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The effect of crude protein content of the diet on renal energy losses in horses

Marleen Kuchler[1] | Annette Zeyner[2] | Andreas Susenbeth[3] | Ellen Kienzle[1]

[1]Chair of Animal Nutrition and Dietetics, Department of Veterinary Science, Ludwig-Maximilians-University, Munich, Germany
[2]Institute of Agricultural and Nutritional Sciences, Animal Nutrition Group, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany
[3]Institute of Animal Nutrition and Physiology, Christian-Albrechts-University, Kiel, Germany

Correspondence
Ellen Kienzle, Lehrstuhl für Tierernährung und Diätetik, Veterinärwissenschaftliches Department, LMU München, Schönleutner Str. 8, D 85764 Oberschleißheim, Germany.
Email: kienzle@tiph.vetmed.uni-muenchen.de

Abstract
Renal energy losses of horses are high in comparison with other species. In the present study, more data were obtained on this parameter to improve predictive equations for renal energy losses. Four adult ponies (247–344 kg body weight [BW]) were fed with eight different diets based on first cut hay, second cut hay, early first cut fresh grass, late cut herbs–grass mix, early cut clover–grass mix, sugar beet pulp, rice bran and straw. Feed intake was measured, and urine and faeces were quantitatively collected for 3 × 12 hr at daytime and afterwards 3 × 12 hr at nighttime. Feed was analysed for crude nutrients, gross energy, amino acids and neutral-detergent-insoluble crude protein (NDICP); faeces were analysed for crude nutrients and gross energy; and urine was analysed for nitrogen (N) and gross energy. Renal energy losses per gram dry matter (DM) intake (y; kJ/g DM) were strictly correlated to protein content in DM (x; g/kg DM): y = 0.325 + 0.00431x; r^2 = .81; n = 38; p < .001. The data suggest that the intercept represents energy losses by detoxification products such as hippuric acid and the regression coefficient by protein metabolites such as urea.

KEYWORDS
energy evaluation, horses, metabolizable energy, renal energy excretion

1 | INTRODUCTION

Renal energy losses in horses are relatively high, when compared to other mammals (Kienzle & Zeyner, 2010). Hipp, Südekum, Zeyner, Goren, and Kienzle (2017) showed that renal energy losses of horses depend mainly on renal hippuric acid and renal nitrogen excretion. Renal nitrogen excretion is predominantly caused by crude protein (CP) intake. By contrast, hippuric acid excretion is less closely related to an individual crude nutrient. Hippuric acid is a metabolite of available phenolic acids from plant cell walls (Jung & Fahey, 1983; Oestmann, Südekum, Voigt, & Stangassinger, 1995). However, content and availability of these acids vary considerably. As a general rule, their availability is reduced with plant age while their content in dry matter (DM) is increased. Especially in gramineae, protein content in DM decreases with plant age (Jeroch, Flachowsky, & Weißbach, 1993). Hipp et al. (2017) demonstrated for gramineae that all three factors are linked together and that renal energy excretion per gram protein intake (y; kJ/g crude protein) can be predicted by crude protein content (x; g/kg DM) according to the following Equation 1:

$$y = 0.034x^2 - 1.254x + 17.509\,(r^2 = 0.64; n = 18, p < 0.01). \quad (1)$$

Such an equation can be used to estimate renal energy losses per g CP and to improve the accuracy of the equation of GfE (GfE, 2014) to calculate the ME value of feeds, where a constant value of 8 kJ/g CP is used. The aim of the present study was to obtain more data for renal energy losses in feeding gramineae and other feedstuffs and to develop a better verified prediction equation for horse feeds. Since the study of Hipp et al. (2017) showed high values for

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TABLE 1 Dry matter (DM) intake, DM content and nutrient composition of the ration, intake of crude protein (CP) and percentage of non-protein nitrogen (NPN) and neutral-detergent-insoluble CP (NDICP) of CP and intake of aromatic amino acids

Test ingredient	Acronym	*n*	DM Intake	Test ingredient	DM of ration	Crude protein	Crude fat	Crude fibre	Crude ash	NfE[2]	NDF[3]	CP Intake	Aromatic amino acid intake	NDICP	NPN
			g/kg $BW^{0.75}$	% of DM	%	% DM						g/kg $BW^{0.75}$	mg/kg $BW^{0.75}$	% CP	
Hay, first cut	HAY1	4	47.3 ± 1.4	100	86	8.3	0.6	33.3	5.5	52.3	66.0	3.9 ± 0.1	276	29.9	7.9
Hay, second cut	HAY2	4	51.3 ± 1.7	100	89	13.7	1.4	35.2	6.3	43.4	61.3	7.0 ± 0.2	490	42.5	17.4
Grass[1]	GRASS	4	51.1 ± 1.5	52	32	11.4	1.2	25.3	7.9	54.2	53.8	5.8 ± 0.2	763	18.8	3.7
Herbs–grass mix[1]	HERBS	4	71.4 ± 0.7	62	45	11.5	1.2	33.0	7.5	46.8	62.0	8.2 ± 0.1	946	28.3	13.9
Clover–grass mix[1]	CLOV	4	51.8 ± 1.6	52	29	13.0	1.2	31.5	5.8	48.4	51.1	6.8 ± 0.2	1,090	16.5	5.0
Sugar beet pulp[1]	SBP	4	38.2 ± 1.1	35	87	8.3	0.5	28.3	7.5	55.5	58.0	3.2 ± 0.1	251	42.5	8.0
Rice bran[1]	RIBR	4	39.4 ± 1.2	33	90	8.7	1.8	23.2	20.2	46.2	41.4	3.4 ± 0.1	299	24.3	13.2
Straw[1]	STR	3	55.1 ± 1.3	43 ± 6	85	6.0	0.8	38.9	4.4	49.9	74.1	3.3 ± 0.1	203	30.7	7.3

[1]Mixed with HAY1.
[2]NfE nitrogen-free extracts.
[3]NDF neutral detergent fibre.

renal energy excretion in fresh grass, the present study was focused notably on this aspect.

2 | ANIMALS, MATERIALS AND METHODS

For the study, four adult crossbred ponies (2 geldings, 2 mares: 247–344 kg body weight (BW)) were available. They were fed with eight different test ingredients: first cut hay (HAY1), second cut hay (HAY2), early first cut fresh grass (GRASS), late cut herbs–grass mix (HERBS), early cut clover–grass mix (CLOV), sugar beet pulp (SBP), rice bran (RIBR) and straw (STR). HAY1 and HAY2 were fed exclusively, and all other feedstuffs were combined with the HAY1 to prevent digestive or behavioural problems. GRASS and CLOV were cut in May and HERBS in August. The herbs–grass mix contained mainly different herbs like ribwort, dandelion, daisy, stinging nettle and cornflowers, but also small amounts of clover and different grass varieties. During the trial, no minerals or other supplements were added. One pony was medicated with pergolide because of pituitary pars intermedia dysfunction (PPID). Food was offered in three meals: at 8 a.m. and 8 p.m., the respective test ingredient and hay, and at 3 p.m., only a small amount of hay (max. 0.5 kg) because the ponies were used to a feeding time at the afternoon. Feed intake and diet composition are given in Table 1 and nutrient composition of the test ingredients in Table 2. All diets were eaten completely, except of the straw, where two ponies left over between 0.1 and 0.3 kg per day. Because of colic, one pony could not take part in the trial with the straw, so only the results of three ponies could be evaluated. Water was offered free choice.

The trials lasted between 10 and 16 days depending on the test ingredient and the time for adaptation. For hay, the ponies were switched to the test batch and fed this for four days prior to the trial. For green fodder, this started with a small amount which was slowly increased. The quantity used in the trial was achieved four days prior collection period. Urine and faeces were collected during the last six days of the trial. For animal welfare reasons, the faeces and urine collection was split. The first three-day faeces and urine were collected completely during 12 hr of the day; thereafter, the same procedure was repeated during 12 hr of the night for three nights. Urine was collected manually with a bucket as described earlier (Goren, Fritz, Dillitzer, Hipp, & Kienzle, 2014). During the 12 hr of collection, the ponies were kept outside their boxes on rubber mats where they did not urinate because of splashing. They were allowed to return into their boxes with hemp bedding minimum every four hours. Urine and faeces were frozen at −20°C until analysis.

Urine and faeces were analysed as described by Hipp et al. (2017): to determine the gross energy content of urine and faeces, bomb calorimetry was used. Nitrogen in urine was determined by the Kjeldahl method (VDLUFA, 2012). Dry matter and crude nutrients and non-protein nitrogen in feed and faeces were determined according to VDLUFA (2012). Neutral-detergent-insoluble CP (NDICP) was determined according to VDLUFA (2016; method 4.13.1). Feed proteins were hydrolysed with hydrochloric acid, and AA were analysed using ion-exchange chromatography (Biochrom 30 with

TABLE 2 Nutrient composition of the test ingredients, percentage DM

Test ingredient	DM (% wet weight)	Crude protein (% DM)	Crude fat	Crude fibre	Crude ash	NfE	NDF	NDICP	Gross energy (MJ/kg)
Hay, first cut	86.3	8.3	0.6	33.3	5.5	52.3	66.0	2.5	18.9
Hay, second cut	88.6	13.7	1.4	35.2	6.3	43.4	61.3	5.8	19.0
Grass	20.1	14.1	1.7	18.1	10.1	56.0	42.2	1.8	18.2
Herbs–grass mix	34.8	13.5	1.6	32.8	8.7	43.4	59.6	3.7	18.3
Clover–grass mix	18.3	17.4	1.8	29.8	6.1	44.9	38.0	1.9	18.6
Sugar beet pulp	88.3	8.3	0.2	19.4	11.0	61.1	43.1	5.4	16.2
Rice bran	96.3	9.2	3.8	6.8	43.8	36.4	15.8	1.5	12.7
Straw	85.2	2.6	1.0	47.2	2.8	46.4	86.2	0.9	18.3

TABLE 3 Apparent digestibility of dry matter (DM), gross energy (GE) and crude protein (CP) in % in total rations and for test ingredients

Acronym	DM[1]	DM[2]	GE[1]	GE[2]	CP[1]	CP[2]
HAY1	46.5 ± 5.9[a]	—	46.7 ± 5.3[ab]	—	61.2 ± 4.9[ab]	—
HAY2	56.2 ± 4.9[a]	—	54.7 ± 5.0[ab]	—	73.0 ± 1.9[ab]	—
GRASS	60.8 ± 4.9[a]	73.9 ± 3.8[a]	61.4 ± 4.6[b]	75.2 ± 3.7[a]	70.0 ± 1.7[ab]	75.5 ± 3.1[a]
HERBS	54.8 ± 9.0[a]	59.8 ± 11.6[ab]	53.5 ± 8.8[ab]	57.8 ± 11.6[a]	76.2 ± 3.7[b]	81.9 ± 4.1[ab]
CLOV	59.2 ± 6.6[a]	70.6 ± 8.6[ab]	60.8 ± 6.3[b]	72.6 ± 8.1[a]	73.4 ± 2.2[ab]	78.6 ± 1.7[a]
SBP	46.7 ± 9.2[a]	47.1 ± 20.5[ab]	47.0 ± 9.1[ab]	47.4 ± 22.1[a]	50.1 ± 3.3[a]	30.2 ± 6.9[ab]
RIBR	40.5 ± 9.4[a]	30.6 ± 22.7[b]	42.1 ± 9.6[ab]	30.8 ± 28.2[a]	59.2 ± 3.4[ab]	56.4 ± 10.3[ab]
STR	42.0 ± 15.2[a]	38.2 ± 27.5[ab]	34.7 ± 17.4[a]	15.7 ± 37.8[b]	42.9 ± 17.1[a]	−33.7 ± 65.9[b]

Note: Means in the same column not sharing a superscript letter are significantly different ($p < .05$).
[1]Total ration.
[2]Test ingredient (calculated by difference assuming no interaction between compounds of ration).

PEEK-Sodium Prewash Column, 100 × 4.6 mm, and PEEK-Oxidised Feedstuff Column, 200 × 4.6 mm, Biochrom Ltd., Cambridge, UK) according to the protocol of VDLUFA (2012, method no. 4.11.1). Tryptophan (Trp) was hydrolysed with phosphoric acid and hydrochloric acid and analysed using high-performance liquid chromatography (HPLC; Agilent 1,100 Series with ZORBAX Eclipse XDB-C8, 150 × 4.6 mm, 5 µm, Agilent Technologies Inc.) according to Fontaine, Bech-Andersen, Butikofer, and Froidmont-Görtz (1998).

The digestibility of the test ingredients was calculated by difference. Renal energy excretions caused by HAY1 were subtracted from total excretions according to its proportion in the diet, and the difference was attributed to the test ingredient.

The study was approved by the appropriate ethical committee, that is the ethical commission of the Veterinary Faculty of the Ludwig-Maximilians-University of Munich, Germany. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes.

Means and standard deviation were calculated. Comparison of means was carried out after 1-way ANOVA for factor feed by the Holm–Sidak method using the software SigmaStat 3.0 (SPSS). A p-value of <.05 was considered significant. Simple linear regressions were calculated to describe a relationship between parameters (software SigmaStat 3.0 [SPSS]).

3 | RESULTS

All ponies finally included in the evaluation remained clinically healthy during the trials. One pony had constipation when diet STR was introduced and was excluded from this trial. In the rice bran diet (RIBR), the apparent digestibility of dry matter was low (Table 3) as well in the straw diet (STR). The diet with the early first cut fresh grass (GRASS) had the highest dry matter digestibility. The results for digestibility of dry matter and energy were very similar (Table 3). The highest crude protein digestibility was observed in the herbs–grass mix (HERBS) and the lowest in the straw diet. All diets with fresh green fodder (GRASS, HERBS, CLOV) as well as second cut hay (HAY2) had a high digestibility of crude protein (Table 3).

The total amount of urine was highest in the rice bran diet (RIBR) and lowest in the straw diet (STR) (Table 4). Total renal energy excretion in kJ per kg metabolic BW was highest in the clover–grass mix diet (CLOV) and lowest in the sugar beet pulp diet (SBP) (Table 4).

TABLE 4 Total daily urine weight, renal excretion of energy and nitrogen (N), calculated renal energy excretion from test ingredient and N Balance

Acronym	Total daily urine weight in g/kg $BW^{0.75}$	Renal energy excretion in kJ/kg $BW^{0.75}$	Renal N excretion in g/kg $BW^{0.75}$	Renal energy excretion in kJ/g DM intake	Calculated renal energy excretion from test ingredient[1] in kJ/g DM intake	Renal energy excretion in kJ/g CP intake	Calculated renal energy excretion from test ingredient[1] in kJ/g CP intake	N balance in g/kg $BW^{0.75}$
HAY1	73.0 ± 38.1^{a}	36.4 ± 11.9^{ab}	0.82 ± 0.35^{a}	0.77 ± 0.3^{ab}	–	9.2 ± 2.9^{a}	–	−0.4 ± 0.3^{a}
HAY2	125.4 ± 57.6^{a}	46.7 ± 4.4^{ab}	0.71 ± 0.23^{a}	0.91 ± 0.1^{ab}	–	6.7 ± 0.7^{ab}	–	0.1 ± 0.2^{ab}
GRASS	122.8 ± 47.6^{a}	43.6 ± 7.2^{ab}	0.68 ± 0.09^{a}	0.85 ± 0.2^{ab}	0.54 ± 0.2^{a}	7.4 ± 1.2^{ab}	6.5 ± 1.8^{ab}	0.0 ± 0.2^{ab}
HERBS	86.7 ± 20.5^{a}	45.0 ± 5.3^{ab}	0.58 ± 0.05^{a}	0.63 ± 0.2^{ab}	0.93 ± 0.4^{a}	5.5 ± 0.6^{b}	4.0 ± 0.8^{a}	0.4 ± 0.1^{b}
CLOV	142.0 ± 75.6^{a}	49.2 ± 6.2^{a}	0.67 ± 0.07^{a}	0.93 ± 0.2^{a}	1.07 ± 0.4^{a}	7.2 ± 0.9^{ab}	6.4 ± 1.2^{ab}	0.1 ± 0.2^{ab}
SBP	163.7 ± 38.8^{a}	31.2 ± 5.3^{b}	0.52 ± 0.12^{a}	0.77 ± 0.2^{ab}	0.78 ± 0.5^{a}	9.8 ± 1.6^{a}	11.0 ± 4.3^{bc}	−0.3 ± 0.2^{a}
RIBR	166.7 ± 58.5^{a}	33.0 ± 1.6^{ab}	0.53 ± 0.05^{a}	0.80 ± 0.2^{ab}	0.86 ± 0.4^{a}	9.6 ± 0.3^{a}	10.3 ± 0.8^{bc}	−0.2 ± 0.2^{a}
STR	63.1 ± 33.7^{a}	31.5 ± 6.9^{ab}	0.33 ± 0.07^{a}	0.57 ± 0.2^{b}	0.46 ± 0.4^{a}	9.6 ± 2.0^{a}	9.9 ± 10.9^{c}	−0.1 ± 0.1^{ab}

Note: Means in the same column not sharing a superscript letter are significantly different (p < .05).

[1]See Table 1.

Renal N excretion in g per kg metabolic BW (Table 4) was highest in HAY1 and lowest in the straw diet (STR). Renal energy excretion in relation to DM intake was highest in the clover–grass mix diet (CLOV) and lowest in the straw diet (STR). In relation to protein intake, the renal energy excretion, however, was highest in the sugar beet pulp diet (SBP) and lowest in the herbs–grass mix diet (HERBS). This parameter was not only calculated for the mixed rations, but also for the test ingredients and showed a similar ranking (Table 4). The nitrogen balance was not significantly different from zero for all diets (Table 4).

When the equation (Equation 1) of Hipp et al. (2017) was used for the prediction of renal energy excretion per gram crude protein intake in rations containing mainly gramineae (HAY1, HAY2, GRASS, STR), there was a very close correlation between predicted and measured values (r^2 = .90). When the data for the ingredients which were calculated by difference were included, there was still a remarkably good prediction (Figure 1). The same was true for the prediction of renal N excretion from protein intake (data not shown). The relationship between renal excretion of energy per g protein and protein content is shown in Figure 2 (own data). The renal excretion of energy per g DM intake, however, correlated strictly with protein content in DM (Figure 3, own data).

4 | DISCUSSION

The results of the present study confirm the findings of Hipp et al. (2017) on renal energy excretion in horses. Renal energy losses per g crude protein intake decrease with increasing protein content in the diets. The reason could be that in high protein diets, the renal

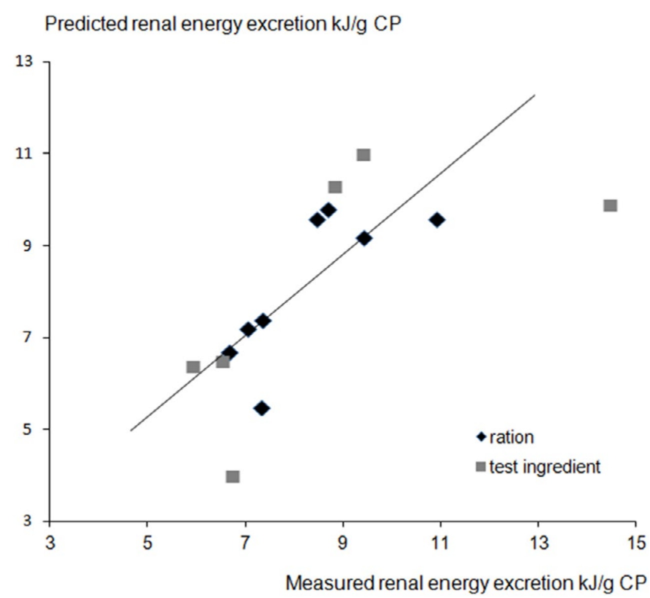

FIGURE 1 Comparison of measured renal energy excretion in total rations and test ingredients (x; kJ/g CP intake) and predicted renal energy excretion (y; kJ/g CP intake) according to the equation of Hipp et. al. (2017) for grass products. (y = 0.8859x + 0.8219; r^2 = 0.60; *SEM* = 1.71; *n* = 16; *p* < .01) [Colour figure can be viewed at wileyonlinelibrary.com]

excretion of hippuric acid, which contains high amounts of energy, decreases in relation to urea excretion, which contains less energy. The present study shows that in general green fodder does not lead to exceptionally high renal energy losses. This is in contrast to the results of Hipp et al. (2017) for fresh grass, which was considered to be an outlier, possibly due to mild diarrhoea when the ponies were eating only fresh grass. In the present study, diarrhoea was avoided by mixing fresh fodder with hay.

The renal energy losses per gram protein intake decreased in the present study with increasing crude protein content. When literature data were included, the relationship was clearly non-linear (Figure 2, all data). By contrast, the renal energy losses per g DM intake increased in a linear regression with crude protein content in DM. This was confirmed when literature data were included (Figure 3, all data). The resulting regression Equation 2:

$$y = 0.325 + 0.00431x \; (r^2 = 0.81; n = 38; p < 0.001) \quad (2)$$

with protein content in dry matter (x; g/kg DM) as independent and renal energy excretion (y, kJ/g DM intake) as dependent

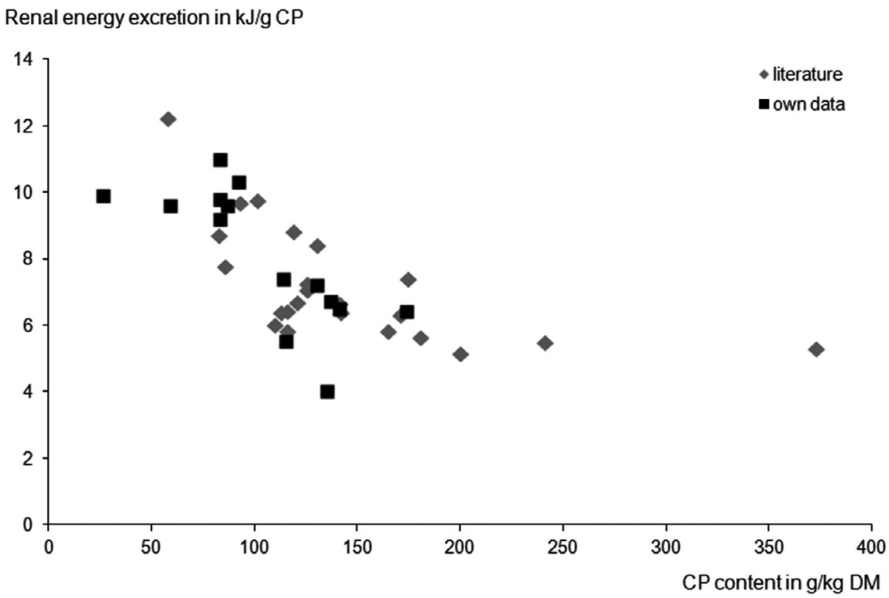

FIGURE 2 Relationship between crude protein content in feed dry matter and renal energy excretion per g crude protein intake (own and literature data: Fingerling 1931–1939; Hipp et al., 2017; Kienzle et al., 2009; Pagan & Hintz, 1986; Ragnarsson, 2009; Vermorel, Martin-Rosset, Martin-Rosset, & Vernet, 1997; Vermorel, Vernet, Vernet, & Martin-Rosset, 1997) An equation was not calculated because it would be a broken line model which is rather impractical for use in a predictive equation

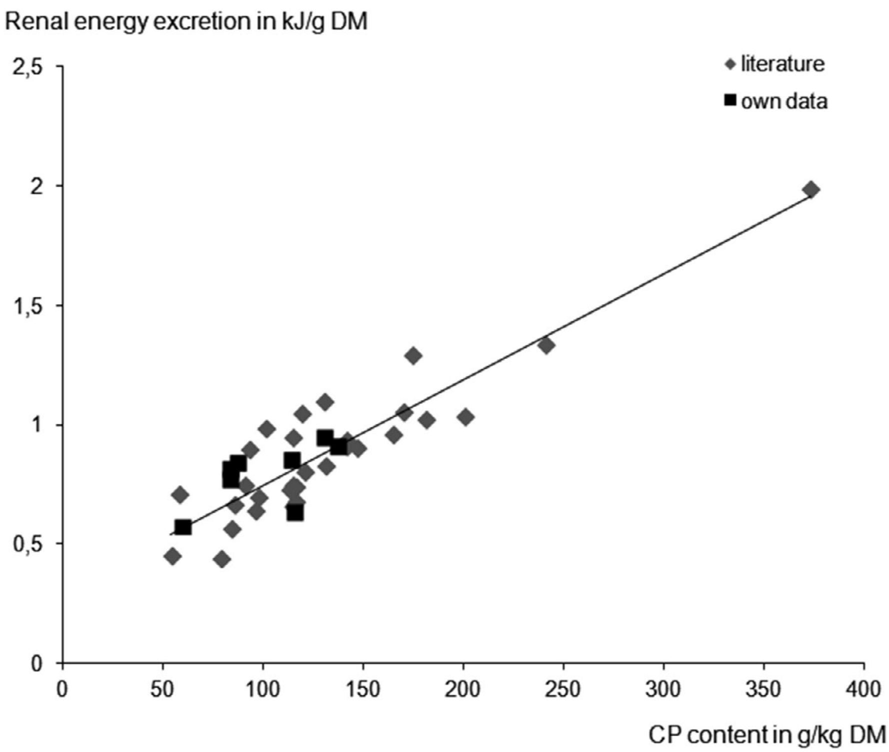

FIGURE 3 Relationship between crude protein content in feed dry matter (x; g/kg DM) and renal energy excretion per g DM intake (y; kJ/g DM) (own and literature data: Hipp et al., 2017; Kienzle et al., 2009; Pagan & Hintz, 1986; Ragnarsson, 2009; Vermorel, Martin-Rosset, et al., 1997; Vermorel, Vernet, et al., 1997) (y = 0.325 + 0.0043x; r^2 = 0.81; n = 38; p < .001)

TABLE 5 Examples for DE and ME content (MJ/kg wet weight) in typical horse feed calculated with the mean protein correction of 8 kJ/g crude protein (GfE 2014) and the new modified equation (Equation 4)

Feed[1]	CP in % DM[1]	DE[3]	ME[4]	ME calculated with Equation 4[5]
Hay, late first cut, extensive[2]	6.4	7.5	6.5	7.0
Corn	10.5	13.6	12.8	12.9
Oats	12.0	12.1	11.1	11.4
Mixed feed for adult horses	15.9	12.4	11.1	11.5
Hay, second cut	16.3	8.9	7.4	8.0
Grass, early first cut	20.0	1.7	1.4	1.5
Mixed feed for foals	20.5	12.5	11.0	11.4
Linseed meal	43.1	12.0	8.7	10.1
Soya bean meal	51.0	12.4	8.7	10.2

[1]Meyer and Coenen (2014).

[2]Unpublished data from Coenen and from Zeyner, Kienzle, Möllmann, Nater, Wanner, and Wichert (2008).

[3]DE (MJ/kg DM) = −3.54 + 0.0209 crude protein + 0.0420 crude fat + 0.0001 crude fibre + 0.0185 NfE.

[4]ME (MJ/kg DM) = −3.54 + 0.0129 crude protein + 0.0420 crude fat − 0.0019 crude fibre + 0.0185 NfE.

[5]ME (MJ/kg DM) = −3.865 + 0.0166 crude protein + 0.0420 crude fat − 0.0019 crude fibre + 0.0185 NfE.

variable can be used to predict renal energy losses for all diets and feeds tested so far. It is remarkable that the regression coefficient is very close to the renal energy losses per g/CP in dogs (NRC, 2006), who excrete very little hippuric acid, and in sheep and cattle (Schiemann, Jentsch, & Wittenburg, 1971). The regression coefficient might represent renal energy losses by urea and other N-containing metabolites such as creatinine and allantoin. The intercept might then represent losses by hippuric acid and other high energy compounds of plant detoxification. Compared to Equation 1 and also compared to a broken line model which could be obtained from the data in Figure 2, Equation 2 has practical advantages. The curvilinear model of Equation 1 has its deepest point at a value of around 170 g crude protein content per kg DM, and with higher protein content, it is no longer valid. The broken line model would result in comparatively large differences in the energy losses per g protein in feed with small differences in protein content, depending on where the protein content is with regard to the breaking point.

Equation 2 can be used to estimate renal energy losses of diets and feedstuffs, which enables to calculate their ME content from their DE content, when methane losses are known. The latter was estimated with 2.0 kJ per g of crude fibre or 2.2 kJ per g acid detergent fibre (Kienzle & Zeyner, 2010).

In line with this the equation of Kienzle and Zeyner (2010) to estimate the DE content Equation 3:

$$\begin{aligned}\mathrm{DE}(\mathrm{MJ/kg\,DM}) = -3.54 \quad &+ \mathit{0.0209}\ \text{crude protein}\\ &+ 0.0420\ \text{crude fat}\\ &+ 0.0001\ \text{crude fibre}\\ &+ 0.0185\ \mathrm{N} - \text{free extract}\end{aligned} \tag{3}$$

(crude nutrients in g/kg DM).

can be accordingly modified to estimate the ME content as follows Equation 4:

$$\begin{aligned}\mathrm{ME}\,(\mathrm{MJ/kg\,DM}) = -3.54 - \mathbf{0.325} = -3.865\\ &+ (0.0209 - \mathbf{0.0043}) = \mathit{0.0166}\ \text{crude protein}\\ &+ 0.0420\ \text{crude fat}\\ &+ (0.0001 - \mathbf{0.002}) = -0.0019\ \text{crude fibre}\\ &+ 0.0185\ \mathrm{N}-\text{free extract}\end{aligned} \tag{4}$$

(crude nutrients in g/kg DM; correction for renal losses in bold print, new intercept and new factor for protein in italics).

In Table 5, the estimated DE values according to Equation 3, estimated ME values according to GfE (2014), where a constant factor for renal energy excretion with 8 kJ/g crude protein is used, and ME values estimated according to Equation 4 are presented. This illustrates the importance of a more precise determination for renal energy losses realized with Equation 4.

ANIMAL WELFARE STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes.

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