,500}$	
Exp. nr.	Animal	Weight (kg)	Feed (kcal)	Faeces (kcal)	Digested (kcal)	Urine (kcal)	Methane (kcal)	Metabol. energy (kcal)	Milk (kcal)	Heat exp. (kcal)	Energy balance (kcal) A	C-bal. (g)	N-bal. × 6.25 (g)	Energy bal. CN-method (kcal) B	$\frac{1}{2}$A + $\frac{1}{2}$B	B–A	Days before calving	p=0.8 c=1.43(—) c=1.61(+) (therms)	p=0.8 c=1.67(—) c=2.00(+) (therms)
R 1 I	Witschoft	496	36 850	9 740	27 111	1 654	3 033	22 424	6 161	15 590	+ 673	+ 96	— 84	+ 1 250	+ 961	577	225	11.71	
R 1 II	,,	485	36 850	9 366	27 484	1 661	3 081	22 742	5 885	15 406	+ 1 451	+ 137	— 33	+ 1 723	+ 1 587	272		11.64	
R 1 I	Zwartschoft	606	39 057	10 854	28 203	1 821	2 920	23 462	4 230	18 308	+ 924	+ 150	— 15	+ 1 865	+ 1 394	941	92	12.75	
R 1 II	,,	594	39 304	10 615	28 690	1 706	2 920	24 063	3 960	17 697	+ 2 406	+ 254	+ 78	+ 3 083	+ 2 744	677		11.94	
R 2 I	Annie 7	637	33 327	10 284	23 043	1 577	2 684	18 782		21 618	— 2 836	— 189	+ 25	— 2 358	— 2 597	478	55	18.54	
R 2 II	,, 7	616	33 306	10 530	22 776	1 558	2 646	18 572		20 295	— 1 723	— 114	+ 33	— 1 431	— 1 577	292		17.62	
R 3	,, 7	659	37 758	10 783	26 975	1 653	3 175	22 147		19 829	+ 2 318	+ 220	+ 150	+ 2 616	+ 2 467	298	34	14.57	
R 2 I	Alie 1	602	31 477	9 653	21 824	1 494	2 552	17 778		20 246	— 2 468	— 151	+ 1	— 1 871	— 2 170	597	39	18.00	
R 2 II	,, 1	609	31 469	9 451	22 017	1 522	2 646	17 849		20 638	— 2 789	— 177	+ 35	— 2 213	— 2 501	576		18.30	
R 3	,, 1	624	35 844	10 471	25 373	1 564	2 882	20 927		21 212	— 285	+ 23	+ 211	+ 131	— 77	416	18	17.63	
R 10 I	,, 1	570	25 175	7 315	17 861	1 311	2 241	14 309		14 380	— 71	+ 5	+ 18	+ 50	— 11	121	77	12.90	12.91
R 10 II	,, 1	565	25 175	7 326	17 850	1 320	2 175	14 354		14 121	+ 233	+ 39	+ 24	+ 462	+ 348	229		12.51	12.38
R 4 I	Clara	561	31 413	8 142	23 271	1 193	2 769	19 310		17 360	+ 1 950	+ 182	+ 128	+ 2 160	+ 2 055	210	58	14.58	13.85
R 4 II	,,	557	31 392	8 134	23 259	1 240	2 821	19 197		17 107	+ 2 090	+ 212	+ 127	+ 2 528	+ 2 309	438		14.20	13.37
R 4 I	Klaske	562	31 425	8 760	22 665	1 244	2 679	18 742		16 587	+ 2 155	+ 200	+ 112	+ 2 396	+ 2 275	241	52	13.73	12.92
R 8	,,	536	21 921	5 723	16 198	1 818	1 715	12 664		13 986	— 1 321	— 97	— 141	— 1 092	— 1 207	229	—	13.61	
R 5 I	Zwartkop 2	541	31 299	8 420	22 879	1 390	2 613	18 876		16 364	+ 2 512	+ 266	+ 112	+ 3 205	+ 2 859	693	65	13.41	12.36
R 5 II	,, 2	548	31 299	8 876	22 423	1 319	2 570	18 534		15 814	+ 2 720	+ 275	+ 100	+ 3 332	+ 3 026	611		12.69	11.60
R 6	,, 2	590	37 312	10 944	26 368	1 536	2 927	21 906		19 496	+ 2 410	+ 308	+ 189	+ 3 671	+ 3 040	1 261	14	14.91	
R 5 I	Coba 6	511	30 008	7 945	22 063	1 345	2 604	18 114		15 176	+ 2 939	+ 296	+ 45	+ 3 636	+ 3 287	697	69	12.59	11.33
R 5 II	,, 6	511	30 008	7 892	22 115	1 311	2 627	18 177		15 380	+ 2 796	+ 274	+ 64	+ 3 344	+ 3 070	548		13.01	11.84
R 6	,, 6	551	35 639	10 072	25 567	1 504	2 851	21 212		18 536	+ 2 676	+ 351	+ 162	+ 4 222	+ 3 449	1 545	18	14.50	
R 7 I	Eke 42	446	22 504	6 265	16 239	1 250	1 970	13 018		12 130	+ 889	+ 97	— 28	+ 1 216	+ 1 052	327	69	12.42	11.97
R 7 II	,, 42	448	22 504	6 377	16 127	1 222	2 074	12 831		12 136	+ 695	+ 85	— 24	+ 1 071	+ 883	376		12.46	12.09
R 7 III	,, 42	453	21 827	6 052	15 775	1 218	1 980	12 577		12 197	+ 380	+ 63	— 16	+ 795	+ 588	415		12.60	
R 9	,, 42	486	24 237	7 658	16 578	1 333	2 001	13 245		14 232	— 987	— 62	+ 45	— 795	— 891	192	11	14.85	
R 7 I	Jansje	490	23 548	6 607	16 941	1 284	2 027	13 630		12 813	+ 818	+ 99	+ 21	+ 1 213	+ 1 016	396	75	12.18	11.78
R 7 II	,,	493	23 548	6 922	16 627	1 253	2 046	13 328		13 334	— 6	+ 35	+ 22	+ 416	+ 205	421		13.15	13.07
R 7 III	,,	493	22 839	7 086	15 753	1 155	1 928	12 670		12 872	— 202	— 7	+ 35	+ 66	— 68	268		12.91	
R 9	,,	505	25 197	7 768	17 429	1 428	2 016	13 985		14 631	— 646	— 22	+ 73	— 331	— 488	314	17	14.57	
R 8	Lamkje	494	19 716	4 937	14 779	1 556	1 616	11 607		13 669	— 2 062	— 173	— 85	— 2 083	— 2 072	— 21	44	14.72	
R 8	Roosje	505	20 614	5 364	15 250	1 647	1 479	12 124		13 218	— 1 094	— 74	— 56	— 875	— 985	220	78	13.41	
R 10 I	Klaasje 3	530	24 227	6 586	17 641	1 297	2 128	14 216		12 642	+ 1 574	+ 134	+ 19	+ 1 644	+ 1 609	70	95	11.10	10.49
R 10 II	,, 3	539	24 227	6 921	17 306	1 292	2 046	13 967		12 440	+ 1 528	+ 124	+ 31	+ 1 511	+ 1 519	— 17		10.85	10.30
R 12 I	Kee 2	606	26 238	8 085	18 154	1 377	2 166	14 610		14 955	— 344	— 20	+ 12	— 262	— 303	82	62	12.89	12.95
R 12 II	,, 2	603	26 238	7 973	18 266	1 318	2 334	14 613		15 161	— 548	— 51	+ 26	— 656	— 602	— 108		13.32	13.44
R 12 I	Alke	533	23 866	7 511	16 355	1 162	1 876	13 317		14 650	— 1 332	— 97	+ 38	— 1 230	— 1 281	103	57	14.41	14.69
R 12 II	,,	534	23 866	7 228	16 638	1 186	2 001	13 452		14 106	— 655	— 38	+ 38	— 494	— 575	161		13.54	13.67
R 13 I	Betsy	521	24 159	7 550	16 610	1 215	1 987	13 408		14 432	— 1 024	— 76	— 21	— 961	— 993	63	90	14.36	14.58
R 13 II	,,	525	24 159	7 605	16 554	1 247	1 890	13 417		14 268	— 851	— 48	+ 0	— 596	— 723	256		13.90	14.06
R 14	,,	556	28 020	9 262	18 758	1 528	2 077	15 153		17 434	— 2 282	— 158	+ 45	— 1 994	— 2 138	288	39	16.73	
R 13 I	R. Willy	624	27 103	8 541	18 562	1 210	2 202	15 151		15 580	— 430	— 11	+ 55	— 181	— 305	249	81	13.06	13.13
R 13 II	,, ,,	626	27 103	8 570	18 533	1 218	2 138	15 178		16 540	— 1 363	— 85	+ 79	— 1 110	— 1 236	252		14.17	14.42
R 14	,, ,,	651	30 644	10 440	20 204	1 480	2 302	16 422		17 604	— 1 182	— 60	+ 119	— 832	— 1 007	350	30	14.47	