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Title: Evaluation of the True Metabolizable Energy Bioassay for Poultry, and Determination of Energy Values of Selected Feedstuffs

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Several experiments were conducted with adult, S.C.W.L. roosters, and in one case Wrolstad poults, to evaluate the True Metabolizable Energy (T.M.E.) bioassay, and to determine the T.M.E. values of ten selected feedstuffs. The experimental procedure used was described by Sibbald (1976a, Poultry Sci., 55:303). Examination of the digestive tracts of roosters following 24, 36 and 48-hour starvation periods showed the presence of undigested feed and grit particles in the gizzards. No correlation was found between body weights and endogenous energy ($FE_m + UE_e$) excretion of the fasted birds. Screening of the dried excreta samples did little to improve the correlations, but reduced the standard errors of $FE_m + UE_e$ outputs.

In two experiments, the effect of free-choice grit intake on $FE_m + UE_e$ outputs and T.M.E. values of corn and sunflower seed

products; ground whole seeds and hulls were measured. Grit feeding exerted only a weight contribution effect on excreta outputs of control and fed birds, and yielded misleading fecal gross energy values. However, the T.M.E. values, with the exception for the dehulled sunflower seeds, were not influenced by the presence of grit in digestive systems of the assay birds. The significant differences observed between the inorganic residue (ash) contents of fecal samples from control and fed birds, was assumed to be the cause of obtaining a non-significant abrasive effect of grit on T.M.E. values. The screening process, when applied to dried excreta samples of control birds, was found to be useful in reducing the standard errors associated with $FE_m + UE_e$ outputs.

With the exception of the questionable value for corn oil, the T.M.E. values obtained from trials, where the gelatin capsules were used as an alternative method of administering the feed ingredient, were comparable to those obtained from regular force-feeding technique.

There were no significant differences between the adult roosters and 10-week old poults, in terms of utilizing the T.M.E. value of three varieties of triticale. Extending the excreta collection period to 48 hours did not effect the T.M.E. value of triticale varieties significantly indicating a complete passage through the digestive tract of roosters and poults, within 24 hours. Whereas soybean meal, meat and bone meal and fish meals required more than 24 hours to pass through the birds.

The T.M.E. values of ten selected feedstuffs are presented.

The T.M.E. value of corn, when assayed as a control ingredient throughout the course of this study, did not show any trend associated with the seasonal changes. Adult, S.C.W.L. roosters were not susceptible to short-term changes in light and temperature regimens.

Evaluation of the True Metabolizable Energy Bioassay for Poultry, and Determination of Energy Values of Selected Feedstuffs

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Typed by Donna Lee Norvell-Race for Sacit Faruk Bilgili

Dedicated to My Brother

• *Sedat Bilgili* •

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I. INTRODUCTION

The energy requirement of birds is met by the chemical energy contained in the feed. Birds eat to satisfy their energy needs; consequently, nutrients are included in diets in proportion to available energy. Since protein and energy are the most expensive nutrients in practical rations, accurate and precise knowledge of the available energy content of feedstuffs is necessary to formulate the most economical, least-cost rations and to achieve profitable production levels.

There is a high degree of heterogeneity in the methods for the determination, calculation and the designation of energy values for animals. Currently, the Apparent Metabolizable Energy (A.M.E.) system is the most widely used method for evaluating poultry feedstuffs for available energy. However, since Sibbald (1976a) developed a bioassay for True Metabolizable Energy (T.M.E.), a considerable amount of research has been conducted to investigate its applicability.

Sibbald's method has several advantages over the previous A.M.E. assays; it is simple, rapid and inexpensive. Furthermore, the T.M.E. system can be extended to permit measurement of bioavailable amino acids (Likuski and Dorrel, 1978; Sibbald, 1979c,f)

and lipids (Sibbald and Kramer, 1980a) in feedstuffs. The assay has already been endorsed by several investigators, organizations and feed manufacturers. Boldaji et al. (1981) and Roush (1979), from Oregon State University, Poultry Science Department, have recently used this assay. However, despite its reported flexibility, reproducibility and data quality, there is a need for additional information before the T.M.E. system can be fully accepted.

Therefore, the purpose of this study was to evaluate some factors that may effect the assay and to determine the T.M.E. values of some selected feedstuffs available in the Pacific Northwest.

II. REVIEW OF LITERATURE

According to Brody (1964), bioenergetics may be said to have been initiated by Lavoisier in 1777, when he applied the first law of thermodynamics to animals 65 years before the formulation of the law by R. J. Mayer. Later, the studies of Rubner in 1885 and 1902 on dogs, Atwater and Benedict in 1903 on man and Armsby in 1903 on cattle proved the applicability of the first law by comparing the heat of oxidation of nutrients in vivo and in vitro.

Since energy cannot be created or destroyed but can only be transformed from one state to another, determination of heat production of animals may be used to (1) determine the energy content of feedstuffs that is available to the animal for maintenance and growth and (2) estimate the energy requirement of animals.

Armsby (1917) indicated that the measurement of the true value of feeds as sources of energy to animals involves the determination of the energy content of the feed and of all losses of energy during its utilization by the animal. If the latter are subtracted from the former, the "net-energy" value of the feed is obtained. Armsby (1916) defined and formulated net-energy as the maximum proportion of the feed energy convertible to work, milk, eggs, meat and maintenance. Armsby calculated the net-energy in terms of therms (one therm equals 1000 kcal), by multiplying each pound of digestible organic matter by factors which varied according to the composition of feedstuffs and by correcting its total dry matter.

Fraps (1916a) reported a method for calculating the net-energy values of feeds from their chemical composition by using several production coefficients (productive energy values of the digestible nutrients, calories per gram). Later (1916b, 1925, 1928, 1929, 1932a,b), Fraps reported the energy production coefficients of feedstuffs for ruminants, sheep and pigs.

Fraps (1928) reviewed several early reports (Kalugin in 1896; Von Knierem in 1900; Brown in 1904; Bartlett in 1911; Gerhartz in 1913; Hari in 1917; Hari and Kriwuscka in 1918) regarding digestion trials in poultry despite the difficulties associated with combined excretion of feces and urine. Investigators mainly used one of the three methods to determine the digestion coefficients. The most extensively used method was to estimate the uric acid and ammonia content of the mixed feces and subtract it from the total. This method assumed that the urinary products consist entirely of uric acid and ammonia. The second method, which was used by Paraschtschuk in 1902, Lehman in 1904, Voltz in 1909 and Katayama in 1924 required operating on the birds to make an artificial anus to separate the urine from undigested feed residues. Later Katayama in 1924 and Halnan in 1926 used the average factors obtained by the operative method to correct the results from the analysis of mixed excreta. Fraps (1928) conducted extensive digestion experiments with poultry, in order to obtain the necessary digestion coefficients for the calculation of net-energy values. He modified the first method by

maintaining the birds in wire cages and by using excreta collection trays. The digestability and production coefficients of 63 poultry feeds were reported by Fraps (1928). Fraps and Carlyle (1939a) published a new method for determining the efficiency of feed by substituting an ingredient for corn in a basal diet. The procedure was based on the difference in energy gain as a result of feeding one group of chickens the experimental diet ad libitum, and a second group restricted to about 50 percent of ad libitum. The energy retention as determined by the initial and final body composition (protein and fat) and by using conversion factors (5.66 cal/gm for protein and 9.35 cal/gm for fat). Simultaneous equations based on energy intake, energy gain and weight gain enabled them to calculate the maintenance energy requirement and the net-energy of the diets. The net-energy values of several feedstuffs for poultry have been published by Fraps and Carlyle (1939b,c, 1941, 1942). Later, with the use of digestibility data and assumed "productive energy values of the effective digestible nutrients," Fraps (1946) calculated the net-energy values of feed ingredients for poultry from their average composition data. These values have been used extensively and found to be useful guides for comparing the energy content of feedstuffs. However, practical difficulties regarding the excretion of both feces and urine via the common cloaca, prevented the derivation of precise digestibility values.

Axelsson (1939) and Halnan (1951) have argued strongly against

the use of net-energy values. Despite the modifications on techniques by Bolton (1955) and Ariyoshi and Morimoto (1956), experiments conducted later by Davidson et al. (1957) and Hill and Anderson (1958) showed that the determined net-energy value of a diet varied considerably from the values calculated by using Fraps' energy coefficient tables. Davidson (1963) criticized the net-energy measurements because of the fact that the maintenance energy requirement of growing chickens was not proportional to the body weight, which was assumed by Fraps. Hill and Anderson (1958) showed that by expressing body weight to the 0.7 power rather than body weight alone, the variation in productive energy of diets was marginally reduced. More serious criticism of Fraps' work was that frequently the metabolizable energy of experimental diets was not determined, but calculated from the digestible nutrients. Also there is a possibility that chickens given restricted amounts of feed initially lose body weight (Davidson et al., 1957) but the contribution of tissue energy to energy balance during this period of low feeding is unknown.

Determination of the net-energy of poultry rations requires specialized equipment and expertise in its operation. Only a limited number of calorimetric measurements can be made each year. Above all, the net-energy of a diet is not constant but will vary with the level of intake (Burlacu et al., 1969), with ambient temperature (O'Neil et al., 1971), and according to whether the energy is being used to promote egg production (Waring and Brown, 1965, 1967;

Grimbergen, 1970; Burlacu and Baltac, 1971) or the synthesis of meat and fat (Peterson, 1970). When Davidson et al. (1957), Potter et al. (1959a,b), Hill and Anderson (1958) and Davidson (1963) concluded that metabolizable energy is more precisely measured than the productive energy values, many researchers turned to the evaluation of feeds in terms of metabolizable energy.

Metabolizable Energy

Metabolizable Energy (ME) is the difference between the gross energy of the feed eaten and the gross energies of feces and urine. In poultry, the energy losses through the gases of fermentation are negligible and ignored. ME is evaluated either directly by means of feeding trials or indirectly through digestibility measurements. The excretion of feces and urine together in poultry made the direct method technically very simple. Apart from the determination of the heats of combustion of samples of feed and excreta, it requires only the quantitative collection of excreta output and minimum chemical analysis. The ME values of several feedstuffs for poultry have been reported by several investigators using the direct method (Mitchell and Haines, 1927a,b, 1930; Fraps et al., 1940; Olsson, 1950; Halnan, 1951; Anderson, 1955; Carpenter and Clegg, 1956) and by others (Fraps and Carlyle, 1941, 1942; Axelsson and Erikson, 1951; Titus, 1955) indirectly using the available digestible data. There are more common ME assays, described in detail by Hill et al. (1959), Potter

et al. (1959a), and by Sibbald and Slinger (1963a), whereby the ME value of the test ingredient is calculated from the difference in the measured ME values of diets in which the test ingredient is supplied at two or more levels. Other methods have also been introduced (Metta and Mitchell, 1954; Bernstein et al., 1956) but their application requires the use of appropriate statistical procedures.

In order to reduce the error involved in direct ME assays, regarding the quantitative collection of excreta output per unit weight of feed eaten, several index methods have been proposed (Olsson, 1950; Kane et al., 1950; Dansky and Hill, 1952). Hill (1960) and Sibbald and Slinger (1963a) used chromium sesquioxide (Cr_2O_3) as an index substance. The advantages of using an indicator are that it eliminates the need for measurement of feed intake and total collection of excreta. Sibbald et al. (1960) considered the indicator method to be more precise, whereas Halloran (1972) suggested that the Cr_2O_3 analysis was the major source of error in ME determinations.

ME values are corrected to nitrogen-equilibrium to eliminate the variations arising from variable retention. The proportion of the dietary protein nitrogen retained in the body decreases with age. Usually the correction for nitrogen-retention is made on the assumption that the oxidation of tissue protein will yield energy containing compounds. Hill and Anderson (1958) applied 8.22 Kcal/g nitrogen, whereas Titus et al. (1959) used the factor 8.73 Kcal/g. However, the use of these different factors exert only a minor effect on

ME values.

All the ME assays have yielded useful energy values and ME has become the generally accepted method of expressing the available energy content of feedstuffs and energy requirements in poultry nutrition. However, the ME and nitrogen-corrected ME (ME_n) values are often criticized because they may be influenced by many factors, such as: method of determination (Matterson et al., 1958; Kalmbach and Potter, 1959); strain and species (Sibbald and Slinger, 1963b; Slinger et al., 1964; Foster, 1968b; Bayley et al., 1968; Proudman et al., 1970; March and Biely, 1971; Fisher and Shannon, 1973); age (Carew et al., 1963; Bayley et al., 1968; Zelenka, 1968; Lodhi et al., 1969; Rao and Clandinin, 1970; Burlacu and Baltac, 1971); steam pelleting (Blakely et al., 1965; Bayley et al., 1968); antibiotic supplementation (March et al., 1972); toxicants (Vohra, 1972); and deficiency of B-vitamins (Lockhart et al., 1966). Furthermore, the direct ME bioassay is slow, equipment and facilities are not widely available and labor requirements are high (Sibbald, 1975a).

Development of alternative methods, such as the chick growth assays proposed by Yoshida and Morimoto (1970) and Squibb (1971), have not been widely accepted, because of the high variability and labor requirement. More recently, DeGroote (1974) transformed ME values into net-energy by the use of conversion coefficients for carbohydrates (75%), fat (90% and proteins (60%), but this procedure has not gained general acceptance.

True Metabolizable Energy System

It is well known that the excreta of animals contain energy which is not derived directly from the diet. In 1962 and 1966, Harris illustrated in a "Ture Energy Distribution Scheme" that the Fecal Metabolic Energy (FE_m) and Endogenous Urinary Energy (UE_e) were part of the net-energy requirement and should be considered as part of the maintenance energy (Figure 1). The FE (fecal energy of metabolic origin, derived from abraded intestinal mucosa, bile, digestive juices, etc.) and UE_e (urinary energy of endogenous origin, derived from the products of tissue catabolism) should be subtracted from Metabolizable Energy (ME) to obtain the "True Metabolizable Energy." The absence of practical methods for measuring these energy losses prevented researchers from deriving true metabolizable energy (TME) values.

Later, Guillaume and Summers (1970) observed and estimated the combined FE_m and UE_e losses of adult roosters by measuring the average energy content of feces and urine excretion of fasting birds. They concluded in their studies that these losses should be taken into account for correcting ME values when feed intake is close to the maintenance requirement.

Sibbald (1975a) observed that the energy in the excreta of roosters decreased in a linear manner as the feed intake decreased. It was noted that the intercept of the regression line showed a small but significant amount of energy. The slope of the regression line

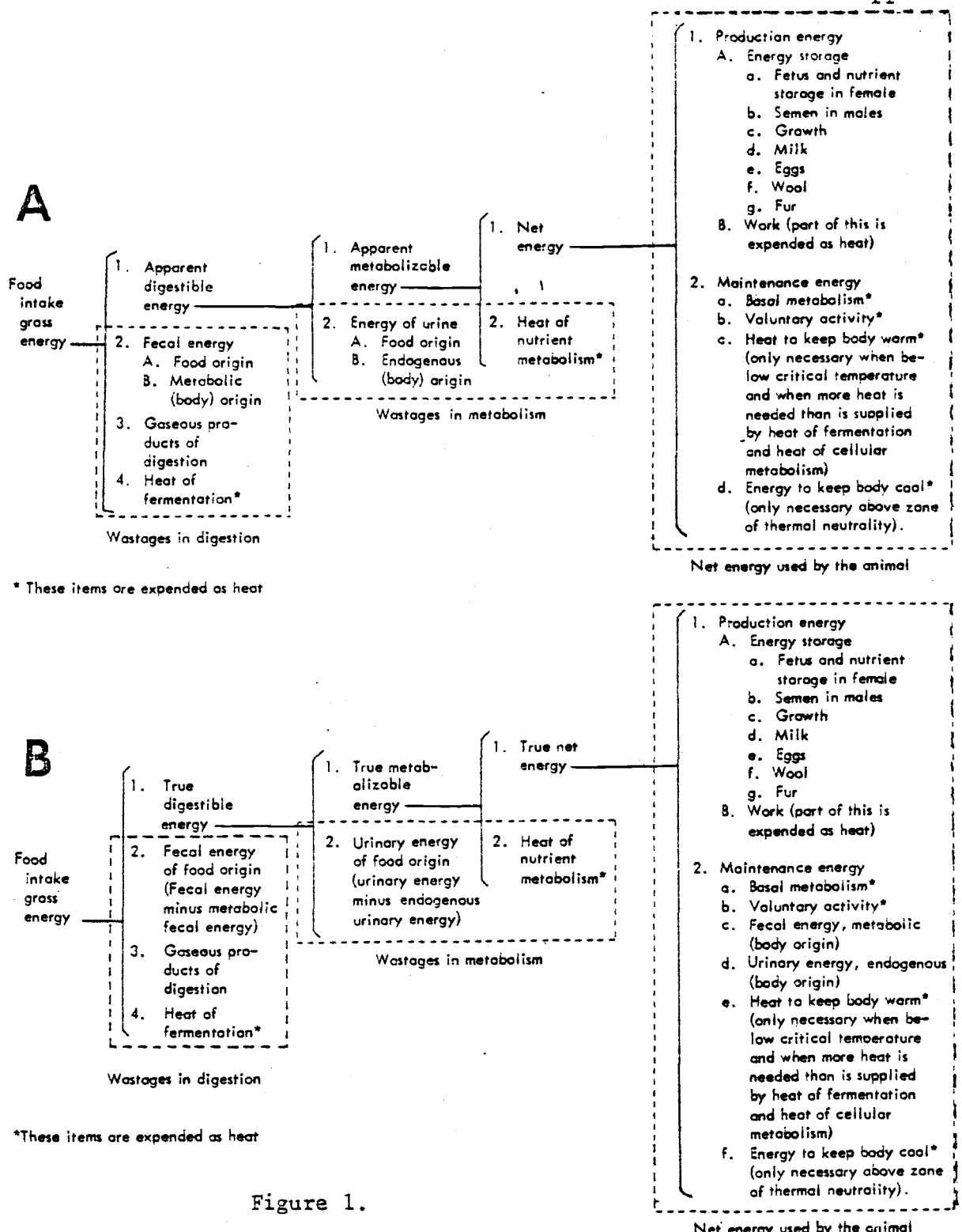

Figure 1.

The utilization of energy (scheme to show where various portions originate). Since some of the fecal energy is of metabolic origin and some of the urinary energy is of endogenous origin, the scheme shown in 1A has been modified to give 1B. Since the metabolic energy and endogenous energy are part of the net energy requirements under this scheme these items are shown as part of the maintenance energy (Harris, 1966).

gave the estimation of the $FE_m + EU_e$, when the level of feed intake was zero. The T.M.E. value of wheat, when calculated, was found to be independent of the variation in intake, whereas Apparent Metabolizable Energy (AME)--apparent because it does not take $FE_m + UE_e$ losses into account--values were increased in a curvilinear manner with intake.

By assuming that there is a linear relationship between feed intake and excreta energy output, Sibbald (1976a) developed a simple and rapid bioassay for T.M.E. The assay involves force-feeding a known quantity of feed in the crop of a starved adult rooster and collecting the excreta resulting therefrom. The effect of level of feed intake is controlled when the excreta is corrected for $FE_m + UE_e$. Subsequent experiments have provided additional information about the variables that may influence the T.M.E. assay.

Sibbald (1976c) performed three experiments to measure the effects of the type of assay bird on the T.M.E. values of feedstuffs. There was no sex effect on T.M.E. values. When yellow corn, soybean meal, wheat sorts and fish meal were assayed using S.C.W.L. roosters, S.C.W.L. laying hens of different strain and adult Small White Turkey hens, the only significant difference was for the values for soybean meal measured with the turkey hens. In the final experiment, wheat bran and a laying hen diet were assayed with roosters, laying hens, broiler hens and turkeys. There were no differences attributable to the type of assay bird. However, for

routine assay work the adult (S.C.W.L.) cockerel is preferred, because it tends to be in metabolic equilibrium, has good livability and has sufficient feed capacity to minimize experimental errors. Meat-type males become heavy and obese and have higher mortality. Laying hens are not suitable because the starvation period, followed by suboptimal feed input, often causes soft-shelled eggs that break and contaminate excreta. Chicks and growing birds are not considered due to the fact that they have less feed capacity and lose down feathers, which contaminate the excreta (Sibbald, 1979g).

Dale and Fuller (1980a) later reported that there was good agreement between the T.M.E. values of yellow corn, dehulled soybean meal, corn gluten meal, fish meal and poultry by-product meal measured with S.C.W.L. roosters, six-week-old poults (Large White) and six-week-old broiler males (Cobb), with the exception of the slightly lower values obtained from broilers. The same investigators (Dale and Fuller, 1980b) also observed a consistent relationship between the A.M.E. and T.M.E. contents of five broiler diets, determined during the final week of a 21-day growth study with broiler chicks.

Halloran (1980) reported ME_n and T.M.E. values on identical samples of corn, dehydrated alfalfa meal and menhaden fish meal, using two-week-old Hubbard males. He indicated that TME:ME_n ratios were in agreement with the previous published results.

Sibbald (1978c) conducted two experiments to measure the effect

of age of the assay bird on the T.M.E. values of four diets. A basal diet supplemented with 10 percent of beef tallow, rapeseed oil and wheat sorts assayed with S.C.W.L. males aged 29, 43 and 57 days, together with adults. Although significant age effects were observed within certain diets, they were not consistent and attributed to difficulties of excreta collection voided by the growing chicks and to the contamination of excreta with down and scales. Many of the age effects were explained by the atypical value of a single treatment group within the diet group. It was observed that the T.M.E. values for adult birds on the same diet were less variable than those for chicks.

Shires et al. (1980) reported that T.M.E. values of soybean meal and rapeseed meal for four-week-old S.C.W.L. chicks were 104 percent and 90 percent of the values obtained by S.C.W.L. roosters. The authors suggested that, with the exception of high-glucosinolate rapeseed meal, T.M.E. values obtained with adult roosters could be used in the formulation of diets for young growing birds.

The first step in the assay involves starving the birds to empty their alimentary canals of feed residues. A starvation period of 24 hours is adequate but, according to Sibbald (1976d), lengthening the starvation period from 24 to 96 hours by 24-hour intervals had no significant effect on T.M.E. values. Shires et al. (1979) also reported that a starvation period of 12 hours before force-feeding resulted in a significant decrease of four percent in the

T.M.E. value of corn, whereas fasting periods of 24 or 48 hours had no significant effect.

The bioassay for T.M.E. also involves measuring the $FE_m + UE_e$ losses of one or more unfed birds in each replication (Sibbald, 1976a). The precision of the T.M.E. assay depends on accurate measurement of $FE_m + UE_e$ losses of control birds. The rate of excretion of $FE_m + UE_e$ by adult S.C.W.L. roosters decreases during the period of starvation prior to the initiation of excreta collection (Sibbald, 1976a; 1979a). It is, therefore, essential that the duration of both the starvation period and experimental period is identical for control and force-fed birds.

Sibbald and Price (1978) analyzed the collection of 300 $FE_m + UE_e$ losses of adult S.C.W.L. roosters from 38 experiments conducted over three years. The $FE_m + UE_e$ values ranged from 5.97 to 16.57 kcal/bird per 24 hours. Multiple regression analysis showed that 23 percent of the variation could be explained by the body weight and weight change, and there was no evidence of any relationship between the year, or the time of year, and the magnitude of the energy loss. Shires et al. (1979) found that the output of excreta energy tended to vary with body weight and suggested that the assay birds be paired on the basis of body weight. They also reported that the protein content of the diet fed prior to fasting affected $FE_m + UE_e$. This latter observation is also supported by the work of Sibbald (1980c) who observed that amino acid excretion by starved birds

varied with the composition of the preceding diet.

Teneseca and Sell (1978) reported that when a totally indigestible material (silica gel) was fed to adult roosters up to 13.68 g. per rooster $FE_m + UE_e$ increased with increasing intake of silica gel. When corn was fed with silica gel, the apparent digestibility of the grain decreased. They concluded that T.M.E. of a feedstuff may change when the feedstuff is fed in combination with an indigestible of poorly digested material. Later, Sibbald (1980c) conducted two experiments to measure the effects of inert materials on $FE_m + UE_e$ excretions. In the first experiment, a laying hen diet and corn supplemented with up to 4 g. cellulose were fed. The T.M.E. values of the laying hen diet and corn were unchanged with the presence of cellulose, and cellulose did not influence the $FE_m + UE_e$ output. In the second experiment, four grains and four levels of sand (0, 1, 2, 3 g./bird) were used. Feeding sand had no effect on gross energy outputs. When the T.M.E. values were determined for the grains, a number of significant differences were observed, but there was no evidence that the sand altered the T.M.E. values.

Sibbald and Price (1980) investigated the variability in $FE_m + UE_e$ losses of adult cockerels. They fed the birds alternatively with one of two samples of dehydrated alfalfa meal, then starved them to serve as a negative control. Each bird was fed twice and served as a control twice, thus allowing them to estimate the $FE_m + UE_e$ from the same bird, at a different period, or from a different

bird at the same period. The correlation of the first observation with the second observation for each bird was 0.744 (44df.). Variation in body weight did little to explain the variation in FE_m + UE_e ($r = 0.69$,90df). They indicated that the most precise T.M.E. estimates were obtained by using each bird as its own negative control. Using a group mean value for FE_m + UE_e was found to be somewhat inferior, but the decrease in precision was proposed to be offset by reduced time and cost. The T.M.E. values provided additional evidence that the FE_m + UE_e output was largely characteristic of the bird.

The usual procedure is to assay several feedstuffs simultaneously, to minimize the work, by using the same control group. Sibbald (1979g) proposed using the formula:

(Number of test materials + 1) x the number of replications, to plan the number of birds required.

Birds are force-fed in order to insure the amount and time of the food entering the bird. The amount of feed input depends upon the size of the bird and the form, availability and nature of the sample feedstuff. Sibbald (1977d) measured the T.M.E. value of a diet at levels of feed input ranging from 10 to 100 g. by 10 g. increments. At feed input levels above 40 g. per bird there was regurgitation which tended to increase in incidence and severity with the level of input. The standard errors of mean T.M.E. values decreased as the level of input increased from 10 to 40 g. per bird.

Therefore, it was concluded that to obtain maximum precision in T.M.E. measurements, the optimum level of feed input should be approximately 40 g. of pelleted feed when the assay bird is an adult S.C.W.L. rooster. However, later Sibbald (1977f) indicated that when the feed is not pelleted, the amount of feed input should be reduced to about 25-30 g. per bird, because of the difficulties associated with force-feeding. Farrel (1978, 1980) discussed the effect of low levels of feed input and proposed a new and rapid method for determining the ME values of feedstuffs. In his method, Farrel trained the adult roosters to consume their daily feed allowance of about 100 g. in one hour. Chami et al. (1980) used Farrel's method of training roosters to eat their feed in one hour, to calculate the T.M.E. values of grains, rather than force-feeding them.

The usual excreta collection period of T.M.E. assay is 24 hours. However, the feedstuffs with slow rates of passage (dehydrated alfalfa, peanut skins, meat and fish meals, rapeseed meal) require longer excreta collection periods (Sibbald, 1979a,b). Varying input levels and diluting with a carrier had minor effects on clearance times (Sibbald, 1979d). It is recommended that the excreta collection period be extended beyond 24 hours for materials having slow rates of passage (Sibbald, 1979d). Muztar and Slinger (1979) reported that at feed input levels of 30 g. or more, a collection period in excess of 36 hours was required in order to obtain reliable T.M.E. values for rapeseed meals.

In order to test the possibility that a change in water intake would accelerate the rate of clearance time of alfalfa residues, Sibbald (1980b) fed 30 g. dehydrated alfalfa supplemented with varying levels (up to 0.60 g.) of sodium chloride to adult cockerels. However, the rate of clearance of alfalfa residues were not affected by the supplemental salt. The T.M.E. value of the alfalfa was unchanged by the salt.

Between assays the birds were fed a maintenance ration ad libitum. The effect of the duration of the time interval between the assays on T.M.E. values was investigated by Sibbald (1978a). It was concluded that while a bird may be used for a second bioassay 24 hours after completion of the first assay, a rest period should be planned between the assays to permit recovery of body weight. Adult cockerels have been used for as many as 30 assays, spaced 14 days apart without any adverse effects (Sibbald, 1979g).

T.M.E. value is calculated using the formula:

$$\text{T.M.E. (kcal/g)} = \frac{(GE_f \times F_i) - (Y_f - Y_e)}{F_i}$$

where: GE_f is the gross energy of the feedstuff (kcal/g); F_i is the feed input (g); Y_f is the energy excreted by the fed bird and Y_e is the energy excreted by the unfed bird (Sibbald, 1979g).

The T.M.E. values of 235 samples representing 45 feedstuffs

(Sibbald, 1977b) as well as the T.M.E. values of the seeds of Brassica campestris, B. birta and B. napus (Sibbald and Price, 1977a), sprouted wheat (Wehner et al., 1980), rapeseed, rapeseed meal and oil (Sibbald, 1977c) are reported in literature.

There is evidence regarding the additivity of T.M.E. values (Sibbald, 1976a). Simple mixtures of wheat with fish meal and corn with soybean meal had T.M.E. values similar to those calculated from the T.M.E. values of the component parts. Later, Sibbald (1977a) observed that the T.M.E. values of 10 diets prepared from five feedstuffs were similar to those calculated from individual T.M.E. values. Dale and Fuller (1980a) formulated three mixtures using 50 percent ground yellow corn, 25 percent soybean meal and 25 percent of either poultry by-product meal, corn gluten meal or fish meal, and determined the T.M.E. values using adult roosters, broilers, and poults. Values for the ingredient combinations assayed with the three classes of birds showed less than three percent difference between predicted and determined values, indicating a satisfactory degree of additivity.

Since the determination of available energy content of fats and fat mixtures poses difficulties, Sibbald conducted several experiments in order to obtain valid T.M.E. values for fats. The A.M.E. values of fats show high degree of variation due to: the age of the bird which is fed (Fedde et al., 1960; Renner and Hill, 1960, 1961; Young, 1961; Whitehead and Fisher, 1975); the interaction with other dietary components (Young, 1961; Sibbald et al., 1961a,b,c, 1962; Artman, 1964; Lewis and Payne, 1966; Lall and Slinger, 1973) and the

position of fatty acids on the triglyceride molecule (Renner and Hill, 1961). Sibbald and Kramer (1977) observed that several fats (corn oil, soybean oil, oleic acid) had T.M.E. values greater than their gross energy values. The observed T.M.E. values of several fat mixtures were greater than the sum of the T.M.E. values of their component parts. Using fatty acid composition as a predictor of T.M.E. was found to be valid for only certain groups of fats. They concluded that the fats interact to allow for increased utilization of the energy of other dietary components. Also, there are reports in the literature indicating that some fats had higher A.M.E. values than their gross energy contents (Cullen et al., 1962; Jensen et al., 1970). Later Sibbald (1978b) showed that the addition of soybean oil (as little as two parts per 98 parts of tallow) increased the T.M.E. value of the tallow, but the addition of lard had little or no effect. Further studies with lipids showed that the level of dietary inclusion affected the T.M.E. values attributed to tallow and to soybean oil when fed in conjunction with a high calcium basal diet (Sibbald and Price, 1977b). An increase in the level of dietary calcium decreased the T.M.E. value of tallow but had a much smaller effect on the T.M.E. value of soybean oil. However, the effects were complex and varied with the level of inclusion of the fats. Sibbald and Kramer (1978) also found that the T.M.E. value of tallow decreased with the level of dietary inclusion and also differed according to the basal diet with which it was fed. There

was a significant linear relationship between the T.M.E. value of the tallow and the amounts of phospholipid and linoleic acid per unit weight of dietary fat. In another study, Sibbald and Kramer (1980a) again observed that the T.M.E. value of tallow differed between basal diets and with the level of tallow input. A corn-type basal diet permitted greater T.M.E. values than a wheat basal, but a supplement of soybean lecithins made the wheat basal diet as effective as the corn basal. The authors suggested that linoleic acid and phospholipid concentrations in the diet appeared to influence tallow utilization. Sibbald and Kramer (1980b) later used corn products (degermed corn, germ, extracted germ, crude oil, soapstock and refined oil) to substitute for wheat in the basal diet at levels similar to those in the corn basal. All fractions except soapstock had some ability to enhance tallow utilization. Mateos and Sell (1980) reported an inverse relationship between the level of yellow grease supplementation and the T.M.E. and $A.M.E._n$ values, when determined with White Leghorn laying hens in egg production.

Sibbald (1976b) conducted experiments to determine the effect of cold pelleting on the T.M.E. values of cereal grains. Cold pelleting increased the T.M.E. values of the wheats (3.5%) and barleys (0.9%), but decreased the values of the oats (3.5%). On the other hand, steam pelleting did not effect the T.M.E. values of 10 diets (Sibbald, 1977e).

Chami et al. (1980) observed that T.M.E. values of corn and soybean meal were significantly reduced in the presence of two percent guar gum or one percent tannic acid. T.M.E. value of soybean meal was reduced to some extent by 0.05 percent gossypol.

III. MATERIALS AND METHODS

A series of experiments was conducted involving the "T.M.E. Bioassay" described by Sibbald (1976a, 1977f), using dubbed, adult Single Comb White Leghorn (S.C.W.L.) roosters and ten-week-old Medium White (Wrolstad) poults.

The birds of similar body weight were kept in individual wire cages (30 x 45 cm), equippped with water and feed troughs. Alternate cages were left vacant to prevent any cross-contamination of excreta. During and between assays the roosters were kept on a rooster maintenance ration (1241D). The composition of the ration is described in Table I. Prior to each experiment the birds were starved for 24 hours to empty their digestive tracts. At the start of the assay, the birds were weighed and force-fed 25-30 g. of the test ingredient under study. Force-feeding was accomplished using a transparent polyvinyl tube and a rod, described in detail in Boldaji et al. (1981). A plastic funnel was fused in one end of the tube to facilitate the flow of feed. After force-feeding, the time was recorded and the birds were returned to their cages. An aluminum tray was placed under each cage to collect the excreta. In each trial a sufficient number of birds of similar body weight were fasted over the same period of time to serve as a control for endogenous energy ($FE_m + UE_e$) losses. Only water was provided during the 24-hour fast and after the force-feeding. Exactly 24 and/or 48 hours after the force-feeding, the excreta collection trays were removed,

TABLE I. COMPOSITION OF ADULT ROOSTER RATION (1241D)[a]

Ingredient	Percent
Yellow corn, ground	94.35
Alfalfa meal, dehydrated	2.00
Defluorinated phosphate	1.50
Limestone flour	1.50
Salt, iodized	.40
Trace mineral mix DD-65[b]	.05
Vitamin premix 1-75[c]	.20
Total	100.00
Calculated analysis	
Crude protein, %	8.75
Crude fat, %	3.73
Crude fiber, %	2.37
Apparent metabolizable energy, kcal/kg.	3260.47

[a]Fed to S.C.W.L. roosters during and between the assays.

[b]Trace mineral mix provides per kg ration: Mn, 50 mg; Fe, 2 mg; Cu, 0.2 mg; Zn, 27.5 mg; Co, 0.2 mg.

[c]Vitamin premix provides per kg ration: Vit A, 3300 IU; Vit D-3, 1100 ICU; riboflavin, 3.3 mg; d-pantothenic acid, 5.5 mg; niacin, 22.0 mg; choline, 190.0 mg; Vit B-12, 5.5 mcg; Vit E, 1.1 IU; Vit K, 0.55 mg; folic acid, 0.22 mg; ethozyquin, 0.06 g.

cleaned for feathers and scales and the excreta voided were collected from the trays quantitatively with the aid of a water sprayer. The excreta were dried in a forced-air oven at 102°C for 24 hours, allowed to come to equilibrium with atmospheric moisture, weighed and ground with a mortar and pestle. Subsamples of ground feed and excreta were analyzed for gross energy using a Parr-adiabatic bomb calorimeter, equipped with a digital thermometer. The moisture content of the feed, together with gross energy values of feed, and excreta, were used to calculate T.M.E. values (Sibbald, 1976a).

Means and standard errors were computed for the treatments and the data were statistically treated using analysis of variance. Least significant differences were computed as described by Snedecor and Cochran (1967).

EXPERIMENT I. The Effect of Duration of Starvation Period Prior to Initiation of the Assay on Gut Clearance

Eighteen dubbed, adult S.C.W.L. roosters (Babcock)* were assigned randomly into three replicated groups. The birds were provided with 14 hours of artificial light each day. Water was supplied for eight 15-minute periods at about equal intervals between 4:15 a.m. and 5:45 p.m. The birds received the maintenance ration ad libitum prior to initiation of the experiment for 21 days. The birds were sacrificed at intervals of 24, 36 and 48 hours from the

*Male chicks from a hatch involving Babcock B-300 pullet chicks.

time feed was withdrawn. The digestive tract of each bird was ligated between the anatomical sections and the crop; gizzard, small and large intestines and cecum were cut open to collect any feed residue being retained. The residue was carefully washed with distilled water, oven-dried and weighed.

EXPERIMENT II. Observations on the Effect of Management Factors; Environmental Temperature and Photoperiodic Regimens on Endogenous Energy and T.M.E. Values

Adult, S.C.W.L. roosters (Shaver)* were assigned to each of the pretreatments that are outlined in Tables V and VI.

The birds were kept on the described pre-treatments for 21 days prior to initiation of the assay. The test material for both treatments was ground corn, but, in addition, some birds on the temperature trial were also fed ground triticale. Treatments involved force-feeding each bird 25 g. of test material and collecting the excreta voided during the next 24 hours. Water was provided continuously during these trials. T.M.E. values for corn and triticale were determined for the respective treatments.

EXPERIMENT III. Correlations Based on Endogenous Energy Output of the S.C.W.L. Roosters

One hundred adult, S.C.W.L. roosters (Dekalb)** were starved overnight for 24 hours and weighed to the nearest 10 grams. Twenty

*Male chicks from a hatch involving Shaver Starcross-288 pullet chicks.

**Male chicks from a hatch involving Dekalb XL pullet chicks.

pairs of birds in same body weight, ranging from 1.99 to 3.22 kg, were selected and starved for another 24 hours as control birds. The excreta voided during the subsequent period were collected quantitatively, dried, equilibriated with atmospheric moisture and ground to pass through a 20-mesh sieve. Gross and net excreta weights were calculated for each bird by excluding the screened material. Pearson correlation coefficients were calculated between the variables (Snedecor and Cochran, 1964).

EXPERIMENT IV. The Effect of Free Choice Grit Feeding on Excreta Weights, Endogenous Energy and T.M.E. Value of Corn

Twenty-four adult, S.C.W.L. roosters (Shaver) were randomly divided into two replicated groups. One group received free choice medium-size granite grit for eight weeks, whereas the other group was kept on a grit-free regimen. Both groups received the rooster maintenance ration ad libitum. Water was provided for eight 15-minute periods during the light period. At the end of the pre-treatment, both groups were starved for 24 hours. Six birds from each group were randomly assigned as control birds. The remaining birds were force-fed 25 g. ground corn. Excreta from both groups were collected quantitatively for a 24-hour period. At the end of the excreta collection, the grit-fed birds were sacrificed to determine the amount of grit that was retained in the gizzard. Excreta from both groups were dried in an oven, equilibriated with atmospheric moisture and

weighed for gross excreta weight. In order to separate the grit, excreta from each bird was made into a slurry with water and poured through a 20-mesh sieve under a jet of water. The grit that was removed from the excreta was dried, weighed and the excreta weights corrected to a grit-free basis. T.M.E. values for corn were determined for each group.

EXPERIMENT V. The Effect of Using Screened Excreta on 24- and 48-hour T.M.E. Values of Wheat, Soybean Meals, Meat and Bone Meal and Fish Meal

Samples of wheat, soybean meal (44%), soybean meal (47.5%), meat and bone meal and fish meal (Herring) were assayed for T.M.E. using adult S.C.W.L. (Shaver) roosters. T.M.E. values for each feedstuff were calculated after 24- and 48-hour collection periods. Excreta from control and fed birds were ground to pass 20-mesh sieve after drying in the oven. The screened portion of the excreta were discarded and gross and net excreta weights were computed for each bird. By using these adjusted values three different calculations were made for each of the feedstuffs.

EXPERIMENT VI. T.M.E. Values of Three Varieties of Triticale as Measured with Roosters and Poults

Adult, S.C.W.L. roosters (Shaver) and ten-week-old Medium White Turkeys (Wrolstad) were housed in wire cages in a windowless room where they received 14 hours of light daily. The roosters were kept on the maintenance ration previously described, whereas poults

received OSU turkey developer ration. The composition of the turkey developer ration is presented in Table II. All birds were starved for 24 hours and were force-fed 25 g. of air dry feed. The excreta voided by the control and the fed birds were collected at 24 and 48 hours after feeding. The T.M.E. values of three varieties of triticale were measured using the energy excretion values for both the 24- and 48-hour periods. Five birds of each type were assigned to each variety.

EXPERIMENT VII. The Use of Gelatin Capsules as an Alternative Method of Force-feeding in T.M.E. Bioassay

Four different feedstuffs were analyzed for T.M.E. using adult S.C.W.L. roosters (Shaver). The test materials were weighed into empty gelatin capsules (Lilly No. 000) and delivered into the crop of fasted birds with the aid of force-feeding tube. Ten capsules were fed to each bird with the approximately gelatin:feed ratio of 1:7.5. Instead of determining the T.M.E. value of gelatin, the control birds were force-fed the mean gelatin consumption (1.61 g.) of fed birds. The excreta voided by the control birds were used to correct the fed birds' energy output for the endogenous losses. T.M.E. values of ground corn, corn oil, freeze-dried hake meal and spray-dried hake meal were determined by using the gross energy values of feed and excreta (48-hour collection) for each individual bird.

TABLE II. COMPOSITION OF TURKEY DEVELOPER RATION.[a]

Ingredient	Percent
Yellow corn, ground	60.10
Soybean meal, solvent (47.5% protein)	20.00
Meat and bone meal (50% protein)	10.00
Alfalfa meal, dehydrated (17% protein)	5.00
Whey, dried	2.50
Limestone flour	1.00
Dicalcium phosphate	.50
Salt, iodized	.50
Trace mineral mix[b]	.05
Vitamin premix[c]	.30
d, l-methionine	.05
Calculated analysis	
Crude protein, %	21.09
Crude fat, %	3.80
Crude fiber, %	3.68
Apparent metabolizable energy, kcal/kg.	2790.00

[a]Fed to medium white (Wrolstad) male turkeys between 9-13 wks. of age when the assays made.

[b]Trace mineral mix provides per kg ration: Ca, 195 mg; Mn, 120 mg; Fe, 40 mg; Cu, 4 mg; I, 2.4 mg; Zn, 55 mg.

[c]Vitamin premix provides per kg ration: Vit A, 5782 IU; Vit D, 1927 ICU; riboflavin, 5.78 mg; d-pantothenic acid 9.64 mg; niacin, 38.54 mg; choline, 334 mg; Vit B_{12}, 9.64 mg; Vit E, 1.92 IU; Vit K, 0.96 mg; folic acid, 0.38 mg.

EXPERIMENT VIII. The Effect of Water Treatment and the Addition of Corn on T.M.E. Value of Western Barley

Twenty-four adult, S.C.W.L. roosters (Dekalb) of similar body weight were randomly assigned into four replicated groups. The birds were starved overnight for 24 hours and force-fed 25 g. of either ground Western Barley (Klages), Western Barley (water treated) or Western Barley with 10 percent added corn. Water treatment of ground barley was accomplished by adding one part tap water (50°C) to one part of the ground barley, mixing, holding in room temperature for half an hour, drying in a forced-draft oven at 70°C and regrinding before using. While drying, the barley was stirred in the pan at least once (Leong et al., 1962). T.M.E. values of respective treatments were measured as described previously.

EXPERIMENT IX. T.M.E. Values of Sunflower Seed Products: Dehulled Seeds, Ground Seeds and Hulls, Measured with S.C.W.L. Roosters Kept on a Grit and Grit-free Regimens

Forty-eight adult, S.C.W.L. roosters (Dekalb) were randomly assigned with two pre-treatments for two weeks. One group was provided free choice, hen-size granite grit, whereas other group received only the rooster maintenance ration, mentioned previously. Both groups were kept in a windowless room where they received 14 hours of light, and eight 15-minute periods of water during the light phase. At the end of the pre-treatment period, both groups were starved for 24 hours and were randomly divided into four

replicated sub-groups. One sub-group from each pre-treatment was assigned as the control, and the other sub-groups were force-fed either 25 g. dehulled sunflower seeds, 15 g. ground sunflower seeds or 10 g. sunflower seed hulls.

The sunflower seeds used in this experiment were cleaned from foreign particles by using an E.S.M. Pneumatic Seed Cleaner. The dehulling process was accomplished by passing the seeds through pinch rollers, belt threshers and pneumatic seed cleaners. After the force-feeding the excreta from both groups were collected for 48 hours, dried in a forced-air oven, equilibriated with atmospheric moisture, weighed and ground to pass through a 20-mash sieve to remove the grit. Subsamples of feed and excreta were analyzed for energy and T.M.E. values were calculated by using both gross and net (grit-free) excreta weights.

IV. RESULTS AND DISCUSSION

EXPERIMENT I. The Effect of Duration of Starvation Period Prior to Initiation of the Assay on Gut Clearance

Table III summarizes the dry matter retention (g.) in various portions of the digestive tracts of 24, 36 and 48-hour starved roosters, previously fed ad libitum. There were significant differences among the gizzard ($P < 0.05$), small and large intestine contents ($P < 0.05$) and the cumulative dry matter retention ($P < 0.01$) of 24- and 36-hour starved roosters. However, extending the starvation period from 36 to 48 hours was not sufficient enough to change the rate of clearance of ingesta significantly. The feed residues had mostly disappeared from the crop and cecum at the end of the 24-hour fast and their contents were unchanged with the duration of starvation. Table IV shows the dry matter retention in individual birds used in this experiment. Close examination of the data reveals the movement of feed particles from gizzard to the posterior portions of the digestive tract with the duration of fast. One may readily speculate that, if the excreta collection period had been extended beyond 48 hours, most of the particles in the small and large intestines of 48-hour starved birds would have cleared the gastrointestinal system completely.

Sibbald (1979d) suggested that the rate (%) of clearance of feed residues was inversely related to input levels. If this is

TABLE III. EFFECT OF DURATION OF STARVATION, PRIOR TO FORCE-FEEDING, ON GUT CLEARANCE.[1]

Duration of starvation (hr.)	No. of birds	Crop contents (g.)	Gizzard contents (g.)	Large and small intestine contents (g.)	Cecum contents (g.)	Cumulative dry-matter retention
24	6	.04 ± .01[a]	4.10 ± .79[a*]	1.46 ± .17[a*]	.34 ± .06[a]	5.94 ± .76[a**]
36	6	.02 ± .004[a]	2.22 ± .33[b]	.84 ± .11[b]	.27 ± .03[a]	3.35 ± .36[b]
48	6	.03 ± .01[a]	2.15 ± .29[b]	1.25 ± .09[b]	.31 ± .05[a]	3.74 ± .33[b]

[1]Data expressed on a dry-matter basis.

[2]Means with standard errors.

[a,b,c]Means within a column with different superscripts are significantly different.

*$P < .05$.

**$P < .01$.

TABLE IV. THE DISAPPEARANCE OF THE INGESTA FROM THE GASTROINTESTINAL TRACT OF STARVED ROOSTERS.

Starvation period (hr.)	Animal	Crop Contents (g.)	Gizzard Contents (g.)	Small and large intestine contents (g.)	Cecum contents (g.)	Total (g.)
24	1	.09	2.74	.81	.27	3.91
	2	.02	7.85	1.29	.16	9.32
	3	.01	2.74	1.59	.30	4.64
	4	.05	3.16	2.10	.40	5.71
	5	.04	4.26	1.42	.58	6.30
	6	.02	3.84	1.55	.32	5.73
36	1	.04	2.55	.90	.32	3.81
	2	.02	2.02	.61	.38	3.03
	3	.04	2.72	.50	.20	3.46
	4	.01	.79	.76	.22	1.78
	5	.02	2.08	1.26	.31	3.67
	6	.01	3.16	.99	.20	4.36
48	1	.01	2.66	1.40	.48	4.55
	2	.04	2.39	1.44	.18	4.05
	3	.02	2.99	1.03	.42	4.46
	4	.04	1.95	1.46	.28	3.73
	5	.04	.99	1.18	.22	2.43
	6	.01	1.94	.98	.29	3.22

true, high variation observed in cumulative dry matter content of digestive tracts of the birds may be the result of variable amounts of feed intake during the ad libitum feeding period.

It is essential in the T.M.E. bioassay that the starvation period, prior to force-feeding, is sufficient enough for the feed residues to clear the digestive tracts of assay birds. Otherwise the corrections based on energy losses of the unfed birds, during the subsequent periods, will not be valid estimates for combined metabolic plus endogenous energy ($FE_m + UE_e$) losses. The precision of the T.M.E. assay depends on how closely the losses of control birds estimate the $FE_m + UE_e$ losses of the assay birds (Sibbald and Price, 1980).

In this experiment birds retained appreciable amounts of dry matter in their digestive tracts, despite the recommended 24-hour starvation period (Sibbald, 1979a; Shires et al., 1979; Parsons and Potter, 1980). None of these investigators sacrificed the assay birds for direct evidence. Kessler and Thomas (1980) and Kokue and Hayama (1972) presented direct evidence regarding the retention of undigested feed residues in cecums and gizzards of 24-hour starved chicks and roosters, respectively. The observed dry matter retention in various portions of the digestive tracts of starved roosters raises the question whether the variation observed in $FE_m + UE_e$ excretion of control birds (Sibbald and Price, 1978, 1980) is due to incomplete passage of previously fed maintenance diet. Obviously

the dry matter contents of the digestive tract, observed in this experiment, are a mixture of engodenous excretions as well as digested and undigested residues. During the experiment it was noted that some of the birds had variable amounts of grit in their gizzards, despite the fact that they were kept on a grit-free regimen. Muztar and Slinger (1979b) also observed the presence of grit in gizzards of roosters, kept on grit-free diets. Consequently, the gizzard dry matter (g.) measured in this experiment may have been overestimated by the existence of grit. The gizzard contents of the individual birds were not screened to calculate the dry matter retention on a grit-free basis. However, pooled gizzard contents, which were composed of coarse fibrous residues, had a gross energy value of 4.3 kcal/g. when analyzed. This value is high enough to conclude that part of the residues retained in gizzards of starved roosters were not of endogenous origin, and that birds do show a tendency to retain coarse particles and grit which originates from the feed.

Sibbald (1979b) measured the effects of the duration of starvation period on the T.M.E. value of a laying hen diet. Lengthening the starvation period from 24 to 96 hours by 24-hour intervals had no significant effect on the T.M.E. values. However, the excretion of $FE_m + UE_e$ by the control birds tended to decrease with the duration of starvation. Shires et al. (1979) found a slight increase in the excreta outputs of the unfed birds, when the fasting period was increased from 24 to 48 hours. Extending the fasting

period resulted in increases in the T.M.E. value of the corn by 1.5 and 3.8 percent, respectively. The difference between the 12- and 48-hour fast in the T.M.E. values were significant, whereas 24- to 48-hour values were not.

The above mentioned evidence in the literature suggests that 24-hour starvation is sufficient enough to obtain reliable results, despite the small amount of retention reported in the literature and in this experiment. However, there is a need for further research regarding the effect of retained feed residues and grit on $FE_m + UE_e$ losses, which are assumed to be a characteristic of the bird (Sibbald and Price, 1980).

EXPERIMENT II. Observations on the Effect of Management Factors: Environmental Temperature and Photoperiodic Regimens on Endogenous Energy and T.M.E. Values

Endogenous energy excretion ($FE_m + UE_e$) and the T.M.E. value of ground corn, measured by adult roosters kept on either (1) constant temperature, different photoperiodic regimes, or (2) constant light, different temperatures are shown in Tables V and VI, respectively. Varying the management factors, light and temperature, did not influence the $FE_m + UE_e$ outputs and T.M.E. value of corn significantly in this experiment. The T.M.E. value of triticale, measured for two temperature regimens, were also not different (Table VI).

There are several reports in the literature regarding the effects of environmental temperature on energy metabolism of the

TABLE V. THE EFFECT OF PHOTOPERIODIC REGIMENS ON ENDOGENOUS ENERGY EXCRETION AND T.M.E. VALUES[1]

	Treatment[2]	
	14L:10D	24L
Endogenous energy ($FE_m + UE_e$) Excretion (kcal/24 hr.)	7.33 ± .3[a](7)[3]	7.98 ± .3[a](7)
	T.M.E. (kcal/g.)	
Corn, yellow, ground	3.99 ±.02[b](7)	4.00 ±.01[b](5)

[1]Values are expressed on a dry-matter basis; room temperature, 22°C.

[2]L:light period; D:dark period.

[3]Number of replications.

[a,b]Means with the same superscript are not significantly different ($P < .05$).

TABLE VI. THE EFFECT OF ENVIRONMENTAL TEMPERATURE ON ENDOGENOUS ENERGY AND T.M.E. VALUES.[1]

	Treatment	
	58°F (14.4°C)	78°F (25.6°C)
Endogenous energy (FE_m + UE_e) Excretion (kcal/24 hr.)	6.62 ± .5[a](7)[2]	6.54 ± .2[a](7)
	T.M.E. (kcal/g.)	
Corn, yellow, ground	3.94 ± .03[b](10)	4.01 ± .04[b](10)
Triticale	3.48 ± .04[c](5)	3.58 ± .05[c](4)

[1]Values are expressed on a dry-matter basis; provided with 24-hr. light.

[2]Number of replications.

[a,b,c]Means with the same superscript are not significantly different ($P < .01$).

fowl. Peterson et al. (1969) observed increase in fecal fat with decreasing environmental temperature, from 21.2° to 4.4°C. Consequently, the metabolizable energy (M.E.) content of laying hen diet increased with increasing temperature. Olson et al. (1970) also reported improvements on the M.E. values of corn and cassava with the higher (25.5° to 42.5°C) temperatures. O'Neill et al. (1971) and Swain and Farrel (1975) reported decreasing urinary nitrogen with increasing environmental temperature. On the other hand, Waring and Brown (1967) did not observe any change in M.E. of a laying diet, measured at 22° and 29°C.

Shannon and Brown (1969) observed a significant decrease in the fasting metabolic rate of White Leghorn hens and Light Sussex cockerels following an increase in environmental temperature from 22° to 28°C. No significant decline in the fasting metabolic rate of the cockerels occurred during the first seven days at the higher temperature. After 28 days no further decline occurred, indicating that the period of adaptation was between 7 and 28 days. These authors associated this full acclimatization to the thyroid gland and thyroxine secretion.

In the present experiment, the temperature was raised from 14.4°C, which is considered to be under the accepted thermoneutral zone of 18° to 26°C (Sturkie, 1965), to a point of 25.6°C which approaches the upper zone. The lack of temperature chambers limited the use of more extreme temperatures, which might have given more

conclusive results in this experiment.

Wilson and McFarland (1969) studied the diurnal changes in digestive systems of Coturnix as related to photoperiodic regimens. They reported fluctuations in the digestive system with the light treatment. Crop fullness was maximal during the light phase. The gall bladder was emptied during the light phase in relation to the time feed was being assimilated, and filled during the dark phase. The ceca also underwent progressive filling during the light period as food passed down the gut. Most of the quail emptied their ceca during the darkness.

Tyler (1958) reported variations in the quantity of gastrointestinal excretion that occurred in poultry and related the variations to the influence of the starting time (zero hour) and to the length of time period.

The attempt of reducing the influence of light regimes on assay birds by using constant illumination was also found to be without effect in this experiment. Had the assay birds been reared under different photoperiodic regimens, more pronounced effects on their digestive system and excretion patterns might have been observed.

These observations suggest that the adult, S.C.W.L. roosters tend to maintain a steady state and are not susceptible to short-term changes in light and temperature management. Also, corrections for $FE_m + UE_e$, obtained from the control birds under the same

experimental conditions, make T.M.E. values less susceptible to other causes of variation.

EXPERIMENT III. Correlations Based on Endogenous Energy Output of the S.C.W.L. Roosters

Definition of variables, means, ranges and the variances are summarized in Table VII. By applying the Sibbald's procedure (1976a) of passing the dried excreta through a 20-mesh sieve, it was possible to calculate both gross (GExWt) and net (NExWt) excreta weights for each bird. The difference between the GExWt and NExWt, termed as the correction in excreta weight (Corr.), consisted of scales, down feathers and grit particles that adhered to wet excreta. Obviously this screened portion, which ranged from .18 to .71 g., was not of endogenous origin and was excluded from the excreta samples. Resulting new variables (NExWt, NEE/B, NEE/BWt, and NexWt/BWt) were also included in correlations.

There were reductions in variances of excreta weights and endogenous energy excretions when the correction in excreta weights were employed (Table VII). By excluding the weight contribution of this screened material, mean GEE/B dropped from 7.99 to 6.70 kcal (NEE/B). These values are relatively lower than the mean $FE_m + UE_e$ values of 10.44 kcal (Sibbald and Price, 1978), 8.92 to 9.57 kcal (Shires et al., 1979), 9.32 to 10.0 kcal (Muztar and Slinger, 1980), 9.6 to 11.1 kcal (Sibbald, 1980c), 8.5 to 10.6 kcal (Chami et al., 1980) reported in the literature.

TABLE VII. DEFINITION OF VARIABLES USED IN CORRELATIONS.

Variables		Minimum	Maximum	Variance
BWt: Initial body weight (kg)	2.52	1.99	3.22	.096
GExWt: Gross excreta weight/bird/24 hr. (g)	2.78	2.05	4.37	.222
NExWt: Net excreta weight/bird/24 hr. (g)	2.33	1.64	3.71	.192
GEE/B: Gross endogenous energy/bird/24 hr. (kcal)	7.99	5.63	12.55	2.064
NEE/B: Net endogenous energy/bird/24 hr. (kcal)	6.70	4.51	10.67	1.712
GEE/BWt: Gross endogenous energy/kg BWt/24 hr.(kcal)	3.22	2.34	6.31	.589
NEE/BWt: Net endogenous energy/kg BWt/24 hr. (kcal)	2.70	1.78	5.36	.501
GExWt/BWt: GExWt/kg BWt/24 hr. (g)	1.12	.82	2.19	.072
NExWt/BWt: NExWt/kg BWt/24 hr. (g)	.94	.63	1.87	.062
Corr: Correction in excreta weight (g)	.45	.18	.71	.015

[1]Expressed on an oven-dried basis.

Presently there is no clear explanation for this difference, however, these reported values fall into the range of GEE/B (5.63 to 12.55 kcal) and NEE/B (4.51 to 10.67 kcal) observed in this experiment. This variation may partly be due to differences in experimental conditions. Sibbald and Price (1978) discussed the lack of agreement among the data describing the magnitude of $FE_m + UE_e$ losses of poultry.

Pearson correlation coefficients among the variables presented in Table VII are summarized in Table VIII. The correlation between the variables BWt and GEE/B had a coefficient of -.005, which is not significantly different from zero ($P < 0.01$). The use of the correction in excreta weights did little to improve this coefficient (-.121). When the "metabolic body weight" instead of simple body weight was used, the correlation coefficients were essentially the same. Scattergrams of variables also revealed the absence of any significant correlation. Additional correlation coefficients are also displayed in Table VIII.

When Sibbald and Price (1978) analyzed a collection of 300 $FE_m + UE_e$ values from 38 experiments conducted over three years, linear regressions on body weight and weight change explained only 23 percent of the variation. Shires et al. (1979) also reported a weight effect on $FE_m + UE_e$ losses. However, later Sibbald and Price (1980) observed a correlation of .069 between body weight and $FE_m + UE_e$ and concluded that the endogenous energy output was

TABLE VIII. PEARSON CORRELATION COEFFICIENTS BETWEEN THE VARIABLES.[a]

	BWt	GExWt	NExWt	GEE/B	NEE/B	GEE/BWt	NEE/BWt	GExWt/BWt	NExWt/BWt	Corr
BWt	1.000									
GExWt	-.091	1.000								
NExWt	-.205	.965	1.000							
GEE/B	-.005	.962	.917	1.000						
NEE/B	-.121	.945	.968	.967	1.000					
GEE/BWt	-.536	.860	.878	.836	.866	1.000				
NEE/BWt	.572	.831	.893	.798	.871	.984	1.000			
GExWt/BWt	-.599	.841	.869	.761	.805	.978	.971	1.000		
NExWt/BWt	-.621	.812	.882	.730	.814	.960	.982	.986	1.000	
Corr	.377	.388	.133	.412	.171	.164	-.001	.125	-.030	1.000

[a]The variable labels are defined in Table VII.

largely characteristic of the bird. Arvat et al. (1980) also failed to observe any correlation between the above mentioned variables.

The above findings support the results of this experiment, where the $FE_m + UE_e$ losses were found to be independent of the body size. Sibbald and Price (1980) obtained the most precise T.M.E. estimates by using each bird as its own negative control. However, this would introduce extra work and lengthen the assay time, which is considered to be one of the biggest advangages of this technique.

EXPERIMENT IV. The Effect of Free Choice Grit Feeding on Excreta Weights, Endogenous Energy and T.M.E. Value of Corn

The gross and net excreta weights of the birds which were kept on grit and non-grit regimens are summarized in Table IX. There were significant ($P < 0.05$) differences in gross excreta outputs of control and fed birds in both grit and non-grit treatments. These differences were associated with significantly ($P < 0.01$) greater amounts of grit excretion of the birds on the grit regimen. The screening process removed the differences between the excreta weights of fed birds (net excreta weight), whereas excreta outputs of control birds in both treatments were unaffected by this procedure. Appreciable amounts of grit were found in gizzards of birds on grit treatment. Gizzard grit and the dry matter content of control and fed groups on grit treatment were not significantly different.

The $FE_m + UE_e$ losses and the calculated T.M.E. values of

TABLE IX. SUMMARY OF MEANS AND APPROPRIATE STANDARD ERRORS FOR THE GRIT EXPERIMENT.

	Treatment			
	Grit		No grit	
	Control group	Fed group	Control group	Fed group
Bird weight (kg.)	2.21 ± .09	2.22 ± .11	2.56 ± .11	2.45 ± .08
Weight loss (g.)	79.3 ± 5.7	61.8 ± 21.6	50.8 ± 2.7	37.4 ± 12.3
Dry gross excreta output/24 hr. (g.)	6.26 ± .99^{a*}	7.61 ± .20^{ac**}	3.20 ± .21^{b}	5.06 ± .25^{d}
Dry net excreta output/24 hr. (g.)	4.84 ± .79^{a*}	6.09 ± .46^{ad*}	2.79 ± .17^{b}	4.61 ± .21de
Correction for the grit (g.)	1.42 ± .45^{f}	1.51 ± .31^{g}	.42 ± .08^{fh}	.45 ± .07^{h**}
Gizzard grit (g.)	11.34 ± 1.14	11.39 ± 1.53	-	-
Gizzard dry matter (g.)	.72 ± .16^{a}	1.67 ± .83^{a}	-	-

^{a,b,c,d,e,f,g,h}Values with different superscripts are significantly different.

$^{*}p < 0.05$.

$^{**}p < 0.01$.

ground corn are presented as means in Table X. As a consequence of differences in gross excreta outputs of control birds on both treatments, endogenous energy excretion of control birds was also found to be significantly ($P < 0.05$) different. However, when the net excreta weights were used in calculation of $FE_m + UE_e$ output, the observed differences were removed. The T.M.E. value of corn measured with the grit fed birds was 4.22 kcal/g. as compared to 4.17 kcal/g. obtained from grit-free birds. Adjusting the excreta weights of all the birds (control and fed) in both treatments did not change the T.M.E. value of corn. Statistical analysis of the T.M.E. data indicated that the numerical differences between the grit and non-grit treatments were not significant.

From the results obtained from this experiment, it is not possible to state that administration of totally indigestible material (silica gel) to adult cockerels, increases their $FE_m + UE_e$ outputs (Teneseca and Sell, 1978). The effects of grit on endogenous energy outputs of control birds were mainly associated with weight contribution of excreted grit on gross energy calculations. Numerical increases observed in $FE_m + UE_e$ outputs of grit-fed birds were not statistically significant, and may have been the result of pulverized grit particles that had escaped from the 20-mesh sieve. Teneseca and Sell (1978) also reported a decrease (4%) in the T.M.E. value of corn when it was fed with silica gel. Once again, grit feeding exerted no effect on T.M.E. value of corn in this experiment.

TABLE X. THE EFFECT OF FREE CHOICE GRIT CONSUMPTION ON ENDOGENOUS ENERGY AND T.M.E. VALUE OF CORN.

	Treatment	
	No grit	Grit
Endogenous energy (FE_m + UE) Output, kcal/24-hr.	$9.48 \pm .73^{a}$ (6)[4]	$15.68 \pm 2.20^{b*}$ (6)
Corrected FE_m + UE_e[1] kcal/24-hr.	$8.24 \pm .55^{a}$ (5)	$12.19 \pm .80^{ab}$ (5)
T.M.E. (kcal/g.)[2]	$4.17 \pm .1^{ab}$	$4.22 \pm .03^{a}$
Corrected T.M.E.[3] (kcal/g.)	$4.17 \pm .05^{ab}$	$4.22 \pm .03^{a}$
A	$4.12 \pm .06^{ad}$	$4.07 \pm .03^{bd*}$
B	$4.40 \pm .06^{c**}$	$3.99 \pm .03^{d}$
C	$4.24 \pm .06^{c**}$	$3.95 \pm .05^{d}$

[1]Correction is made by adjusting the excreta weight on a grit-free basis.

[2]T.M.E. values are calculated without the correction.

[3]T.M.E. values are calculated with the adjusted excreta weights of both the control and the fed groups of each treatment.

[4]Number of replicates.

A: T.M.E. values are calculated by using corrected E.E. of control groups.

B: T.M.E. values for each group are calculated by using the uncorrected E.E. value of other group.

C: T.M.E. values for each treatment are calculated by using the corrected E.E. value of other group.

^{a,b,c,d}Values with different superscripts are significantly different.

$^{*}P < 0.05$.

$^{**}P < 0.01$.

Sibbald (1980c) reported that supplemented cellulose (alpha-floc) at levels up to 4 g./bird, and sand (up to 3 g./bird) had no effect on the $FE_m + UE_e$ excretions of adult cockerels, and on T.M.E. values of corn, wheat and oats. There was an evidence, however, that the added sand may have delayed the clearance of wheat residues from the alimentary canal. This effect had disappeared when the excreta collection period was extended to 48 hours. The cumulative weight of excreta voided by the birds did not increase with the sand input. Although some sand was detected during the grinding of excreta, it was concluded that little passed the length of alimentary canal within 48 hours. The absence of an effect on $FE_m + UE_e$ outputs may have been due to the use of low levels of sand and to the high degree of retention of this material.

Muztar and Slinger (1980) were also unsuccessful in their attempt to increase the $FE_m + UE_e$ outputs of adult cockerels, by using alpha-floc and pure corn cob meal as sources of inert materials, due to high degree of retention in digestive tract.

Sibbald (1976b, 1980d) mentioned the importance of using cockerels, which were reared on a grit-free regimen for T.M.E. assays. He emphasized the possibility of obtaining misleading values for excreta weights and damaging the grinding equipment. Many researchers (Brown, 1904; Buckner and Martin, 1922; Kaupp and Ivey, 1923a,b; Keith, 1927; Muztar and Slinger, 1979) observed the presence of varying amounts of grit in gizzards of birds, despite the fact that

they were on a grit-free ration. Their studies also show that grit may normally be found in all sections of the digestive tract, through its consumption with feed. Thus, passing the dried excreta samples through a 20-mesh sieve after the grinding may be a useful effort to reduce the variation in gross energy outputs of the assay birds.

T.M.E. values of corn, when calculated by only using the adjusted excreta weights of control groups (Table X;A), was 4.12 and 4.07 kcal/g. for non-grit and grit treatments, respectively. The use of net excreta weights of control birds did not change the T.M.E. value of corn in the non-grit treatment, whereas the T.M.E. value obtained from grit treatment was significantly ($P < 0.05$) lower (Table X). The T.M.E. value of corn was also influenced significantly ($P < 0.01$) when all the possible cross-calculations (using the uncorrected and corrected $FE_m + UE_e$ of other groups) were employed (B,C), suggesting the contribution of grit on $FE_m + UE_e$ estimations, and the misleading values that may be obtained with the presence of grit in excreta.

EXPERIMENT V. The Effect of Using Screened Excreta on $T.M.E._{24}$ and $T.M.E._{48}$ Values of Wheat, Soybean Meals, Meat and Bone Meal, and Fish Meal

Table XI summarizes the $T.M.E._{24}$ (24-hour excreta collection) and $T.M.E._{48}$ (48-hour excreta collection) values for wheat, soybean meals (44 and 47.5%), meat and bone meal, and fish meal, obtained

TABLE XI. EFFECT OF EXCRETA CORRECTION ON T.M.E.$_{24}$ AND T.M.E.$_{48}$[1] VALUES, OBTAINED BY USING ROOSTERS.

	24-hr. collection		48-hr. collection		Difference between means
	Mean[2]	SEM[3]	Mean	SEM	
	(kcal/g.)	±	(kcal/g.)	±	(kcal/g.)
Wheat, ground[a]	NS		NS		NS
I	4.00	.03	4.07	.03	.07
II	3.99	.04	4.07	.03	.08
III	3.99	.03	4.05	.03	.06
Soybean meal (44%)[b]	NS		NS		*
I	3.23	.07	2.97	.03	.26
II	3.30	.07	3.06	.02	.24
III	3.22	.07	2.95	.03	.27
Soybean meal (47.5%)[b]	NS		NS		NS
I	3.15	.13	2.85	.09	.30
II	3.29	.11	3.01	.10	.28
III	3.12	.13	2.81	.09	.31
Meat and Bone meal[b]	NS		NS		*
I	2.99	.09	2.69	.04	.30
II	3.06	.07	2.77	.05	.29
III	2.97	.08	2.66	.05	.31
Fish meal (Herring)[a]	NS		NS		*
I	4.40	.11	4.01	.11	.39
II	4.50	.09	4.14	.09	.36
III	4.38	.11	3.98	.11	.40

[1] kcal/g. dry matter.

[2] Mean of five replicates.

[3] Standard error of the mean

I: T.M.E. values calculated without correction.
II: T.M.E. values calculated by correcting the excreta of both the control and the fed birds.
III: T.M.E. values calculated by only correcting the excreta of control.

[a,b] Feedstuffs with different letters are significantly different ($p < .01$) in terms of T.M.E.$_{48}$.

* $P < .05$.

NS: nonsignificant.

by using three types of calculation. Calculating the T.M.E. values by using either unadjusted (gross) excreta weights (I), adjusted (net) excreta weights (II) of both control and fed birds, or only net excreta weights of control birds (III) did not influence the T.M.E. values of the respective ingredients significantly. The T.M.E. value of wheat was not affected by the duration of excreta collection period, indicating that undigested feed residues cleared the digestive tract with 24 hours. On the other hand, soybean meal (44%), meat and bone meal, and fish meal residue took more than 24 hours to clear the digestive system and resulted in significantly ($P < 0.05$) lower T.M.E. values for the 48 hours. The standard errors associated with mean T.M.E. values of soybean meal (44%), meat and bone meal, and fish meal were also reduced with longer excreta collection periods. It was interesting to observe that $T.M.E._{24}$ and $T.M.E._{48}$ values for soybean meal (47.5%) were not significantly different. There is no apparent explanation for this result, besides the higher variation observed in T.M.E. value of this feedstuff.

The results of this experiment confirm the observations (Sibbald, 1979b) that excreta collection periods of the feedstuffs with slow rates of passage (soybean meal, meat and bone meal and fish meal) should be extended beyond 24 hours. The attempt of screening the excreta samples to reduce the variability associated with the T.M.E. values was unsuccessful, despite the small but nonsignificant changes in standard errors. On the other hand, applying this process

may be useful in cases where the grit-feeding history of the assay birds is unknown (Exp. IV). The screening process, when applied to excreta samples from control birds, was found to be useful (Exp. III) in reducing the variation associated with $FE_m + UE_e$ outputs of cockerels. The T.M.E. values obtained from this experiment also confirms the possibility of using such a correction on excreta samples of fed birds without affecting the quality of the data.

EXPERIMENT VI. T.M.E. Values of Three Varieties of Triticale, as Measured with Roosters and Poults

The mean $T.M.E._{24}$ and $T.M.E._{48}$ values of three varieties of triticale, as measured with adult roosters and poults, are presented in Table XII. With the exception of the relatively lower value for the variety A (VT-75 229), the T.M.E. values for the triticale varieties for each species were in good agreement. Analysis of variance confirmed the absence of any effect either due to the type of bird, to the variety, or to the duration of excreta collection period. The high variability in T.M.E. values (as noted in S.E.M.), obtained from turkey poults, may be attributed to differences in the amount of excreta voided. It was interesting to note the liquid appearance of the excreta collected from poults. The excreta collection trays were completely filled with watery droppings. It is possible that some of the wet excreta may have been voided beyond the capacity of the trays to contain them. The watery consistency of the droppings

TABLE XII. COMPARISON OF THE MEAN $T.M.E._{24}$ AND $T.M.E._{48}$ VALUES[a] (KCAL/G. DRY MATTER) OF TRITICALE VARIETIES, MEASURED WITH ROOSTERS AND TURKEYS.

	ROOSTERS			TURKEYS		
	A	B	C	A	B	C
24-hr. collection						
Mean[c]	3.61	3.58	3.54	3.28	3.58	3.56
S.E.M.[d]	.05	.04	.16	.17	.13	.12
48-hr. collection						
Mean	3.45	3.48	3.33	3.23	3.58	3.54
S.E.M.	.09	.07	.14	.22	.24	.14
Difference between means	.16	.10	.21	.05	.00	.02

[a]Calculated by using corrected endogenous energy values.

[b]Variety A: VT-75 229
" B: Palouse
" C: 1776-8651 02

[c]Each value is the mean of 5 replicates.

[d]Standard error of the mean.

is assumed to be related to increased water consumption during the feed withdrawal period.

Triticale varieties used in this experiment cleared the digestive tract within 24 hours. Extending the excreta collection period to 48 hours resulted in increased standard errors of the mean T.M.E. values.

Sibbald (1976d) reported that the endogenous energy voided per unit of time by the negative control birds decreased with the duration of starvation. Thus the small differences between the 24- and 48-hour T.M.E. values for the triticale varieties obtained from roosters may be associated with the differences in $FE_m + UE_e$ outputs of the control versus fed birds.

Results of this experiment are in agreement with Sibbald's comparative study (1976c), with birds of different species, breeds and sexes. The correction for $FE_m + UE_e$ makes T.M.E. data applicable to a wide variety of birds in addition to the preferred assay bird, S.C.W.L. cockerels (Sibbald, 1980d).

EXPERIMENT VII. The Use of Gelatin Capsules as an Alternative Method of Force-feeding in T.M.E. Bioassay

$T.M.E._{48}$ (48-hour collection) values of spray and freeze-dried hake meal, corn and corn oil fed in gelatin capsules, together with their mean intakes (g.), are presented in Table XIII. The mean T.M.E. values for spray-dried hake meal, freeze-dried hake meal,

TABLE XIII. $T.M.E._{48}$ VALUES OF SPRAY AND FREEZE-DRIED HAKE MEAL, CORN AND CORN OIL, MEASURED WITH ADULT S.C.W.L. ROOSTERS BY THE AID OF GELATIN CAPSULES.

	FEEDSTUFF			
	Spray-dried hake meal	Freeze-dried hake meal	Yellow corn	Corn oil
$T.M.E._{48}$[1] kcal/g.	5.26 ± .11 (6)[2]	3.99 ± .08 (3)	3.97 ± .08 (4)	7.35 ± .3 (3)
Mean intake (g.)	12.5	11.5	12.9	11.7
Mean gelatin intake (g.)	1.63	1.58	1.64	1.55

[1]Expressed on a dry-matter basis.

[2]Number of replications.

corn and corn oil were 5.26, 3.99, 3.97 and 7.35 kcal/g. dry matter, respectively. Mean gelatin consumption through the capsules were 1.61 g., with an average gelatin:feed ratio of 1:7.5. Comparison of the T.M.E. values of freeze-dried hake meal and corn (Table XIV) fed either by the regular force-feeding method or by the aid of gelatin capsules, confirmed the absence of statistical differences associated with the method of administration. High standard errors observed in mean T.M.E. values are the result of low level of feed input. Sibbald (1977d) reported a decrease in standard errors of mean T.M.E. values as the level of feed input increased from 10 to 30 g./bird. However, there was no trend associated with the effect of level of input on the ability of birds to extract energy from the feed. The low level of feed input used in this experiment was mainly due to difficulties of administering large quantities of gelatin capsules into the crop of the cockerels. Average amount of capsules used were 10 caps./bird.

Physical (powdery, sticky, hygroscopic) characteristics of freeze-dried hake meal and related difficulties in achieving quantitive delivery into the crop caused a high degree of variation when this feedstuff was fed alone (Table XIV). Dudley et al. (1980) also proposed the use of gelatin capsules for measuring the T.M.E. values of liquid (fats and oils) and sticky (blood meals) ingredients. Their method required accurate determination of the T.M.E. value of gelatin and necessary corrections on the T.M.E. value of feed plus

TABLE XIV. COMPARISON OF T.M.E.$_{48}$ VALUES OF FREEZE-DRIED HAKE MEAL AND CORN, OBTAINED BY EITHER FEEDING THEM ALONE OR IN GELATIN CAPSULES.

Feedstuff	T.M.E.$_{48}$, kcal/g. dry matter	
	Force-fed alone	Force-fed in gelatin capsules
Freeze-dried hake meal	4.00 ± .11[a] (9)[1]	3.99 ± .08[a] (3)
Yellow corn, ground	4.09 ± .003[a](4)	3.97 ± .08[a] (4)

[1]Number of replications.

[a]Values with same superscript are statistically non-significant ($P < 0.05$).

gelatin, thus introducing a new source of variation. However, they did not report any T.M.E. values in their abstract regarding the results of their studies. The high T.M.E. value for spray-dried hake meal is due to the higher gross energy content of this product compared to freeze-dried one.

The only surprising result in this experiment was the comparatively low T.M.E. value (7.35 kcal/g.) of corn oil. There are difficulties associated with accurate delivery of fat into the crop (Sibbald and Price, 1977b). Thus, the usual procedure of assaying fats for T.M.E. has been feeding them in conjunction with other feedstuffs or basal diets and calculating the T.M.E. value of fats by the difference (Sibbald, 1977b). However, since the statistical error must be applied against a smaller portion of test ingredient, the confidence interval of the absolute energy value of the test ingredient is very large (Dudley et al., 1978). Sibbald and Kramer (1977) and Sibbald (1978b) had observed T.M.E. values for fats and oils which were higher than their gross energy contents and suggested an interaction with other dietary components, thus causing an increase in their utilization. T.M.E. values for corn oil, reported in literature ranges between 9.40 to 9.87 kcal/g. (Sibbald, 1977b; Sibbald and Kramer, 1977) being considerably higher than the 7.35 kcal/g. obtained in this experiment. The possible explanation for this difference may be the adverse effects of oil alone on the digestive processes and metabolic systems or the prevention of

possible interaction with other dietary components (Sibbald and Price, 1977b).

The method of administering the feed into the crop by gelatin capsules and the values obtained therefrom, with the exception of the questionable value for corn oil, was found to be useful and valid in terms of assaying feedstuffs which possess different physical characteristics.

EXPERIMENT VIII. The Effect of Water Treatment and the Addition of Corn on T.M.E. Value of Western Barley

The experimental data are summarized in Table XV. The T.M.E. values were 3.51, 3.43, and 3.44 kcal/g. of dry matter for ground western barley, water-treated barley and and barley + 10 percent corn, respectively. Statistical analysis of the data showed no differences between the treatment groups. Also, efficiency of energy utilization, expressed as percentages of the gross energy of the respective treatments, did not show any trend associated with treatment of barley.

There are several reports in literature regarding the increases in metabolizable energy (M.E.) content of western barley, either by water treatment (Leong et al., 1962; Potter et al., 1965) or by addition of corn (Arscott, 1963). The differences in utilization of energy content of western barley may be associated with the age of the birds, since the above mentioned M.E. determinations were conducted with chicks. If the ability of birds to extract energy from

TABLE XV. RESULTS FROM ANALYZING GROUND BARLEY, WATER-TREATED BARLEY AND CORN ADDED BARLEY, EXPRESSED ON A DRY-MATTER BASIS.

Dietary variable	Gross energy (kcal)	T.M.E.[1] (kcal/g.)	Percent metabolized
Barley,[3] ground	4.36	3.51 ± .06 (5)[2]	80.5
Water-treated barley	4.41	3.43 ± .05 (5)	77.8
Barley + 10% corn	4.41	3.44 ± .03 (6)	78.0

[1]Means and standard errors.

[2]Numbers in parentheses are the number of replications.

[3]Variety, Klages.

feed ingredients increases with the development of digestive systems, adult birds which were used in this study would be expected to be more efficient in utilizing the energy of western barley. Also, growth improvements in chicks fed barley are reported to be related to the presence of the enzyme, β-glucanase, isolated from B. subtilis (Rickes et al., 1962). Hence, the digestive enzymes of adult birds or other factors (β-glucanase) produced by microorganisms in their digestive tract may be, in part, the reason for the lack of differences between the barley treatments.

Sibbald (1975a, 1978c) suggested that the differences in the M.E. values associated with age, strain and species may be attributable in part to variations in the $FE_m + UE_e$ losses relative to outputs of energy of feed origin. Shires et al. (1980) also concluded that, with the exception of high-glucosinolate rapeseed meal, T.M.E. values obtained with adult roosters were applicable to the formulation of diets for young, growing birds.

At this point, the use of T.M.E. value of western barley obtained with adult birds is questionable when applied to chick rations. Further work and evidence is necessary to avoid errors that may be associated with such an application.

EXPERIMENT IX. T.M.E. Values of Sunflower Seed Products; Dehulled Seeds, Ground Seeds and Hulls, Measured With Roosters Maintained on Grit and Non-grit Regimens

Table XVI summarizes gross (unadjusted) and net (adjusted)

TABLE XVI. SUMMARY OF GROSS AND NET EXCRETA WEIGHTS[1] OF CONTROL AND FED ROOSTERS, KEPT EITHER ON GRIT OR NON-GRIT REGIMENS.

	Grit			Non-grit		
	Gross excreta weight	Net excreta weight	Adj. on excreta weight	Gross excreta weight	Net excreta weight	Adj. on excreta weight
	grams					
Control birds	7.19 ± .61[a]	5.72 ± .24[b]	1.47 ± .45[c]	5.43 ± .29[b]	5.31 ± .29[b]	0.12 ± .03[d]
Fed birds:						
Sunflower seeds dehulled	13.16 ± .46[a]	12.06 ± .48[a]	1.10 ± .24[b]	12.51 ± .25[a]	12.25 ± .19[a]	0.26 ± .07[c]
Sunflower seeds ground	11.00 ± .57[a]	10.00 ± .30[a]	1.00 ± .31[b]	10.26 ± .24[a]	9.99 ± .22[a]	0.27 ± .03[c]
Sunflower seeds hulls	17.67 ± 1.0[a]	12.44 ± .39[b]	5.23 ± .95[c]	11.70 ± .26[b]	11.17 ± .26[b]	0.53 ± .07[d]

[1]Means and standard errors (48-hr. collection).

[a,b,c,d]Means with different superscripts, with a row, are significantly ($P < 0.05$) different.

excreta weights of control and fed roosters maintained on either grit or non-grit regimens. Adjusting the excreta weights on a grit-free basis by the screening process reduced the variability associated with the grit feeding. Statistical analysis of the data revealed significant ($P < 0.05$) difference between gross and net excreta weights of control and sunflower seed hulls fed roosters on the grit treatment, whereas the fecal samples from the birds fed dehulled and ground sunflower seeds were not affected by the screening process significantly. Adjustments made on excreta weights (g.) between the grit and non-grit treatments were significantly different for both control and the fed birds. It was noticed that in both treatments the adjustments (g.) on excreta weights of birds that had been fed sunflower seed hulls were considerably higher than that of birds receiving the other sunflower seed products. This difference could be associated with the higher (37.22%) fiber (acid detergent fiber) content of the hulls (Table XVII). It seems probable that the hulls may have forced the movement of grit and other particles that were retained in gizzards of the birds (Exps. I and III) to the posterior portions of the digestive tract. Even though they were kept on a grit-free regimen, the same mechanism could also be true for the birds on non-grit treatment. Keith (1927) mentioned the high retaining characteristics of birds in their gizzard, when there was no grit available. Thus, it is logical to assume the presence of some grit in digestive tracts of birds

TABLE XVII. THE PROXIMATE ANALYSIS[1] OF THE SUNFLOWER SEED PRODUCTS USED IN EXPERIMENT IX.

	Dry matter (%)	Crude protein (%)	ADF[2] (%)	Gross energy (kcal/g.)
Sunflower seeds:				
dehulled	94.93	24.38	8.99	6.98
ground	93.26	19.39	21.25	6.53
hulls	90.95	8.58	37.22	4.98

[1]Values are expressed on air dry basis.

[2]Acid detergent fiber.

on non-grit group. The results obtained in Experiment I would also agree with this assumption.

Endogenous energy ($FE_m + UE_e$) outputs of control birds and the T.M.E. values of sunflower seed products obtained from birds in both treatments and calculated by using gross and net excreta weights are presented in Table XVIII. $FE_m + UE_e$ outputs of both control birds on grit and non-grit treatments showed a high degree of variability. The significant differences observed in gross and net excreta weights of control birds on grit treatment (Table XVI) were not reflected to their $FE_m + UE_e$ output. Numerical difference between "as is" and "corrected" values were not significant. This result is contradictory to the results obtained in Experiment IV, where the $FE_m + UE_e$ outputs of control birds on grit treatment were significantly affected with the use of gross excreta weights. One possible reason for this difference may be the shorter (two weeks) exposure to grit feeding employed in this experiment. The reductions in standard errors of the mean $FE_m + UE_e$ outputs of control birds on grit treatment once again confirmed the usefulness of screening process (Exp. IV).

The "as is" T.M.E. values (calculated by using gross excreta weights) of sunflower seed products, namely; dehulled seeds, ground seeds and hulls, on grit treatment, were 6.37, 5.79, and 1.43 kcal/g. dry matter, respectively. Adjusting the excreta weights on a grit-free basis (corrected T.M.E. values) only affected the T.M.E.

value of hulls significantly ($P < 0.05$). This result was expected because of relatively high amount of grit excretion of these birds (Table XVII). The $FE_m + UE_e$ outputs of control birds and the T.M.E. values obtained from fed birds on non-grit treatment did not change significantly with the excreta adjustments. This is in agreement with the results of previous experiments (Exp. IV).

When compared, only the T.M.E. values of dehulled sunflower seeds were found to be significantly ($P < 0.05$) higher for the grit treatment. The difference, even though small, may be associated with the grinding action of grit in the gizzard, and the consequent enhancement of energy utilization. McIntosh et al. (1962) discussed other possible action(s) of grit in addition to grinding the feed in gizzard, such as (1) to stimulate the flow of digestive juices, (2) to present a greater surface for enzyme action, (3) to slow down the rate of passage of feed in digestive tract, thus allowing more complete digestion. Even though the excreta collection period had been extended to 48 hours in this experiment, delayed passage may be the reason for overestimated values. This raises the question of the need for precise knowledge of passage rates of feedstuffs that are used in assays. On the other hand, Muztar and Slinger (1979b) speculated that presence of grit in gizzards of birds may increase the birds' ability to move fibrous feeds through the gizzard. If this theory was true, one would have expected to observe higher T.M.E. values for ground sunflower seeds and sunflower seed hulls

TABLE XVIII. ENDOGENOUS ENERGY OUTPUTS OF CONTROL BIRDS, TOGETHER WITH THE "AS IS" AND "CORRECTED" T.M.E. VALUES OF SUNFLOWER SEED PRODUCTS, OBTAINED FROM ROOSTERS KEPT EITHER ON GRIT OR NON-GRIT REGIMENS

	Grit		Non-grit	
	As is	Corr.[1]	As is	Corr.
Endogenous Energy (FE_m+UE_e)[2]				
Mean	17.85	14.20[a] (6)[3]	15.87[a]	15.51[a] (5)
SEM	1.56	.63	.94	.91
	T.M.E. (kcal/g. dry matter)			
Sunflower seeds:				
dehulled	6.37±.1[a]	6.37±.1[a](6)	6.06±.09[b]	6.08±.09[b] (5)
ground	5.79±.13[a]	5.75±.08[a](5)	5.61±.06[a]	5.66±.05[a] (6)
hulls	1.43±.16[a]	2.78±.16[b](6)	2.57±.09[b]	2.71±.12[b] (6)

[a,b]Means with different superscripts, within a row, are significantly ($P < .05$) different.

[1]Correction is made by adjusting the excreta weights on a grit-free basis.

[2]kcal/bird/48 hr.

[3]Number of replications.

from the birds on non-grit treatment, since these feed ingredients contain much higher percentage of ADF (Table XVII). However, if the excreta collection period had been kept 24 hours, the T.M.E. values of these high-fiber sunflower seed products may have been overestimated.

Throughout the experiments in this study, one interesting observation was the presence of variable amounts of inorganic residue (assumed to be ash) on combustion capsules after the combustion. In order to find out the possible relationship between the amount (g.) of residue and the energy determinations, these ash portions of the samples were carefully weighed after each combustion. The mean ash content of the fecal samples from both grit and non-grit treatments are summarized in Table XIX. In terms of their ash content, fecal samples of control birds in both treatments differed significantly ($P < 0.05$), being 0.23 and 0.11 percent for grit and non-grit treatments. Similar significant differences among the two treatments were also observed in fecal samples of fed birds. Birds on non-grit treatment showed a uniform trend in their fecal ash content, with the exception of birds which were fed sunflower seed hulls. Presence of grit in digestive tracts of control and fed birds resulted in variability in their fecal ash contents. Obviously, this new source of error was mainly due to pulverized grit particles that had passed through the 20-mesh sieve. The nonsignificant effect of grit treatment on control birds may have been the

TABLE XIX. THE MEAN ASH CONTENT[1] OF THE FECAL SAMPLES, COLLECTED FROM THE STARVED AND FED ROOSTERS.

	Grit	Non-grit
	ash (%)	
Control[2]	0.23 ± .01[a]	0.11 ± .01[b]
Sunflower seed products:		
dehulled seeds	0.21 ± .02[a]	0.09 ± .01[b]
ground seeds	0.14 ± .01[c]	0.08 ± .003[b]
hulls	0.13 ± .03[c]	0.05 ± .01[d]

[1]Determined by weighing the inorganic residue remained after the combustion of the fecal material.

[2]Starved 48 hr. to estimate the FE_m + UE_e losses.

a,b,c,d
Values with different superscripts are significantly ($P < 0.05$) different.

result of higher ash content and underestimated gross energy values of the fecal samples. The percent ash differences between the fecal samples of control and fed birds may effect the validity of correction and the T.M.E. values. Sibbald (1979b) observed an increase in the T.M.E. values of alfalfa as the sum of crude protein plus ash decreased. One might speculate that had the fecal samples of these birds contained the same amount of ash as the control birds, the gross energy values and resulting T.M.E. values would have been lower in grit treatment. The same mechanism should also decrease the T.M.E. values of sunflower seed hulls obtained from non-grit treatment.

It seems very possible that some of the observed variability in $FE_m + UE_e$ outputs of control and fed birds may be the result of differences in ash content of their excreta. However, there is a need for further research to validate these assumptions.

The amount of inorganic residue that remains in combustion capsules after each energy determination could easily and readily be used to estimate the ash content of feeds and/or excreta samples as an alternative rapid method. Such a technique would allow one to determine the gross energy and the ash content of the samples simultaneously and would greatly reduce the time and cost involved in separate analysis.

Summary of T.M.E. Values of Selected Feedstuffs

The T.M.E. values and proximate analysis of 10 selected feedstuffs determined during the course of this study are presented in Table XX. Some of the feedstuffs are sub-divided to make the data more informative. Samples of spray and freeze-dried hake meal and corn oil were assayed with the aid of gelatin capsules rather than as single ingredients.

Samples of ground corn, drawn from the same lot, were assayed as a control ingredient throughout the course of this study. The T.M.E. value of corn (4.06 ± .04 kcal/g. dry matter), when analyzed separately, did not show any trend associated with seasonal changes. This result further supports the observations made in Exp. II, regarding the effect of light and temperature regimens on T.M.E. values.

TABLE XX. SUMMARY OF ANALYSIS OF SOME SELECTED FEEDSTUFFS AVAILABLE IN THE PACIFIC NORTHWEST.

Feedstuff	Dry matter (%)	Crude protein (%)	Gross energy (kcal/g.)	T.M.E.[1] (kcal/g. dry matter)	
Alfalfa, 17%	90.7	18.0	4.08	1.68 ± .05	(2)[2]
W. Barley (Klages)	88.3	9.0	3.84	3.48 ± .05	(16)
Corn, yellow	87.7	8.7	3.91	4.06 ± .04	(60)
Corn oil	-	-	9.96	7.35 ± .3	(3)
Fish meals:					
- Herring	89.2	65.9	4.87	4.01 ± .11	(5)
- Hake, spray dried	91.2	55.2	5.63	5.26 ± .11	(6)
- Hake, freeze-dried	89.2	58.1	4.93	4.00 ± .10	(12)
Meat and Bone meal	91.6	48.1	3.72	2.66 ± .05	(5)
Soybean meals:					
- 44%	89.6	42.0	4.11	2.95 ± .03	(5)
-47.5%	88.3	46.6	4.22	2.81 ± .09	(5)
Sunflower seeds:					
- dehulled	94.9	24.4	6.98	6.08 ± .09	(5)
- ground	93.3	19.4	6.53	5.71 ± .07	(11)
- hulls	91.0	8.6	4.98	2.75 ± .14	(12)
Triticale:					
- Palouse	88.3	12.1	3.85	3.61 ± .05	(5)
- VT-75 229	88.2	13.8	3.89	3.58 ± .04	(5)
- 1776 8651 02	88.1	12.6	3.78	3.54 ± .16	(5)
Wheat (Yamhill)	87.2	9.3	3.81	4.07 ± .03	(5)

[1]Means and standard errors.

[2]Number of replications.

V. SUMMARY AND CONCLUSIONS

Several experiments were conducted using adult, S.C.W.L. roosters, and in one experiment Wrolstad poults, to evaluate the T.M.E. bioassay of some available feedstuffs in the Pacific Northwest.

The following conclusions were made from the results of these experiments.

1. Direct examination of the gastrointestinal tract of assay birds following 24 hours of starvation, but prior to initiation of the assay, revealed the presence of variable amount of dry matter and grit, mainly in the gizzard, which persisted even up to 48 hours of starvation.

2. The endogenous energy ($FE_m + UE_e$) outputs of negative control birds were found to be independent of body size. The application of the excreta screening process slightly improved the standard errors associated with the mean dry matter outputs of birds but failed to improve the correlations.

3. With adult, S.C.W.L. rooster, the T.M.E. values obtained were found to be unaffected with the short-term changes in light and temperature regimens.

4. The presence of grit in digestive systems of the assay birds significantly affected the gross excreta outputs and gave

misleading fecal gross energy values. The influence of grit feeding on $FE_m + UE_e$ outputs and on T.M.E. values was found to be the result of a weight contribution when voided with excreta. However, the significant differences observed in ash content of the fecal samples was assumed to have been the cause of obtaining a non-abrasive effect.

5. The screening process, when applied to fecal samples from the birds that were fed high-energy feeds, did not influence the T.M.E. values significantly, but failed to reduce the standard errors associated with them.

6. Soybean meal, meat and bone meal, and fish meals required longer excreta collection periods, due to their delayed passage rate.

7. There were no significant differences between roosters and poults, in terms of utilizing the energy of triticale varieties. The T.M.E. values of three varieties of triticale were unchanged with the extension of excreta collection period, indicating a complete clearance of the digestive tract within 24 hours.

8. The use of gelatin capsules, as an alternative method of administering feeds, which poses difficulties in quantitative delivery, yielded comparable data to regular force-feeding method. However, the values obtained for corn oil are presently questionable, due to special characteristics of this feed ingredient.

9. The T.M.E. value of western barley (Klages) was unaffected by water treatment and corn supplementation when assayed with adult roosters. It is questionable, at this point, whether these values could be used in formulation of diets for chicks.

10. A summary of T.M.E. values of some selected feedstuffs determined through the course of this study are presented.

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