ORIGINAL ARTICLE

Effects of ruminally undegradable protein levels on nitrogen and phosphorus balance and their excretion in Saanen goats fed oil palm fronds

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Abstract

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Twelve Saanen goats 3-4 months old with initial live weight (mean±SD) of 18.8±2.2 kg were used in a 4×4 Latin square design. Diets were isonitrogenous (15% CP) and isocaloric (3.6 Mcal/kg DM) and were fed *ad libitum*. Total mixed diets were composed of steamed oil palm fronds (OPF) and concentrate [urea, soybean meal (SBM), cassava waste, molasses, sulphur and commercial mineral and vitamin mix]. The treatments were four levels of ruminally undegradable protein (RUP) supplementation viz, 0, 2, 4 or 6% (0%RUP, 2%RUP, 4%RUP or 6%RUP, respectively). Nitrogen (N) and phosphorus (P) intakes tended to be increased linearly ($p=0.08$ and $p=0.09$) as a consequence of additional of RUP supplementation. Similarly, %N absorption and retention, dry matter intake (DMI) and crude protein digestibility increased linearly ($p>0.05$) as a consequence of the additional of RUP supplementation. There were no effects of dietary treat-

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ment on P balance. However, P intake, absorption and retention tended to be increased as a consequence of additional of RUP supplementation. The linear equation between RUP (%) and P intake (g/d) of goats was P intake = 2.05+0.056RUP; (p=0.07). Nitrogen and P retention also tended to increase with increasing levels of RUP supplementation. Manipulation of diet by supplementation of RUP in the rations can be used to reduce the amount of N excreted, especially N in urine, which in turn reduces ammonia emissions.

Key words : nitrogen, phosphorus, ruminally undegradable protein, Saanen goat

บทคัดย่อ

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ผลของระดับโปรตีนที่ไม่ถูกย่อยในกระเพาะรูเมนต่อสมดุลไนโตรเจน ฟอสฟอรัส และการขับออก ในแพะซาเนนที่เลี้ยงด้วยทางใบปาล์มน้ำมันเป็นอาหารหยาบพื้นฐาน

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การศึกษาครั้งนี้ใช้แพะนมพันธุ์ซาเนน 12 ตัว อายุระหว่าง 3-4 เดือน มีน้ำหนักตัวเริ่มต้นเฉลี่ย 18.8±2.2 กก. โดยใช้แผนงานทดลองแบบ 4×4 ลาตินสแควร์ อาหารทดลองประกอบด้วย 4 สูตร ที่มีการเสริมโปรตีนที่ไม่ถูกย่อยในรูเมน (Ruminally Undegradable Protein, RUP) ที่ระดับ 0, 2, 4 และ 6% อาหารทุกสูตรที่ใช้ในการทดลองได้ปรับให้มีโปรตีนและพลังงานในระดับเท่ากัน (โปรตีน 15% และพลังงาน 3.6 เมกกะแคลอรี/กก. ตามลำดับ) โดยสัตว์ได้รับอาหารอย่างเต็มที่ สูตรอาหารผสมประกอบด้วย ทางใบปาล์มน้ำมัน ยูเรีย กากถั่วเหลือง กากมันสำปะหลัง กากน้ำตาล กำมะถันและแร่ธาตุ และไวตามินผสมสำเร็จรูป ปริมาณของไนโตรเจนและฟอสฟอรัสที่สัตว์ได้รับมีแนวโน้มเพิ่มขึ้นแบบเส้นตรงตามระดับของโปรตีนที่ไม่ถูกย่อยในรูเมนในอาหาร เปอร์เซ็นต์ไนโตรเจนที่ถูกดูดซึมและสมสมในร่างกาย ปริมาณของวัตถุแห้งที่สัตว์ได้รับ และการย่อยได้ของโปรตีนเพิ่มขึ้นแบบเส้นตรง (p>0.05) ตามระดับของโปรตีนที่ไม่ถูกย่อยในรูเมน การเสริมโปรตีนที่ไม่ถูกย่อยในรูเมนในอาหารไม่มีผลต่อสมดุลของฟอสฟอรัส อย่างไรก็ตามปริมาณของฟอสฟอรัสที่สัตว์ได้รับ (P intake, g/d) มีแนวโน้มเพิ่มขึ้นตามระดับของโปรตีนที่ไม่ถูกย่อยในรูเมน (RUP, %) ดังสมการ P intake = 2.05+0.056RUP; (p=0.07) จากผลการทดลองสามารถกล่าวได้ว่า ปริมาณไนโตรเจนและฟอสฟอรัสที่สะสมในร่างกายเพิ่มขึ้นตามระดับของโปรตีนที่ไม่ถูกย่อยในรูเมน และการจัดการด้านการให้อาหารโดยการเสริมแหล่งของโปรตีนที่ไม่ถูกย่อยในรูเมน นอกจากสามารถเพิ่มผลผลิตของสัตว์แล้วยังสามารถลดการขับออกของไนโตรเจน โดยเฉพาะอย่างยิ่งไนโตรเจนในปัสสาวะซึ่งเป็นการลดการปลอดปล่อยแอมโมเนียต่อสิ่งแวดล้อม

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Nitrogen (N) and phosphorus (P) pollution by animal farming systems are rising due to increased feeding of protein and P supplement. Thus improving N and P utilization in ruminants and especially reducing N and P output in excreta is desirable due to global concerns about agricultural contribution of N and P to environmental pollution, particularly as ammonia emissions or P losses (Knowlton *et al.*, 2001; Kebread *et al.*, 2002). Many published data (Kertz *et al.*, 1987; Daccarett *et al.*, 1993) suggest benefits of increasing dietary N to ruminants. In contrast, excessive feeding of N is problematic. Excessive feeding of N to ruminants results in increased faeces and urine N excertion (Wilkerson *et al.*, 1993; Hoffman *et al.*, 2001), which is an environmental concern, while P losses from livestock farms account for as much as 47% of P loading to bodies of surface water, depending on watershed (Smith and Alexander, 2000). Generally, P intake is also strongly correlated with P excretion in ruminant animals (Morse *et al.*, 1992) and significant overfeeding of P is common practice on ruminant farm areas (Ternouth *et al.*, 1996). Nevertheless, the linear decrease of faeces and urine N as the true protein concentration of the diet increased is due to increased efficiency of microbial utilization of the available N (Dewhurst and Thomas, 1992). Wright *et al.* (1998), Sutter and Beever (2000) and Haig *et al.* (2002), showed experimentally that addition of true protein to the rumen increased the amount of microbial N and by-pass protein entering the small intestine.

The objective of the study was to evaluate partitioning of N and P excretion in growing goats fed the synchronization of N and energy based on steamed oil palm fronds (OPF).

Materials and Methods

Animals and management

Twelve male dairy goats (Saanen), 3-4 months of age and average (±SD) body weight (BW) of 18.8±2.2 kg, were allocated in a 4×4 Latin square design (4 dietary treatments and 4 periods). The goats were housed in individual pens and allowed 3 weeks to adapt to experimental conditions during which the animals were treated against anthelmintics and intestinal parasites using 0.2 mg/kg Invermectin. Animals were fed the diets according to the NRC (1981) values for maintenance and gain (150 g/d). The metabolizable protein (ME) and crude protein (CP) requirements were calculated to be 9.1 MJ ME (3.6 Mcal/kg DM) and 83 g CP (15.0% CP).

The experiment was conducted to determine the supplementation of ruminally undegradable protein (RUP) of 0, 2, 4 or 6% (0%RUP, 2%RUP, 4%RUP or 6%RUP) of DM in the rations. The goats were offered total mixed rations (TMR): ingredients consisting steamed OPF mixed with concentrate (based on SBM, urea, cassava waste, molasses sugarcane, and commercial mineral and vitamin mixed) (Table 1). The diets were offered to the respective goats *ad libitum* twice daily at 0830 am and 1630 pm. Drinking water was freely available.

Experimental Procedure

The experiment consisted of four treatment periods, of 30 days each, three weeks of adjustment followed by 9 days of measurements. The latter consisted of 2 days of adaptation, 7 days of digestibility and N and P balance studies. During the digestibility trial, samples of feed refusal, faeces and urine were collected before feeding morning to determine digestibility and N balance.

Sampling Methods

Feed samples were collected twice a week. The daily fecal output of each animal was measured and a 10% sub-sample was collected and stored at -20°C. The samples were dried in a forced draught oven at 60°C, ground through 1 mm sieve and stored for chemical analysis. Daily urine output was collected into a plastic container (contained 25 ml of 10% H_2SO_4). Ten percent of the urine was later sampled and frozen and stored at -20°C until the analysis.

Chemical Analysis and Calculations

Representative samples of feed and faeces (collected during digestibility trial) were analyzed for dry matter (DM), ash, ether extract (EE), gross energy, Kjeldahl-N, phosphorus (P) (AOAC, 1985), and fiber components (Goering and Van Soest, 1970). Urine was analyzed for N and P. Apparent digestion coefficients were calculated using equations of Schnieder and Flatt (1975).

Statistical Analysis

The data were analyzed using the general linear model procedure of the Statistical Analysis System Institute SAS (1988). Treatment means were compared using Duncan's New Multiple Range Test and Orthogonal Contrast Analysis (Steel and Torrie, 1980).

Results and Discussion

Ingredients and chemical compositions of the feed are reported in Table 1. The ash, CP, and hemicellulose contents were similar in all treatments. As anticipated, NDF decreased with increasing levels of true protein substitution, because of the proportions of OPF in the rations.

Nitrogen intake tended to be increased ($p = 0.08$) with increasing levels of RUP supplementation (Table 2). Similarly to faeces N excretion, N absorption and retention (g/d) increased linearly ($p<0.01$, $p<0.05$ and $p<0.05$, respectively) as a consequence of additional of RUP supplementation. While urine N excretion decreased linearly ($p<0.01$) or quadratically ($p<0.05$) as a consequence of additional of RUP supplementation. Urine N was significantly ($p<0.05$) lower (4.07, 3.71 and 3.48 g/d) for goats fed 2%RUP, 4%RUP and 6%RUP as compared to the 0%RUP diet (4.92 g/d).

There were no differences between the treatments in P intake, P excretion or P absorption and retention (Table 2). However, P intake, P absorption (g/d, %) and P retention (%) tended to be increased linearly with increasing levels of RUP supplementation.

Intake of DM in terms of g/d tended ($p = 0.15$) to be increased with the additional of RUP supplementation. These differences were significant linearly ($p<0.01$) when DMI was expressed in terms of %BW and metabolic live weight. Similarly, BW gain increased linearly ($p<0.01$) or quadratically ($p<0.05$) as a consequence of additional of RUP supplementation. Dry matter digestibility was not affected by the treatments. However, the values tended ($p = 0.06$) to increase with increasing levels of RUP supplementation,

Table 1. Ingredient and chemical composition of the experimental diets (%DM basis)

	Dietary treatments			
	0%RUP	**2%RUP**	**4%RUP**	**6%RUP**
Ingredient				
Steamed OPF	38.1	34.7	31.4	29.7
Soybean meal	0.0	5.8	11.5	17.3
Urea	3.0	2.1	1.1	0.2
Cassava waste	45.0	44.7	44.8	42.5
Molasses	11.8	10.6	9.0	8.2
Salt	0.5	0.5	0.5	0.5
Sulphur	0.1	0.1	0.1	0.1
Commercial premix	1.5	1.5	1.5	1.5
Total	100	100	100	100
Chemical composition, %DM basis				
Ash	16.1	16.3	16.5	16.4
Crude protein	15.3	15.8	15.7	15.8
Neutral detergent fiber	49.2	48.4	47.6	48.3
Acid detergent fiber	23.4	22.6	22.8	22.5
Hemicellulose	25.8	25.5	24.8	25.8
Phosphorus	0.279	0.281	0.284	0.285
Calculated RUP supplementation	0.0	2.0	4.0	6.0

RUP = ruminally undegradable protein

Table 2. Daily N and P balances, dry matter intake (DMI), digestibility and body weight (BW) gain of dairy goats fed total mixed diet with different levels of ruminal undegradable protein (RUP)

	Dietary treatments					Contrast*	
	0%RUP	2%RUP	4%RUP	6%RUP	SEM	L	Q
N intake, g	17.85	20.00	20.54	20.96	0.06	0.08	ns
N excretion							
Faeces N, g	9.02[b]	10.80[ab]	11.29[a]	11.57[a]	0.359	0.01	ns
Urine N, g	4.92[a]	4.07[b]	3.71[b]	3.48[b]	0.152	0.01	0.05
Total N excretion, g	13.93	14.86	15.00	15.06	0.355	ns	ns
N absorption, g	8.83	9.20	9.25	9.39	0.330	ns	ns
N retention, g	3.92	5.13	5.54	5.91	0.376	0.06	ns
N retention, %	20.36[b]	23.69[ab]	26.59[ab]	28.03[a]	1.256	0.02	ns
P intake, g	2.03	2.22	2.30	2.36	0.070	0.09	ns
P excretion							
Faeces P, g	0.91	0.95	1.01	1.02	0.031	ns	ns
Urine P, g	0.69	0.64	0.64	0.67	0.022	ns	ns
Total P excretion, g	1.59	1.59	1.66	1.69	0.037	0.07	ns
P absorption, g	1.13	1.27	1.28	1.34	0.043	0.09	ns
P absorption, %	55.42	56.58	55.92	57.00	0.456	0.08	ns
P retention, g	0.44	0.63	0.64	0.67	0.049	ns	ns
P retention, %	19.64	25.92	27.28	27.86	1.522	0.08	ns
DMI,							
g	729.0	790.9	817.7	829.2	24.71	ns	ns
%BW	3.2[b]	3.9[ab]	4.0[a]	4.1[a]	0.05	0.01	ns
g/kg BW 0.75	76.4[b]	81.7[ab]	85.6[a]	85.9[a]	1.08	0.01	ns
Digestibility (%)							
Dry matter	53.1	53.5	53.8	54.1	0.19	0.06	ns
Crude protein	50.6[b]	54.8[a]	54.9[a]	54.9[a]	0.62	0.02	0.08
BW gain, g	69.1[c]	100.0[b]	127.4[a]	130.1[a]	5.19	0.01	0.04

[a, b, c]*Values on the same row under* **each main effect with different superscripts differ significantly ($p<0.05$).**
*** contrast effects (L = linear, Q = quadratic).**
SEM = standard error of means.
ns = not significantly different ($p>0.05$).

while CP digestibility increased linearly ($p<0.05$) or quadratically ($p<0.08$) as a consequence of the addition of increased RUP supplementation.

There was a *linear relationship between* proportion of RUP supplementation (%) and N intake (g/d) that can be expressed by the following equation: N intake = 18.39+0.47RUP; $R^2 = 0.06$. Nitrogen degradability had a major effect on urine N output because of excess soluble N in the rumen from diets of a high rapidly RDP (Tamminga, 1996; Castillo *et al.*, 2001). In addition, in the present study, urine N excretion decreased ($p<0.01$) with additional RUP from true protein substitution. The *linear equation between* RUP *(%) and urine* N excretion (g/d) of goats was Urine N = 4.74-0.233 RUP; $R^2 = 0.25$, ($p<0.001$) (Figure 1). The linear decrease of urine N as true protein (RDP and RUP) in the diet increased is due to increased efficiency of microbial capture of the available N, which contributes to increased amount of microbial N

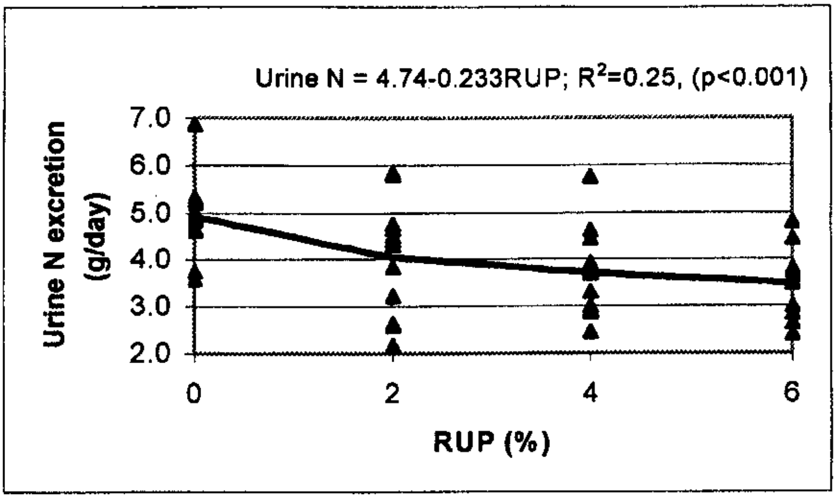

Figure 1. Relationship between ruminally undegradable protein (RUP, %) and urine N (▲) excretion of goats fed differences of RUP supplementation.

supply. The slight decrease in faeces N output as RUP increased was due to higher proportions of indigestible protein associated with RUP. Nevertheless, CP digestibility had a major effect on urine N output because of excess N in the rumen for diets with a higher proportion of rapidly of RDP (Kebreab *et al.*, 2002).

Phosphorus intake, absorption and retention tended to be increased as a consequence of additional of RUP supplementation. The linear equation between RUP (%) and P intake (g/d) of goats was P intake = 2.05+0.056RUP; ($p = 0.07$). Nitrogen and P retention also tended to be increased with increasing levels of RUP supplementation.

Regarding BW gain, it is well established that increasing levels of true protein as RUP source elevates BW gain. The present study also showed that BW gain increased with increasing the substitution of RUP from true protein. Especially supplementation of RUP at 4% and 6% gave significantly ($p<0.05$) higher BW ($p<0.05$) than fed 0%RUP and 2%RUP. However, at the two high levels of substitution, the results were not significantly different possibly due to the host animals not differ to received protein (amino acids and peptides) from microbial protein or RUP. Kempton *et al.* (1979) observed that the addition of a small amount of RUP increased DMI, growth rate and improved the efficiency of feed utilization in growing lamb fed low protein-cellulose diets. Crude protein digestibility gave positive significant linear response to the RUP substitution. This is in agreement with ørskov *et al.* (1974) who succesfully observed increasing CP digestibility of pelleted barley in lambs supplemented with dietary RUP. Lallo (1996) also found that the rate of CP and fibre digestion in ruminants supplemented with true protein was greater than in those supplemented with urea.

Conclusions

Supplementation of RUP from true protein for urea at 2, 4 and 6% of RUP in goats fed steamed OPF based diet, significantly improved in DMI, DM and CP digestibilities, N and P absorption (g/d) and retention. All the above values tended to be increased with increased RUP supplementation. However, the same parameters at the high two levels of the supplementation (4%RUP and 6% RUP) were not significantly different. Thus it is not necessary to substitute at the highest level (6% RUP) because of the cost of diets increased with increasing levels of RUP supplementation. Nevertheless, urine N decreased with increasing levels RUP supplementation. Thus goats fed 4%RUP could represent the optimum improvement in terms of animal performances, economic and reduction of urine N.

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