Evaluation of the Relationship between Urine Purine Derivatives with Metabolizable Energy and Metabolizable Protein in Lactating Holstein Cows

Chunbo Wei[1,2], Zhibo Wang[1], Yongli Qu[1,*]

[1]*College of Animal Science and Veterinary Medicine*
Heilongjiang Bayi Agricultural University
Daqing 163319, China
E-mails: weicb298@163.com, andywangdge@163.com, ylqu007@126.com

[2]*Animal Science and Technology College*
Northeast Agricultural University
Harbin 150030, China

[*]*Corresponding author*

Received: February 01, 2016 **Accepted: June 01, 2016**

Published: June 30, 2016

Abstract: *The objective of this experiment was to evaluate the relationship between purine derivatives (PD) excretion in total collection or spot sampling of urine and metabolizable energy (ME) and metabolizable protein (MP) of dairy cattle. Twenty-seven dairy cows were selected to investigate the changes of PD, creatinine (C), and purine derivative/creatinine ratio (PD/C) in the urine of cows fed different diets using the total pooled urine samples collected throughout 7 days of trial period (total collection) or spot urine collection at 800-900 and 1800-1900 on the days 20 and 21. The rations were formulated with three levels of ME (127% ME/ME required, 100% ME/ME required, 75% ME/ME required) and three levels of MP (123% MP/MP required, 100% MP/MP required, 80% MP/MP required) using the CPM-Dairy 3 model. Our data demonstrated a strongly linear relationship between the concentration of PD (and PD/C) and ME and MP level of the diets using total collection or spot sampling. It was concluded that the concentration of PD and PD/C may have potential to predict the ME and MP supply in the dairy rations, and that the PD/C could be determined by spot urine sampling instead of total urine collection.*

Keywords: *Purine derivatives, Metabolizable energy, Metabolizable protein, Spot urine sampling, Total urine collection.*

Introduction

Plasma urea N and milk urea N are helpful indicators in the determination of dietary protein and energy efficiency in lactating dairy cattle [1, 12]. However, conventional indicators in milk could not fully reflect dietary nutrient digestion and they may not be considered as an ideal index of diagnosing energy and protein balance in dairy farms [19].

Monitoring the urinary excretion of purine derivatives (PD) might be considered as an alternative noninvasive technique since the urinary PD is derived from microbial nucleic acid flowing out from rumen [20]. Researchers have suggested that the urinary excretion of PD could be used as a predictor of the microbial protein supply in intact animals [2, 24]. Another extensive research conducted by Chen and Ørskov [5] demonstrated that there was a linear relationship between allantoin excretion and level of feed (N or dry matter) intake. For ruminants, physiological stage, diet type and nutrient levels in diets were the primary factors that affected the excretion of PD [15, 19]. The amount of purine derivatives excreted in the urine was highly influenced by nutrients supply [7]. The highest rates of urinary

allantoin and uric acid excretion were observed in ewes receiving a high protein, high energy diet; while the amounts of allantoin and uric acid excretion were lowest in ewes fed a low protein, low energy diet. However, the rates of excretion of hypoxanthine and xanthine were similar in all the treatments [17].

Previous studies have reported that creatinine (C) excretion, no matter on a daily basis or on a BW basis, was not significantly affected by the diets [9]. Urinary creatinine excretion is relatively a constant function of BW and therefore it might be used as a possible marker for estimating urine output [3]. Vagnoni et al. [23] reported that there was a linear relationship between the ratio of urinary allantoin to creatinine and total daily purine flow, regardless of a significant cow effect on creatinine excretion.

The CPM-Dairy v.3 is a diet formulation and evaluation software system, which resulted from the development of CNCPS v.5 including expanded carbohydrate fractions and a lipid sub-model [22]. It had been demonstrated that the system can accurately predict dietary nutritive value and improve energy and nitrogen balance and animal performance in dairy farms in China [18, 21].

In this experiment, nine diets with different levels of metabolizable energy (ME) and metabolizable protein (MP) were formulated using the CPM model to evaluate the variation between concentration of PD, C, and PD/C in urine under production condition. The main objective of the present experiment was conducted to determine if there is a relationship between PD and supplies of ME and MP in dairy diets.

Materials and methods

Cows and diets

Twenty-seven multiparous Holstein cows with similar parity (3.5 ± 0.4), days in milk (203 ± 15d), body weight (595 ± 52 kg), body condition score (3.1 ± 0.08) and age in month (76 ± 4) were randomly assigned to nine groups (n = 3). Cows in the experiment were fed individually and daily individual feed intake was recorded. The averaged milk yield of these cows was 20.30 ± 1.6 kg/d. These cows were housed at HeXing dairy farm in Daqing city of Heilongjiang province. Nine diets were formulated using CPM-Dairy v.3 to contain three levels of ME (127% ME/ME required, 100% ME/ME required, 75% ME/ME required) and three levels of MP (123% MP/MP required, 100% MP/MP required, 80% MP/MP required). The nine diets were high ME high MP (127% ME/ME required and 123% MP/MP required, HEHP), high ME balanced MP (127% ME/ME required and 100% MP/MP required, HEBP), high ME low MP (127% ME/ME required and 80% MP/MP required, HELP), balanced ME high MP (100% ME/ME required and 123% MP/MP required, BEHP), low ME high MP (75% ME/ME required and 123% MP/MP required, LEHP), balanced ME balanced MP (100% ME/ME required and 100% MP/MP required, BEBP), balanced ME low MP (100% ME/ME required and 80% MP/MP required, BELP), low ME balanced MP (75% ME/ME required and 100% MP/MP required, LEBP), low ME low MP (75% ME/ME required and 80% MP/MP required, LELP), which are presented in Table 1.

The experiment was conducted for 21 days, the first 14 days (1-14) were served as the adaptation period and urine samples were collected from 15-21 days. Each group of cows was fed one of nine treatment diets during the experimental period. Diets were fed three times daily. The forage was offered first, and followed by the concentrate with 5% orts were expected for the total diet.

Table 1. Ingredient and nutrient composition of diets (% of DM)

Ingredient	HEHP	HEBP	HELP	BEHP	LEHP	BEBP	BELP	LEBP	LELP
Corn meal	14.28	43.27	52.07	5.12	0	17.95	36.25	0	20.27
Cottonseed meal	4.9	1.49	0	7.53	10.62	5.03	0.26	8.57	5.16
DDGS	3.84	1.17	0	5.59	8.31	3.93	0.2	6.71	4.04
Rice bran meal	2.49	0.45	0	2.49	3.23	1.53	0.08	2.61	1.57
Rapeseed meal	2.13	0.65	0	3.27	4.62	2.18	0.11	3.73	2.25
Corn gluten meal	1.07	0.32	0	1.64	2.31	1.09	0.06	1.86	1.12
Soybean meal	15.52	1.94	0	22.64	31.44	9.23	0.33	30.16	7.63
Premix	1.52	0.38	0	1.69	2.38	1.14	0.1	1.92	1.16
$NaHCO_3$	0.91	0.68	0.58	1.2	1.61	0.9	0.42	1.37	0.92
$CaCO_3$	0.24	0.27	0.29	0.27	0.35	0.23	0.21	0.30	0.32
Corn silage	22.59	26.16	26.76	25.41	11.62	29.58	41.16	0	29.47
Chinese ryegrass	30.51	23.22	20.3	23.15	23.51	27.21	20.82	23.77	26.09
Corn straw	0	0	0	0	0	0	0	19.00	0
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Dry matter intake, (kg/d)	20.50	18.10	17.20	18.30	16.00	17.20	16.80	14.80	12.60
Nutrient	**HEHP**	**HEBP**	**HELP**	**BEHP**	**LEHP**	**BEBP**	**BELP**	**LEBP**	**LELP**
NE_L, (mcal/kg)	1.56	1.67	1.67	1.57	1.66	1.57	1.53	1.66	1.63
Crude protein	18.48	10.83	9.16	23.07	28.59	16.12	9.35	26.02	15.57
ME/ME required, (%)	127	127	127	100	75	100	100	75	75
MP/MP required, (%)	123	100	80	123	123	100	80	100	80

Sample collection

Cows were milked thrice daily in their stalls at 0400, 1200, and 2000 h, and milk yield was recorded at each milking. Total urine was collected using indwelling bladder catheters, which were inserted on 15 day. Daily urine output samples were pooled for 7 days as the total urine collection (total collection). Spot urine samples were collected with the aid of a device adapted from a fraction collector. Spot urine sample (twice per day) was collected on the days 20 and 21 at 800-900 and 1800-1900, then were pooled as spot urine collection (spot sampling). The total collection and spot sampling were acidified with H_2SO_4

(50% vol/vol) to maintain a pH < 3.0. A representative sample (2%) was taken and immediately diluted with distilled water. The diluted samples were filtered through surgical gauze and kept frozen at −20°C for subsequent analyses of purine derivatives (PD) and creatinine.

Analytical determinations

Urine samples were analyzed for allantoin, uric acid and creatinine. Allantoin was measured according to the colorimetric method. In this procedure, allantoin is hydrolyzed firstly under a weak alkaline condition at 100 °C, for allantoic acid which is further degraded to urea and glyoxylic acid in weak acid solution. The glyoxylic acid then reacts with phenylhydrazine hydrochloride to produce a phenylhydrazone of the acid. The product can then form an unstable chromophore with potassium ferricyanide. The colour is read at 522 nm. Commercial kits were used to analyze uric acid (Sigma procedure no. 686; Sigma Chemical Co., St. Louis, MO). Uric acid, which absorbs light at 293 nm, is converted by uricase to allantoin, which is nonabsorbing at 293 nm. The change in absorbance at 293 nm due to the disappearance of uric acid is directly proportional to the concentration of uric acid in the urine. Creatinine was measured with a kit designed specifically for the analysis (555-A Sigma Chemical Co., St. Louis, MO). Creatinine reacts with picric acid under alkaline conditions to form a yellow-orange complex. The color is derived from creatinine as well as certain other non-specific substances. Upon the addition of acid, the color contributed by creatinine is destroyed, while that produced by non-specific substances remains. The difference in color intensity measured at 500 nm before and after acidification is proportional to the creatinine concentration.

Statistical analysis

The data were analyzed using the GLM procedure of SAS program package (SAS Institute, version 9.1). The following model was used:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij},$$

where Y_{ij} is the dependent variables; μ is the overall mean; T_i is the fixed effect of group or treatment; ε_{ij} is the error term.

Duncan's multiple range tests were used to detect statistical significance between treatment groups. Linear regression analysis procedure of SAS program package was used to examine the relationships between the concentrations of allantoin, uric acid, total PD, the ratio of PD/C in the urine and the different level of ME and MP diets.

The following model was used:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \varepsilon,$$

where y is the dependent variables; β_0 is intercept; β_1 and β_2 are regression coefficient; x_1 and x_2 are independent variables, ε is the random error.

Results and discussion

Concentration of PD, creatinine, and PD/C in total urine collection of cows fed different level of ME and MP diets

Table 2 presents the total excretion of uric acid, allanatoin, total PD, Creatinine and PD/C from cattle fed different diets in total urine collection. The data demonstrated that concentrations of uric acid, allantoin and PD were increased with increasing MP supply when

cows were fed similar ME levels (Table 2). The concentrations of uric acid, allantoin and PD from cattle fed the HEHP or HEBP diets were greater than those of cows fed the HELP diet ($p < 0.05$). The concentrations of uric acid, allantoin and PD were increased with increasing of ME intakes when cows were fed similar MP level diets. There was numerical difference between cattle fed the BEHP diet and the LEHP diet ($p > 0.05$), but the excretion of PD was greater in the cattle fed the HEHP diet ($p < 0.05$). The value of C was the highest when cows were fed the HELP diet and lowest in cattle fed the LELP diet ($p < 0.05$) and showed a linear response among the cattle fed the other diets. The PD/C was increased with increasing of ME supply when cows were fed diets with similar MP levels ($p < 0.05$) (Table 2).

Table 2. Excretion of uric acid, allantoin, PD, creatinine, and PD/C with different level of ME and MP diets using total urine collection

Level of energy and nitrogen	Urinary purine derivatives				
	Uric acid (mmol/d)	Allantoin (mmol/d)	PD (mmol/d)	C (mmol/d)	PD/C
HEHP	22.75±0.72^{a}	137.1±4.28^{a}	159.86±4.99 ^{a}	54.10±6.00^{abc}	2.95±0.17^{a}
HEBP	20.49±2.30^{ab}	123.46±13.89^{ab}	143.95±16.2 ^{ab}	53.36±3.98^{abc}	2.70±0. 06^{ab}
BEHP	19.26±1.01^{bc}	116.04±6.09^{bc}	135.30±7.09 ^{bc}	57.28±5.03^{ab}	2.36±0.36^{bc}
BEBP	18.36±1.11^{bcd}	110.64±6.68^{bcd}	129.01±7.79 ^{bcd}	56.24±4.20^{ab}	2.29±0.19^{c}
LEHP	17.24±0.48^{cde}	103.91±2.92^{cde}	121.16±3.40 ^{cde}	57.87±4.37^{ab}	2.09±0.23^{c}
HELP	14.72±1.61^{ef}	66.74±7.28^{f}	81.47±8.89 ^{f}	59.16±3.18^{a}	1.38±0.15^{de}
BELP	14.07±2.15^{fg}	63.81±9.73^{fg}	77.89±11.87 ^{fg}	46.70±3.56^{c}	1.67±0.11^{d}
LEBP	12.75±0.92^{fgh}	54.22±3.90^{fgh}	66.98±4.81 ^{fgh}	51.07±5.18^{bc}	1.31±0.26^{e}
LELP	8.24±1.76^{i}	49.61±10.56^{h}	57.85±12.31^{i}	54.22±3.90^{abc}	1.07±0.15^{e}

$^{a-i}$ means within a column with different superscripts differ ($p < 0.05$).

Linear relationship between urine acid, allantoin, PD, C, and PD/C in total urine collection with different Level of ME and MP diets are shown in Table 3 and Fig. 1. The regression equation showed that there was a linear relationship between concentrations of uric acid, allantoin, PD and PD/C with changes of ME and MP level in the diets using total urine collection ($p < 0.01$), except for that of C ($p = 0.35$).

The results showed that when the MP supply was constant, the concentration of PD (uric acid, allantoin, and total PD) was increased with the increasing level of ME, and that of uric acid, allantoin and total PD was greatest in the greatest ME level group. It was indicated that the available carbohydrate for fermentation was one of main factors affecting the growth of microorganisms and ME supply. The findings were similar to studies discussed by Gonda et al. [10] and Carro et al. [8].When ME supply was at a similar level, the contents of uric acid, allantoin and PD increased with increasing MP level, the value of them was greatest in the highest MP level , suggesting that when cattle were either deficient in rumen nitrogen or in available fermentable carbohydrate, microbial protein production was decreased [13, 14].

Table 3. Linear relationship between urine acid, allantoin, PD, C, and PD/C in total urine collection with different Level of ME and MP diets

Urinary purine derivatives	Model Adj R-Sq	Parameters	Estimates	Standard error	*p*-value
urine acid	0.8588	Intercept	–15.39	2.55	< 0.0001
		x_1	0.16	0.02	< 0.0001
		x_2	0.15	0.03	< 0.0001
allantoin	0.7643	Intercept	–130.15	24.09	< 0.0001
		x_1	0.81	0.23	0.0017
		x_2	1.32	0.26	< 0.0001
PD	0.7817	Intercept	–145.54	26.23	< 0.0001
		x_1	0.97	0.25	0.0007
		x_2	1.47	0.28	< 0.0001
C	–0.0277	Intercept	–43.81	7.96	0.5842
		x_1	0	0.08	0.9986
		x_2	0.10	0.09	0.2326
PD/C	0.7098	Intercept	–2.34	0.54	0.0002
		x_1	0.02	0.01	0.0011
		x_2	0.02	0.01	0.0007

x_1 = ME/ME required; x_2 = MP/MP required; PD = Purine Derivatives; C = Creatinine.

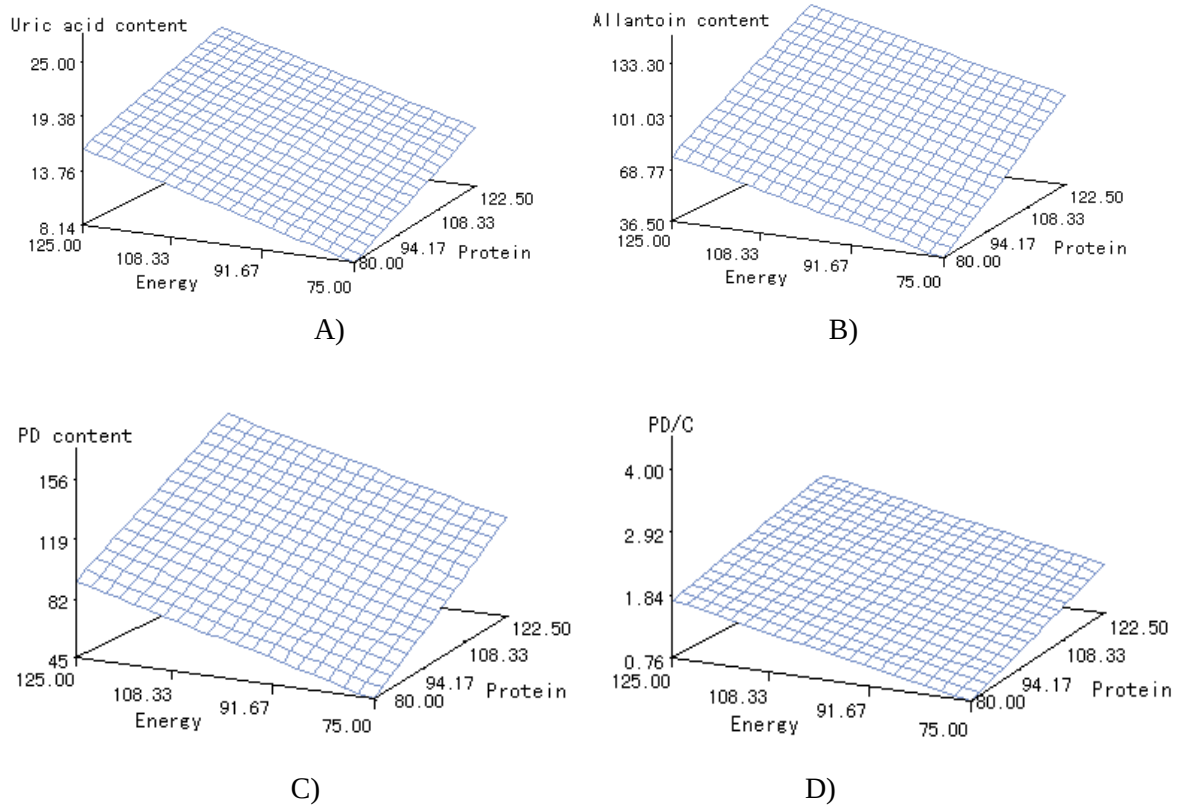

Fig. 1 Three-dimensional chart of the change of uric acid, allontoin, PD, and PD/C with different level of ME and MP diets using total urine collection

The results also revealed that the concentration of PD (uric acid, allantoin, and total PD) had significant linear correlation with ME/ME required (x_1) and MP/MP required (x_2). The *p*-value of uric acid, allantoin, and total PD by linear regression analysis were less than 0.001, which meant that there were linear relationships between content of PD and dietary ME and MP levels. Moreover, it also indicated that uric acid, allantoin and PD could be predicted by the changes of ME and MP level of diets in dairy farm at the range of ME/ME required (127% to 75%) and MP/MP required (123% to 80%). Similarly, at the same range of ME/ME required and MP/MP required, it could be possible to use the concentration of PD to predict the ME and MP level in dairy diets by the three-dimensional curves established in the present study.

Responses in concentrations of PD, creatinine, and PD/C in spot urine sampling with different level of ME and MP diets

The changes of uric acid, allanatoin, total PD, Creatinine and PD/C in spot urine sample of animals fed different diets are shown in Table 4.The excretion data showed that concentrations of the uric acid, allantoin and PD in the spot urine sample had similar variation compared with those in the total collection data. The concentrations of uric acid, allantoin and PD was increased with increasing of MP intakes when cows were fed diets with similar ME levels. The concentrations of the uric acid, allantoin and PD of cows fed HEHP diet were greater than those of cows fed HEBP diet ($p < 0.05$), and both of them were greater than those of cows fed HELP diet ($p < 0.05$). The spot sample data also showed that the concentrations of uric acid, allantoin and PD in the urine were increased with increasing of ME intakes when cows were fed similar MP level diets. The concentrations of the uric acid, allantoin and PD in the cows fed HEHP diet were greater than those in the cows fed BEHP ($p < 0.05$), and they were also greater than those of cows fed LEHP diet ($p < 0.05$).

Table 4. Changes of uric acid, allantoin, PD, creatinine, and PD/C with different level of ME and MP diets using spot urine sampling

Level of energy and nitrogen	Urinary purine derivatives				
	Uric acid (mmol/L)	Allantoin (mmol/L)	PD (mmol/L)	C (mmol/L)	PD/C
HEHP	3.49±0.12^{a}	19.59±0.23^{a}	23.08±0.36^{a}	9.08±0.05^{cde}	2.54±0.34^{a}
HEBP	3.13±0.19^{b}	15.40±0.63^{b}	18.53±0.82^{b}	8.39±0.13^{ef}	2.21±0.32^{ab}
BEHP	3.16±0.11^{b}	15.02±0.13^{b}	18.18±0.19^{bc}	9.87±0.58^{c}	1.84±0.22^{bc}
BEBP	1.31±0.06^{e}	7.67±0.10^{e}	8.98±0.16^{e}	9.49±0.49^{cd}	0.95±0.08^{d}
LEHP	2.54±0.10^{c}	14.45±0.39^{c}	16.99±0.50^{c}	11.01±0.42^{b}	1.54±0.34^{c}
HELP	1.66±0.06^{d}	9.44±0.11^{d}	11.10±0.17^{d}	13.40±0.58^{a}	0.82±0.17^{d}
BELP	1.20±0.05^{ef}	6.23±0.13^{f}	7.43±0.17^{fg}	7.72±0.73^{f}	0.96±0.10^{d}
LEBP	1.28±0.05^{e}	6.43±0.29^{f}	7.71±0.33^{f}	8.03±0.55^{f}	0.96±0.14^{d}
LELP	1.06±0.12^{f}	5.86±0.21^{g}	6.92±0.32^{g}	8.65±0.86^{def}	0.80±0.04^{d}

$^{a-g}$ means within a column with different superscripts differ ($p < 0.05$).

The PD/C was increased with the increasing of ME intakes when cattle were fed similar MP level diets, except for low MP diets. The PD/C in the cows fed the HEHP diet was the highest and greater than those of cows fed the BEHP and LEHP diets ($p < 0.05$). The PD/C of cows fed the HEBP diet was greater than those of cows fed the BEBP diet, which was also greater than those cows fed the LEBP diet ($p < 0.05$).

Relationships between PD, creatinine, and PD/C in spot urine sampling and levels of ME and MP are shown in Table 5 and Fig. 2. The regression equation showed that there was a linear relationship between concentrations of uric acid, allantoin, PD and PD/C with changes of ME and MP level in the diets using spot urine sampling ($p < 0.01$), except for that of C ($p = 0.40$). The use of urinary purine derivatives requires a total collection of urine for 7 days. Total urine collection is laborious and difficult in grazing animals or under farm conditions and generates discomfort in the animals due to the presence of catheters or collecting funnels. Subsequent studies were carried out by Chen et al. [6] in steers, Gonda and Lindberg [11] in dairy cows and Chen et al. [4] in sheep to examine the diurnal variations in spot urine measurement. These studies concluded that PD/C ratio correlates to feed intake and intestinal flow of microbial purines, and thus could be used as an indicator of microbial protein availability. These reports also pointed out that sufficient number of measurements should be made in order to reduce errors; otherwise the variability of spot measurement would be greater than that based on total urine collection [5, 16]. Our results showed that uric acid (1.06-3.49 mmol/L), allantoin (5.86-19.59 mmol/L) and total PD content (6.92-23.08 mmol/L) in the spot urine sample had the same tendency as the data in total urine collection. There were linear relationships between contents of uric acid, allantoin and PD with dairy ME and MP levels, which could provide the basis of evaluation of the ME and MP level in dairy diets by spot urine measurement instead of by the total urine collection.

Table 5. Linear relationship between PD, creatinine, and PD/C in spot urine sampling with different Level of ME and MP diets

Urinary purine derivatives	Model Adj R-Sq	Parameters	Estimates	Standard error	*p*-value
Urine acid	0.7409	Intercept	–4.24	0.74	< 0.0001
		x_1	0.02	0.01	0.0147
		x_2	0.04	0.01	< 0.0001
allantoin	0.7708	Intercept	–22.04	3.56	< 0.0001
		x_1	0.09	0.03	0.0111
		x_2	0.23	0.04	< 0.0001
PD	0.7706	Intercept	–26.32	4.25	< 0.0001
		x_1	0.11	0.04	0.0109
		x_2	0.27	0.05	< 0.0001
C	–0.0047	Intercept	6.59	2.70	0.0223
		x_1	0.03	0.03	0.2395
		x_2	0.00	0.03	0.8930
PD/C	0.5945	Intercept	–2.60	0.64	0.0005
		x_1	0.01	0.01	0.0546
		x_2	0.03	0.01	0.0008

x_1 = ME/ME required; x_2 = MP/MP required; PD = Purine Derivatives; C = Creatinine.

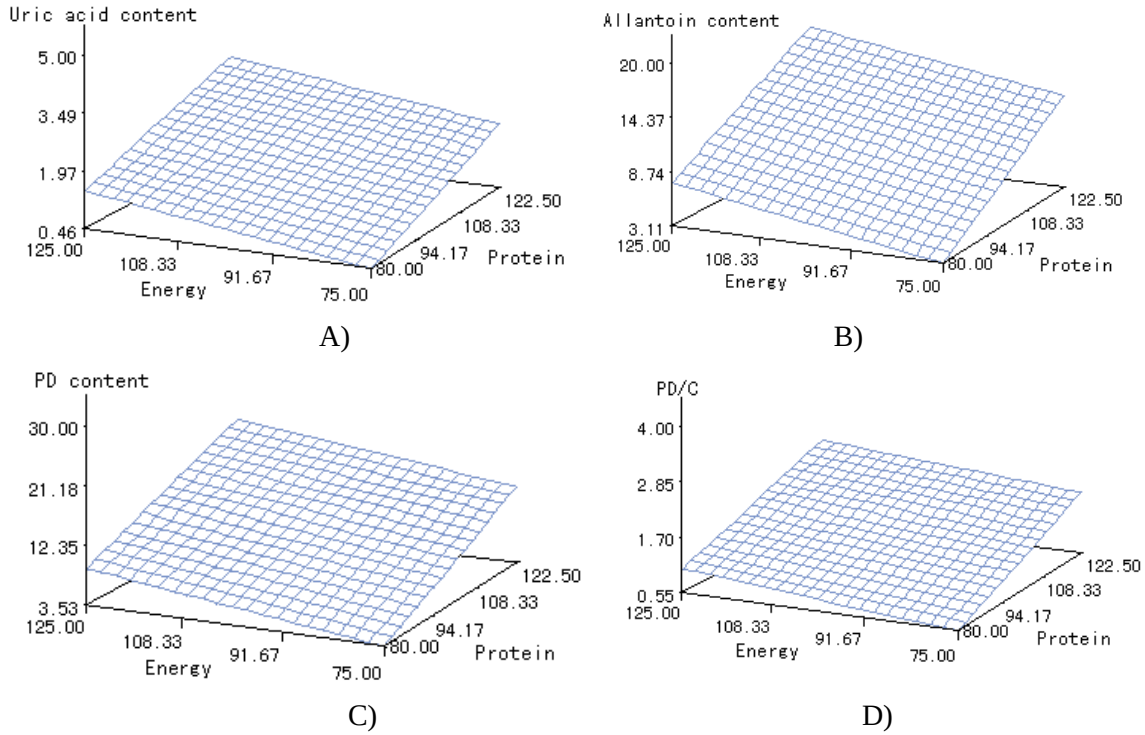

Fig. 2 The three-dimension chart of change of uric acid, allontoin, PD, and PD/C with different level of ME and MP diets using spot urine sampling.

Relationship of PD/C between total urine collection and spot sample collection

The relevance of the PD/C between total urine collection and spot samples is shown in Fig. 3. The correlation coefficient (*R*) was 0.89. The PD/C in spot urine samples was highly correlated with daily PD excretion, along with the relative diurnal stability of the ratio, indicated the validity of this ratio in predicting the daily PD excretion for the estimation of microbial protein supply. The results and discussion may be combined into a common section or presented separately. They may also be broken into subsections with short, informative headings.

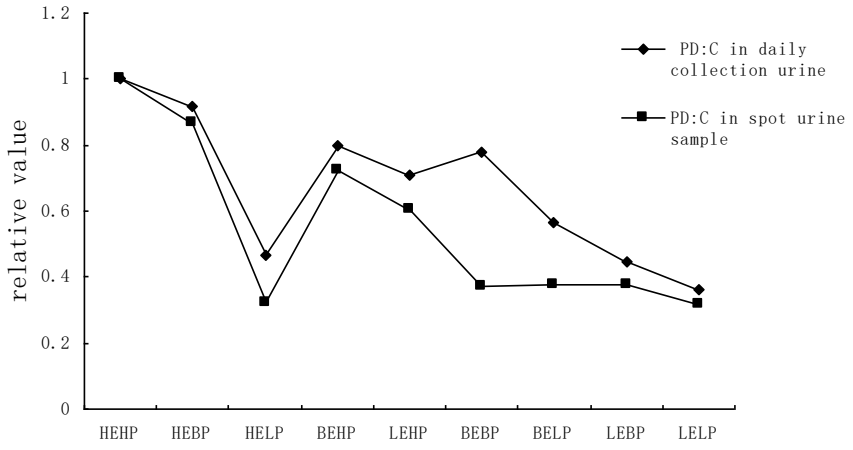

Fig. 3 The relationship of PD/C between total urine collection and spot urine sampling among cattle fed nine diets differing in metabolizable energy and metabolizable protein

Conclusion

Our data demonstrated a strongly linear relationship between the concentration of PD (and PD/C) with ME and MP level of the diets using total collection or spot sampling. The PD/C measured by spot urine collection had a strong correlation with those measured using total urine collection (R = 0.89). It was concluded that the concentration of PD and PD/C may have potential to predict the ME and MP supply in the dairy rations, and that the PD/C could be determined by spot urine sampling instead of total urine collection.

Acknowledgements

This work was supported by National Natural Science Foundation of China (Grant No. 31340031), Synergetic innovation center of food safety and nutrition, National Key Technologies R&D Program (No. 2012BAD12B05-1), Natural science foundation of Heilongjiang province (No. QC2013C031).

References

1. Patton R. A., A. N. Hristov, H. Lapierre (2014). Protein Feeding and Balancing for Amino Acids in Lactating Dairy Cattle, Veterinary Clinics of North America-food Animal Practice, 30(3), 599-621.
2. Arndt C., J. M. Powell, M. J. Aguerre, P. M. Crump, M. A. Wattiaux (2015). Feed Conversion Efficiency in Dairy Cows: Repeatability, Variation in Digestion and Metabolism of Energy and Nitrogen, and Ruminal Methanogens, Journal of Dairy Science, 98(6), 3938-3950.
3. Blagoeva R., A. Nedev (2011). An Approach to Modeling Protein Release from Lipid Implants, International Journal Bioautomation, 15(1), 25-32.
4. Chen X. B., A. T. Mejia, D. J. Kyle, E. R. Ørskov (1995). Evaluation of the Use of the Purine Derivative: Creatinine Ratio in Spot Urine and Plasma Samples as an Index of Microbial Protein Supply in Ruminants: Studies in Sheep, Journal of Agricultural Science, 125, 137-143.
5. Chen X. B., E. R. Ørskov (2004). Estimation of Microbial Protein Supply in Ruminants Using Urinary Purine Derivatives, Springer Netherlands.
6. Chen X. B., G. Grubic, E. R. Ørskov, P. Osuji (1992). Effect of Feeding Frequency on Diurnal Variation in Plasma and Urinary Purine Derivatives in Steers, Animal Production, 55(02), 185-191.
7. Chibisa G. E., P. Gorka, G. B. Penner, R. Berthiaume, T. Mutsvangwa (2015). Effects of Partial Replacement of Dietary Starch from Barley or Corn with Lactose on Ruminal Function, Short-chain Fatty Acid Absorption, Nitrogen Utilization, and Production Performance of Dairy Cows, Journal of Dairy Science, 98(4), 2627-2640.
8. Carro M., C. Valdes, M. Jranilla, J. Gonzalez (2000). Effect of Forage to Concentrate Ratio in the Diet on Ruminal Fermentation and Digesta Flow Kinetics in Sheep Offered Food at a Fixed and Restricted Level of Intake, Animal Science, 70(1) ,127-134.
9. Coto G., M. Rodriguez, F. Perez-Infante, P. Melgarez, M. Perez (1989). Evaluation of the N Fractions in the Rumen of Rams Fed Increasing Levels of Concentrates, Revista Cubana de Ciencia Agricola, 23, 187-191.
10. Gonda H. L., M. Emanuelson, M. Murphy (1996). The Effect of Roughage to Concentrate Ratio in the Diet on Nitrogen and Purine Metabolism in Dairy Cows, Animal Feed Science and Technology, 64(1), 27-42.
11. Gonda H. L., J. E. Lindberg (1994). Evaluation of Dietary Nitrogen Utilization in Dairy Cows Based on Urea Concentrations in Blood, Urine and Milk, and on Urinary Concentration of Purine Derivatives, Acta Agriculturae Scandinavica A-Animal Sciences, 44(4), 236-245.

12. Hammond A. (1998). Use of BUN and MUN as Guides for Protein and Energy Supplementation in Cattle, Revista Corpoica, 2(2), 44-48.
13. Hindrichsen I., P. Osuji, A. Odenyo, J. Madsen, T. Hvelplund (2002). Effects of Supplementation of a Basal Diet of Maize Stover with Different Amounts of Leucaena Diversifolia on Intake, Digestibility, Nitrogen Metabolism and Rumen Parameters in Sheep, Animal Feed Science and Technology, 98(3), 131-142.
14. May D., J. Fcalderon, V. Manuelgonzalez, M. Fmontano, A. Plascencia, J. Salinaschavira, N. Torrentera, R. Azinn (2014). Influence of Ruminal Degradable Intake Protein Restriction on Characteristics of Digestion and Growth Performance of Feedlot Cattle During the Late Finishing Phase, Journal of Animal Science and Technology, 56(1), 1-7.
15. Mandok K. M., J. K. Kay, S. L. Greenwood, J. P. Mcnamara, M. Crookenden, R. Gwhite, S. Shields, G. R. Edwards, J. R. Roche (2014). Efficiency of Use of Metabolizable Energy for Body Weight Gain in Pasture-based, Nonlactating Dairy Cows, Journal of Dairy Science, 97(7), 4639-4648.
16. Prasitkusol P., E. Ørskov, X. Chen, F. Hovell, D. Kyle (2002). Variation between Sheep in Renal Excretion of [^{14}C] Allantoin, British Journal of Nutrition, 87(6), 561-568.
17. Puchala R., G. Kulasek (1992). Estimation of Microbial Protein Flow from the Rumen of Sheep Using Microbial Nucleic Acid and Urinary Excretion of Purine Derivatives, Canadian Journal of Animal Science, 72(4), 821-830.
18. Qu Y., J. Wu, S. Li, Y. Zhang (2010). Evaluation for Increasing Dairy Cattle Performance and Improving Diet Energy and Nitrogen Balance Applying CPM Model, Chinese Journal of Animal Nutrition, 22(2), 310-317 (in Chinese).
19. Roseler D., J. Ferguson, C. Sniffen, J. Herrema (1993). Dietary Protein Degradability Effects on Plasma and Milk Urea Nitrogen and Milk Nonprotein Nitrogen in Holstein Cows, Journal of Dairy Science, 76(2), 525-534.
20. Stefanon B., C. Mills, M. Spanghero, P. Susmel (1995). An Evaluation of Purine Derivatives as Indicators of Tureen Microbial Protein Synthesis in Dry and Lactating Cows, Publication-European Association for Animal Production, 81, 147-152.
21. Stergiadis S., M. Rallen, X. Jchen, D. Wills, T. Yan (2015). Prediction of Nutrient Digestibility and Energy Concentrations in Fresh Grass Using Nutrient Composition, Journal of Dairy Science, 98(5), 3257-3273.
22. Tedeschi L., W. Chalupa, E. Janczewski, D. Fox, C. Sniffen, R. Munson, P. Kononoff, R. Boston(2008). Evaluation and Application of the CPM Dairy Nutrition Model, The Journal of Agricultural Science, 146(2), 171-182.
23. Vagnoni D., G. Broderick, M. Clayton, R. Hatfield (1997). Excretion of Purine Derivatives by Holstein Cows Abomasally Infused with Incremental Amounts of Purines, Journal of Dairy Science, 80(8), 1695-1702.
24. Wang C. (2015). A Modified Machine Learning Method Used in Protein Prediction in Bioinformatics, International Journal Bioautomation, 19(1), 25-36.

Chunbo Wei, Ph.D. Student
E-mail: weicb298@163.com

Chunbo Wei was born in Jixi city, Heilongjing Province of China, in 1977. He got his M.Sc. degree in Heilongjiang Bayi Agricultural University (Daqing) in 2005. He is studying for his doctorate of Animal Nutrition and Feed Science in Northeast Agricultural University and he plans to graduate in June 2016.

Zhibo Wang, Ph.D.
E-mail: andywangdge@163.com

Zhibo Wang graduated and received his Ph.D. in College of Northeast Agricultural University. He is a lecturer at College of Animal Science and Veterinary Medicine of Heilongjiang Bayi Agricultural University. His interests are in dairy cattle nutrition and feeding science.

Prof. Yongli Qu, Ph.D.
E-mail: ylqu007@126.com

Yongli Qu graduated and received his Ph.D. in College of Northeast Agricultural University. He is a Professor at College of Animal Science and Veterinary Medicine of Heilongjiang Bayi Agricultural University. His interests are in dairy cattle nutrition and feeding science.