The Effects of Seasonal Variation on the Microbial-N Flow to the Small Intestine and Prediction of Feed Intake in Grazing Karayaka Sheep

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Abstract—The objectives of the present study were to estimate the microbial-N flow to the small intestine and to predict the digestible organic matter intake (DOMI) in grazing Karayaka sheep based on urinary excretion of purine derivatives (xanthine, hypoxanthine, uric acid, and allantoin) by the use of spot urine sampling under field conditions. In the trial, 10 Karayaka sheep from 2 to 3 years of age were used. The animals were grazed in a pasture for ten months and fed with concentrate and vetch plus oat hay for the other two months (January and February) indoors. Highly significant linear and cubic relationships (P<0.001) were found among months for purine derivatives index, purine derivatives excretion, purine derivatives absorption, microbial-N and DOMI. Through urine sampling and the determination of levels of excreted urinary PD and Purine Derivatives / Creatinine ratio (PDC index), microbial-N values were estimated and they indicated that the protein nutrition of the sheep was insufficient.

In conclusion, the prediction of protein nutrition of sheep under the field conditions may be possible with the use of spot urine sampling, urinary excreted PD and PDC index. The mean purine derivative levels in spot urine samples from sheep were highest in June, July and October. Protein nutrition of pastured sheep may be affected by weather changes, including rainfall. Spot urine sampling may useful in modeling the feed consumption of pasturing sheep. However, further studies are required under different field conditions with different breeds of sheep to develop spot urine sampling as a model.

Keywords—Karayaka sheep, spot sampling, urinary purine derivatives, PDC index, microbial-N, feed intake.

I. INTRODUCTION

THE Karayaka sheep is one of the indigenous breeds reared on the coastline of the Black Sea region of Turkey. The breed is mainly kept for its high meat quality because its milk production is lower than other native breeds. Karayaka sheep are well adapted to the wet climate of the region and there is a total population of approximately one million [1]. The quality of grazing in the areas of its distribution is much better than in most of the other regions and the grazing season is longer.

The use of spot urine sampling has been proposed to predict protein nutrition and digestible organic matter intake in grazing sheep [2] and goats [3]. Microbial protein flow to the small intestine has been estimated total of purine derivatives excreted in the urine of ruminants [4], [5]. Rumen microbes constitute the major source of protein supply to the ruminants. The purines from the rumen microbes are metabolized and excreted in the urine as their derivatives, hypoxanthine, xanthine, uric acid and allantoin. Nucleic acids leaving the rumen are essentially of microbial origin. That is because ruminant feeds usually have a low purine content, most of which undergoes extensive degradation in the rumen as the result of microbial fermentation. In sheep, hypoxanthine and xanthine are converted to uric acid by xanthine oxidase, and uric acid is further converted to allantoin by uricase. All four compounds are excreted in the urine of sheep. The synthesis of microbial protein is dependent on ruminal ammonia nitrogen supply [6] and digestible organic matter intake [7], [8].

Reports that estimate microbial-N flow to the small intestine are mostly from European sheep breeds [2], [8]-[11]. The objectives of the present study were to examine the microbial-N flow to the small intestine and to predict DOMI in grazing Karayaka sheep on the basis of urinary excretion of PD by the use of spot urine sampling under field conditions and to investigate the effects of seasonal variation on ruminal microbial synthesis.

II. MATERIAL AND METHODS

A. Animal and Feed Materials

A total of 10 Karayaka sheep aged between 2 and 3 years, and with live weight ranging from 42.4 to 47.6kg, were used in this study that was undertaken in Akyaz village of Bafra town, Samsun province, Turkey. The animals were grazed in a pasture for ten months and fed a concentrate and vetch plus oat hay for the other two months (January and February) indoors.

Meadow and pasture samples were collected by using the visual estimation method. Plant samples were taken by hand clipping to ground level at the beginning of the grazing experiment and repeated every month. They were collected from one square meter area in six different locations in a 0.5-1 ha area [12]. The important part of meadow and pasture was formed by Agropyron cristatum, Lotus corniculatus L., Agropyron elongatum, Bromus inermis, Convolvulus sp., Trifolium pretense, Trifolium repense, and Dactylis glomerata. All plant samples were dried to a constant weight in a forced-air oven at 65°C for 48h. Dry matter (DM), nitrogen (N), neutral detergent fibre (NDF), and acid detergent fibre (ADF) were determined after the grinding through a 1mm screen. Ash
content was determined by heating in a muffle furnace at 550°C [13]. Organic matter (OM) was calculated as DM–ash. N content was analyzed with the Kjeldahl method, with the use of a semi-automated N analyzer. NDF and ADF were determined according to the methodology of van Soest et al. [14].

The levels of purine derivatives were determined according to the methodology of Chen et al. [15] using a spectrophotometer (Shimadzu UV-1700). Allantoin is first hydrolyzed under weak alkaline conditions and at 100°C to allantoic acid which is further hydrolysed to urea and glyoxylic acid in a weak acid solution. The glyoxylic acid is reacted with phenylhydrazine hydrochloride to produce a phenylhydrazone derivative of the acid. The product forms an unstable chromophore with potassium ferricyanide. The colour was read at 522nm. Xanthine and hypoxanthine are converted to uric acid by treatment with xanthine oxidase and are thus determined as uric acid, the amount of which is determined by its absorbance at 293nm, although other compounds may also absorb at this wavelength. When samples are treated with uricase, uric acid is converted to allantoin and other compounds that do not absorb UV at 293 nm. Therefore, the reduction in absorbance reading after treatment with uricase is correlated with the concentration of uric acid in the sample. After treatment, the absorbance of the standards should be zero, if the conversion is complete. Creatinine reacts with picate ion formed in alkaline medium and a red-orange colour develops. The colour produced from the sample is then compared in a colorimeter at 505nm with that produced by a specific breed of animals, which should have been previously measured from the complete urine collection. Average daily creatinine excretion was taken to be 503μmol/kg CA0.75 for sheep [5].

D. Estimation of PDC Index

PDC index is determined by calculating the creatinine concentrations and total purine derivatives in the urine. The following equation is used to index the PDC:

\[ \text{PDC index} = \frac{(\text{PD})}{C} \times W^{0.75} \]

where W is the body weight (kg), and PD and C are purine derivatives and creatinine concentrations, respectively in mmol/L.

\[ \text{PD excretion (mmol/d)} = \text{PDC index} \times C \]

where C is the daily creatinine excretion (mmol/kg W0.75) for a specific breed of animals, which should have been previously measured from the complete urine collection. Average daily creatinine excretion was taken to be 503μmol/kg CA0.75 for sheep [5].

E. Estimation of Digestible Organic Matter Intake (DOMI)

The spot measurement of the PDC index can provide an estimate of feed intake. Digestible organic matter intake was calculated with the following equation [5]:

\[ \text{DOMI (g /d)} = 59.7x\text{PDC–678} \]

F. Statistical Analysis

Data were summarized with descriptive statistics for means, and the standard errors of the means were analyzed with analysis of variance (ANOVA), using the Least Square Method of the GLM procedure of the SAS [17]. The differences between the groups were analyzed via 3rd order orthogonal polynomials. All results were summarized as mean ± standard error of mean (SEM). Ordinary linear regression and Pearson correlation analyses were performed with the use of variables, namely allantoin, microbial-N, DOMI and purine derivatives excretion, and digestible organic matter digested in the rumen.

III. Results

The dry matter (DM), organic matter (OM), crude protein (CD), neutral detergent fiber (NDF) and acid detergent fiber (ADF) values of meadow and pasture samples (g/kg DM) collected monthly are presented in Table I. Levels of creatinine and allantoin, uric acid, hypoxanthine and xanthine in spot urine samples are shown in Table II. The purine derivatives index (PDC index), purine derivatives excretion, purine derivatives absorption, microbial-N and DOMI for spot urine samples are shown in Table III. Monthly variations in both mean values of the PDC index and microbial-N (Fig. 1) and mean values of DOMI are presented in Fig. 2.
**Table I**

| Period | DM (g/kg DM) | OM (g/kg DM) | CP (g/kg DM) | NDF (g/kg DM) | ADF (g/kg DM) |
|--------|--------------|--------------|--------------|---------------|---------------|
| June   | 913.0        | 838.4        | 134.0        | 437.2         | 351.7         |
| July   | 931.2        | 866.5        | 148.0        | 476.8         | 337.7         |
| August | 892.5        | 801.2        | 106.5        | 500.2         | 392.1         |
| September | 909.3   | 830.9        | 143.7        | 479.4         | 334.9         |
| October | 905.6        | 824.5        | 131.1        | 442.1         | 313.5         |
| November | 913.0       | 837.6        | 107.5        | 468.4         | 345.5         |
| December | 904.3        | 827.3        | 101.2        | 543.7         | 415.5         |

**Table II**

| Grazing period | Months | n | Allantoin (mmol/L) | Uric acid (mmol/L) | Hypoxanthine + Xanthine (mmol/L) | Creatinine (mg%) | Purine derivatives |
|----------------|--------|---|-------------------|-------------------|---------------------------------|-----------------|-------------------|
| June           | 9      | 7.67±0.37 | 1.76±0.18 | 0.65±0.06 | 6.39±0.19 | 10.08±0.44 |
| July           | 9      | 7.71±0.38 | 1.40±0.08 | 0.68±0.06 | 6.30±0.32 | 9.79±0.43 |
| August         | 10     | 6.17±0.18 | 1.25±0.06 | 0.61±0.06 | 6.75±0.23 | 8.02±0.18 |
| September      | 10     | 6.81±0.29 | 1.77±0.09 | 0.75±0.07 | 5.74±0.21 | 9.32±0.29 |
| October        | 10     | 6.62±0.58 | 1.59±0.12 | 0.68±0.04 | 6.49±0.28 | 10.90±0.66 |
| November       | 9      | 7.28±0.27 | 1.64±0.19 | 0.35±0.03 | 4.72±0.22 | 9.27±0.31 |
| December       | 10     | 5.04±0.22 | 1.96±0.10 | 0.32±0.02 | 5.44±0.27 | 7.32±0.21 |
| January        | 9      | 4.84±0.18 | 0.87±0.05 | 0.42±0.04 | 5.00±0.16 | 6.13±0.20 |
| February       | 8      | 6.31±0.31 | 1.29±0.13 | 0.53±0.03 | 6.64±0.28 | 8.12±0.31 |

Significance of main effects

- **Linear**
  - Allantoin: ***
  - Uric acid: NS
  - Hypoxanthine + Xanthine: NS
  - Creatinine: NS
  - Purine derivatives: ***

- **Quadratic**
  - Allantoin: NS
  - Uric acid: ***
  - Hypoxanthine + Xanthine: ***
  - Creatinine: NS
  - Purine derivatives: ***

- **Cubic**
  - Allantoin: NS
  - Uric acid: NS
  - Hypoxanthine + Xanthine: NS
  - Creatinine: NS
  - Purine derivatives: NS

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**Fig. 1** Monthly changes of mean values of PDC index and Microbial-N (g N/d) for grazing Karayaka sheep

**Fig. 2** Monthly changes of mean values of DOMI (g/d) for grazing Karayaka sheep
TABLE III
MEAN LEVELS OF PURINE DERIVATIVES INDEX (PDC INDEX), PURINE DERIVATIVES EXCRETION, PURINE DERIVATIVES ABSORPTION, MICROBIAL-N SUPPLY AND DOMI IN SPOT URINE SAMPLES COLLECTED FROM GRAZING KARAYAKA SHEEP

| Grazing period | Year | Months | n  | PDC index | PD excretion (mmol/d) | Purine absorption (mmol/d) | Microbial-N supply (g of N/kg of DOMR) | DOMI g/d |
|----------------|------|--------|----|-----------|-----------------------|---------------------------|----------------------------------------|---------|
|                |      |        |    |           |                       |                          |                                        |         |
|                | June | 9      |    | 32.29±1.51| 16.24±0.76            | 19.28±0.92                | 14.02±0.67                             | 20.87±0.73 |
|                | July | 9      |    | 31.90±1.02| 16.04±0.52            | 19.06±0.62                | 13.86±0.45                             | 20.86±0.78 |
|                | August | 10   |    | 24.36±0.74| 12.25±0.37            | 14.48±0.45                | 10.53±0.33                             | 21.06±0.70 |
| 2010           | September | 10  |    | 33.27±1.17| 16.74±0.59            | 19.89±0.70                | 14.46±0.51                             | 20.98±0.70 |
|                | October | 10   |    | 33.98±1.40| 17.09±0.70            | 20.31±0.85                | 14.77±0.62                             | 19.76±0.73 |
|                | November | 9    |    | 40.26±1.20| 20.25±0.60            | 24.10±0.72                | 17.52±0.52                             | 18.12±0.73 |
|                | December | 10   |    | 28.00±1.14| 14.08±0.57            | 16.69±0.69                | 12.14±0.50                             | 17.49±0.73 |
|                | January | 9     |    | 24.90±0.69| 12.53±0.35            | 14.81±0.43                | 10.77±0.31                             | 21.24±0.70 |
|                | February | 8    |    | 25.04±0.99| 12.60±0.50            | 14.89±0.61                | 10.83±0.44                             | 17.26±0.70 |
| 2011           | March | 10    |    | 24.71±0.92| 12.43±0.46            | 14.69±0.56                | 10.68±0.41                             | 17.29±0.70 |
|                | April  | 10    |    | 25.23±0.94| 12.69±0.47            | 15.01±0.57                | 10.91±0.42                             | 15.69±0.73 |
|                | May    | 9     |    | 27.14±0.96| 13.65±0.48            | 16.18±0.59                | 11.76±0.43                             | 19.34±0.70 |

Significance of main effects

- Linear
- Quadratic
- Cubic

**P<0.001, *P<0.05, NS: Non Significant, DOMR: Digestible organic matter fermented in the rumen calculated as 0.65DOMI (g of N/kg of DOMR), DOMI: Digestible organic matter intake g/d.**

IV. DISCUSSION

Measurement of microbial protein supply to sheep has been a major area of study in the context of their protein nutrition. An estimate of microbial protein contribution to the intestinal protein flow is incorporated into the new protein evaluation systems already being used in a number of countries. The supply of microbial protein to the animal per unit of feed ingested varied from 14 to 60 g microbial-N/kg digestible dry matter. The excretion of creatinine is affected minimally by the amount of protein and non-protein nitrogen consumed. In the current study, creatinine concentrations in the spot urine samples were higher in August, October and February than in other months (Table II). That phenomenon may reflect the impact of vegetation changes due to higher than average

catabolites are proportionally recovered in urine, mostly as allantoin, but also as hypoxanthine, xanthine and uric acid 18. The present study reports 69-84%, 9-27% and 4-8% for allantoin, uric acid and xanthine respectively. For the same catabolites, Chen and Gomes [11] reported the proportions to be 60-80%, 10-30% and 5-10% for allantoin, uric acid and xanthine respectively. Furthermore, allantoin excretion in urine was reported to be 80-85% of total purine derivatives. The profile of PD excretion in grazing Karayaka sheep was similar to that reported in previous studies [19]-[21]. The results obtained may indicate that the proportion of purine derivatives is independent of diet.

In the current study, the levels of allantoin in the urine of grazing sheep ranged from 4.84-8.62 mmol/L on a monthly basis across the sampling period. The study also determined a positive correlation (r=0.615, P<0.01) between the amount of allantoin excreted in urine and rumen microbial protein flowing into the small intestine, as described by the equation:

\[ Y = 0.378X + 1.594 \]

whereby is the amount of allantoin excreted in urine and x is rumen microbial protein flowing into the small intestine. This equation showed that allantoin excretion was able to enhance duodenal flow of microbial protein. Studies of cattle [22], [23] and sheep [7] have indicated a high correlation between the excretion of purine derivatives and rumen microbial protein flow into the small intestine (R² = 0.97). In the present study, the average allantoin amounts in spot urine samples for sheep were lowest in January, April and December, whereas the highest values were determined in June, July and October. In 2010, allantoin amounts in June, July and October were higher than in other months (Table II). That phenomenon may reflect the impact of vegetation changes due to higher than average
The higher excretion of PD in October and November clearly indicates enhanced microbial protein synthesis, since significant relationships have already been reported between urinary PD excretion and the levels of nucleic acid infused in the abomasums [4], [15] and duodenum [9], [24]. Orellana-Boero et al. [25] found that the excretion of purine derivatives in the urine increased linearly (r = 0.867) with digestible organic matter intake. The principle is that duodenal purine bases are efficiently absorbed in the small intestine [24], [26]. Urinary PD excretion is used to predict ruminal microbial protein synthesis. The daily excretion of purine derivatives and the microbial-N supply in grazing Karayaka sheep were found to be in the range of 12.25-20.25 and 10.53-17.52 mmol/d, respectively (Table III). These values for Karayaka sheep are within the range of those published for different sheep breeds [2], [10]. The urinary PD excretion values obtained in goats [3] and wethers are similar to those observed in sheep [10], [27], [28]. Hence, purine derivatives in spot urine samples may provide a practical indicator of microbial protein supply status in grazing ruminants.

The estimated average monthly DOMI values (Table III) in grazing Karayaka sheep were within the range of 776 to 1725 g/day. The estimation of DOMI from PDC index (Table III) through the use of spot urine sampling from grazing Karayaka sheep showed that this technique may be applied in grazing animals where DOMI cannot be measured directly. Many reports have confirmed a linear relationship between allantoin excretion and both the level of feed intake and flow of nucleic acids in the duodenum [29]. Laurent et al. [30] determined that allantoin excretion is correlated with digestible organic matter intake, and also that allantoin excretion (r=0.54, P<0.01) can be used as an index of rumen microbial protein synthesis, as also reported by Jetana et al. [31] and Laurent et al. [30].

The present study also determined that digestible organic matter fermented in the rumen was converted to a similar proportion of microbial-N in all months, ranging from 16 to 21 g N/kg of digestible organic matter apparently digested in the rumen (Figs. 1, 2; Table III), which was similar the range reported by Yu et al. [32]. The higher amount of urinary allantoin reported in the present study in June, July, September, October and November was due to the increased digestible organic matter intake. The present study also determined that there were linear and cubic relationship between the PDC index, urinary excretion of purine derivatives, microbial-N and DOMI and month (Table III), and that there was a seasonal influence on these parameters.

In the current study, the estimated microbial-N values appear insufficient for adequate protein nutrition. Monthly mean values of PDC index, microbial-N and DOMI were relatively stable. However, fluctuations were observed between June and December (Figs. 1, 2). The lowest values for DOMI, PDC index and microbial-N were observed in August and December (Figs. 1, 2). This is due to meadow and pasture conditions reflecting the driest period of the year (Fig. 1). In conclusion, protein nutrition of pastured sheep may be affected by weather changes. Spot urine sampling may serve as the basis for modeling their feed requirements. Furthermore, it may provide a basis for the preparation of balanced diets to meet the protein requirements of sheep by closely approximating the amount of rumen microbial-N flowing into the small intestine. However, further studies are required under different field conditions and with different breeds of sheep to develop the spot urine sampling technique into a model.

ACKNOWLEDGMENT

This study was supported by OndokuzMaysis University Research Fund (Project number: PYO.VET.1901.10.03, 2010). Correspondence: +903623121919/2591, msalman@omu.edu.tr

REFERENCES

[1] Turkish Statistical Institute (TurkStat): Livestock Statistics, http://www.turkstat.gov.tr, 2008.
[2] Chen XB, Mejia AT, Kyle DJ, Orskov ER: 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. J AgricSci, 125:137-143, 1995.
[3] Cetinkaya N, Salman M, Genè B: Estimation of the microbial N flow to small intestine in Saanen goats and kids based on urinary excretion of purin derivatives by the use of spot urine sampling technique. KalkasUniv Vet Fak Derg,16(1):75-79, 2010.
[4] Fujihara T, Orskov ER, Reeds PI, Kyle DJ: The effect of protein infusion on urinary excretion of purine derivatives in ruminants nourished by intragastic infusion. J AgricSciCamb, 109:7-12, 1987.
[5] JAEA: A training package the technique for estimating microbial protein supply in ruminants based on determination on purine derivatives in urine. Joint FAO/IAEA Division of Nuclear Applications in Food and Agriculture. Vienna, Austria, 2003.
[6] Wanapat M, Pimpaa, O: Effect of ruminal NH3-N levels on ruminal fermentation, purine derivatives, digestibility and rice straw intake in swamp buffaloes. J AnimSci, 12: 904, 1999.
[7] Puchala R, Kulasek GW: Estimation of microbial protein flow from the rumen of sheep using microbial protein flow from the rumen of sheep using microbial nucleic acid and urinary excretion of purine derivatives. Can J AnimSci, 72, 821-830, 1992.
[8] Makkar HPS, Chen XB: Estimation of microbial protein supply in ruminants using urinary purine derivatives, Springer 1 ed. VII, p.212, 2004.
[9] Antoniewiez AN, Hienemann WW, Hanks EM: The effect of changes in the flow of nucleic acids on allantoin excretion in the urine of sheep. J AgricSci, 95: 395-400, 1980.
[10] Chen XB, Chen YK, Franklin MF, Orskov ER, Shand, WJ: The effect of feed intake and body weight on purine derivative excretion and microbial protein supply. J AnimSci, 70:1534–1542, 1992.
[11] Chen XB, Gomes IM: Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives and overview of the technical detail. International feed resources unit. Rowett Research Institute, Bucksburn Aberdeen, UK. Occasional publication, pp.1-21, 1992.
[12] Karabulut A, Canbolat O: Feed evaluation and analyze methods. Uludag University Publ., no: 2,05.048,0424, Bursa, 2005.
[13] AOAC: Official Methods of Analysis, 15th ed. Association of Official Agricultural Chemists, Washington, DC, 1995.
[14] Van Soest PJ, Robertson JB, Lewis, BA: Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci, 74:3583, 1991.
[15] Chen XB, Hovell FD, De B, Orskov ER, Brown DA: Excretion of purine derivatives by ruminants: effect of exogenous nucleic acid supply on purine derivative excretion by sheep. Br J Nutr, 63:131-142,1990.
[16] Agricultural Research Council (ARC): The nutrient requirements of ruminant livestock. Suppl. No.1, Commonwealth Agricultural Bureaux, Slough, 1984.
[17] SAS Statistical Software: SAS Compus drive, Cary, NC 27513 USA, 2009.
McAllan AB, Smith RH: Degradation of nucleic acids in the rumen. Br J Nutr, 29:331-345, 1973.

Brun-Bellut J, Linberg JE, Hadjipanayiotou M: Protein Nutrition and Requirements of Adult Dairy Goats. In, Morand-Fehr P (Ed): Goat Nutrition. EAAP Publ. No.46, PudocWageningen Netherlands, pp. 82-93, 1991.

Linberg JE: Urinary allantoin excretion and digestible organic matter intake in dairy goats. Swed J Agric Res, 15, 3137, 1985.

Ma T, Deng K, Jiang C, Tu Y, Zhang N, Liu J, Zhao Y, Diaoa, Q: The relationship between microbial N synthesis and urinary excretion of purine derivatives in Dorper×thin-tailed Han crossbred sheep, http://dx.doi.org/10.1016/j.smallrumres.2012.09.003, 2012.

Cetinkaya N, Yaman S, Baber NHO: The use of purine derivatives/creatinine ratio in spot urine samples as an index of microbial protein supply in Yerli Kara crossbred cattle. LivestSci, 100:91-98, 2006.

Gonzalez-Ronquillo M, Balcells J, Belenguer A, Castrillo C, Mota M: A comparison of purine derivatives excretion with conventional methods as indices of microbial yield in dairy cows. American Dairy Science Association. J Dairy Sci, 87:2211-2221, 2004.

Balcells J, Guada JA, Peiro JM: Simultaneous determination of allantoin and oxytpurines in biological fluids by high performance liquid chromatography. J Chromatogr, 575:153-157, 1992.

Orellana-Boeroa P, Seradjb AR, Fondevillac MJ, Noland J, Balcells J: Modelling urinary purine derivatives excretion as a tool to estimate microbial rumen outflow in alpacas (Vicugnapacos), Small Rumin. Res., 107, 101-104, 2012.

Belenguer A, Yanez D, Balcells J, Oxdemir BNH, Gonzalez-Ronquillo M: Urinary excretion of purine derivatives and prediction of rumen microbial outflow in goats. Livest Prod Sci, 77:127-135, 2002.

Carro MD, Cantalapiedra-Hijar G, Ranilla MJ, Molina-Alcaide E: Urinary excretion of purine derivatives, microbial protein synthesis, N utilization, and ruminal fermentation in sheep and goats fed diets of different quality. J AnimSci, 2011-4577, 2012.

Hindrichsen IK, Osuji PO, Odeny AA, Madsen J, Hvelplund T: Effects of supplementation of a basal diet of maizestover with different amounts of Leucaenadiversifolia on intake, digestibility, nitrogen metabolism and rumen parameters in sheep. Anim Feed SciTechnol, 98: 131-142, 2002.

Kreutzer M, Kirchgessner M, Kellner RJ, Roth FX: Effect of varying protein and energy concentration on digestibility of nutrients, nitrogen metabolism and allantoin excretion in wethers. German J AnimPhysiolAnimNatr 55:144-159, 1986.

Laurent F, Vignon B: Factors of variations in urinary excretion of allantoin in sheep and goats. AnimSci, 64:281-282, 1983.

Jetana T, Suthikrai W, Usawang S, Kijsamrej S, Sophon S: The use urinary purines excreted in the urine for prediction microbial protein production form rumen: Using spot sampling for the prediction microbial protein from the rumen of Brahman Cattle. The 4th Chulalongkorn University Veterinary Annual Conference 60th Veterinary Anniversary Building, Chulalongkorn University, 2005.

Yu P, Boon-ek L, Leury BJ: Effect of dietary protein variation in term of net truly digest intestinal protein (DVE) and rumen degraded protein balance (OEB) on the concentration and excretion of urinary creatinine, purine derivatives and microbial N supply in sheep: comparison with the prediction from the DVE/OEB model. Anim Feed Sci Tech, 93:71-91,2001.