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PURINE DERIVATIVE EXCRETION AND MICROBIAL PROTEIN SYNTHESIS IN SHEEP FED A ROUGHAGE-BASED DIET SUPPLEMENTED WITH OR WITHOUT GRASS SILAGE.

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Target Audience: Ruminant nutritionist, nutritional biochemist, farmers

ABSTRACT

In a 3 x 3 Latin square design experiment, urinary excretions of purine derivatives (allantoin N, Uric acid N, Xanthine + Hypoxanthine N) were measured and used to estimate microbial N yield in 9 sheep fed roughage- based diet supplemented with 0, 150 and 300g DM grass silage respectively.

Daily urinary excretions of allantoin N, uric acid N, xanthine + hypoxanthine were 3.66, 3.77, 4.51, 0.95, 1. 18, 1.24; 1.01, 1.21 and 1.34 mmol d^{-1} for sheep fed roughage-based diet and supplemented with 0, 150 and 300g DM silage respectively. Allantoin N, Uric acid N and Xanthine + Hypoxanthine N excretion increased significantly ($P<0.01$) with increasing amount of silage.Dry matter intake which increased with level of supplementation significantly ($P< 0.01$) influenced estimated microbial protein yield, which ranged from 4.86 to 6.14 gN d^{-1} . The efficiency of conversion of dietary N into microbial N which ranged from 48.17 to 57.22 g N/100gDN was highly significant ($P<0.01$) as the level of supplementation increased. Microbial N yield per kg of OM fermented (gN/kg DOMR) increased linearly.

The results indicate that the amount of microbial N biomass made available to the host animal per unit of feed fermented could be influenced by feed type, level of intake and size of animal. The results also showed that allantoin constituted the highest proportion of purine derivatives excreted.

Key Words: Microbial protein, purines, sheep, roughage, silage

DESCRIPTION OF PROBLEM

Microbial protein is the major protein source to ruminant animals. If dietary feed intake is adequate in energy, microbial protein produced in the rumen could be adequate to meet the animal's requirement of protein for maintenance(21). Dietary nucleic acids are extensively degraded within the rumen and the nucleic acids that escape into the small intestine are largely of microbial origin. In the intestine, unabsorbed purines not utilized are degraded to allantoin, uric acid, xanthine and hypoxanthine (19). Their urinary excretion has been proposed to be used as an index of rumen microbial protein available to the ruminant (23,25,26,29).

Allantoin is quantitatively the most important of the purine derivatives (PD) and its excretion in cattle has been reported to be linearly related to digestible dry matter intake (31). In sheep nourished by total intragastric infusion, the proportion of allantoin, uric acid, Xanthine and hypoxanthine of excreted purine derivatives were 0.49,0.41, 0.02 and 0.10 respectively (18). In sheep fed purified and normal rations, Xanthine constituted 0.04 – 0.025 of excreted purine derivatives (17). Previous workers (1,8,16) have shown in sheep and in cattle (30) that excretion of purine derivatives responds rapidly to changes in the supply of microbial nucleic acids.

It is possible to quantify the production of microbial protein under various feeding conditions. Report (28) showed that microbial nitrogen yields ranged from 15.3 to 53.1 gN/kg (average 31g N/kg) organic matter fermented in the rumen (DOMR). A value of 32g N/kg DOMR was also adopted by Agricultural Research Council (ARC) (3). Other workers (14) have indicated that the influence of feed intake on efficiency of microbial protein production was not significant in dairy cows. Little is known about the distribution of various PD in urine as well as microbial N synthesis, when increasing amounts of silage are fed to sheep as supplement to a roughage- based diet.

Hence in the present experiment, the utilization by sheep of roughage - based diet enriched with urea and supplemented with varied levels of grass silage was studied. The effects of dry matter intake and supplementation level on excretion of purine derivatives and microbial N synthesis, were also assessed.

MATERIALS AND METHODS

Animals:

Twelve female sheep approximately six months old with a mean live-weight of 38 " 2.4kg were used. The lambs were of Black face X Suffolk breeds.

Design and Treatments:

The twelve sheep were allocated to three groups of four animals balanced for body weight and were allocated at random to either the basal roughage -based diet only (control) or to roughage- based diet supplemented with 150 or 300 g DM silage/head/day. The animals in each dietary group received three treatments according to a Latin square design.

Diet Preparation :

The roughage- based diet (Table1) consisted of barley straw, grass hay, molasses, urea and propionic acid. Both barley straw and grass hay were shredded through a bale grinder with a 40mm screen into a mixer trailer and the remaining constituents added as a liquid mix. The inclusion of urea was to ensure that adequate soluble N was present to support maximum microbial protein synthesis in the rumen. The rye grass silage fed as supplement was treated with 61/t (fresh material) of Maxgrass additive at the time of ensiling to preserve carbohydrate content of silage. The grass with the additive was ensiled in big bale bags.

Table 1. Composition of basal diet

Ingredients (g Kg^{-1})	
Barley Straw	570
Hay	280
Molasses	90
Urea	30
Propionic acid	30

Proximate composition of basal diet and grass silage fed to sheep

	Basal diet	Silage
Dry Matter (g Kg^{-1})	927.6	203
Content in dry matter (gKg^{-1}DM)		
Crude Protein	149.09	111.97
NDF	671.73	706.41
ADF	397.48	373.11
Hemicellulose	274.25	433.31
Gross energy (MJ Kg^{-1})	18.02	18.49

NDF = Neutral detergent fibre
ADF = Acid detergent fibre

Feeding and Management:

Before the experiment commenced, sufficient quantity of silage was removed from bale bags and stored at –20°C. Appropriate amount needed to feed animals the following day was removed and allowed to thaw for 10-12 hours before feeding. The animals were individually penned on a slatted floor which allowed free drainage of urine. The diets were offered simultaneously in separate feed troughs once daily at 09.00h. The roughage based diet was offered in sufficient amount to allow for 10% refusal. All animals received 0.04% of body weight of a low-copper vitamin-mineral mixture sprinkled on their constituted roughage diet at the time of feeding.

The animals had free access to water at all times. Fresh feed was introduced daily and unconsumed feed was collected and weighed to determine daily feed intake. Each experimental period lasted for 21 days. Animals were weighed weekly.

Digestibility Trials:

During the last 7 days of each experimental period, 3 animals randomly selected from each dietary group totalling 9 animals were placed in individual metabolism stalls to facilitate collection of total faeces and urine voided. Urine was collected daily into buckets containing 100ml of 1M H_2SO_4 to keep the pH below 3. The 7 – day urine collected from each sheep was diluted with distilled water to 5 liters out of which two samples were taken, one stored at 4^0C for the determination of N and the other stored at -20^0C for analysis of purine derivatives. The bulked faeces collected for 7 days was mixed, sampled and kept frozen at -20^0C until analysed. Samples of feeds and residues collected for 7 days were similarly aggregated and kept frozen until analysed.

Chemical Analysis:

Dry matter in the straw diet and faeces was determined by drying a weighed sample in an oven at 100^0C for 48 hours and organic matter by ashing at 550^0C (2). Dry matter in silage was determined by toluene distillation method (11). Total nitrogen (Kjeldahl N) was analysed (10). Water-soluble carbohydrate (WSC) of the silage was determined in the juice extracted from the silage. Fermentable acids and NH_3 – N in silage were determined (15).

Purine derivatives in the urine were determined as allantoin, uric acid and xanthine plus hypoxanthine (8). Allantoin was dertemined by a modified method based on Pentz, (22). Uric acid in urine was measured using the phosphotungstic acid method adopted for the Technicon Auto analyser described in the Technicon method file (27) . Xanthine plus hypoxanthine were determined as uric acid after treatment of the urine samples with xanthine oxidase.

Statistical Analysis:

Data were subjected to analysis of variance based on Latin square design to examine the effects of the following variables, intake level, animal and period on the measurements obtained in the experiment. The means of different treatments were compared using T. test after a significant F. test.

RESULT

Voluntary feed intake data are presented in Table 2. The intakes of both dry matter (DM) and organic matter (OM) increased with increasing level of silage supplementation. The average daily intakes of DM,OM and N were 37.65, 38.31, 44.15; 35.12, 35.71, 41.15;0.9, 0.86 and 0.94 gkg $^{-1}W^{0.75}$ for control diet supplemented with 0, 150 and 300g DM silage respectively. The digestible energy intakes were 5.67, 6.35 and 7.86 MJ/head/day for the respective treatments. Except for N, the DM, OM and digestible energy intakes increased significantly (P#, 0.05).

Table 2: Intake of sheep fed on a roughage-based diet with or without supplement of grass silage (g/d)

	Supplement Level (g DM/h/d)				
Dry Matter	0	150	300	SE	Statistical Significant of treatment effect
Silage	0	148	295	1.45	
Straw	586	452	401	37.67	
Total	586	600	694	37.77	*
Total DM/Met wt.	37.65	38.31	44.15	2.19	*
Organic Matter					
Silage	0.00	138.04	272.85	1.84	
Straw	546.2	421.7	374.2	35.13	
Total	546.2	559.7	647.00	31.91	*
Total OM/Met wt.	35.12	35.71	41.15	2.08	*
Nitrogen					
Silage	0.00	2.66	5.25	.05	
Straw	13.97	10.79	9.57	.06	
Total	13.97	13.44	14.82	.90	NS
Gross Energy (MJ/d)					
Silage	0.00	2.74	5.42	.03	
Straw	10.55	8.15	7.20	.65	
Total	10.53	10.89	12.65	.66	*

NS = Not Significant:
*P< 0.05:
SE=Standard error
OM= Organic matter
Met wt=Metabolic weight $KgW^{0.75}$

Mean excretions of purine derivatives are given in Table 3. There was a significant (P< 0.05) increase in total purine excretion when silage inclusion level was increased. Total excretion of purine derivatives excretion averaged 5.62, 6.16 and 7.09 mmol/day for sheep fed roughage- based diet supplemented with 0, 150 and 300g DM silage respectively. The relative contribution from allantoin N, uric acid N and xanthine + hypoxanthine increased with mean values of 3.66, 3.77, 4.51; 0.95, 1.18, 1.24; 1.01, 1.21 and 1.34m mol/day for the respective treatment.

Table 3: Urinary excretion (m mol/d) of purine derivatives by sheep in response to feeding of roughage- based diet with or without supplement of grass silage

	Supplement (gDM/h/d)				F.Test		
Components	0	150	300	SE	Diet	Animal	Period
Allantoin	3.66	3.77	4.51	0.67	***	*	NS
Uric acid	0.95	1.18	1.24	0.35	**	*	NS
Xanthine + hypoxanthine	1.01	1.21	1.34	0.37	**	*	NS
Total Purine Excreted	5.62	6.16	7.09	0.75	***	*	NS

NS =Not Significant;
SE = Standard Error
* - P<0.05
** - P<0.01
*** - P< 0.001

Table 4: Estimated microbial yield and efficiency of microbial production in sheep fed roughage- based diet with or without supplements of grass silage

	Supplement Level (gDM/h/d)				F .Test		
	0	150	300	SE	Diet	Animal	Period
DM Intake g/W $^{0.75}$	35.12	35.71	41.15		*	*	NS
Purine absorbed, mmol/d	6.69	7.33	8.45	0.41	***	**	NS
Estimated microbial N yield, gN/d	4.86	5.33	6.14	0.40	***	**	NS
Estimated microbial N yield, g N/kg DOMR	26.68	26.82	28.55	0.81	*	NS	NS
Estimated microbial N Yield, gN/100g digestible nitrogen	48.17	54.06	57.22	3.74	**	**	NS

NS, Not significant;
* = P# 0.05
**,= P # 0.01,
***, = P #0.001.
SE = Standard Error
DOMR = Digestible organic matter fermented in the rumen taken as 0.65 of the digestible OM intake.
DM = Dry Matter.

Both total DM intakes and silage inclusion level had a positive effect on purine derivative excretion. Animal effect on all purine derivatives excreted was significant ($P < 0.05$) except between periods ($P > 0.05$).

The means of purine absorbed, estimated microbial N production and efficiency of microbial N production into the tissue nucleic acid in the liver ranged from 6.69 to 8.45 mmol per day (Table 4). These values which increased with increasing level of silage supplement and feed intake were highly significant ($P < 0.01$). The estimated microbial N yields ranged from 4.86 to 6.14g per head per day favouring the supplemented groups.

Microbial growth was also closely related to measures of digestible energy, which increased with increase in the digestible energy.The efficiency of microbial N production expressed as gN/kg digestible organic matter fermented in the rumen (DOMR) assumed to be 0.65 of the digestible organic matter intake, (3) ranged form 26.63 to 28.55 gN/kg DOMR. The efficiency was positively influenced by DM, OM and energy intakes. The efficiency of conversion of dietary N into microbial N improved significantly ($P < 0.01$) with increasing level of N intake and level of supplementation. The values ranged from 48.17 to 57.72 g microbial N/ 100g digestible N intakes.

DISCUSSION

Results of this study support published findings (4)which showed that allantoin,a purine derivative, increased with incremental intake of dry matter or organic matter. In this study the relative contributions from allantoin, uric acid and xanthine plus hypoxanthine of total purine excreted were 0.65, 0.62, 0.64; 0.17, 0.19, 0.17; 0.18, 0.20, 0.19 for roughage based diet supplemented with 0, 150 and 300 g DM silage respectively. For animals nourished by intragastric infusion, the relative contributions from allantoin, uric acids, xanthine and hypoxanthine as reported were 55, 0.31 and 0.14 respectively (8,9,13). The present results for normally fed animals indicated higher excretion of allantoin and xanthine plus hypoxanthine and lower uric acid than for intragastric infusion. Variation in allantoin excretion could be due to differences in the microbial biomass reaching the intestine (7). The range in allantoin excretion cannot be accounted for by variation in the contribution from endogenous allantion which should have been in the range of 1.1 – 1.7 mmol day $^{-1}$ nor differences in the proportion of plasma allantoin recycled to the gut (8).

It is however suggested (26) that the net outflow of microbial protein synthesized in the rumen is positively correlated with the OM apparently fermented in the rumen provided that 30g of rumen degradable N are available per Kg OM fermented in the rumen . The provision of 30g urea/

kg of basal diet satisfies the dietary N need for microbial growth. In cattle, other workers (23) also reported that efficiency of bacterial N yield increased with intake (17.4, 20.7, 31.6 and 38.2 gN/Kg DOMR for the intake level of 56, 99, 138 and 162g DM/Kg $W^{0.75}$ respectively). Improved efficiency of microbial protein synthesis for a higher level of intake was also reported (24). Calculated microbial nitrogen yields (g/Kg DOMR)were lower with unsupplemented diet and increased with the level of supplementation. These results agree with other findings (4,6).

The results of the present studies demonstrated that the level of intake can profoundly influence the supply of microbial protein to sheep provided adequate substrates are made available. Increase in total dry matter intake for 150 and 300 g DM silage supplemented groups resulted in an increase in the microbial N yield by 8.8 and 20.85% over the control group respectively. The efficiency of microbial protein production was improved by the increased level of dry matter intake. Promotion of microbial protein synthesis resulted from the feed stuffs being sources of ammonia, sulphide, branched chain volatile fatty acids and amino acids or peptides all of, which are essential for microbial growth (5,12).

CONCLUSION AND APPLICATIONS

In conclusion, data obtained in this study provide further validity for the use of excreted purine derivatives in urine of ruminants as an index of absorbed microbial biomass.. The results also showed that the amount of microbial biomass made available to the host animal could be influenced by feed type, level of intake and animal size. Furthermore the present work indicates that feeding of silage as supplement to straw based diet provided some improvement in the yield of microbial N per unit of organic matter fermented in the rumen. Therefore, methods of silage preservation either by wilting or using additives which influence the extent of silage fermentation can also be a meaningful factor of microbial N yield.

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