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The Effect of Different Level of Concentrates in Diet on Microbial Protein Synthesis in Iranian Native Buffaloes

Research Article

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ABSTRACT

This research was conducted to estimate the amount of microbial protein synthesis in Iranian buffaloes' rumen. Four male swamp buffaloes with average live body weight of 140±10 kg were used in this study. Four diets of 0 (control diet, 100% forage), 15, 30 and 45% concentrate in a 4 × 4 Latin Square were tested. The results indicated that by increasing the concentrate level in the diet of swamp buffaloes. the amount of purine derivatives (PD) excreted in urine. The amount of allantoin excreted in urine increased by increasing concentrate level form 0 to 45% (13.2 and 21.8 mmol/day; $P<0.01$). There was no significant difference between uric acid excretion from buffaloes fed with diets contained 15 and 30% or 30 and 45% concentrate ($P>0.01$). However, there was a significant difference between diets containing 45 and 15% concentrate level ($P<0.01$). The difference between control diet and other diets containing different levels of concentrates were significant ($P<0.01$). The total purine derivatives excretion and microbial nitrogen synthesis in rumen increased from 14.5 in control diet to 18.11, 21.58 and 24.55 mmol/day and from 38.76 in control diet to 60.44, 81.48 and 99.43 g/day in buffalos fed diets containing 15, 30 and 45% concentrate, respectively. The results of this experiment indicated that increasing the concentrate level in diet of swamp buffaloes increases the amount of microbial protein synthesized in rumen.

KEY WORDS buffalo, microbial protein, purine derivatives, rumen.

INTRODUCTION

To digest the feed, ruminants depend on the microbial activity in their rumen. The microbes can degrade the feed components with their special enzymes. In addition to degradation of the feed, when passing through rumen and reticulum to get to the lower parts of the alimentary tract like abomasum and intestines, the micro-organisms will be degraded and turn into proteins which will be used by the host animal. Therefore, there are two sources of metabolizable protein available to the ruminants: the true protein of the diet that escapes degradation in the rumen, and the microbial protein produced in the rumen. The latter can provide the animal with 42 to 93% of the protein requirements (Djouvinov *et al.* 1994). Thus rumen micro-organisms constitute the major source of protein supply to the ruminant (Khorshidi *et al.* 2007; Alipoor *et al.* 2009 and Ashabi *et al.* 2009). Rumen micro-organisms are rich in nucleic acid: around 18% of total nitrogen is present as nucleic acids or 11% as purines. The purines are metabolized and excreted in the urine as their end products: hypoxanthine, xanthine, uric acid and allantoin. In buffalo and cattle, because of high xanthine oxidase activity in intestine and blood, hypoxanthine and xanthine are converted to uric acid. Therefore, uric acid and allantoin are solely purine derivatives excreted in urine (Chen and Ørskov, 2004). There are various methods to estimate microbial protein synthesis in the rumen and most of them are based on estimation of microbial indices. The ordinary feeds of ruminants contain minor amounts of nucleic acids. Microorganisms in the rumen degrade the feed nucleic acids almost completely (McAllen *et al.* 1973). Therefore, it can be assumed that the nucleic acids entering the small intestine, have microbial origins. In the intestine, purine nucleic acids turn into purine nucleosides and free radicals both of which can be absorbed by the intestine. These products can be either used to produce nucleic acids or turn into hypoxanthine, xanthine, uric acid and allantoin. These purine derivatives (PD) are often excreted in urine.

Therefore, if we know the proportion of purine to microbial nitrogen, as well as digestibility of purines, we can estimate the microbial nitrogen absorbed through the intestines based on observing the amount of absorbed purine in the excreted urine. Chen (1989) proposed another method based on the mentioned procedure. This method provides an index to estimate the microbial protein available to the animal. One of the advantages of this method is its simplicity; all the urine needs to be collected and analyzed to estimate the purine derivatives. Furthermore, no surgery is needed in this method. Chen (1989) proposed equations to estimate the amount of absorbed microbial purines in the intestine based on the purine constituents excreted in the urine. Also, Chen *et al.* (1990); Verbic *et al.* (1990) and Liang *et al.* (2004) suggested a formula to estimate the amount of synthesized microbial nitrogen (g/day) based on the amount of the absorbed purines. There have been vast researches by the scientists on different animal species in this regard including: sheep (Chen *et al.* 1990; Balcells *et al.* 1993 and Mupangwa *et al.* 2000), cattle (Verbic *et al.* 1990; Orellana Boero *et al.* 2001; Soejono *et al.* 2004 and Vo *et al.* 2006), goats (Lindberg, 1985 and Belenguer *et al.* 2002), buffaloes (Chen *et al.* 1996; Liang *et al.* 2004; Soejono *et al.* 2004 and Vo *et al.* 2006), camel (Mura *et al.* 1986; Guerouali *et al.* 2004 and Vo *et al.* 2006) and rabbit (Balcells *et al.* 1998). Nonetheless, the data does not seem to be adequate yet. This research was carried out to use excretion of PD namely allantoin and uric acid as a parameter to estimate the microbial protein synthesis in the rumen of native swamp buffaloes in north of Iran.

MATERIALS AND METHODS

Animals, experimental periods and diets

This study was conducted in 2005-2006 at Animal Research Center of Islamic Azad University, Ghaemshahr Branch in Iran. Four Iranian male swamp buffaloes with the average live body weight of 140±10 kg were used. The experiment duration was 84 days including four periods and each period lasted 21 days (11 days for adaptation and 10 days for urine collection). Animals were housed in four metabolic cages and fitted with urine collection instrument. Four diets consisting of 0 (all forage diet), 15, 30 and 45 percent concentrate (on % dry matter basis) in a Latin Square design, in four periods were experimented. The concentration included: Corn grain (10%), barley (27%), wheat bran (32%), soybean meal (18%), beet pulp (4%), beet molasses (4%), calcium carbonate (1%), mineral and vitamin premix (0.5%), salt (1%), and zeolite (2.5%). However, the concentration included 18% CP and 2.71 ME (Mcal/kg DM). Diets were offered twice daily at 8:00 AM and 4:00 PM, in two equal meals. Water was available *ad libitum*. Housing and management conditions were the same for all animals.

Urine collection, dilution, and analysis

Urine was collected in a container each day during sampling period. To prevent microbial degradation of purines, urine was acidified by 10% H_2SO_4 to a pH of 2-3. Urine was diluted by distilled water to prevent the precipitation of purine derivatives during the storage period.

A sub sample of 40 ml was taken and stored at -20 ^{o}C for further analysis. In order to measure the uric acid, the urine samples were diluted to become readable for the spectrophotometer.

Then, using a uric acid kit, the amounts of uric acid in the samples were determined. In addition, the method of Chen *et al.* (1992) was implemented in order to measure allantoin. Afterwards, the obtained amounts of uric acid and allantoin in the samples (mg/dL) were converted into mg/day and then into millimol/day (mmol/day). The conversions were done so that the units could be used in equations. Then the amounts of uric acid and allantoin in each sample were added to each other to obtain total purine derivatives (mmol/day). Then applying the total of excreted purine derivatives in the urine (Y, mmol/day) in the Liang *et al.* (2004) equation, the amount of absorbed external purine for each buffalo (X, mmol/day) was obtained.

$$Y= 0.12X + 0.20\ W^{0.75}$$

Then applying X in the Chen *et al.* (1990) formula, the amount of synthesized microbial nitrogen as g/day was measured. Consequently, multiplying the results by 6.25, the amount of microbial protein was estimated.

Statistical analysis

The model used to analyze the data was:

$$Y_{ij(k)}= M+ A_i+ P_j+ T_k+ E_{ijk}$$

Where:

$Y_{ij(k)}$: related sample to the ith animal in the jth period under the effect of kth diet.

M: treatment average
A_i: effect of ith animal
P_j: effect of jth period
T_k: effect of kth treatment
E_{ijk}: experimental error

Data were statistically analyzed using the General Linear Model procedure of SAS (1996) with the Duncan's multiple range test.

RESULTS AND DISCUSSION

Excretion of purine derivatives

The results of this research revealed that the excreted purine derivatives in the urine (mmol/day) consisted of 89 percent allantoin and 11 percent uric acid, on average (Table 1).

Table 1 Excretion model of purine derivatives in Iranian buffaloes

Ration	Allantoin (% of total PD)	Uric acid (% of total PD)
1*	90.8	9.2
2	87.4	12.6
3	88.6	11.4
4	88.7	11.3
Average	88.8	11.2

* 1: Control, Groups; 2, 3, 4: Diets contained 15%, 30% and 45% concentrate respectively.
PD: Purine derivatives.

Microbial nitrogen produced, absorbed purines and excreted purine derivatives

The amount of allantoin, uric acid, total of excreted purine derivatives and produced nitrogen (Table 2), were increased significantly (P<0.01) by the increase of the level of diet concentrate 0 to 45% (P<0.01).

The researchers observed the increase of 13.2 to 21.7 (mmol/day) for the allantoin, 14.5 to 24.5 (mmol/day) for the total of purine derivatives, 53.3 to 136.7 (mmol/day) for the absorbed microbial purines, 38.7 to 99.4 (gr/day) for the microbial nitrogen, and 242.2 to 621.4 (gr/day) for the microbial protein.

The amount of uric acid also increased 1.32 to 2.75 (mmol/day) with the increase of the level of concentrate (0% to 45%). However, this increase between the levels of 30% to 45% of the concentrate, also between the levels of 15% and 30% concentrate were not statistically significant (P<0.01), but the differences between the levels of 15% and 45 % of concentrate, and between the level of 30 and an all forage diet were statistically significant (P<0.01). Moreover, the differences among an all forage and the three diets of 15%, 30% and 45% concentrate were also significant (P<0.01).

Daily dry matter intake in relation with excreted purine derivatives

The amount of daily dry matter intake (DMI, kg day^{-1}) increased significantly (P<0.01) by the increase of the level of the dietary concentrate. Moreover, there was a high and significant correlation (r^2=0.99) between DMI with excreted purine derivatives (Figure 1) and produced microbial nitrogen protein in the rumen (Figure 2). The results were also analyzed by regression where X= daily Dry Matter Intake (DMI, kg day^{-1}) and Y=. Total purine derivatives excretion (mmol/day) or microbial protein synthesized in rumen (g/day).

Purine derivatives

In buffalo and cattle, the xanthine oxidase activity in intestinal mucosa and liver is too high to permit any uptake of salvageable PD, xanthine and hypoxanthine (Guerouali *et al.* 2004).

Therefore, hypoxanthine and xanthine are converted to uric acid leaving only uric acid and allantoin excreted in urine way (Chen and Ørskov, 2004). However, the amount of excreted allantoin as part of total excreted purine derivatives, 89% of the total purine derivatives revealed higher than those reported by Chen *et al.* (1992), 80 to 85% for cow and 60 to 80% for sheep. Also, the amount of excreted uric acid was a little lower than that was reported by them, 15 to 20% for cow and 5 to 10% for sheep.

Table 2 Estimated microbial nitrogen synthesis in rumen of Iranian buffaloes

Purine derivatives (PD)	Diets concentrate*			
	45%	30%	15%	0%
Allantoin (mmol/day)	21.79±0.73a	19.15±1.44^{b}	15.84±1.26^{c}	13.21±0.31^{d}
Uric acid (mmol/day)	2.75±0.30a	2.44±0.12a^{b}	2.28±0.22b^{c}	1.326±0.16^{d}
Total PD (mmol/day)	24.55±0.96a	21.59±1.50^{b}	18.11±1.44^{c}	14.54±0.36^{d}
Microbial nitrogen synthesis (g/day)	99.43±5.02a	81.48±7.36^{b}	60.44±7.99^{c}	38.76±2.49^{d}

Values in table are mean±standardeviation (SD).
* The means in the same row that have at least one common letter, do not have significant difference (P>0.05).

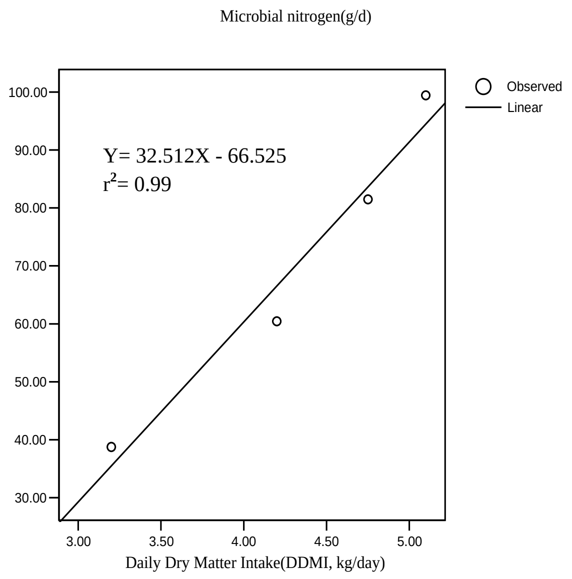

Figure 1 Microbial nitrogen

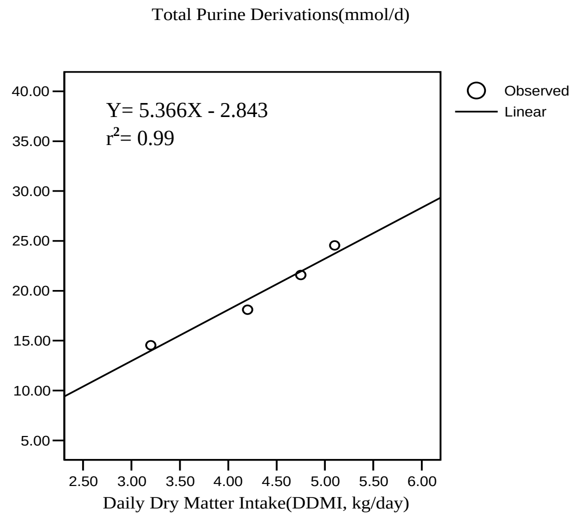

Figure 2 Total purine derivation

The proportional difference between allantoin and uric acid in the total purine derivatives can be due to the differences between species, since they almost remain alike in the same species (Chen *et al*. 1992; 1996).

Differences in allantoin excretion

Allantoin was the main excretion product of purine metabolism in agreement with the results of Watts (1980); Lindberg (1989) and Chen *et al*. (1992). The increase of excreted allantoin in the urine along with the increased level of dietary concentrate can be related to the increase in daily amount of digestible organic matter fermented (DOMR) in agreement with the result of Lindberg (1985).

The increase in concentrate level of diet causes the increase in DMI (kg day^{-1}), and this in turn, can increase the excreted allantoin in the urine. The significant difference among the diets containing 15, 30 and 45% concentrate, as well as with the all forage diet can be related to the sensitivity of this purine derivative to diet compounds and the level of feed intake (Khorshidi *et al*. 2007 and Khorshidi *et al*. 2010).

Differences in uric acid excretion

The increase in the uric acid concentration in the urine can be related to daily DMI, as well as increase in the amount of DOMR. The lack of significance in the increase in the uric acid concentration between the diets containing 30 and 45% concentrate, also between the diets containing 15 and 30% concentrate, as well as significance in the amounts of excreted uric acid between diets containing 15% and 30% concentrate as well as significance in the amounts of excreted uric acid between diets containing 15% and 45%, also significance in the amount of 15%, 30%, 45% with an all forage diet can be explained as the following comparing to allantoin, uric acid has more stability (Grootveld *et al*. 1987). Thus, in order to make significant changes, it needs more portions in the diet i.e. the increase of the amount of concentrate from 30% to 45% cannot make significant differences in the excreted uric acid; whereas, the increase of concentrate from 15% to 45% causes significant difference in the amount of excretion uric acid in urine (Khorshidi *et al*. 2007 and Khorshidi *et al*. 2010).

The increase in total of excreted purine derivatives can be justified similar to allantoin with the increase of DOMR in agreement with the result of Chen *et al*. (1990); Daniels *et al*. (1994) and Cetinkaya *et al*. (1999; 2001; 2006). On the other hand, as mentioned before, because allantoin makes the biggest proportion of the excreted purine derivatives in buffaloes, we can expect that the differences between the amounts of total excreted purine derivatives, like allantoin amounts, be significant in case of all for diets. That is so because the allantoin, being large proportion, affects the amount of uric acid and gives significance to the total excreted purine derivates in the urine.

Microbial nitrogen and synthesized microbial protein

Since the produced microbial nitrogen can be estimated from the total amount of the excreted purine derivatives in the urine, it can be expected that the amounts of nitrogen or synthesized microbial protein also increase, and this increase is significant in all levels. This increase can be explained that with the increase in proportion of concentrate, daily DMI also increases. Also, with the increase in the proportion of concentrate, increased digestibility of the diet as well as DOMR is expected. The increase of the amount of daily digestible organic matter, increases the amount of DOMR, and that ARC (1980; 1984) has used it to estimate the produced microbial nitrogen in the rumen (32 g/kg DOMI), ARC (1984) has used 0.65 coefficients in order to turn DOMI to DOMR. We can also expect that by increasing the concentrate in the diet, total digestible organic matter (TDOM) also increases which in turn can increase the synthesis in microbial protein. Increasing the concentrate level will also increase the fermentable metabolizable energy (FME) in the diet, as well as increasing the synthesized microbial protein in the rumen (AFRC, 1993).

CONCLUSION

The results of this experiment indicated that increasing the concentrate level in diet of swamp buffaloes would increase the amount of synthesized microbial protein in rumen. The reason can be related to increasing the amount of digestible organic matter fermented in rumen (DOMR), as a result of increasing concentrate to forage ratio. However, the results revealed that there was a high and significant correlation between daily dry matter intake with excreted purine derivatives and produced microbial nitrogen in rumen.

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