Impact of Ambient Temperature and Dietary Crude Protein in Wethers: Nitrogen Metabolism and Feed Efficiency

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ABSTRACT : Young lambs (Suffolk wethers, n=18), which were 22 to 26 kg average BW, were chronically exposed to temperatures of +1 to +4°C (cold) or +21 to +24°C (warm) during 10-wk experimental periods. The sheep were closely shorn and were housed in individual metabolism crates in controlled environment rooms. Sheep consumed pelleted diets *ad libitum*, which consisted of mainly barley grain and brome grass, and contained 7, 11, or 14% CP. The experimental design consisted of a 2×3 factorial with a single crossover of environment treatment. Feed intake, BW, feces, and urine excretion were measured. Apparent digestibilities were not affected by diet CP concentration or temperature treatments; however, voluntary intake per kg BW was increased ($p<0.05$) by diet CP content in both environments. Growing lambs gained weight slightly faster in a cold environment when N intake was above 27 g/d. Nitrogen excretion and N balance were positively related ($p<0.01$) with diet CP content, and fecal N excretion was significantly increased ($p<0.05$) in the cold environment. Therefore, dietary CP content strongly influenced N metabolism; however, cold exposure did alter only fecal N excretion. The higher DM intake per kg BW at 11% CP diet in the cold environment permitted ADG comparable to 14% CP diet in the warm environment. The results of this study do support the hypothesis that lambs are better able to utilize a moderate reduction in the CP content of the diet in a cold environment. *(Asian-Aust. J. Anim. Sci. 2001. Vol 14, No. 9 : 1221-1227)*

Key Words : Nitrogen Metabolism, Feed Efficiency, Cold, Sheep

INTRODUCTION

The imposition of cold stress on ruminants usually results in less efficient utilization of feedstuffs due, in part, to depressed digestibilities of DM and OM. However, ruminant animals appear to maintain a high rate of flow of non-ammonia nitrogen to the intestine in the cold. Also, cold exposure of ruminants usually results in faster rates of passage of digesta from the rumen and enhanced motility of reticulo-rumen (Christopherson, 1976; Kennedy et al., 1986). In cold ambient temperatures, there is reduced degradation of protein to ammonia in the rumen and increased escape of dietary N to the intestine. In severe cold, decreases in both animal production and feed efficiency have been observed (Young, 1981) as well as decreases in the extent of digestion of the diet (Christopherson, 1976). The growth rate of young ruminants exposed to cold environments can be reduced by about 12% by a lack of nutrients to meet both maintenance and growth requirements (Williams and Innes, 1982). Therefore, insufficient nutrient availability combined with an increased maintenance cost during periods of cold exposure may restrict animal productivity (McBride and Christopherson, 1984). In one study, more endogenous urea was shown to enter the rumen of cold-exposed sheep compared to that of warm-exposed sheep (Kennedy and Milligan, 1978). Therefore, animals in a cold environment may be better able to utilize a low CP diet compared to animals in a warm environment. To examine this question in the present study, measurements were made of N balance and digestibilities of DM, OM, and N at different dietary CP levels in lambs exposed to warm and cold environments.

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MATERIALS AND METHODS

Eighteen Suffolk wethers (initially 30 to 45 d old with BW ranging from 10 to 20 kg) were chronically exposed to temperatures of +1 to +4°C (cold) or +21 to +24°C (warm) during a 10-wk experiment. The sheep were shorn to a fleece depth of approximately 4-6 mm at the beginning of the experiment and after 2-wk of each 5-wk period. Animals were housed in individual metabolic crates in controlled environment rooms. During the temperature adaptation periods, sheep were accustomed to wearing urine collection funnels, which were attached by harness to the abdomen. Urine collection funnels and harness were carefully adjusted in order to promote comfort and to avoid stressing the animal. Animals were weighed weekly. The metabolic crates were designed for sheep involved in nutrient experiments.

Sheep were fed pelleted diets (table 1) containing 7, 11, or 14% CP *ad libitum*. Diets contained barley grain (*Hordeum vulgare*) and brome grass (*Bromus inermis Leyss*) which contained 8.4 and 4.8% CP, respectively. The vitamin, mineral, and energy contents of the diets were calculated to approximate the requirements of the sheep (NRC, 1985), based on BW at the start of the experiment.

Table 1. Composition and diet energy content of feeds fed to lambs

Ingredient (%)	DM	CP	Ration 1	Ration 2	Ration 3
			%		
Barley grain	89	8.4	61.78	52.16	44.95
Brome grass	92	4.8	37.72	37.72	37.72
Soybean meal	89	50	-	9.62	16.83
Vit.-Min. Mix.[1]			0.3	0.3	0.3
Trace Min. Salt[2]			0.2	0.2	0.2
CP (%)			7.0	11.0	14.0
DE (Mcal/kg)			3.03	3.05	3.06

[1] Vit-min. mix : 120 mg Zn, 13 mg Mn, 250 mg Fe, 10 mg Cu, 0.11 mg Se, 5500 IU Vit A, Vit D 550 IU, 25 IU Vit E, 13 mg riboflavin, 50 mg niacin, 30 mg calcium pantotenate, 301 g Vit B_{12}, 550 mg choline per kg.

[2] Trace mineral mixture (%) : 96.5 NaCl, 0.4 Zn, 0.16 Fe, 0.12 Mn, 0.0033 Cu, 0.007 I, 0.004 Co.

Water and cobalt-iodized salt blocks were also available *ad libitum*. Feed was weighed immediately prior to feeding and offered once daily at 10:00 at a rate of 10-15% in excess of the voluntary feed consumption of the previous day. Feed not consumed was collected and weighed back daily during the course of the experimental period in order to determine daily feed intakes.

The experimental design consisted of 2×3 factorial with a single crossover of environment treatments. The treatments were designated as W7, W11, W14, C7, C11, and C14 to denote warm-exposed (W) and cold-exposed (C) sheep, and 7, 11, and 14% CP diets, respectively. Nine sheep (three fed each level of CP) were exposed to each ambient temperature for 35 d, after which they were transferred to the alternative temperature treatment for 35 d. Total feces and urine output were collected once daily between 10:00 and 11:00. The daily collection for each sheep was mixed and weighed, and a 10% sub-sample was dried in an forced-air oven for 48 h at 60°C. The dried samples were finely ground using a Christie-Norris grinder. Urine samples were collected in a bucket containing 25 mL of 25% HCl solution in order to protect against bacterial contamination, to prevent loss of free ammonia, and to maintain the pH below 2.0. Daily samples were combined to form a composite sample for each sheep for each digestion trial prior to proximate analysis. Samples of the diet and any feed refusals were collected daily.

Nitrogen contents of feed, feces, and urine samples were determined by a Kjeldahl procedure (AOAC, 1980). Organic matter was determined on dry samples by ignition in a combustion furnace at 550±50°C overnight. Average daily gain was calculated from weight change weekly during each 35-d period. When significant treatment effects were observed, the Student-Newman-Keuls test was used to test for significance of differences between means (Steel and Torrie, 1980). Linear and quadratic relationships, with computation of correlation coefficients, slopes with their standard errors, and intercepts were conducted.

RESULTS

There were no significant differences ($p>0.05$) in intakes of feed, DM, and OM among the treatments (table 2). However, there were positive relationships between DM and OM intakes and diet CP content, with the highest intakes occurring for the 11% CP diet during cold exposure. In both environments, there was a small negative relationship between DM digestibility and dietary CP content. Although somewhat lower DM and OM digestibilities were observed for the three diets in the cold environment compared to those of the warm environment, the effects of diet and temperature were not significant. Diet CP content significantly affected apparent N digestibility ($p<0.01$) with the highest apparent N digestibility observed in the 14% CP diet in both environments.

Average DMI per unit BW increased with increasing diet CP content, resulting in a positive ($p<0.05$) linear relationship in both environments (table 2). The DMI per kg of BW was also higher in the cold environment when the 11% CP diet was fed. Mean BW was not different among treatments.

Average daily gain was increased ($p<0.10$) with increasing dietary CP levels. Weight gain was depressed in both environments when the 7% CP diet was fed. The cold-exposed animals gained faster than the warm-exposed animals above a N intake of about 27 g/d (fig. 1). However, below this point, cold-exposed animals gained more slowly. Also there was a significant ($p<0.05$) difference in the regression coefficients between the warm and cold environments. The OM intake linearly affected ADG in the cold environment but there was a curvilinear relationship in the warm environment (fig. 2). The ADG for the warm-

Table 2. Feed intake and digestibilities in the young lambs fed three different CP levels diet in a warm or cold environment

Diet CP (%)	Warm			Cold			SEM
	7	11	14	7	11	14	
Intake, g/d							
Total	1,114	1,173	1,389	1,131	1,472	1,376	149
DM	1,028	1,088	1,283	1,044	1,365	1,272	141
OM	972	1,027	1,206	986	1,289	1,195	133
Digestibilities, %							
DM	65.1	65.5	64.6	63.9	61.4	62.0	1.48
OM	68.4	69.3	68.3	67.1	64.9	65.7	0.88
Apparent	51.1^{ab}	58.6^{c}	66.3^{d}	48.2^{a}	54.0b	63.8^{d}	1.24
BW, kg	22.9	23.3	24.3	22.1	26.6	24.2	2.42
ADG, g/d	206^{a}	266^{ab}	279^{ab}	195^{a}	311^{b}	302^{b}	22.8
Feed/gain, g/d	5.53	5.06	4.99	5.70	4.66	4.61	0.54
Feed/BW, g/kg	48.6^{a}	50.4^{a}	57.2^{b}	51.3^{a}	55.3b	56.9^{b}	2.12

^{a,b,c,d} Means in the same row that do not have a common superscript differ ($p<0.05$). Mean values with six replications.

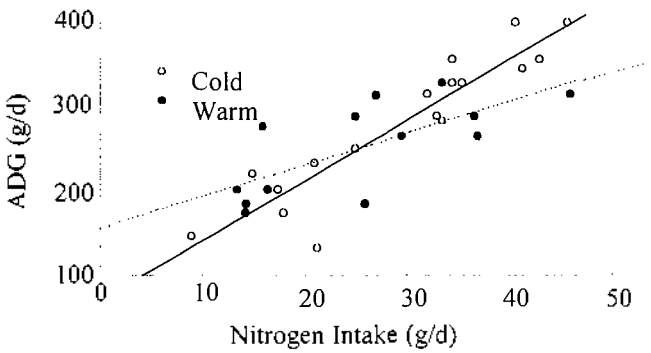

Figure 1. The relation between average daily gain and nitrogen intake in the cold environment (○), (ADG)= 70.90+6.91(N intake), (R^2=0.86), and in the warm environment (●) (ADG)=155.45+3.61(N intake) (R^2=0.50)

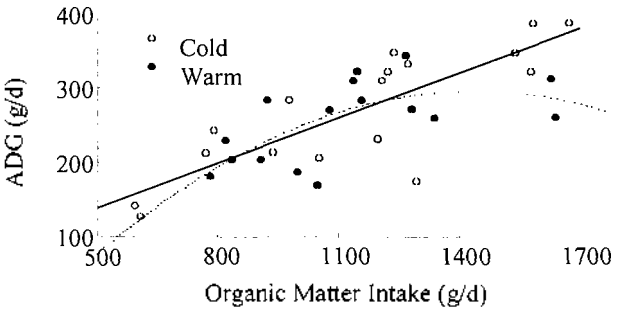

Figure 2. The relation between average daily gain and OM intake in the cold environment (○), (ADG)=37.58+0.20 (OM intake) (R^2=0.68), and in the warm environment (●), (ADG)=-220.83+0.72 (OM intake)-0.0002 (OM intake)^2 (R^2=0.43).

exposed sheep plateaued at about 290 g/d. Feed to gain ratios were inversely related to diet CP content in both environments, and there was no significant difference between the cold and warm environments.

The parameters of N metabolism are shown in table 3. Nitrogen intake, fecal N, urinary N excretion, N retention, and apparent N absorption were all positively related ($p<0.01$) to diet CP content, but they were not significantly influenced by temperature except for fecal N excretion, which was higher ($p<0.05$) in the cold environment for the 11% CP diet. Nitrogen intake substantially increased from 13.7 to 21.5 and 32.0 g/d in the warm and from 13.9 to 27.2 and 31.8 g/d in the cold as diet CP content increased from 7 to 11 and 14%. There was no significant difference in N intake between cold and warm temperature treatments, respectively. However, the daily N intake of the 7% CP diet was significantly ($p<0.01$) depressed compared with that of 11% and 14% CP diets in both environments.

Fecal N output was significantly ($p<0.05$) lower in the warm compared to the cold environment based on regression analysis. A significantly greater amount of fecal N was excreted in the C11 treatment (12.5 g/d) compared to that of the W11 treatment (8.9 g/d). The relationship between fecal N output and N intake is shown in figure 3. There was a linear relationship between fecal N and N intake in both warm- and cold-exposed animals. The amount of N apparently digested was increased in response to increasing CP % in the diet, but there were no significant differences due to ambient temperature.

Table 3. Nitrogen metabolism in the young lambs fed three different CP levels diet in a warm or cold environment

Diet CP (%)	Warm			Cold			SEM
	7	11	4	7	11	14	
N intake, g/d	13.7^{a}	21.5^{ab}	32.0^{b}	13.9^{a}	27.2^{b}	31.8^{b}	2.80
g/kg of $BW^{0.75}$	1.21^{a}	1.78^{ab}	2.67^{c}	1.28^{a}	2.10^{bc}	2.63^{c}	0.23
Fecal N, g/d	6.7^{a}	8.9^{ab}	10.8^{abc}	7.2^{a}	12.5^{c}	11.5^{bc}	0.52
g/kg of $BW^{0.75}$	0.59^{a}	0.74^{a}	0.90^{b}	0.66^{a}	0.97^{b}	0.95^{b}	0.17
% of N intake	48.9^{d}	41.4^{bc}	33.8^{a}	51.8^{d}	46.0^{cd}	36.2^{ab}	2.57
N Digestibility, g/d	7.0^{a}	12.6^{b}	21.2^{c}	6.7^{a}	14.7^{b}	20.3^{c}	1.64
g/kg of $BW^{0.75}$	0.62^{a}	1.04^{b}	1.66^{c}	0.62^{a}	1.14^{ab}	1.68^{c}	0.21
Urinary N, g/d	2.9^{a}	5.9^{ab}	9.8^{b}	3.5^{a}	6.4^{ab}	8.7^{b}	1.07
g/kg of $BW^{0.75}$	0.26^{a}	0.45^{a}	0.82^{b}	0.32^{a}	0.49^{a}	0.72^{b}	0.04
% of N intake	21.2^{a}	27.4^{bc}	30.6^{c}	25.2^{ab}	23.5^{ab}	27.4^{bc}	1.78
Total N excretion, g/d	9.6^{a}	14.8^{ab}	20.5^{b}	10.8^{a}	18.9^{b}	20.2^{b}	1.44
g/kg of $BW^{0.75}$	0.85^{a}	1.23^{ab}	1.71^{c}	0.99^{a}	1.46^{bc}	1.67^{c}	0.14
% of N intake	70.1^{b}	68.4^{b}	64.1^{a}	77.7^{c}	69.49^{b}	63.52^{a}	2.06
N retained, g/d	4.0^{a}	6.7^{ab}	11.5^{b}	3.2^{a}	8.3^{ab}	11.6^{b}	1.67
% of N intake	29.2^{ab}	31.1^{bc}	35.9^{c}	23.0^{a}	30.5^{b}	36.5^{c}	3.26
% of N digested	57.1^{b}	53.2^{ab}	54.2^{b}	47.8^{a}	56.5^{b}	57.1^{b}	4.15

^{a,b,c,d} Means in the same row that do not have a common superscript differ ($p<0.05$). Mean values with 6 replications.

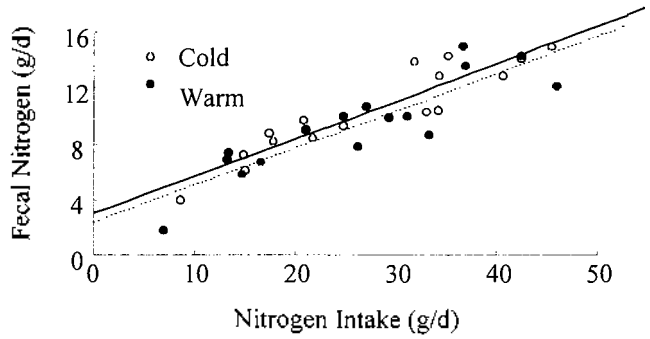

Figure 3. The relation between fecal nitrogen excretion and nitrogen intake in the cold environment (○) (Fecal N)= 2.99+0.27 (N intake) (R^2=0.86), and in the warm environment (●) (Fecal N)=2.39+0.26 (N intake) (R^2=0.78)

Urinary N excretions were not significantly different across environmental treatments (table 3). Approximately 20 to 30% of the N intake was excreted into the urine. Urinary N excretion was numerically increased in a curvilinear fashion as a result of increasing N intake in both environments (fig. 4). The amount of N retained was directly related to the CP content of the diet in both environments. In the warm environment, as CP content increased, the N retained as a percentage of N absorbed was decreased from 57.1 to 54.2, whereas the percentage was increased from 47.8 to 57.1 in the cold temperature treatment. The relationship between N intake and N balance is shown in fig. 5. The regression coefficients did not differ between warm and cold environments.

DISCUSSION

The voluntary feed intake (VFI) expressed per unit BW was 9.7% higher ($p<0.05$) for 11% diet in the cold compared to the warm environment. This result is consistent with other reports that VFI often increases for ruminants fed *ad libitum* in a cold environment (Webster et al., 1970; Minson and Ternouth, 1971; Baile and Forbes, 1974). Minson and Ternouth (1971) reported that the VFI was increased 5 to 13% in shorn sheep fed a concentrate diet at ambient temperatures near 13°C. Also, hay intake of growing heifers increased by approximately 21% during cold exposure (Webster et al., 1970). The VFI should increase to a greater extent at temperatures below the lower critical temperature (LCT). Christopherson (1985) reported that the LCT was around 4°C in the growing lambs. In the present study, young lambs were exposed to temperatures of 2°C. Therefore, the degree of cold stress experienced by the lambs in this study was not very severe. However, the 7% CP diet was expected to be below the optimal CP level for growth of the lambs and might have made these lambs more susceptible to cold stress. Increased feed intake when it

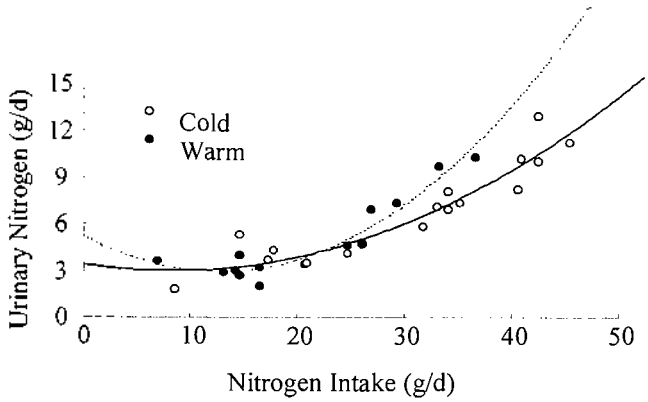

Figure 4. The relation between urinary nitrogen excretion and nitrogen intake in the cold environment (○) (Urinary N)=5.28-0.36 (N intake)+0.014 (N intake)^2 (R^2=0.93), and in the warm environment (●) (Urinary N)=3.45+0.11 (N intake)+0.006 (N intake)^2 (R^2=0.89).

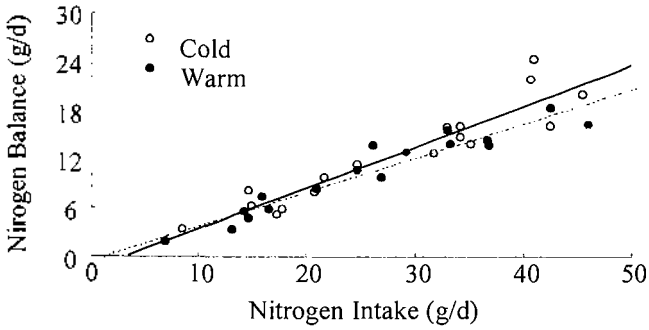

Figure 5. The relation between nitrogen balance and nitrogen intake in the cold environment (○), (N balance) =-1.56+0.50 (N intake) (R^2=0.87), and in the warm environment (●) (N balance)=-0.46+0.42 (N intake) (R^2=0.91)

occurs may be partly due to a faster rate of passage of residues through the digestive tract and increased energy demand of a cold environment (Baile and Forbes, 1974; Christopherson, 1976).

Diet source and type may affect the VFI. In this study, sheep were fed a pelleted diet that was composed mainly of brome grass (37.7%), barley grain (from 45.0 to 61.8%), and soybean meal substituted for barley at a rate of 9.6 and 16.8% in the 11 and 14% CP diets. Norton et al. (1982) reported that the addition of readily digestible carbohydrate such as barley to ruminant diets usually increases digestible OM intake. Kennedy (1985) reported that there was a 13% increase in VFI of chopped hay but no significant increase in VFI of ground and pelleted diets in the cold. Chai et al. (1985) reported that VFI increased 10% at 10°C and 27% at -5°C. For those diets showing an increased VFI, the quantitative availability of nutrients may be enhanced in the

cold (Kennedy and Milligan, 1978; Dixon and Milligan, 1984; Egan et al., 1986).

The small non-significant decreases in DM and OM digestibilities agree with the results of several previous studies (Moose et al., 1970; Christopherson, 1976). In the present experiment the diet contained 62% concentrate. Although the apparent digestibility was depressed for 50% and 70% concentrate diets fed to cold-exposed steers and lactating ewes (Christopherson, 1976), several studies have suggested that the effect of temperature may not occur with high concentrate diets (Kennedy et al., 1982; McBride and Christopherson, 1984a, b; Williams and Innes, 1982).

Apparent N digestibility was increased ($p<0.01$) with increasing diet CP likely because soybean meal containing a higher quality protein was substituted for barley grain. In addition, the contribution of endogenous N as a proportion of total fecal N is likely larger for the low CP diet, resulting in a lower apparent digestibility. Even though there may be increased heat production and decreased digestibilities in the cold (Christopherson, 1976; Young, 1981), these may be compensated by increased intakes of N and energy in the cold environment. In the present study, ADG was enhanced due to increased diet CP content likely because of the higher OM intakes. It is possible that a high urea recycling rate in the C14 treatment also helped to maintain N supply and urea recycling (Webster, 1974) and, at the same time, helped to maintain the growth rate of the animals in the cold. Average daily gain was affected more by diet CP content than temperature treatments in the present experiment and was depressed for the 7% CP diet in both environments.

The steeper relationship between ADG and N intake in the cold environment indicates that the lambs were able to achieve a higher rate of gain in the cold than in the warm environment but only when the dietary CP content was 11% or higher and N intake was above 27 g/d (fig. 1). However, below this point, cold exposure depressed the growth rate. In addition, OM intake up to 1,400 g/d appeared to linearly affect animal growth in the cold environment. On the other hand, the ADG plateaued at around 290 g/d at OM intakes above 1,200 g/d in the warm environment (fig. 2). The lambs fed the 7% CP diet may have been more stressed by cold because of the lower intake, whereas the higher intakes of the 11 and 14% CP diets may have improved their cold tolerance sufficiently to permit faster growth. The growth rate of young ruminants exposed to cold environments can be limited by a lack of nutrients and insufficient nutrient availability to meet both maintenance and growth requirements (Gibb and Penning, 1972; Young, 1981; McBride and Christopherson, 1984b). The present results also support the result of Wellard and Hume (1981), who observed increased ADG when daily soybean meal supplementation was increased from 0.45 to 0.68 kg per head. Also, a high level of protein supplementation resulted in increased ADG in calves fed soybean meal (Orskov and MacLeod, 1986; Davenport et al., 1987).

The increase in fecal N excretions with diet CP are consistent with results of other studies (Kelly and Christopherson, 1989; Kennedy and Milligan, 1978). The nitrogen values are somewhat higher than the results of several other studies in sheep fed a variety of roughages (Egan and Ulyatt, 1980; Mousa et al., 1983). The high values in the present study may have been affected by microbial protein formed in the large intestine from undigested dietary N compounds and OM of the barley grain and soybean meal. Also there is a possibility of heat damage during the pelleting process.

Estimates from the data of the present experiments with sheep give a fecal excretion value of approximately 0.55 to 0.73 g N per 100 g DM intake when diet CP content was increased from 7 to 14%. This result agrees with Maynard and Loosli (1962) suggestion for ruminants of a metabolic fecal excretion factor of 0.5 g N per 100 g DM and for sheep (0.53 g N per 100 g DM) (NRC, 1985). Fecal N excretion was significantly higher ($p<0.05$) in the cold environment. This might have been due, in part, to increased escape of dietary N from the rumen to the intestine in cold-stressed sheep (Kennedy and Milligan, 1978; Kennedy et al., 1982) but might also have been due to the higher N intake on the 11% CP diet.

The present results agree with the report of Bunting et al. (1987, 1989) in which the N retention was 4.1 and 9.7 g/d for intakes of 12 and 21 gN/d, respectively, in calves. About 26.9 and 16.2% of intake N were retained in these treatments. Similarly, Mousa et al. (1983) observed a concomitant increase in N balance in sheep and goats when they were fed high CP diets. When combinations of low-quality roughages in high concentrate pelleted diets were fed to sheep, about 20% of N intake was retained (Kinser et al., 1988). However, there was low N retention (-1.0 and 2.3 g/d) in roughage-fed sheep consuming 21 and 33 gN/d, respectively (Egan and Ulyatt, 1980). In addition, as much as 34 and 46% of intake N was retained in calves fed roughage diets (Bunting et al., 1987). Probably, the barley-containing diet in the present experiment greatly increased N retention in the lambs. This might have been partly due to a high proportion of urea recycling, which would be favored by the supply of readily fermentable OM in the rumen. In addition, the higher digestible energy supply to the animal would promote body growth and N retention.

Lowering diet CP within certain limits to animals in a cold environment appears to be beneficial. Animals in cold maintained as high a level of intake at 11% CP as the warm animals did at 14% CP. The benefits in the cold environment include reduced feed cost (since CP is expensive) and also reduced N excretion per kg of growth

(thus providing benefit for the environment).

In conclusion, diet CP content was the predominant factor affecting the overall nitrogen retention in young lambs. This suggests that any increased energy requirement in the cold was satisfied by metabolism of substrates other than protein or amino acids. The principal energy substrates would include lipids and volatile fatty acids. The results of this study do support the hypothesis that lambs are better able to utilize a moderate reduction in the CP content of the diet in a cold environment.

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