Journal of Animal and Veterinary Advances 6 (9): 1140-1145, 2007
ISSN: 1680-5593

Estimation of Microbial Protein Synthesis and Urinary Excretion of Purine Derivatives in Sheep Offered Alfalfa

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Abstract: The aim of the present study was to estimate the Microbial N yield (MN), Efficiency of Microbial Protein Supply (EMNS), amounts of Purine absorbed (P_a) and Purine Derivatives excretion (PD_e) in gezel sheep fed Hamedani (HAM) and Kareyonge (KAR) hays. Digestible Organic Matter fermented in the Rumen (DOMR) of KAR (0.473 kg d^{-1}) was lower than that of HAM hay (0.713 kg d^{-1}). The MN was higher for HAM (22.8 g d^{-1}) hay, than that of KAR hay (15.1 g d^{-1}); but EMNS was similar (32 g N kg^{-1} DOMR). The P_a and PD_e contents of HAM hay were higher than that of KAR hay (31.6 vs. 20.8 and 28.5 vs. 19.5 mmol d^{-1}, respectively). In conclusion,it seems that, HAM hay can have a higher inclusion than of KAR hay in diets for sheep because of greater MN and PD_e.

Key words: Microbial protein, purine derivative, alfalfa, sheep, urinary excretion, estimation

INTRODUCTION

It is well known that in ruminants, endogenous urea is partly recycled in the forestomech. This process is nutritionally advantageous for ruminants because ruminal bacteria are able to use urea nitrogen to synthesize proteins that will be absorbed in the small intestine (Cirio and Bovin, 1990). Microbial protein synthesis is important in ruminants because microbial protein synthesized in the rumen provides from 50% to nearly all amino acids required for beef cattle depending on the undegraded crude protein concentration of the diet. Synthesis of microbial protein and growth of ruminal microbes depends on Adequate energy (ATP), resulting from fermentation of organic matter in the rumen and N resulting from degradation of non-protein and protein nitrogen sources. Other nutrients such as, sulfur, phosphorus and other minerals and vitamins are also required for microbial protein synthesis. It is estimated that between 40-80% of the total flow of the protein reaching to intestine of from microbial protein (Sniffen and Robinson, 1987; Clark *et al*., 1992; McDonald *et al*., 1995; NRC, 1996). In Fig. 1 and 2 show the various nutrients required for microbial growth and transformation of dietary nitrogen into microbial protein in the rumen.

A widely used method for the estimation of microbial protein production requires ruminal and duodenal cannulas and microbial and digesta flow markers. However, the microbial protein entering the duodenum can be estimated by quantification of urinary allantoin. The nucleic acids synthesized by rumen micro-organisms are enzymatically degraded to purine and pyrimidine bases which are absorbed; their final products are excreted in the urine with allantoin being in the greatest proportion (Condon and Hatfield, 1970; Faichney, 1975; Macrae, 1975; Puchala and Kulasek, 1992). Topps and Elliott (1965) were among the earliest investigators to suggest that urinary allantoin and uric acid excretion rates reflect the amount of microbial protein flowing into the small intestine. Later, several authors have confirmed that urinary Purine Derivatives (PD) can be an accurate index of rumen microbial protein flowing into the small intestine (McAllen and Smith, 1973; McAllen, 1980; Razzaque *et al*., 1981). Giesecke *et al*. (1984) suggested the use of purine derivatives as an effective measure for rumen microbial growth, when they found a significant relationship between the amounts of purine metabolites excreted in urine in sheep, maintained by intragastric infusions. Chen *et al*. (1990) have shown that purine derivatives (allantoin, xanthine, hypoxanthine and uric acid) could be used to estimate the supply of microbial protein from the rumen to the intestine.

The aim of this study was to estimate microbial protein yield and some purine derivatives of Gezel sheep fed two alfalfa varieties.

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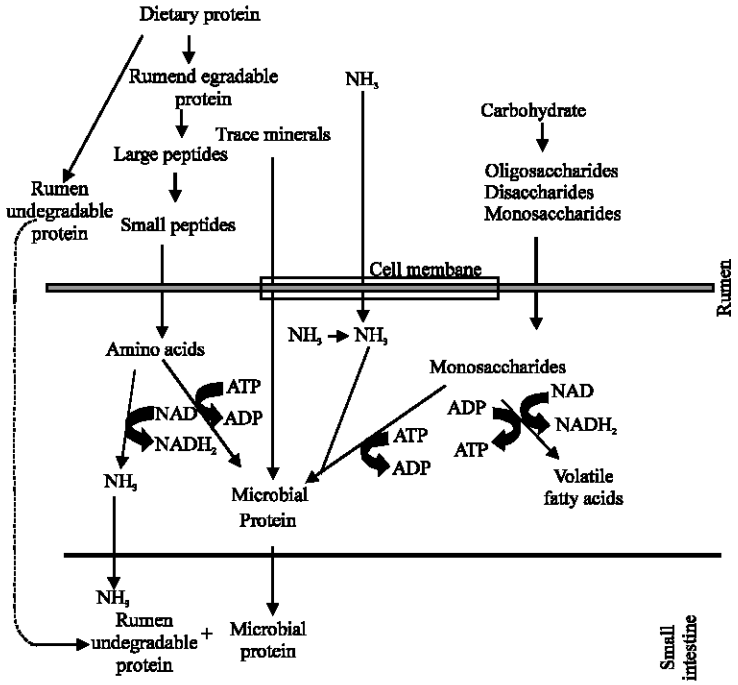

Fig. 1: Nitrogen metabolism and microbial protein synthesis in ruminant animals. Adapted from Nocek and Russell (1988)

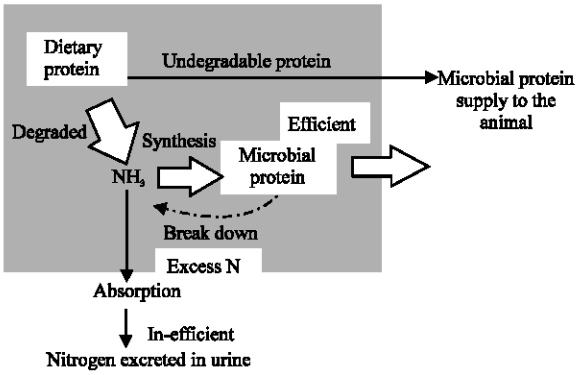

Fig. 2: Inter-conversion of dietary nitrogen in to microbial protein. Adapted from Bahadur-Subba (1997)

MATERIALS AND METHODS

Forage: Two alfalfa varieties (Hamedani and Kareyonge) used in this study were randomly sampled from ten alfalfa farms at near West Azerbaijan, Iran (located in the Urmia and Miandoab cities) in summer 2005. The samples were transported to the laboratories of Islamic Azad University-Shabestar Branch.

Both alfalfa, at harvested, were estimated to be at late maturity (mid to late bloom). Samples were collected, oven-dried at 60°C for 48 h, ground (5 mm screen) and prepared for chemical analysis.

Chemical analysis: Dry Matter (DM) was determined by drying the samples at 105°C overnight and ash by igniting the samples in muffle furnace at 525°C for 8 h and Nitrogen (N) content was measured by the Kjeldahl method (AOAC, 1990). Crude Protein (CP) was calculated as N×6.25. Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL) were determined by procedures outlined by Georing and Van Soest (1970) with modifications described by Van Soest *et al.* (1991), sulfite was omitted from NDF analysis.

Data collection procedures: The calculations are illustrated as follows:

Calculation of Digestible Organic Matter fermented in the Rumen (DOMR):

$$DOMR\ (kg\ d^{-1}) = Feed\ intake \times DM\ content \times OM\ content \times OM\ digestibility \times 0.65 \quad (1)$$

(Chen and Gomes, 1995)

Feed intake and OM digestibility adapted from Maheri-sis *et al.*, (2007).

Calculation of Microbial N (MN) yield:

$$MN\ (g\ d^{-1}) = 32\ g\ kg^{-1} \times DOMR \quad (2)$$

(Chen and Gomes, 1995)

Calculation of Efficiency of Microbial Protein (nitrogen) Supply (EMNS): EMNS in rumen was expressed as grams of microbial N per kilogram of digestible organic matter apparently digested in the rumen (Khampa and Wanapat, 2006).

$$EMNS = MN\ (g\ d^{-1}) / DOMR\ (g) \times 1000\ (g) \quad (3)$$

Calculation of the equivalent amounts of Purine absorbed (P_a) by the animal:

$$P_a\ (mmol\ d^{-1}) = MN\ (g\ N\ d^{-1}) / 0.727$$
(Chen and Gomes, 1995) (4)

Calculation of total Purine Derivatives excretion (PD_e):

$$Pd_e\ (mmol\ d^{-1}) = 0.84P_a + 2\ (assume$$
the endogenous contribution = 2 mmol d^{-1})
(Chen and Gomes, 1995) (5)

Calculation of Allantoin excretion (A_e):

$$A_e\ (mmol\ d^{-1}) = PD_e \times 0.85\ (i.e.\ 85\%\ of$$
the PD_e is allantoin) (Chen and Gomes, 1995) (6)

Calculation of Uric Acid excretion (UA_e):

$$Ua_e\ (mmol\ d^{-1}) = PD_e \times 0.15\ (i.e.\ 15\%\ of\ the$$
PD_e is uric acid) (Chen and Gomes, 1995) (7)

Equations: Digestible Energy (DE) and Digestible Neutral-Detergent Fiber (DNDF) values were determined using of equations:

$$DE\ (MJ\ kg^{-1}\ DM) = 0.019 \times DOMD\ (Mirzaei-$$
Aghsaghali, 2006) (8)

DOMD (g kg^{-1} DM) = Digestible Organic Matter in Dry matter (Maheri-sis *et al*., 2007).

$$NFC\ (\%) = 100-(\%NDF + \%CP + \%Fat + \%Ash)$$
(NRC, 2001) (9)

$$INDF\ (\%) = (1000 \times (ADL/NDF))^{0.76}$$
(Carpino *et al*., 2003) (10)

$$DNDF\ (\%) = 100 - INDF\ (Carpino\ et\ al., 2003)\ (11)$$

NFC = Non-Fibrous Carbohydrate.
NDF = Neutral-Detergent Fiber.
ADL (%) = Acid-Detergent Lignin.
INDF = Indigestible Neutral-Detergent Fiber.
DNDF = Digestible Neutral-Detergent Fiber.

RESULTS AND DISCUSSION

There was variation between forages in terms of chemical composition and some estimated parameters of HAM and KAR hays (Table 1). The DNDF and INDF contents ranged from 55.2-55.9 and 44.1-44.8%, respectively. The DNDF and INDF contents of both hays were similar (Table 1; $p>0.05$). These values are higher than those found in alfalfa by Tejido *et al*. (2002) and similar with that reported by Teimouri *et al*. (2004). The differences between reports may be due to method type, maturity, variety, environmental conditions, agronomic factors and leaves to stem ratio (Maheri-sis *et al*., 2007). Accurately and precisely predicting the DNDF content of the forage NDF is extremely important in generating a quantitative summative forage energy prediction. The NRC (2001) uses lignin based calculation to predict potential NDF digestibility because lignification within a plant species can be negatively associated with NDF digestibility. There are two primary reasons why forages are evaluated for NDF digestibility. First, DNDF is used in summative equations to estimate energy content of forages (NRC, 2001). Second, a 1 unite rise in DNDF content in the diet results in a 0.37 Ibs d^{-1} rise in dry matter intake (Oba and Allen, 1997). The DE concentration of HAM hay was significantly ($p<0.01$) higher than that obtained at KAR hay whereas NFC content was similar ($p>0.05$) in both forages. The DE content of HAM hay was in line with those reported by NRC (2001). The HAM hay Non-Fibrous Carbohydrate (NFC) content (29.4%) was similar with NFC content of KAR hay (26.8%). These values are in agreement with those found in alfalfa hay by NRC (2001).

Table 1: Chemical composition and some estimated parameters of HAM and KAR hays

Item	HAM	KAR	SE	Sig.
DM (%)	92.93	93.46	0.133	NS
CP (%)	15.8	12.5	0.819	NS
CF (%)	29.2	34	0.519	**
EE (%)	1.33	1.33	0.334	NS
Ash (%)	10.33	10.33	0.493	NS
NDF (%)	43.1	49	0.259	***
DNDF (%)	55.9	55.2	1.154	NS
INDF (%)	44.1	44.8	1.010	NS
ADF (%)	29.4	34.4	0.288	**
ADL (%)	6.3	7.3	0.231	NS
NFC (%)	29.4	26.8	1.195	NS
DE (Mcal kg^{-1} DM)	2.77	2.36	0.047	**

HAM = Hamedani hay; KAR= Kareyonge hay; DM = Dry Matter; OM = Organic Matter; CP = Crude Protein; CF = Crude Fiber EE = Extract Ether; NDF = Neutral-Detergent Fiber; INDF = Indigestible Neutral-Detergent Fiber; DNDF = Digestible Neutral-Detergent Fiber; ADL = Acid-Detergent Lignin; NFC = Non-Fibrous Carbohydrate; GE: Gross Energy; DE = Digestible Energy. SE = Standard Error.Sig. = Significant level; NS = Non-Significant; $^{**}p<0.01$; $^{***}p<0.001$

Table 2: Estimated digestible organic matter fermented in the rumen, microbial N yield, efficiency microbial N supply and urinary excretion of purine derivatives in sheep feed at ad libitum

	HAM	KAR	SE
*DOMD (g kg^{-1} DM)	613.2	520	ND
DOMR (kg d^{-1})	0.713	0.473	0.07
MN (g d^{-1})	22.8	15.1	2.24
EMNS (g N kg^{-1} DOMR)	32	32	0.034
P_a (mmol d^{-1})	31.6	20.8	3.14
PD_e (mmol d^{-1})	28.5	19.5	2.64
A_e (mmol d^{-1})	24.2	16.5	2.24
UA_e (mmol d^{-1})	4.27	2.93	0.395

*Adapted from Maheri-sis *et al.* (2007). ND = Non-Detected; DOMD = Digestible Organic Matter in Dry matter; DOMR = Digestible Organic Matter Fermented in the Rumen; MN = Microbial N yield; EMNS = Efficiency of Microbial Protein Supply; P_a = Purine absorbed; PD_e = Purine Derivatives excretion; A_e = Allantoin excretion; UA_e = Uric Acid excretion

The DOMD of HAM hay was significantly ($p < 0.01$) higher than that KAR hay (Table 2) (Maheri-sis *et al.*, 2007). DOMR was 0.713 kg d^{-1} for HAM and 0.473 kg d^{-1} for KAR hay. The DOMR concentration for HAM hay in the present experiment was in agreement with DOMR values of alfalfa hay reported by Gosselink (2004). The ruminal OM digestion of Lucerne was limited as a result of the high rumen outflow rates. Probably two mechanisms were also used to deliver energy for the yield of microbial N. At first N was not only used as protein source but also at energy source. Secondly the high outflow rates were favorable for the escape of microbes from the rumen low ruminal retention time of microbes decreases the intra-ruminal recycling of microbes by reducing bacterial breakdown and protozoal engulfment (Leng and Nolan, 1984).

The Microbial Nitrogen (MN) supplies as calculated from Digestible Organic Matter Fermented in the Rumen (DOMR) were from 15.1-22.8 g N d^{-1}. The value of MN in HAM hay (22.8) was close to the value (18.2) obtained from the Gosselink (2004) study. Moreover, EMNS values were 32 g N kg^{-1} DOMR for both forages and lower than that reported by Gosselink (2004). This difference may be due to the maturity (late maturity in our experiment vs. first cut and beginning of flowering in the experiment of Gosselink, 2004) and technique used in experiment.

In study, similar trends in the efficiency of microbial N synthesis in the rumen with Lucerne with different presentation forms are found (Gosselink, 2004). High efficiency (> 45 g microbial N kg^{-1} OM apparently digested in the rumen) were found with Lucerne hay in steers (Elizalde *et al.*, 1999) and with fresh Lucerne in lambs in combination with high outflow rates of Ru and Cr, respectively 12.0 and 17.7 % h^{-1} (Cruickshank *et al.*, 1992).

Numerous studies have been conducted to determine microbial protein synthesis in the rumen under various conditions (Salter *et al.*, 1979; Clark *et al.*, 1992; Beever and Cottrill, 1994). The efficiency of microbial protein synthesis varies significantly among studies. Some of these variations were attributed to the techniques used in these experiments. But there are other factors that caused differences in microbial protein synthesis among the studies. These factors include nitrogen concentrations, nitrogen sources, rates of nitrogen and carbohydrate degradation, carbohydrate sources, the ratio of forage to concentrate in the diets, dry matter intake and synchronization of nitrogen and simultaneous release of energy. Other factors such as rates of solid and liquid passage and dietary sulfur concentrations must also be considered (Rode *et al.*, 1985; Hoover and Stokes, 1991).

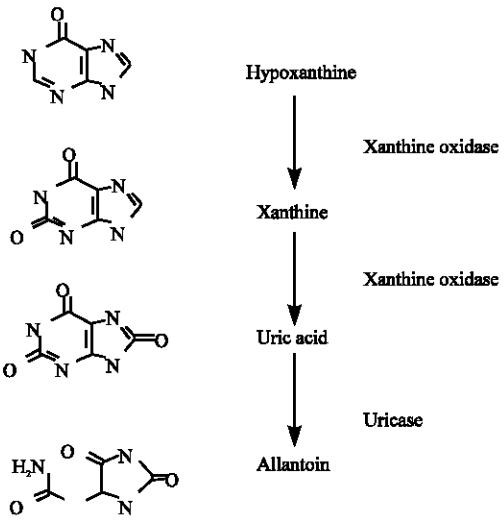

Fig. 3: Chemical structures and the enzymes involved in the conversion of the derivatives. Adapted from Bahadur-Subba (1997)

The individual values for daily absorption and excretion of purine derivatives (A_e and UA_e) and Chemical structures and the enzymes involved in the conversion of the derivatives are shown in Table 2 and Fig. 3. Purine absorbed (P_a) ranged from 20.8-31.6 (S.E. 3.14). The PD_e ranged from 19.5-28.5 mmol d^{-1}. The PD_e concentrations in the present experiment were in a greet agreement with PD_e values of alfalfa hay reported by Belenguer *et al.*, (2002). This difference may be due to the feeding level and animal species (ad libitum vs. maintenance level and sheep vs. goat in our experiment and in the experiment of Belenguer; respectively). A close relationship was found between urinary excretion of purine derivatives and duodenal supply of purines, as previously in other species as sheep (Chen *et al.*, 1990; Balcells *et al.*, 1993) or cattle (Colucci *et al.*, 1984). The A_e and UA_e contens of Ham hay were higher than that KAr hay (Table 2).

Allantoin and other purine derivatives are degraded by rumen bacteria (McAllen and Smith, 1973) and N could be used for microbial synthesis (Belasco, 1954).

CONCLUSION

In overall conclusion, results of this study showed that DE of Hamedani hay was higher than that of Kareyonge hay and the DNDF was similar with it. The DOMR, MN, EMNS and purine derivatives in HAM were higher than that KAR hay. Estimations based on equations indicated that Hamedani hay can have a higher inclusion than Kareyonge hay in diets for ruminants because of greater DE, MN and PD_e contents.

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