

# ENERGY AND PROTEIN REQUIREMENTS FOR NELLORE STEERS ESTIMATED WITH THE DEUTERIUM OXIDE MARKER

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## Abstract

The aim of this study was determine the energy and protein requirements for maintenance and gain of Nellore steers. Thirty six Nellore steers with a average weight and age of  $359 \pm 13$  kg and 20 months at the beginning of the trial were individually fed for 56. The steers were fed the same diet (76.43% TDN and 13.62% CP) in three levels of dry matter (DM) intake, *ad libitum*, 75 g DM/kg  $BW^{0.75}$  and 60 g DM/kg  $BW^{0.75}$ . The initial and final body composition was estimated with the marker deuterium oxide that allowed repeated water estimate in the same animal. Deuterium in blood samples was analyzed by mass spectrometry. The effects of intake levels, comparison of intercept and slope among feeding level, and equations were evaluated by the analysis of variance, adopting  $P < 0.05$  as a significant level. The net energy for maintenance was 75 kcal/kg  $EBW^{0.75}$  or 70 kcal/kg  $BW^{0.75}$ . The net energy for gain for steers with 350 to 450 kg weight was 5.1 to 6.1 Mcal. The efficiency of energy utilization for maintenance,  $k_m$  was 0.7492 and for weight gain,  $k_g$  was 0.3404. The metabolizable protein requirement for maintenance was  $4.32 * BW^{0.75}$  and the net protein for gain was estimated with the equation  $NP_g = (254.68 * EBWG) - (29.38 * RE)$ . The technique of deuterium oxide marker used to estimate energy and protein requirement for maintenance and weight gain at Nellore steers confirm the hypothesis presented in NRC, that  $NE_m$  is lower than the requirement for taurine cattle. On the other hand, the reverse was true for the NP requirement for maintenance and for weight gain. We consider that indirect method using  $D_2O$  for estimate body composition in zebu cattle was suitable to determine nutrient requirements.

## Key words

beef cattle, deuterium oxide, nutritional requirement, ruminant

## INTRODUCTION

The knowledge of the nutritional requirements of cattle is a key component for the success of beef production systems, once it allows the adoption of nutritional and management practices that can impact on the efficiency and profitability of beef chain.

According to NRC (1996) the nutritional requirements of finishing beef cattle is commonly differentiated in maintenance and gain/growth. One of the most important steps to determine those requirements is the estimate of animal's body composition (LOFGREEN and GARRET, 1968).

The body composition is main variable that influences the accuracy of the prediction of nutritional requirements (TEDESCHI, 2015; SILVA et al., 2017). However, obtaining data on body

composition that allow a good estimate of the requirements is difficult, time consuming and expensive.

One technique that can be used to determine the beef cattle body composition without slaughter animals is through dilution techniques using isotopes as markers (LEME et al., 1994). Deuterium is a hydrogen isotope which replaces H in H<sub>2</sub>O molecules and initially was applied to estimate the amount of water in the human body (PINSON, 1952; FALLER et al., 1955a; 1955b). This technique allows repeated measurements in the same individual without undesirable effects, and still used for research (WANG et al., 2014; BANDARA et al., 2015; FOWLER et al., 2020).

The use of deuterium dilution for beef cattle began in the 1980s (ARNOLD et al., 1985; LEME et al., 1994; GOMES et al., 2012) and also has been used for studies with swine (ZHANG et al., 2017), sheep and goats (AL-RAMAMNEH, et al.; 2010), dairy cattle (CHAPMAN et al., 2017; SCHAFF et al., 2017) and equine (DUGDALE et al., 2011; FERJAK et al., 2017).

Like others, the deuterium dilution technique to determine body composition hopes to avoid the use of comparative slaughter and its implications (RESENDE et al., 2017; FONSECA et al., 2017), at the same time that considers the importance of accuracy in estimating body composition. Thus, a study was carried out to compare the estimates of energy and protein requirements obtained by using the deuterium oxide technique with data from the literature obtained by other techniques.

## MATERIAL AND METHODS

The experiment was carried out at the Faculdade de Zootecnia e Engenharia de Alimentos of Universidade de São Paulo. The Research Ethics Committee of the FZEA/USP, under N<sup>o</sup> 6706080515, approved the procedures.

### Animal management and feeding

Thirty-six Nellore steers (*Bos taurus indicus*) with 20 months old and initial body weight of 359±13 kg were housed in individual pens and submitted to 28 d of adaptation to the facilities and diet.

The animals were fed for 56 days, with the same diet ([Table 1](#)) supplied in three levels of dry matter (DM) intake to obtained different body composition: *ad*

*libitum* (TAL), 75 g DM/kg BW<sup>0.75</sup> (T75) and 60 g DM/kg BW<sup>0.75</sup> (T60). The concentrate and the roughage were weighted, mixed and distributed daily in the morning.

Table 1 - Diet composition on dry matter basis

Ingredient	g/100g
Corn grain	20.00
Soybean hulls	57.29
Soybean meal	0.71
Urea	0.92
Mineral salt	0.60
Limestone	0.49
Rumensin®	0.027
Sorghum silage	20.00
Nutrient	
Total digestible nutrients	76.43
Crude protein	13.62
Rumen degradable protein	8.63
Ether extract	2.42
MM	4.26
Neutral detergent fiber	54.44
Acid detergent fiber	38.55
Nitrogen-neutral detergent fiber	6.02
Nitrogen-acid detergent fiber	3.44
Lignin	2.60
Metabolizable energy <sup>1</sup>	2.76

<sup>1</sup>NRC, 2001.

For the *ad libitum* treatment, the orts were collected before the feeding, three times a week, weighted and mixed to provide a sample composed for week for DM determination. Daily dietary adjustment was performed so that the orts were no more than 5% of the feed offered. In the T75 and T60 treatments the diet supply was constant during the periods between weighing, since it was determined, for each animal, based on the initial metabolic weight of the period. The animals were weighted in 28 days intervals, after 16 h of complete fasting.

The diet was formulated according to beef cattle requirements of NRC (2000), using sorghum silage as roughage and common feedstuffs for Brazilian feedlots as concentrate (Table 1). The average daily gain expected was 1.3 kg/d. The concentrate and silage was sampled and analyzed for dry matter (DM, method 950.46), crude protein (CP, Micro Kjeldahl, method 928.08), ether extract (EE, method 950.46) and mineral matter (MM, method 920.153), according to AOAC (1997); neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin, according to Van Soest et al. (1991);

N-NDF and N-ADF (VAN SOEST et al. (1991) and AOAC (1997), method 928.08; Micro Kjeldahl).

Total digestible nutrients (TDN) from the roughage and concentrate were estimated using equations of NRC (2001),  $TDN = \text{digestible CP} + 2.25 * \text{digestible EE} + \text{digestible NDF without ash and protein} + \text{digestible NFC} - 7$ . The digestible energy (DE) was obtained considering that 1 kg of TDN corresponds to 4.409 Mcal of DE (NRC, 2001) and that metabolizable energy (ME) was obtained by equation  $ME = 0.96 * DE - 0.30$  (GALYEAN et al., 2016).

### Body composition

Initial and final body compositions were estimated with the deuterium oxide (D<sub>2</sub>O) technique and the same animals were used for the two evaluations. The D<sub>2</sub>O (99.8% of purity, MW 20.03) was injected in the right jugular through a catheter, in the proportion of 0.1 g/kg body weight. Then, blood samples were collected before D<sub>2</sub>O application and after six hours with the animals kept without access to water or feed. All blood samples were collected in heparinized *Vacutainer* tubes of 10 mL that were refrigerated and later transferred to plastic tubes resistant to freezing. Samples were stored at -20 °C for later analysis of the marker concentration.

The D<sub>2</sub>O in blood samples were determine by mass spectrometry, where the water is separated from the blood by vacuum distilling, retained in trap at -196 °C, and decomposed by reaction with metallic zinc at 500 °C under vacuum system (COLEMAN et al., 1982). From the quantity of D<sub>2</sub>O diluted in water blood the deuterium space (DS) was calculated as follow:

$$DS = \text{mg D}_2\text{O injected} / (\text{mg/mL D}_2\text{O Final} - \text{mg/mL D}_2\text{O Initial})$$

To compare data in different basis and for the estimative and predictions of animal performance, the relationship between empty body weight (EBW) and shrunk body weight (SBW), and between empty body weight gain (EBWG) and shrunk body weight gain (SBWG) is necessary.

From the DS data and the shrunk body weight was possible to determine the water (Eq. 1) and the ether extract (Eq. 2) in the empty body, using the equations obtained by Leme (1993), for Nellore steers.

Eq. 1:

$$\text{Water (\%)} = 65.9654 + (0.0977 * DS) - (0.0909 * SBW), (R^2 = 0.83 \text{ and } S_{y,x} = 1.33).$$

Eq. 2:

$$\text{Ether extract (\%)} = 93.92968 - 1.27598 * \text{Water (\%)}, (R^2 = 0.97 \text{ and } S_{y.x} = 0.62).$$

The estimation of protein quantity in the empty body was obtained through protein and water relationship equal to 0.3009 and the estimation of ash quantity in the empty body through the relationship between ash and water equal to 0.0747, according to established relationships for Nellore steers by Leme et al. (1994).

### Nutritional requirements

Feeding the same diet in three different levels of intake was necessary to obtain regression equations to estimate the energy and protein requirements for no gain situation, equivalent to the maintenance requirements (CÁRDENAS-MEDINA et al., 2010; POSADA-OCHOA et al., 2017).

Knowing fat and protein in the empty body, and that each kilogram of fat corresponds to 9.367 kcal, and to protein 5.686 kcal (NRC, 1996), it was possible to determine the amount of energy in the empty body of each animal at the beginning and at the end of the experimental period.

The requirement of net energy for maintenance was calculated as the antilogarithm of the intercept in the regression equation between the logarithm of heat production and the metabolizable energy intake (MEI). For this, the heat production (HP) was calculated according to Lofgreen and Garrett (1968) equation (Eq. 3).

$$\text{Eq. 3: } HP = MEI - RE.$$

The requirement of net energy for weight gain ( $NE_g$ ) was determined as the energy deposited in the gain (LOFGREEN and GARRETT, 1968). The equation for prediction of RE was calculated according to NRC (1984) (Eq. 4).

$$\text{Eq. 4: } RE = b * EBW^{0.75} * EBWG^a$$

Were:

$a$  = antilogarithm of intercept in the equation between the logarithm of RE per kilogram of empty body weight and the logarithm of empty body daily gain.

$b$  = slope in the same regression equation.

The slope of the regression equation between the daily retained energy per kilogram of metabolic weight, and the metabolizable energy daily intake per kilogram of metabolic weight, was considered as the efficiency of energy utilization for weight

gain (kg) (VALADARES FILHO et al., 2006).

The metabolizable protein for maintenance was determined from the intercept of the regression equation of metabolizable protein intake (g/day) in function of weight gain (kg/day), which was divided by the average metabolic weight of the animals (WILKERSON et al., 1993; NRC, 1996).

The metabolizable protein intake was calculate considering that the diet had 4.99% of rumen undegradable protein, and that the microbial protein (MP) synthesis corresponds to 120 g MP/kg of TDN, as proposed by Valadares Filho et al. (2006) for zebu animals. The total intake of metabolizable protein was obtained by adding the true protein plus the protein of microbial origin.

The requirement of net protein for weight gain (NP<sub>g</sub>) was estimated by regression equation of retained protein in shrunk weight gain (g/kg of daily EBW<sup>0.75</sup>) on protein intake (g/kg of daily EBW<sup>0.75</sup>) (CHIZZOTTI et al., 2007).

### Statistical analyses

The effects of intake levels and equations were evaluated by the analysis of variance using the SAS program (SAS Institute Inc., Cary, NC, USA), according to following model:  $Y_{ij} = \mu + t_i + e_{ij}$ , where:  $Y_{ij}$  = observation of the  $i^{\text{th}}$  animal on the  $j^{\text{th}}$  treatment,  $\mu$  = mean,  $t_i$  = effect of the DMI level,  $e_{ij}$  = inherent error of each observation  $\sim$  NID (0,  $\delta_2e$ ). The comparison of intercept and slope among feeding level for regressions was performed using PROC GLM with the SOLUTION statement, according to CHIZZOTTI et al. (2007). Significant level adopted was  $P < 0.05$ .

## RESULTS AND DISCUSSION

The *in vivo* chemical composition estimated by isotopic dilution methods requires an estimate of EBW, which was obtained by equation (Eq. 5) from shrunk body weight (SBW) of these animals at the end of the experimental period, when they were slaughtered and empty body weight was determined.

Eq. 5:

$$EBW \text{ (kg)} = - 15.74911 + 0.98517 * SBW \text{ (kg)}, (R^2 = 0.96 \text{ and } S_{y.x} = 8.4).$$

The value assumed to represent the conversion of EBW to SBW was one obtained in treatment without feed restriction. The values for conversion ratio of EBW to SBW were different between animals fed restricted and *ad libitum* ( $P=0.0002$ ; 0.96 and 0.93, respectively),

possibly due to effect of restricted feeding in internal organs as reported by Posada-Ochoa et al. (2017). However, the value of 0.93 is in the range suggested by NRC (2000) and BR-Corte (2016).

The EBWG:SBWG value observed in this work (0.9852), was greater than values previously reported in the literature for *Bos indicus* cattle, which ranged from 0.879 to 0.964 (VÉRAS et al., 2001; SILVA et al., 2002a, 2002b; PAULINO et al., 2004; FREITAS et al., 2006), and with current data 0.943 to 0.971 (BR-CORTE, 2016), probability due to differences in the EBW of basal line at slaughter in the papers cited.

The retained energy, determined from the empty body composition of the animals, increased with feed intake and weight gain, while the quantity of deposited energy as protein decreased with the increase in weight gain, consistent with findings of previous reports (CHIZZOTTI et al., 2007; SILVA et al., 2012).

The heat production (HP) increased with feed intake (T60: 131 kcal/SBW<sup>0.75</sup>, T75: 152 kcal/SBW<sup>0.75</sup>, TAL: 212 kcal/SBW<sup>0.75</sup>). Based on HP values, the regression equation between the logarithm of HP and metabolizable energy intake (MEI) was calculated:

$$\text{Log HP} = 1.87781 \pm 0.01236 + (0.00184 \pm 0.00006 * \text{MEI}), (R^2 = 0.9671, \text{RMSE} = 0.01729).$$

The net energy requirement for maintenance ([Table 2](#)) was 75 kcal/kg EBW<sup>0.75</sup>. When this value is adjusted to body weight, using a conversion factor of 0.93 obtained by *ad libitum* animals, it results in 70 kcal/kg BW<sup>0.75</sup> which is similar to recommended to zebu cattle by NRC (2000), or 72.0 (using 0.96 factor conversion by fed restriction animals) similar to 72.3 kcal/kg BW<sup>0.75</sup> suggested by Valadares Filho et al. (2006), but not according to BR-Corte (2016). Note that 0.93 or 0.96 factor conversion was different of 0.891 adopted by NRC (2000) and Valadares Filho et al. (2006), and can be related to comparative slaughter technique.

The literature data is divergent about the fact that zebu cattle have lower maintenance requirement than taurine cattle. These contradictions may be due differences in environmental factors (NRC, 2000), size and birth season (ARRUDA et al., 2018) degree of activity and roughage-concentrate ratio (VÉRAS et al., 2010), and especially, by the wide variability in nervousness and responsiveness to stress inherent to the temperament of Nellore animals, which alter feed efficiency and energy requirements (GOMES et al., 2013; POSADA-OCHOA et al., 2017).

However, the  $NE_m$  requirement for Nellore animals, obtained using the  $D_2O$  technique is in agreement with the hypothesis that  $NE_m$  requirement is smaller for zebu than for taurine breeds (GOMES et al., 2017), regardless of the magnitude of this difference.

The energy requirement for weight gain is a function of the body weight and

Table 2- Net energy requirement for maintenance ( $NE_m$ ) and empty body weight gain ( $NE_g$ ) as a function of shrunk (SBW) or empty (EBW) body weights.

SBW, kg	EBW, kg	$NE_m$ , Mcal	$NE_g$ , Mcal		
			EBWG, kg/d		
			0.250	0.700	1.100
350	329	5.7	1.07	3.39	5.64
375	354	6.1	1.12	3.57	5.94
400	378	6.4	1.18	3.75	6.23
425	403	6.6	1.24	3.93	6.52
450	428	6.9	1.29	4.10	6.81

weight gain of the animal and was predicted by equation:  $RE = 0.0661 * EBW^{0.75} * EBWG^{1.123}$ . This value (0.0661) was similar from that proposed by the NRC (1984):  $RE = 0.0635 * EBW^{0.75} * EBWG^{1.097}$ , varying in approximately 3.1%. It is interesting to note that the difference in EBWG:SBWG conversion rate observed in this study (0.985) and NRC (0.956) was 2.9 percentage points, which suggests that this difference could be related to how the empty weight gain was determined, *in vivo* or *post mortem*, since body composition is the variable with the greatest influence in accurately predicting animal requirements (TEDESCHI, 2015).

The energy requirement for weight gain in steers varying from 350 to 450 kg SBW (Table 3) and for 1 kg of daily weight gain ranged from 5.1 to 6.1 Mcal. These values are higher than observed by other authors (NRC, 2000; VALADARES FILHO et al., 2006; BR-CORTE, 2016), but expected due to the others parameters, as EBW:SBW, EBWG:SBWG and RE equation, that also were higher. Although greater values have already been reported for Nellore cattle, other results reported different values (CHIZZOTTI et al., 2007; GOMES et al., 2017).

Energy efficiency for maintenance ( $k_m$ ) was 0.7492 ( $R^2=0.9764$ ). This value was greater than that reported by Valente et al. (2013) for pasture animals (0.55) as well as those reported by Chizzotti et al. (2007) and Tedeschi et al. (2002), (71.3 and 69.9, respectively). However, our results followed the same direction proposed by Valadares Filho et al. (2005), who in analysis of several works found a  $k_m$  around 70. The authors also mention that values above this, such as the one cited by ARC (1980)

between 73.8 e 77, may reflect an overestimation, as only with animals below maintenance would be possible to make an accurate determination. Another possibility that can occur is due the diet with high concentrate, like used in this work, to be more digestible by microorganisms that increase propionic acid production and decrease methane production, rumination and increment caloric (POSADA et al., 2011;

Table 3- Net and metabolizable protein requirements per kilogram of empty body weight (EBW) or empty body weight gain (EBWG)

Characteristic	Treatments <sup>1</sup>		
	T60	T75	TAL
Net protein, g/kg EBW	178.32	177.20	174.15
Net protein, g/kg EBWG	134.12	132.50	125.74
Metabolizable protein, g/kg EBW	362.44	360.16	353.96
Metabolizable protein, g/kg EBWG	272.60	269.31	255.58

<sup>1</sup>T60: feed intake 60 g DM/kg BW<sup>0.75</sup>, T75: feed intake 75 g DM/kg BW<sup>0.75</sup>, TAL: feed intake *ad libitum*.

LIU et al., 2017), because grazing animal have higher requirements (VALENTE et al., 2013). It may also be related to the lower maintenance requirement, due to more competent metabolic processes (COSTA et al., 2018), because Zebu animals are more efficient using metabolizable energy to maintenance (MARCONDES et al., 2013).

On the other hand, energy efficiency for weight gain ( $k_g$ ) was 0.3404 ( $R^2=0.7862$ ), similar to the literature data (0.33 by SILVA et al., 2002a; 0.35 by VALADARES FILHO et al., 2006; 0.33 by SILVA et al., 2012). This is in agreement with mentioned by Posada et al. (2011) when describing work of Baldwin et al. (1980), where the maximum theoretical growth efficiency of ruminants is 70-80; however, according to those authors, the value of  $k_g$  are 30-60%, there is a notable variation between animals.

The net and metabolizable protein average requirement for maintenance was 176.5 g/kg EBW and 385.8g/kg EBW, respectively. This result was similar to other studies with Nellore (PUTRINO et al., 2006) but greater than the value observed for Brangus animals (156 g/kg EBW; PUTRINO et al., 2006).

The net and metabolizable protein average requirements for gain were 130.8 g/kg EBWG and 265.8g/kg EBWG, respectively. Net protein for gain ( $NP_g$ ) was estimated with the following regression equation:  $NP_g = (254.68 \pm 0.0079 * EBWG) - (29.38 \pm 0.0018 * RE)$ , ( $R^2= 0.99$ ,  $S_{y,x} = 0.0060$ ). For animals with body weight of 350, 400 and 450 kg the values of  $NP_g$  estimated for 1 kg weight gain were 149, 138 e 128 g/d,

respectively.

The net protein requirement for weight gain were 151, 141 and 130 g/kg EBWG for steers with 350, 400 and 450 kg respectively, higher than the values by NRC (1996) (147, 134 and 121 g/d) or by Valadares Filho et al. (2006) (130, 124 and 119 g/d) and others (FREITAS et al., 2006). However, higher values were observed for zebu (VÉRAS et al., 2001; BONILHA et al., 2014; GOMES et al., 2017), even if considering the genetic diversity among animal of same breed.

Based on net protein data from this work and efficiency of use of 49,2% (NRC, 1996) the metabolizable protein requirement for gain, considering an animal with 450 kg, was estimated as being 5.4% higher than the value recommended by NRC (2000) and 6.9% higher than verified by Valadares Filho et al. (2006).

The metabolizable protein requirement was  $MPI \text{ (g/day)} = 371.68 \pm 35.72 + (335.74 \pm 42.46 * ADG \text{ (kg/day)})$ , ( $R^2 = 0.68$  and  $S_{y,x} = 102.98$ ), (Figure 1). Therefore, the value of 4.32 g/kg  $BW^{0.75}$  was found. The factor of microbial efficiency used was 12% of NDT (VALADARES FILHO et al., 2006 model) and the results were similar to that observed by Watson et al. (2017) with the NRC model (13% TDN), 4.2 g/kg  $BW^{0.75}$ . Therefore, the determination of body protein composition by  $D_2O$  seems to match the literature, including higher value for zebu (4.13 g/kg  $BW^{0.75}$  by VALADARES et al., 1997; 4.0 g/kg  $BW^{0.75}$  by VALADARES FILHO et al., 2006; BR-CORTE, 2016) than that of NRC (1996) (3.8 g/kg  $BW^{0.75}$  by WILKERSON et al., 1993; 3.88 g/kg  $BW^{0.75}$  by SUSMEL et al., 1993).

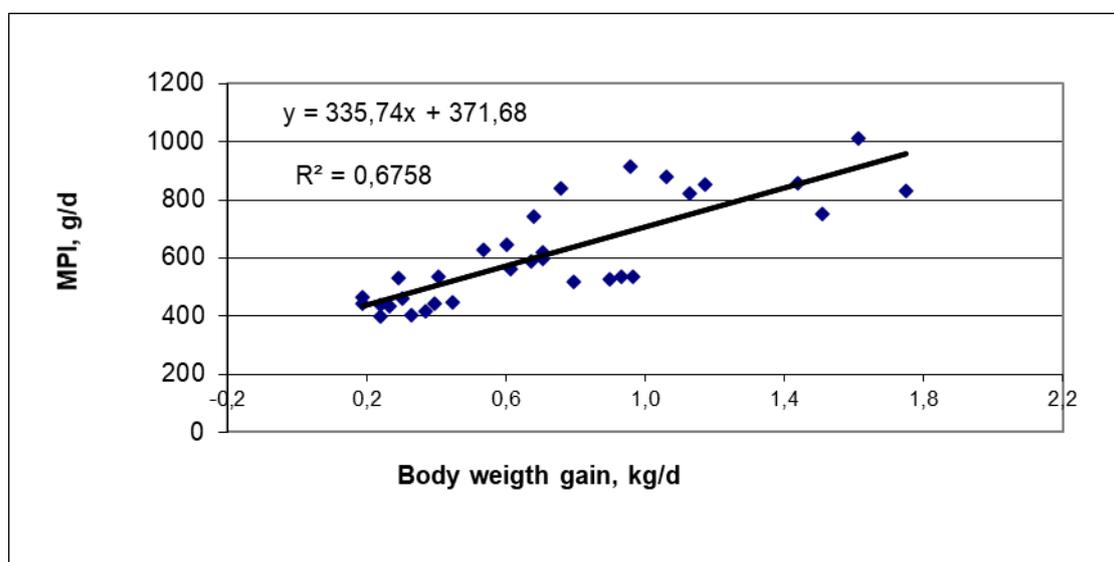


Figure 1. Linear regression between metabolizable protein intake (MPI, g/d) and body weight gain(kg/d).

## CONCLUSIONS

The energy and protein requirement for maintenance and weight gain at Nellore steers, using the deuterium oxide marker technique are in line with the NRC hypothesis that  $NE_m$  is lower than the requirement for taurine cattle, and the reverse was true for the NP requirement for maintenance and for weight gain.

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