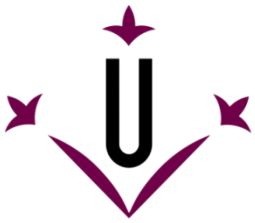

Modelling urinary purine derivatives excretion as a tool to estimate microbial rumen outflow in alpacas (*Vicugna pacos*)

P. Orellana-Boero [a], A.R. Seradj [b], M. Fondevila [c], J. Nolan [d], J. Balcells [b,*]

[a] *Departamento de Ciencias Pecuarias, Facultad de Medicina Veterinaria, Universidad de Concepción, Av. Vicente Mendez 595, Chillán, Chile*

[b] *Departament Producció Animal, ETSEA, Universitat de Lleida, Alcalde Rovira Roure 191, 25198 Lleida, Spain*

[c] *Instituto Universitario de Investigación en Ciencias Ambientales, Departamento de Producción Animal y Ciencia de los Alimentos, Universidad de Zaragoza, Miguel Servet 177, 50013 Zaragoza, Spain*

[d] *School of Rural Science and Agriculture, University of New England, Armidale, NSW 2351, Australia*

Corresponding author: J. Balcells
Tel.: +34 973702903, balcells@prodan.udl.cat

Abstract

Three experiments were carried out to establish a model to estimate the forestomach micro- bial yield based on the urinary excretion of purine derivatives (PD: allantoin, uric acid, xanthine and hypoxantine) in alpacas (*Vicugna pacos*). In Experiment 1, endogenous PD excretion was measured in two fasted adult male alpacas for seven consecutive days. Daily urinary PD excretion (μmol/kg $BW^{0.75}$) decreased during fasting to a minimum value of 194.8 (s.e. 18.4), ranging from 215.3 to 174.3. In Experiment 2, the relationship between purine bases (PB) input and urinary PD output was defined in two alpacas fitted with an infusion catheter at the terminal third compartment of the forestomach (C3). Animals were fed alfalfa hay at a maintenance level, and four RNA-doses (4.2, 8.3, 12.5 and 16.6 mmol PB/day; RNA from Torula yeast) were continuously infused at random into the C3 in four successive 120 h-periods. Urinary recovery of C3 infused purines averaged 0.615 (s.e. 0.0006) mmol/day. In Experiment 3, urinary PD response to levels of feed intake cor- responding to 100, 75, 50 and 20% of the previous voluntary intake was evaluated. The amount of PD excretion in urine increased linearly (r = 0.867) with digestible organic mat- ter intake (DOMI), and the slope of the regression (16.7 mmol PD/kg DOMI) can be assumed as an index of microbial yield.

Keywords: Purine derivatives Microbial protein Camelids

1. Introduction

Urinary excretion of purine derivatives (PD; *i.e.* allan- toin, uric acid, hypoxanthine and xanthine) is proportional to the duodenal inflow of purine bases (PB; adenine and guanine). However, this relationship is always biased by the metabolism of host-endogenous PB. Significant inter- and intra-species differences (Balcells et al., 2004) have been reported in both magnitude of the endogenous excretion and in the response model between absorption and excretion of purine derivatives compounds (Chen et al., 1990; Balcells et al., 1991).

Specific response models have been designed, initially in sheep (Chen et al., 1990; Balcells et al., 1992) and cattle (Orellana Boero et al., 2001), but also in goats (Belenguer et al., 2002) and camels (*Camelus dromedaries*; Guerouali et al., 2004). This technique has also potential for use in other ruminant species of economic relevance such as alpaca (*Vicugna pacos*) (Kadwell et al., 2001) in the high Andean plateau, once adequate equations are defined to take host species differences in purine metabolism into account (Guerouali et al., 2004). The aim of the present approach was to improve our understanding on PB metabolism and to build a response model between duodenal input and urinary output in alpacas.

2. Materials ands methods

According to the Ethic Committee for Animal Experimentation of the University of Zaragoza, the care and use of animals were performed in agreement to the European Union Directive 86/609 on the protection of animals used for experimental and other scientific purposes.

2.1. *Experiment 1: urinary excretion of purine compounds in alpacas during fasting*

The experiment was conducted on two adult alpaca males (41.7 and 48.9 kg body weight, BW and aged between two and three years). The ani- mals were individually penned in metabolic cages and were given alfalfa hay (chemical composition, g/kg; dry matter, DM: 929; crude protein, CP: 166; neutral detergent fibre, NDF: 475; acid detergent fibre, ADF: 364) at a level of 1 kg DM per day, close to *ad libitum* conditions. Alpacas were adapted to the diet for 2 weeks and then the amount of offered feed was reduced stepwise every 2 days (70%, 50%, 30% and 0%). Thereafter, ani- mals were fasted for seven days. Urine was collected directly from the cage using 20 ml of an acidic solution with 1 M H_2SO_4 to ensure a final pH of 2–3 to prevent evaporation of nitrogen compounds. At the end of each 24-h period, tap water was added to each bucket to bring contents to the same weight, samples were mixed and then sub-samples (about 50 ml) were taken and stored at −20 °C for further analysis.

2.2. *Experiment 2: relationship between intestinal absorption of purine bases and urinary excretion of purine derivatives*

Ten days before the onset of the experiment two male alpacas (ini- tial BW of 57.5 and 72.5 kg and aged between two and three years) were fitted with an infusion catheter at the terminal third compartment of the forestomach (C3). Alpacas were kept in individual metabolism cages and were fed the same alfalfa hay as in the previous experiment. Clean and fresh water was freely available throughout the experiment. In order to get different intestinal flow levels of PB, four RNA doses (4.2, 8.3, 12.5 and 16.6 mmol PB/day) as RNA from Torula yeast (SIGMA, St. Louis, USA) were prepared and infused (Orellana Boero et al., 2001) by random into the C3 in four successive 120-h

periods, with 48 h for adaptation to doses changeover and 72 h for continuous urine collection.

2.3. *Experiment 3: urinary excretion of purine derivatives at different levels of feed intake*

The experiment was conducted on three alpacas, two males and one female (73, 67 and 41 kg BW and aged between two and three years) which were kept in metabolic cages. Before the experiment began, the alpacas were individually fed alfalfa hay *ad libitum* for 15 days. The feed was offered twice daily, at 09.00 and 16.00 h in two equal amounts and alpacas had free access to drinking water. A randomised 4 × 3 factorial arrangement was used, with four feeding levels at 100, 75, 50 and 20% of the lowest recorded voluntary intake level from the pre-experimental period were chosen that were given to the 3 alpacas for 21 days feeding periods at random. During the last five days of each period, total collection of urine and faeces were daily performed. A sub-sample (10%) of each daily faecal excretion was kept in polyethylene bags and stored at 4 °C for fur- ther analysis. Urine was acidified to ensure a final pH of 2–3 and collected directly from the cage. In the case of the female, the urine was separated from the faces using an external separators stuck to the vulva (Orellana Boero et al., 2001).

2.4. *Chemical and statistical analysis*

DM, OM and CP were determined following the AOAC (1990) and neutral (NDF) and acid detergent fibre (ADF) following Van Soest et al. (1991). Urinary PD, PB and creatinine concentration were analysed by HPLC following Balcells et al. (1992).

In Experiment 1, results from the study of the effect of animal and time on

the urinary excretion of PD during fasting period were analysed as repeated observations on each animal (experimental unit) following Rowell and Walters (1976). In Experiments 2 and 3, data were analysed using the following model: $Y_{ij} = \mu + A_i + D_j + (A \times D)_{ij} + \varepsilon$; being D_i (PB doses) (in Experiment 2) or feed intake level (in Experiment 3), A_j (animals) and P_k (periods) the main effects that were contrasted against the residual error term (ε). Linear regression models were fitted in Experiment 2 to establish the relationship between C3-input and urinary output of purine compounds (mmol/day), and in Experiment 3 the rate of urinary excretion of PD (y; mmol/day) and digestible organic matter (x; DOM kg/day).

3. Results and discussion

Animal remained healthy throughout the experiment, they behaved adequately and fully consumed the exper- imental diet. Data presented needs to be treated with caution because the small number of animals used.

3.1. *Urinary excretion of purine derivatives during fasting and in response to the different levels of intra-intestinal purines supply*

Total PD excretion in Experiment 1 decreased ($P < 0.05$) with intake along the six days of fasting (Table 1). PD and allantoin excretion (μmol/kg $BW^{0.75}$) decreased from 683 to 195 (s.e. 28.7) and from 593 to 168 (s.e. 39.2), respec- tively. Fasting excretion as an index of endogenous PD losses averaged 194.8 (s.e. 18.4) μmol/kg $BW^{0.75}$ (from d8 to d15 of the experiment) was in the reported range for other Camelidae species, lower than camels (267 μmol/kg $BW^{0.75}$; Guerouali et al., 2004) and similar to llamas (177 μmol/kg $BW^{0.75}$; Bakker et al., 1996). Comparing to other species characterised by a similar urinary PD profile, Belenguer et al. (2002) showed a close level of PD losses in fasted goats (202 μmol/kg $BW^{0.75}$), although Fujihara et al. (1991) found lower values in sheep

(128.8 µmol allan- toin/kg $BW^{0.75}$).

In Experiment 2, allantoin and total PD excre- tion responded positively to the changes in RNA (PB) infusion levels, and a linear model ($y = a + bx$) was fitted to state a relation between duodenal input and urinary output of purine compounds:Total PD = 3.12 (s.e. 2.3)+ 0.619 (s.e. 0.062) PB input; r = 0.905; RSD = 3.26 ; n = 12 ;where "a" is assumed as the endoge- nous component and "b" is the equimolar recovery that was taken as a reference value. Creatinine excretion (µmol/$BW^{0.75}$) remained constant throughout the exper- imental period during fasting (449, s.e. 29.6) also during C3-RNA supply (464, s.e. 11.8). The close relationship (r = 0.905) between intestinal PB infusion and urinary PD excretion confirms the using of urinary PD excretion as a predictive index of microbial output from the forestomach in alpacas if PB are accepted as marker of the microbial mass. The equimolar recovery of intestinally infused PB in alpacas (urinary PD to C3-PB ratio: 0.61 0.062) was similar than values registered in dromedary camels (0.63 0.068; Guerouali et al., 2004) and sheep (from 0.52 to 0.80%; Chen et al., 1990; Balcells et al., 1992), but it is slightly lower than values reported for maintenance and lactating goats (0.76 and 0.69; Liang et al., 1999; Orellana Boero et al., 2001).

The low to moderate levels of endogenous PD excretion and the presence of significant amounts of hypoxanthine and xanthine (salvageable purine compounds) in the urine of alpacas suggest that this South American camelid may behave like sheep in relation to purine excretion modelling. The urinary recovery of infused purines (0.62) suggests that a significant fraction is excreted *via* non-renal routes.

Table 1

Urinary excretion of purine derivatives (PD) and creatinine (μmol/$BW^{0.75}$) in two alpacas (*Vicugna pacos*) under progressive feeding restriction, from *ad libitum* feeding to fasting[a] (Experiment 1, mean values).

Periods	Allantoin	Uric acid	Hypoxanthine and xanthine	Total PD	Creatinine
Ad libitum	593 a	20.2	70.4	683 a	505
Restriction 30%	440 b	14.3	54.8	509 a	516
Restriction 50%	328 b	8.6	40.6	377 b	445
Restriction 70%	193 c	2.3	34.7	229 c	393
Fasting	168 c	2.7	24.8	195 c	402
s.e.	39.2	4.58	6.20	28.7	52.4
P-value	<0.05	–	<0.05	<0.01	–

Mean values within a column with unlike letters (a–c) were significantly different ($P < 0.05$).

[a] Values are for the last two days of *ad libitum*, two for restricted intake, whereas fasting corresponds to a 6 days period.

However, such point should have a minor relevance for predictive purposes, as it has been shown that the parti- tion between renal and non-renal routes is constant despite the level of PB absorption (Balcells et al., 1991; Belenguer et al., 2002). Thus, absorbed purines (*x*, mmol/day) can be simply estimated as PD excretion (*y*, mmol/day) divided by the incremental recovery (0.62), resulting $x = y/0.63$. When absorbed PB are corrected by the true digestibility of intestinal PB (0.92; Chen et al., 1990), then rumen PB out- flow could be calculated. Assuming that most of PB reaching the host digestive system are from microbial origin (Pérez et al., 1997), then the relationship between PB and N deter- mined in microbes extracted from the forestomach camel (1.26 mmol PB/g N; Guerouali et al., 2004) allows calculat- ing the rumen microbial-N outflow.

The resultant equation would be:

$$\text{microbial-N (g/d)} = \frac{x}{0.92 \times 1.26}$$

3.2. *Experiment 3: urinary excretion of purine derivatives at different levels of feed intake*

The PD excretions at the different levels of DOMI are presented in Table 2. The rate of urinary excretion of allantoin as its precursors ($P < 0.05$) and total PD excretion ($P < 0.01$) increased with feed intake. The positive rela- tionship between DOMI (x; kg/day) and PD excretion (y; mmol/day) is showed in the following equation:

y = 2.53 (s.e. 1.20) + 16.72 (s.e. 2.71)x; r = 0.867;
RSD = 3.26; n = 36;

The slope (mmol/kg) can be assumed as an index of microbial yield per unit of DOMI, and the intercept, would be the PD excretion at zero level of DOMI, which corre- sponds to 108.8 (s.e. 11.3) mmol/kg $BW^{0.75}$. This value was lower than the mean value recorded during the fasting period (194, s.e. 18.4 mmol/kg $BW^{0.75}$; Experiment 1).

The relationship between DOMI and urinary PD excre- tion has been previously reported for other ruminant species (Laurent et al., 1983; Han et al., 1992; Balcells et al., 1993), and the slope would be directly related to the effi- ciency of microbial synthesis. Our results confirm such relationship in alpaca (16.7 mmol PD/kg DOMI), which felt between those determined in goats (12.6; Laurent et al., 1983) and sheep (18.9–22.3; Balcells et al., 1993). However, the PD/DOMI ratio as an index of microbial yield efficiency decreases with the level of feed intake.

Table 2

Feeding level, daily purine derivative excretion (allantoin or Total PD), PD/DOMI (PD/DOM) and PD:creatinine (PD/C) ratio in urine samples and predicted microbial N supply in three alpacas (*Vicugna pacos*) fed at 20, 50, 75 or 100% of their previous recorded *ad libitum* intake.

	Ratio (% *ad libitum*)				SEM	*P*-value
	20	50	75	100		
DMI (kg/day)	0.25	0.51	0.76	1.01	044	<0.05
DOMI (kg/day)	0.15	0.35	0.55	0.80	038	<0.05
Feeding level[a]	0.32	0.65	1.00	1.78	100	<0.05
Excretion (mmol/day)						
Allantoin	4.31	6.40	9.65	11.62	0.651	<0.01
Total PD	5.01	7.38	11.06	13.42	0.702	<0.01
Ratio						
PD/DOMI (mmol/kg)	38.53	22.37	22.56	20.65	1.783	<0.001
PD/C (mol/mol)	0.61	0.82	1.07	1.25	0.061	<0.01
Estimated values						
MN[b] (gN/day)	6.85	10.10	15.13	18.36	0.958	<0.001
Microbial MP[c] (gN/day)	4.39	6.46	9.68	11.75	0.509	<0.01
MP requirement[d] (gN/day)	9.70	9,70	9,70	9,70	–	–

[a] Metabolisable energy (ME) intake/ME requirements for maintenance (72.85 Mkca/$W^{0.75}$; Van Saun, 2006).

[b] Microbial nitrogen (MN) outflow = (PD/0.62)/(0.92 × 1.26) = 1.37 PD (mmol/day).

[c] Metabolisable protein supply from microbes = MN × 0.75 × 0.85 (AFRC, 1993).

[d] Basal endogenous nitrogen requirements = 0.38 g N/W 0.75 (Huasasquiche, 1974) and for fleece production 11.5 g or 1.84 gN/day (Van Saun, 2006).

It is known that microbial yield efficiency increases with feed intake (ARC, 1984) but when intake is below the maintenance level, endogenous contribution of PD may lead to an overesti- mation of exogenous rumen PB outflow.

The overall efficiency coefficient for microbial protein outflow (139 g/kg DOMI) was similar to the reported values for cattle and sheep on good-quality mixed diet (130 g/kg DOMI; ARC, 1984) and indicates that microbial growth efficiency in some Camelidae may be similar to the true ruminants, although Guerouali et al. (2004) reported a lower coefficient (95 g/kg DOMI).

To confirm the consistency of our proposed model, the duodenal flow of microbial N was estimated from uri- nary PD excretion and contrasted against the maintenance requirements (Table 2). The net protein requirements for maintenance were assumed to be the sum of the basal endogenous N (3.5 g crude protein/kg $BW^{0.75}$; Huasasquiche, 1974) plus fleece synthesis (11.5 g crude protein/day; Van Saun, 2006). In our study, we also assume that the efficiency of using metabolisable protein at main- tenance level is 1.0, taking into account that 0.75 of microbial N is in amino acidic form, which can be digested with an efficiency of 0.85 (AFRC, 1993). Our results confirm the consistency of the model and indicate that the metabolisable protein from microbes do not meet pro- tein requirements if alpacas are fed below maintenance (72.85 kcal ME/kg $BW^{0.75}$; Schneider et al., 1974). The PD/creatinine values are also presented to confirm the uri- nary ratio of PD/creatinine as a suitable index to detect changes in microbial supply to the digestive system of alpacas.

4. Conclusions

This work defines the relationship between the intesti- nal supply of purine bases and the urinary excretion of their derivatives. The equimolar recovery of administered intestinal purines and the determined endogenous losses by fasting suggest that alpaca may behave like other camelids or even sheep, in terms of purine metabolism.

References

AFRC, 1993. Energy and Protein Requirements of Ruminants. CAB International, Wallingford.

AOAC, 1990. Official Methods of Analysis, 15th ed. AOAC, Arlington, VA. ARC, 1984. The Nutrient Requirement of Ruminant Livestock. Commonwealth Agricultural Bureaux, Wallingford, UK.

Bakker, M.L., Chen, X.B., Kyle, D.J., Ørskov, E.R., Bourke, D.A., 1996. Urinary and plasma purine derivatives in fed and fasted llamas (*Lama glama* and *L. guanacoe*). Comp. Biochem. Physiol. B 113, 367–374.

Balcells, J., Guada, J.A., Castrillo, C., Gasa, J., 1991. Urinary excretion of allantoin and allantoin precursors by sheep after different rates of purine infusion into the duodenum. J. Agric. Sci. 116, 309–317.

Balcells, J., Guada, J.A., Castrillo, C., Gasa, J., 1993. Rumen digestion and urinary excretion of purine derivatives in response to urea supple- mentation of sodium-treated straw fed to sheep. Br. J. Nutr. 69, 721–732.

Balcells, J., Guada, J.A., Peiro, J.M., Parker, D.S., 1992. Simultaneous determination of allantoin and oxypurines in biological fluids by high-performance liquid chromatography. J. Chromatogr. B 575, 153–157.

Balcells, J., Vicente, F., Orellana-Boero, P., Martin Orúe, S.M., González-Ronquillo, M., 2004. Effect of physiological status on endogenous excretion of purine derivatives in cattle. In: Chen, H.M.a.X. (Ed.), Esti- mation of Microbial Protein Supply in Ruminants Using Urinary Purine Derivatives. Kluwer Academic Publishers, Netherlands, pp. 32–41.

Belenguer, A., Yañez, D., Balcells, J., Ozdemir Baber, N.H., Gonzalez Ronquillo, M., 2002. Urinary excretion of purine derivatives and prediction of rumen microbial outflow in goats. Livest. Prod. Sci. 77, 127–135.

Chen, X.B., Hovell, F.D.De.B., Orskov, E.R., Brown, D.S., 1990. Excretion of purine derivatives by ruminants: effect of exogenous nucleic acid sup- ply on purine derivative excretion by sheep. Br. J. Nutr. 63, 131–142.

Fujihara, T., Matsui, T., Harumoto, T., 1991. Urinary excretion of purine derivatives and blood plasma level of allantoin in sheep and goats during fasting. In: Proceedings of 6th International Symposium on Protein Metabolism and Nutrition. Europian Association of Animal Production, Herning, Denmark, pp. 170–172.

Guerouali, A., El Gass, Y., Balcells, J., Belenguer, A., Nolan, J., 2004. Urinary excretion of purine derivatives as an index of microbial pro- tein synthesis in the camel (*Camelus dromedarius*). Br. J. Nutr. 92, 225–232.

Han, D.K., Shin, H.T., Landis, J., 1992. Effect of level of feed intake on the excretion of purine derivative and purine derivatives to creatinine ratio in the urine of sheep. Asian Austral. J. Anim. 5, 465–468.

Huasasquiche, A., 1974. Nitrogen balance and digestibility in alpacas and sheep. Balance del nitrogeno y digestibilidad en alpacas y ovinos. B.Sc. Thesis, Universidad Nacional Mayor de San Marcos, p. 49.

Kadwell, M., Fernandez, M., Stanley, H.F., Baldi, R., Wheeler, J.C., Rosadio, R., Bruford, M.W., 2001. Genetic analysis reveals the wild ancestors of the llama and the alpaca. Proc. R. Soc. B: Biol. Sci. 268, 2575–2584.

Laurent, F., Blanchart, G., Vignon, B., 1983. Relationship between the urinary excretion of allantoin and nitrogen in lactating goats. In: Arnal, M., Pion, R., Bonin, D. (Eds.), IV Symposium International de Metabolisme et Nutrition Azotés. Clermont-Ferrand, France, pp. 175–178.

Liang, J.B., Pimpa, O., Abdullah, N., Jelan, Z.A., Nolan, J.V., 1999. Estimation of rumen microbial protein production from urinary purine deriva- tives in zebu cattle and water buffalo. In: Nuclear Based Technologies for Estimating Microbial Protein Supply in Ruminant Livestock. Inter- national Atomic Energy Agency, Vienna, pp. 35–42.

Orellana Boero, P., Balcells, J., Martín-Orúe, S.M., Liang, J.B., Guada, J.A., 2001. Excretion of purine derivatives in cows: endogenous contri- bution and recovery of exogenous purine bases. Livest. Prod. Sci. 68, 243–250.

Pérez, J.F., Balcells, J., Guada, J.A., Castrillo, C., 1997. Rumen microbial pro- duction estimated either from urinary purine derivative excretion or from direct measurements of ^{15}N and purine bases as microbial mark- ers: effect of protein source and rumen bacteria isolates. Anim. Sci. 65, 225–236.

Rowell, J.G., Walters, D.E., 1976. Analysing data with repeated observa- tions on each experimental unit. J. Agric. Sci. 87, 423–432.

Schneider, W., Hauffe, R., Von Engelhardt, W.,1974. Energy and nitrogen exchange in the llama. In: Proceedings of Sixth Symposium Energy Metabolism of Farm Animals. European Association of Animal Pro- duction, pp. 127–130.

Van Saun, R.J., 2006. Nutrient requirements of South American camelids: a factorial approach. Small Rumin. Res. 61, 165–186.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.