Estimation of Nitrogen-corrected Metabolizable Energy Value of Laboratory Canine Diets

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This study evaluates the reliability of the equation, metabolizable energy (ME) = digestible energy (DE) - $1.25 \times$ digestible crude protein (DCP), as a means of estimating the energy value of non-purified diets for laboratory dogs. To do this, five digestion and nitrogen-balance (NB) trials and determination of the energy value of a diet were conducted using five diets and four mature, male Beagles for each experiment. The resulting digestibility of crude protein, acid ether extract, and nitrogen-free extract of these diets were 79.5%, 91.6%, and 84.8%, respectively. The NB in every diet was positive: 1.4~8.6mg/g dry matter of ingested diet. Of the nitrogen (N) ingested, 21% was excreted into feces, 66% was excreted into urine, and 13% was retained. In terms of dietary gross energy (GE), 18% was lost in feces and 5% was excreted into urine leaving 77% as ME. Retained energy in the body was only 1%, and N-corrected metabolizable energy (MEN) was 76% of GE. The average urinary energy (UE) loss per gram of urinary N was 7.84kcal. The correlation between UE/DCP and NB values is statistically significant: $UE/DCP = 1.24 - 0.03NB$, $r = 0.798$, $P < 0.001$. Therefore, UE/DCP is 1.24kcal/g at N-equilibrium, almost the same as the factor in the above equation. The ME value calculated by the equation is identical to the MEN value, and both are significantly lower ($P < 0.05$) than the empirically determined ME value. We conclude that this equation should be expressed as $MEN = DE - 1.25DCP$, and is a reliable and convenient means of estimating the MEN of diets. Also, this study demonstrates that this revised equation can be applied, in the case of dogs, not only at N-equilibrium but also when N is retained in the body.—KEY WORDS: dietary energy, dog, nitrogen-corrected metabolizable energy, nitrogen-equilibrium, non-purified laboratory diet

Recently, Suzuki *et al.* [14] recommended using nitrogen (N)-corrected metabolizable energy (MEN) to evaluate energy in non-purified diets for laboratory animals, including canine diets. However, the determination of MEN involves laborious and time-consuming processes, such as collection of all urine excreted, removal of hair and dust, freeze-drying of urine, analysis of urinary nitrogen, and determination of urinary energy using an adiabatic bomb calorimeter. To estimate the metabolizable energy (ME) value, researchers (Kendall *et al.* [6], Hirakawa and Baker [5], and Finke [3]) have proposed using the equation of $ME = DE - 1.25DCP$, where DE is digestible energy and DCP is digestible crude protein

determined empirically. Before adopting this equation in the case of dogs, three items should be considered:

- ① how the equation was actually derived as a means of evaluating dietary energy values;
- ② whether the calculated ME value (cME) using the equation is equal to the values of determined ME or MEN;
- ③ use of 1.25 as the DCP factor.

In spite of this, however, Czarnecki-Maulden *et al.* [2] evaluated the validity of this equation in research supported by the Pet Food Institute of the U.S.A.

The present work was designed to ascertain the reliability of the equation as a means of estimating MEN. For this purpose, five diges-

tion and nitrogen-balance (NB) trials and determination of energy value of a diet were conducted using five non-purified, dry diets and four mature, male Beagles for each experiment.

Materials and Methods

Experiments and diets: In this study, experiments were conducted using five diets: one (Experiment (Expt.) 1) was specially prepared, and four (Expts. 2 ~ 5) were commercially available dry diets for laboratory dogs (Nihon Nosan Kogyo K. K.). The formula of the diet used in Expt. 1 is shown in Table 1 and is similar to the proprietary formulation used in Expts. 2 ~ 5. All the diets were obtained by first processing the mash, except for fat component (extra fancy tallow). The mash was processed into expanded pellets using an extruder for 3-4 min. at around 120-130°C under 30 % moisture. The pellets were then dried to approximately 10 % moisture. The surface of the processed diet was then sprayed with liquefied fat. The diets were then crushed using a hammer-mill, and 0.3 % Cr_2O_3 was added as an index for determining digestibility and energy content through a 1.5 mm screen. Table 2 shows the chemical analysis of all five diets. At 10 AM each day, each dog was fed 250 g of the crushed diet, which was first added to 120ml of warm water and then kneaded to form a stiff mixture. Drinking water was available at all times to each animal.

Animals: In Expts. 1 ~ 3, four mature, male Beagles (each weighing an average 15kg) from one litter were used, and in Expts. 4 and 5, four (each weighing an average 12kg) from another litter were used. These two litters were bred in the same colony.

Collection of feces and urine: The animals were individually housed in metabolism cages ($\text{H} \times \text{L} \times \text{W}$: 75 × 70 × 75 cm) equipped with a metal drain-board floor. In each Expt., a 5-day preliminary period for the preparatory test was followed by a 4-day period for the independent collection of feces and urine. Feces were dried at approximately 65°C and then placed in a room to reach equilibrium in terms of moisture content. Hair was then removed from the dried feces by crushing it through a 1 mm screen. Urine was collected in a bottle containing 5 ml of 5 % (v/v) H_2SO_4 solution. A por-

Table 1. Composition of the experimental diet used in Expt. 1

Ingredients	%
Corn	40.00
Milo	7.00
Dried bakery products	5.00
Wheat bran	6.00
Rice bran, solvent extracted	4.00
White fish meal	5.00
Meat and bone meal	10.00
Gelatin by-product	1.00
Soybean oil meal, solvent extracted	3.25
Corn gluten meal	4.00
Torula dried yeast	2.00
Roasted soybean flour (kinako)	7.00
Salt	1.00
Extra fancy tallow	4.00
Vitamin and mineral mixture*	0.75
Total	100.00

* The premix contained per kilogram 3,000,000 I.U. vitamin A, 600,000 I.U. vitamin D₃, 12,000 I.U. vitamin E, 520mg vitamin K₃, 350mg thiamine, 530mg riboflavin, 2,500mg niacin, 250mg pyridoxine, 3.4mg calcium pantothenate, 6.0mg vitamin B₁₂, 20mg biotin, 68mg choline chloride, 45mg folic acid, 1,070 mg Mn, 11.0mg Fe, 500mg Co, 10.0mg Zn and 320 mg I.

Table 2. Chemical analysis (% dry matter basis) of the diets¹⁾

Expt.	Crude protein	AEE ²⁾	NFE ³⁾	Carbohydrate ⁴⁾	Crude ash
1	26.4	11.9	50.7	54.1	7.6
2	26.8	11.9	48.8	52.8	8.5
3	28.6	8.9	50.0	53.3	9.2
4	27.2	9.8	50.7	54.6	8.4
5	24.2	9.5	51.5	56.7	9.6

1) Dry matter(%) of the diets used in Expts. 1-5 was 90.0, 91.5, 90.6, 92.2, and 91.0, respectively,

2) Acid ether extract

3) Nitrogen-free extract

4) NFE+Crude fiber

tion of this collected urine was neutralized with NaOH and then freeze-dried for calorimetry.

Chemical analysis: Proximate analysis was conducted on the diets and collected feces using official Japanese methods [10], with the exception of crude fat analysis, which was determined according to the AOAC [1] as acid ether extract (AEE). Only the N-free extract (NFE) was calculated by subtraction of the

proximate analysis from 100%. As for extruded diets, the usage of AEE in the calculation of NFE is standard for the official Japanese method [10]. Moreover, it is recommended by the AOAC [1]. The Cr_2O_3 contents of the diets and feces were determined at 444 nm in accordance with Hill and Anderson [4] using a spectrophotometer (Spectronic 20A, Shimadzu Co.). The caloric contents of the diets, dried feces, and urine were determined using an adiabatic bomb calorimeter (OSK150, Ogawa Co.).

Determination of MEn: According to the National Research Council (NRC) [12], the MEn (in units of kcal per gram of ingested dry matter (DM)) is defined by

$$\text{MEn} = \text{GE} - (\text{FE} + \text{UE}) - (\text{NB} \times \text{UE} / \text{UN}), \quad (1)$$

where GE is the gross energy of a diet, FE and UE are the energy of feces and urine (kcal/g of ingested DM), respectively, NB is the N-balance or nitrogen (g) retained per gram of in-

gested DM and UN is the urinary nitrogen (g of ingested DM).

Definition of cME: In this work we defined cME as the ME value calculated by [3, 5, 6]

$$\text{cME} = \text{DE} - 1.25\text{DCP}. \quad (2)$$

Results

Apparent digestibility of dietary nutrients: Table 3 shows the apparent digestibility of the dietary nutrients in each diet. The mean and standard deviation of the values for the digestibility of DM, crude protein (CP), AEE, NFE, and carbohydrate were $78.2 \pm 2.0\%$, $79.5 \pm 2.1\%$, $91.6 \pm 1.8\%$, $84.8 \pm 3.6\%$, and $81.4 \pm 3.8\%$, respectively. The mean digestibility values for CP, AEE, and NFE were in good agreement with those adopted by the NRC (i. e., 80%, 90%, and 85%, respectively) [13].

N-balance: As shown in Table 4, the NB in each diet was slightly positive, and the N-

Table 3. Apparent digestibility (%) of dietary nutrients in the diets used in Expts. 1-5 (Means $\pm$ S.D.¹⁾)

Expt.	Number of dogs used	Dry matter	Crude protein	AEE ²⁾	NFE ³⁾	Carbohydrate ⁴⁾
1	4	77.4 $\pm$ 0.4	76.1 $\pm$ 0.4	93.8 $\pm$ 0.4	79.2 $\pm$ 0.7	75.8 $\pm$ 0.8
2	4	79.8 $\pm$ 0.8	82.3 $\pm$ 1.1	92.9 $\pm$ 0.3	86.3 $\pm$ 1.0	83.3 $\pm$ 0.9
3	4	76.3 $\pm$ 0.5	78.3 $\pm$ 1.1	88.6 $\pm$ 0.8	85.2 $\pm$ 0.3	82.7 $\pm$ 0.2
4	4	81.2 $\pm$ 0.8	80.9 $\pm$ 0.9	92.0 $\pm$ 0.9	90.0 $\pm$ 0.8	86.8 $\pm$ 1.1
5	4	76.4 $\pm$ 1.4	79.7 $\pm$ 0.7	90.9 $\pm$ 0.5	83.2 $\pm$ 1.9	78.6 $\pm$ 2.2
Mean $\pm$ SD		78.2 $\pm$ 2.0	79.5 $\pm$ 2.1	91.6 $\pm$ 1.8	84.8 $\pm$ 3.6	81.4 $\pm$ 3.8

1) Standard deviation 2), 3), 4) Same as in Table 2

Table 4. Nitrogen(N)-balance (mg/g dry matter of diet) for each diet (Means $\pm$ S.D.¹⁾)

Expt.	Number of dogs	N-intake	N-excretion		N-retention
			Feces	Urine	
1	4	42.3	10.1 $\pm$ 0.2	23.6 $\pm$ 1.6	8.6 $\pm$ 1.8
2	4	43.0	7.6 $\pm$ 0.5	29.4 $\pm$ 2.4	6.0 $\pm$ 2.8
3	4	45.9	10.0 $\pm$ 0.5	32.7 $\pm$ 1.5	3.3 $\pm$ 2.0
4	4	43.6	8.3 $\pm$ 0.5	26.8 $\pm$ 1.3	8.4 $\pm$ 1.2
5	4	38.6	7.8 $\pm$ 0.2	29.4 $\pm$ 2.7	1.4 $\pm$ 2.8
Mean $\pm$ S.D.		42.7	8.8 $\pm$ 1.1	28.4 $\pm$ 3.0	5.5 $\pm$ 2.8
% of N-distribution		100	21 $\pm$ 2	66 $\pm$ 7	13 $\pm$ 7

1) Standard deviation

Table 5. Urinary energy per urinary nitrogen(N), per urinary N $\times$ 6.25, and per digestible crude protein (kcal/g) (Mean $\pm$ S.D.¹⁾)

Expt.	Number of dogs used	UE/UN ²⁾	UE/UN $\times$ 6.25 ³⁾	UE/DCP ⁴⁾
1	4	8.97 $\pm$ 0.27	1.43 $\pm$ 0.04	1.05 $\pm$ 0.08
2	4	7.77 $\pm$ 0.30	1.24 $\pm$ 0.05	1.03 $\pm$ 0.10
3	4	8.11 $\pm$ 0.19	1.30 $\pm$ 0.03	1.19 $\pm$ 0.05
4	4	6.89 $\pm$ 0.43	1.10 $\pm$ 0.07	0.84 $\pm$ 0.09
5	4	7.48 $\pm$ 0.59	1.20 $\pm$ 0.10	1.14 $\pm$ 0.15
Mean $\pm$ S.D.		7.84 $\pm$ 0.77	1.25 $\pm$ 0.12	1.05 $\pm$ 0.13

1) Standard deviation

2) Urinary energy/urinary N

3) Urinary energy/

urinary N $\times$ 6.25

4) Urinary energy/ingested digestible crude protein

Table 6. Distribution of dietary energy and the calculated metabolizable energy for each diet used in Expts. 1-5 (kcal/g dry matter of diet) (Mean $\pm$ S.D.¹⁾)

Expt.	Number of dogs used	GE ²⁾	FE ³⁾	DE ⁴⁾	UE ⁵⁾	ME ⁶⁾	NB ⁷⁾ $\times$ UE/UN ⁸⁾	ME _N ⁹⁾	cME ¹⁰⁾
1	4	4.96	0.95 $\pm$ 0.02	4.01 $\pm$ 0.02	0.21 $\pm$ 0.01	3.80 $\pm$ 0.03	0.08 $\pm$ 0.01	3.72 $\pm$ 0.02	3.76
2	4	4.80	0.78 $\pm$ 0.03	4.02 $\pm$ 0.03	0.23 $\pm$ 0.02	3.79 $\pm$ 0.04	0.05 $\pm$ 0.02	3.74 $\pm$ 0.02	3.75
3	4	4.65	0.88 $\pm$ 0.04	3.77 $\pm$ 0.04	0.27 $\pm$ 0.01	3.50 $\pm$ 0.03	0.03 $\pm$ 0.02	3.47 $\pm$ 0.02	3.47
4	4	4.54	0.72 $\pm$ 0.03	3.82 $\pm$ 0.03	0.18 $\pm$ 0.02	3.64 $\pm$ 0.03	0.05 $\pm$ 0.01	3.59 $\pm$ 0.03	3.54
5	4	4.49	0.94 $\pm$ 0.06	3.55 $\pm$ 0.06	0.22 $\pm$ 0.02	3.33 $\pm$ 0.07	0.01 $\pm$ 0.02	3.32 $\pm$ 0.06	3.31
Mean $\pm$ S.D.		4.69 $\pm$ 0.17	0.85 $\pm$ 0.09	3.83 $\pm$ 0.17	0.22 $\pm$ 0.03	3.61 $\pm$ 0.18*	0.04 $\pm$ 0.02	3.57 $\pm$ 0.16*	3.57 $\pm$ 0.17*
% of GE		100	18 $\pm$ 2	82 $\pm$ 2	5 $\pm$ 1	77 $\pm$ 2	1 $\pm$ 0	76 $\pm$ 2	—

1) Standard deviation

2) Gross energy

3) Fecal energy

4) Digestible energy

5) Urinary

energy 6) Metabolizable energy

7) Nitrogen balance

8) This overall formula means urinary

energy loss assumed for the metabolism of retained nitrogen

9) Nitrogen-corrected metabolizable energy

10) Calculated metabolizable energy obtained using cME=DE-1.25DCP

* There were significant differences (P<0.05) between ME and ME_N, and between ME and cME.

retention level ranged from 1.4 to 8.6mg/g DM. Of the N ingested, 21 % on average was excreted into feces, 66 % was excreted into urine, and 13 % was retained. The N-balance is affected not only by the physiological condition of an animal but also by dietary factors, such as the biological value of protein. Despite the similar chemical composition of the diets (Table 2), a considerable difference in N-retention was detected among the diets. This is attributable, at least in part, to the difference in dietary ingredients and their formulation.

Dietary energy: Table 5 shows that the UE loss per UN, per 6.25UN and per ingested DCP, were 7.84, 1.25, and 1.05kcal/g, on average, respectively.

Table 6 shows the values of DE, ME and ME_N obtained for each diet, and the values of all parameters in the ME_N equation (1). Of the dietary GE (4.69kcal/g DM), an average of 18

% (0.85kcal/g DM) was lost in feces, 5% (0.22 kcal/g DM) was excreted into urine, and 77 % (3.61kcal/g DM) was ME. A one percent equivalent of the GE was retained in the body, and this portion (0.04kcal/g DM) would be lost in urine without being incorporated into body tissues at N-equilibrium. Therefore, the ME_N was an average of 76 % (3.57kcal/g DM) of the dietary GE. The ME values correlated highly (P<0.01) with the ME_N values (ME=-0.46+1.14ME_N, at r=0.998). But, the ME values were always higher, an average of 40kcal/kg diet, than those of ME_N, and there was a significant difference (P<0.05) between the means. The values of cME correlated highly (P<0.01) with the ME_N values and there was no significant difference between them (cME=-0.30+1.08ME_N, at r=0.988). The cME values obtained using equation (2) are almost identical to the ME_N values.

Fig. 1. Correlation between urinary energy per ingested digestible crude protein (UE/DCP) and nitrogen balance (NB)

In Fig. 1, the UE/DCP is plotted against NB for each dog in every experiment. The correlation between UE/DCP and NB is statistically significant: $r=0.798$ at $P<0.001$. Linear regression analysis yielded the relationship ($UE/DCP=1.24-0.03NB$). According to this relationship, UE/DCP at N-equilibrium ($NB=0$) is 1.24 kcal/g, with 95 % confidence limits ranging between 1.02 and 1.45kcal/g.

Discussion

Other than those of Suzuki *et al.* [14] and of Hirakawa and Baker [5], there are few reports concerning the ME or MEN values of canine diets. Hirakawa and Baker [5] determined GE, FE, UE, and N-retention using growing puppies, however, did not report MEN but cME using the equation (2). They showed that the cME value was in good agreement with the ME value determined empirically, and thus asserted the validity of this equation (2). Although they did not report the MEN value, it can be calculated from their data. The MEN value thus obtained was 91kcal/kg lower on the

average than the determined ME ($P<0.05$), and the difference was equivalent to 3% of MEN. In the present study using mature dogs, the MEN was also significantly lower ($P<0.05$) than the determined ME, but the difference was only 40kcal/kg, which was equivalent to 1 % of MEN. This was due to the fact that N-retention of the total N ingested was 13% in the present study, in contrast to 47% in the work by Hirakawa and Baker [5]. Because N-retention is higher in growing puppies than in mature dogs, the difference between ME and MEN is greater in the former than in the latter. Theoretically, the ME value is affected by the N-retention of each animal. Since the energy value of a diet should not be affected by the physiological condition of an animal, it is recommended that the MEN values be used.

The factor 1.25 in the equation (2) was originally derived from the studies by Atwater, who obtained 7.9kcal of UE per gram of UN in men receiving mixed diets [7,8]. The value of $UE/6.25UN$ is identical to UE per ingested DCP or UE per gram of absorbed protein at N-equilibrium or at zero N-retention ($NB=0$). Thus, the value of 1.25kcal/g DCP was obtained from rounding the value of the ratio, $7.9/6.25$ [7]. This factor may differ depending on respective animals [9,12]. For dogs, the NRC [11] adopted 1.25 for correction of the energy estimation of CP from DE to ME by calculation. Kendall *et al.* [6], however, stated that this application is somewhat arbitrary. According to the NRC [12], Rubner obtained the value of 7.45kcal/g for UE/UN for dogs in 1885. When divided by 6.25, this value becomes 1.19kcal/g for UE/DCP. The discrepancy between these two values (i.e., 1.19 and 1.25kcal/g) may be explained by the difference in N-retention of animals used; Fig. 1 clearly shows that UE/DCP decreases with increased N-retention. The value of UE/DCP at zero N-retention is 1.24kcal/g, in good agreement with the factor 1.25 adopted by the NRC [11]. Fig. 1 illustrates that equation (2) can be applied to animals at N-equilibrium.

When animals are at N-equilibrium, the following relationships are applied:

$$ME = MEN = DE - UE$$

$$UE/DCP = 1.25.$$

Therefore,

$$ME = MEN = DE - 1.25DCP.$$

Moreover, the equation (2) can be applied to

animals which retain N in their body or not at N-equilibrium. By definition,

$$\text{DCP} = (\text{IN} - \text{FN}) \times 6.25, \quad (3)$$

where IN is ingested N and FN is fecal N. Also, by definition,

$$\text{NB} = (\text{IN} - \text{FN}) - \text{UN}. \quad (4)$$

Then, from equation (3) and (4),

$$\text{NB} = \text{DCP}/6.25 - \text{UN}.$$

From this equation,

$$\text{NB}/\text{UN} = \text{DCP}/\text{UN} \cdot 6.25 - 1. \quad (5)$$

By defining DE as $\text{DE} = \text{GE} - \text{FE}$, the equation (1) becomes

$$\text{MEn} = \text{DE} - \text{UE} (1 + \text{NB}/\text{UN}). \quad (1')$$

Then, substituting the equation (5) into this expression yields

$$\text{MEn} = \text{DE} - \text{UE} \cdot \text{DCP}/6.25\text{UN}.$$

If we use the results shown in Table 5, i.e. that $\text{UE}/6.25\text{UN} = 1.25$, then, our final equation is:

$$\text{MEn} = \text{DE} - 1.25\text{DCP}.$$

This transformation demonstrates that when corrected to the N-equilibrium state the total urinary energy loss is constant, even in animals which retain N.

The present study demonstrates that dietary MEn can be estimated conveniently using the equation $\text{MEn} = \text{DE} - 1.25\text{DCP}$ for growing as well as for mature dogs. Strictly speaking, however, further study using growing dogs may be necessary to demonstrate the DCP factor. Moreover, use of this equation still requires collection of feces and determination of fecal energy. For greater convenience, the NRC [13] proposed another method based on proximate analysis of diets and assumed nutrient digestibility. Actually, the assumed digestibility is in good agreement with the determined digestibility shown in Table 3. Further evaluation of our method requires research involving digestion trials of diets with a wider range of energy contents.

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実験イヌ用飼料の窒素補正代謝エネルギーの推定

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実験イヌ用非精製飼料のエネルギー推定法である計算式、〔代謝エネルギー＝可消化エネルギー(DE)－1.25×可消化粗蛋白質(DCP)〕の信頼性を検討すべく、5種類の飼料を4頭のビーグル成犬に給与して、消化試験、窒素(N)出納試験およびエネルギー価の実測試験を行った。粗蛋白質、粗脂肪、可溶無窒素物の消化率は、それぞれ79.5%、91.6%および84.8%であった。窒素出納(NB)はすべて正で、摂取乾物飼料1 g当たり1.4～8.6mgの範囲内であった。摂取された窒素のうち、平均で21%が糞に、66%が尿に、13%が蓄積に回された。飼料の総エネルギーのうち、平均で18%が糞に、5%が尿に排泄され、77%が代謝エネルギー(ME)であった。1%が蓄積分であり、窒素補正代謝エネルギー(MEn)は76%となった。また、尿中窒

素1 g当たりの尿エネルギー(UE)の損失は平均で7.84kcalであった。UE/DCPとNBとの間には高度の相関関係があり(UE/DCP=1.24-0.03NB, $r=0.798$, $P<0.001$), 窒素平衡(NB=0)下ではUE/DCPは1.24kcal/gとなり、上述の計算式の係数とほぼ一致した。この式で求めた代謝エネルギー値は、MEnとよく一致し、両者の値は実測ME値より有意($P<0.05$)に低かった。以上より、上述の式は、本来MEn=DE-1.25DCPと表されるべきものであり、供試飼料のMEnを簡便に推定できる信頼性の高い式と結論した。また、本式はN平衡にあるイヌはもとより、Nを蓄積しているイヌに対しても適用できることが立証された。