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THE UNIVERSITY OF ALBERTA

UREA RECYCLING AND DIGESTIBILITY  
IN WETHERS EXPOSED TO DIFFERENT AMBIENT TEMPERATURES  
AT THREE DIET LEVELS OF CRUDE PROTEIN

by

SANG SOO SUN

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH  
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE  
OF MASTER OF SCIENCE

IN

ANIMAL PHYSIOLOGY

DEPARTMENT OF ANIMAL SCIENCE

EDMONTON, ALBERTA

FALL 1988

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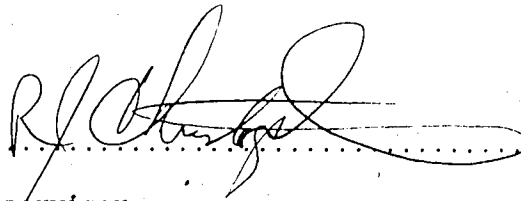
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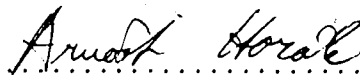
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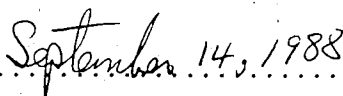
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## ABSTRACT

Eighteen Suffolk wethers, which were 22-26kg average body weight, were chronically exposed to temperatures of +1 to +4°C (cold) or +21 to +24°C (warm) during 10-week experimental periods. The sheep were closely shorn and were housed in individual metabolic crates in controlled environment rooms. Sheep were fed ad libitum pelleted diets, which consisted of mainly barley grain and brome grass, and contained 7, 11 or 14% crude protein (CP). The experimental design consisted of a 2 x 3 factorial with a single crossover of environment treatment. Animals were catheterized via one jugular vein with a PVC catheter and received a single injection of 60 - 65  $\mu\text{Ci}$  of [ $^{14}\text{C}$ ]urea. Feed intake, body weight, feces and urine excretion, plasma urea-N (PUN), urinary urea-N (UUN) and carbon specific activity were measured. Apparent digestibilities were not affected by diet CP content nor temperature treatments, however, voluntary intake per kg body weight was increased ( $P < 0.05$ ) by diet CP content in both environments. Growing lambs gained weight slightly faster in a moderately cold environment when N intake was above 27 g d $^{-1}$ . N 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. Overall urea metabolism was not affected by environment. Percent urea recycling and urea space clearance were highest ( $p < 0.05$ ) on the low nitrogen diet. Urea pool was increased ( $p < 0.10$ ) for the 14% CP diet. Urinary urea-N and plasma urea-N

concentration were positively related ( $p < 0.01$ ) with diet CP content. Therefore, dietary CP content strongly influenced N and urea metabolism, however, cold exposure did not alter those parameters except for fecal N excretion.

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## INTRODUCTION

Nitrogen metabolism in the rumino-reticulum may vary considerably due to dietary and environmental influences. Many studies with ruminants have demonstrated an increase in urea recycling under conditions of low dietary nitrogen intake, or have examined relationships between urea recycling and growth in circumstances of low protein intake (Cocimano and Leng, 1967; Houpt and Houpt, 1968; Nolan and Leng, 1972; Prior et al., 1974; Robbins et al., 1974; Emmanuel et al., 1976; Norton et al., 1978; Obara and Shimbayashi, 1980; Mousa et al., 1983; Chilcott et al., 1985).

The rumen has been assumed to be the principal site of appearance of recycled urea in the digestive tract; however, urea is also recycled to other parts of the digestive system. Urea is not the only small N-containing molecule returned to the tract in this way, but it is by far the most significant, quantitatively. The gastrointestinal tract plays an important role in the degradation of various other endogenous nitrogenous substances, including enzyme secretions, mucous, amino acids and epithelial cells desquamating from the digestive tract mucosa. The recycling of urea to the digestive tract of ruminants and its degradation to provide ammonia for microbial metabolism has been proposed as a mechanism whereby ruminants conserve nitrogen during periods of protein deprivation (Houpt, 1970). The amount of endogenous urea which passes into the gastrointestinal lumen is considerable and represents a large percentage (40 - 60%) of the total daily amount of urea synthesized

in the ruminant body. Ammonia concentrations in rumen fluid have been inversely correlated with urea recycling to the rumen. High proportional degradation of dietary N is usually associated with a low rate of urea recycling, low blood urea concentration and low N intake (Cocimano and Leng, 1967; Ford and Milligan, 1970). The amount of urea degraded in the rumen increases with increasing plasma urea concentration and increasing rate of urea production. In domestic ruminants given a nitrogen deficient ration, urea excretion in the urine is suppressed with the urea being differentially transferred to the digestive tract and converted to microbial protein (Schmidt-Nielsen et al., 1957; Cocimano and Leng, 1967).

The imposition of cold stress on ruminants usually results in less efficient utilization of feedstuffs due, in part, to depressed digestibilities of dry matter(DM) and organic matter(OM). However, ruminant animals appear to maintain a high rate of flow of non-ammonia nitrogen (NAN) 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 the rumino-reticulum (Christopherson, 1976; Kennedy et al., 1986a,b). 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 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 crude protein diet compare to animals in a warm environment. To examine this question in the present study



measurements were made of urea recycling and flux, nitrogen balance and digestibilities of DM, OM, and N at different dietary crude protein (CP) levels in lambs exposed to warm and cold environments.

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## LITERATURE REVIEW

### ANIMAL METABOLISM AND THERMOREGULATION

When environmental temperature drops below the lower critical temperature (LCT), the metabolic rate of animals is increased due to hormonal and sympathetic nervous activity in order to compensate for increased heat loss (Webster et al., 1969; Christopherson and Kennedy, 1983). The cold-exposed animal tries to minimize heat loss and may enhance heat production in order to maintain a state of thermal equilibrium (Webster, 1974) and at the same time, the maintenance requirement of the animal increases (Young and Christopherson, 1974). 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 (Gibb and Penning, 1972; 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, 1984b).

### VOLUNTARY FEED INTAKE AND DIGESTIBILITY

Voluntary feed intake will differ depending on the food species and type (Thornton and Minson, 1973; Moseley, 1981), and may be influenced by digesta passage rate in a cold environment (Baile and

Forbes, 1974). Many studies have concluded that changes in gut motility, rumination activity and passage of feed particles from the rumen may contribute to a changed feed intake during cold stress (Weston, 1970; Minson and Ternouth, 1971; Christopherson and Kennedy, 1983).

In sheep there was a 13% increase in the voluntary feed consumption (VFC) of chopped hay, but there were no significant increases in the VFC of ground and pelleted diets (Kennedy, 1985) during exposure to a cold environment. Norton et al. (1982b) reported that the addition of readily fermentable carbohydrate such as barley or maize to ruminant diets usually has the effect of increasing digestible OM intake (Chamberlain et al., 1980). In another study increased protein absorption in the intestine was accompanied by increases in VFC (Egan, 1977).

Increased feed intake may be due to the faster rate of passage of residues through the tract and the increased energy demand in a cold environment (Baile and Forbes, 1974). Also, Weston (1982) suggested that VFC increases in the cold temperature is a result of changes in digesta load in the rumen in response to the energy deficit caused by increased thermal loss to the environment. Minson and Ternouth (1971) reported that the VFC was increased 5-13% in shorn sheep at ambient temperatures near 13°C. Also, Webster et al. (1970) reported that hay intake of growing heifers increased by approximately 21% during exposure to the cold environment. Therefore, change in VFC is an important response by ruminants to environmental temperature.

The digestibility of forage diets is generally decreased (NAS-NRC, 1981) in a cold environment (Christopherson and Thompson, 1973; Baile and Forbes, 1974; McBride and Christopherson, 1984b). In general, apparent dry matter(DM) digestibility of forages was reduced by approximately 0.2 percentage digestibility units per degree ( $^{\circ}\text{C}$ ) drop in ambient temperature. This occurs even when the level of food intake remains constant across environments. Previous studies on the effects of cold exposure on organic matter (OM) and nitrogen digestion in the rumen and postruminal tract of sheep (Kennedy et al., 1976; Kennedy and Milligan, 1978a) have attributed the decline of OM and N digestibilities in the gut, observed during cold exposure, to increased rumino-reticulum motility with a consequent reduction in turnover time and digestion of dietary material in the rumen. In addition, in the 50% and 70% concentrate diets fed to cold exposed steers and lactating ewes, the apparent digestibility was depressed (Christopherson, 1976; McBride, 1983). Reduced retention time in the cold environment is apparently responsible for the depression of digestibility (Westra and Christopherson, 1976; Kennedy et al., 1976; Kennedy and Milligan 1978a). In spite of a small decrease in digestibility, the total availability of nutrients may be enhanced (Kennedy and Milligan, 1978a) if food intake increases in the cold.

However, in several studies there were no significant differences in the apparent digestibility due to the cold environment (Moose et al., 1970; Christopherson, 1976). Some studies indicated that the digestion of high concentrate diets is not influenced by

temperature (Young and Degen, 1981; Kennedy et al., 1982; Williams and Innes, 1982; McBride and Christopherson, 1984b). This is because the fermentation rate of concentrates is rapid, and a small change in retention time is likely to result in negligible changes in OM digestion (Kennedy et al., 1982).

In forage fed ruminants, there is an inverse relationship between dry matter intake and apparent digestibility of dry matter (Brown, 1966). This has been explained in terms of slower rates of digesta passage through the gut, particularly the rumen, at reduced feed intakes, allowing more time for microbial digestion of refractory constituents such as fiber (Balch et al., 1953). Therefore, animals which increase their voluntary feed intake in a cold environment may experience a further depression in digestibility (Kennedy and Milligan, 1978a).

#### **NITROGEN METABOLISM**

N transformations in the ruminant are modified by the environment (Christopherson and Kennedy, 1983) and diet composition (Preston et al., 1965). Between the digestive tract and the tissue protein and amino acid pool of the organism there is continuous exchange of nitrogen metabolites (Egan et al., 1986). Apart from its main physiological function, which is the breakdown of forage nutrients and subsequent absorption, the gastrointestinal tract plays an important role in the breakdown of various endogenous N substances, including plasma proteins, amino acids, sloughed epithelial cells from the digestive tract mucosa and urea (Boda et al., 1976).

The N supply to the rumen is important for maintaining the viability of the microbial population and its ability to carry out fermentation contributing ultimately to the energy and amino acid supply of the host ruminant. The importance of the N supply to the rumen is illustrated by studies with lambs, in which Ørskov et al. (1972) obtained increased fermentation of organic matter (OM) in the rumen and a 7% increase in non-ammonia-N (NAN) flow when a barley diet was supplemented with urea to give 124g CP per kg.

The ruminant animal derives its protein and amino acid supply from the digesta flowing through the abomasum into the small intestine where amino acid absorption takes place. The N in the abomasum originates from different sources. A major fraction consists of microbial protein formed during the fermentation of carbohydrate in the rumen, while a second major fraction of varying proportion consists of undegraded dietary protein. The relative sizes of the fractions depend largely on the rumen degradability of the dietary protein (Ørskov, 1982). A third fraction derives from endogenous protein contained in enzymes secreted into the abomasum and from epithelial cells desquamating from the respiratory tract, mouth, oesophagus and rumen wall. The third fraction is also subject to partial degradation by microbes associated with rumen ingesta and by bacteria adherent to the rumen wall before entering the abomasum (Wallace et al., 1979). However, quantitative measurements of these transformations have always presented difficulties. A fourth N fraction consists of urea recycled from the blood into the fore-stomach (Kennedy and Milligan, 1980; Mousa et al., 1983).



About two-thirds of the endogenous N in the abomasum is derived from the rumen in steers given protein-free nutrients (Ørskov and MacLeod, 1986). They have estimated that about  $1.2 \text{ g d}^{-1}$  of endogenous N in sheep is included in the total abomasal N. Therefore this quantity is often subtracted from the abomasal N flow in order to calculate the undegraded dietary fraction.

Dixon and Nolan (1982) and Beever et al. (1986) obtained maximum NAN flow through the duodenum when 10-20g urea were added to a low-N diet of oat straw and concentrates. The kinetics of nitrogen(N) in the large intestine of sheep is based primarily on measurements of the concentration of nitrogenous constituents of digesta and their net disappearance, either between the ileum and rectum or within parts of the large intestine.

Nolan et al. (1976) and Dixon and Nolan (1983) reported measurements of nitrogen kinetics in the large intestine of sheep fed a maintenance level of alfalfa hay with a high N content. Those studies showed that production of ammonia in the large intestine by proteolysis, deamination and urea hydrolysis was considerable and that absorption of ammonia was substantially greater than the apparent digestion of total N between the ileum and the rectum. Microbial N constituted about half of the non-urea, non-ammonia-N (NU-NAN) in digesta flowing from the caecum and most of this microbial N was excreted in the feces. A lower N intake ( $10 \text{ gN d}^{-1}$ ) by sheep given bromegrass (Dixon and Milligan, 1984) compared to the  $23 \text{ gN d}^{-1}$  intake by sheep given alfalfa (Dixon and Nolan, 1983) was associated with substantially smaller absolute flows of N

within the caecum and proximal colon.

Metabolic fecal N for rats, pigs and man is commonly estimated to be 0.1 gN per 100g dry matter intake. For ruminants a factor of 0.5 gN per 100g DM is used with normal diets and this is reduced when rations low in roughage are fed (Maynard and Loosli, 1962). Thus metabolic fecal N in ruminants fed highly digestible diets appears to be slightly lower than that suggested for ruminants given regular diets.

N retention was significantly lower in cold-exposed animals suggesting that environmental factors may have an important influence (Christopherson and Thompson, 1973; McBride and Christopherson, 1984b).

## UREA METABOLISM

### A. PLASMA UREA-N:

In ruminants under a normal feeding regime, the plasma urea-N (PUN) concentration primarily depends on dietary protein (nitrogen) content and its quality. Therefore, low diet protein content is of great ecological significance. Values for plasma urea-N concentrations were low ( $7 - 20 \text{ mg } 100 \text{ mL}^{-1}$ ) in cattle on low protein diets (Vercoe, 1969, 1971; Thornton, 1970c). With increasing nitrogen intake, PUN increases, but then plateaus at about  $30 \text{ mg } 100 \text{ mL}^{-1}$  (Thornton, 1970b; Thornton and Wilson, 1972; Godwin and Williams, 1984; Engelhardt et al., 1984). Preston et al., (1965) has shown that plasma urea-N (PUN) did not increase above  $33 \text{ mg } 100 \text{ mL}^{-1}$  in sheep fed increasing levels of crude protein, indicating that there is a physiological limit to PUN levels. Also, McIntyre (1970)

reported similar results when sheep were fed roughage diets with increasing nitrogen intakes from 6.8 to 46.3 g d<sup>-1</sup>. On the other hand, a high energy diet (106 gN d<sup>-1</sup>) decreased plasma urea-N from 10.5 to 4.6 mM in steers (Huntington, 1987).

#### B. UREA SPACE:

As dietary N intake increased, urea pool size, urea entry rate, and urea excretion rate also increased. Cocimano and Leng (1967) reported a decrease in urea space (55 to 100% in sheep as the protein content of the diet decreased (27% to 4% ), and calculations from the data of Mugerwa and Conrad (1971) show that urea space in cattle given high nitrogen diets represented from 85 to 108% of body weight.

#### C. UREA EXCRETION:

The urine is the principle route of urea-N excretion and, in ruminants, has received considerable attention after the studies which demonstrated a renal conservation mechanism for urea in camels (Schmidt-Nielsen et al., 1957) and sheep (Schmidt-Nielsen and Osaki, 1958) given low protein diets. The significance of renal urea conservation depends on the assumption that endogenously produced urea not excreted in urine is degraded in areas of microbial activity in the digestive tract, where the ammonia is converted to amino acids for absorption and utilization by the ruminant animal (Cocimano and Leng, 1967). With increasing nitrogen intake from 6.8 to 46.3 gN d<sup>-1</sup> in sheep fed roughage diets, urinary urea excretion maintained a linear increase in response to N intake (McIntyre, 1970; Thornton, 1970b; Thornton and Wilson, 1972; Godwin and Williams, 1984). On the other hand, a high energy diet decreased urinary urea-N excretion in

steers (Huntington, 1987). Also, values for urinary urea excretion were low and within the range of values found in other studies with cattle on low protein diets (Vercoe, 1969,1971; Thornton, 1970a).

#### **D. UREA RECYCLING:**

##### **a. RUMINO-RETICULUM UREA TRANSFER:**

The interconversion of endogenous urea-N among liver urea, blood urea, rumen ammonia, microbial N, and absorbed amino acids, contributes greatly to the nitrogen economy of ruminants. Endogenous urea provided 29% of N available from dietary and endogenous urea sources in sheep in the warm, compared to 41% for cold-exposed sheep given equal intakes of brome grass pellets (Kennedy and Milligan, 1978b).

Two pathways, which are salivary secretion and direct diffusion through the rumen wall, have been considered to be the major sites for the entry of blood urea into the rumen. The direct diffusion through the rumen wall was suggested to be the principal pathway (Juhasz, 1965; Houpt and Houpt, 1968). On the other hand, Nolan and Leng (1972) suggested that the main pathway for the appearance of urea in the rumen was not direct diffusion through the rumen wall, but salivary secretion. However, the latter findings do not coincide with those of other workers (Kennedy and Milligan, 1978b). Most evidence suggests that its passage across the rumen wall occurs by simple diffusion (Varady et al., 1969; Allen and Miller, 1976). Engelhardt et al. (1978) proposed that the rate of diffusion was influenced mainly by the permeability of the rumen wall and that the permeability in turn depended largely on diet, VFA, CO<sub>2</sub> etc.

(Thorlacius et al., 1971).

Urea passes from plasma to the rumen (Harmeyer and Martens, 1980; Kennedy and Milligan, 1980; Obara and Shimbayashi, 1980), and rumen ammonia and blood urea levels tend to equilibrate after changes in nitrogen intake or intravenous infusion of low levels of urea (McIntyre, 1970; McIntyre and Williams, 1970). Ruminal clearance of urea (the rate of urea degradation per unit of plasma urea concentration) decreased with increasing concentration of rumen ammonia in steers (Kennedy, 1980). Kennedy and Milligan (1980) noted that high concentrations of digesta ammonia and limited ruminal organic matter fermentation are negatively correlated with transfer of endogenous urea into the rumen. Also, bacterial urease activity associated with adherent bacteria on the rumen epithelium has been suggested as a regulatory factor for increasing urea transport from blood to rumen fluid (Houpt, 1970; Cheng and Wallace, 1979).

Total urea transfer from blood to rumen was reported to range from 0.6-2.3 gN d<sup>-1</sup> for sheep given lucerne or low quality diets (Nolan and Leng, 1972; Nolan et al., 1976; Norton et al., 1978; MacRae et al. 1979; Nolan and Stachiw, 1979). These estimates were considerably lower than recycling rates in sheep given high intakes (7.3 - 9.6 gN d<sup>-1</sup>) of a pelleted brome-grass diet (Kennedy and Milligan, 1978a). Norton et al. (1982b) confirmed the latter, since the inclusion of flaked barley in a pelleted-grass diet increased both the total amount of urea degraded in the rumen and the rate of urea entry across the rumen wall.

Many researchers have reported a positive correlation between

plasma urea-N concentration, and a negative correlation between rumen ammonia concentrations and urea entry into the rumen (Weston and Hogan, 1967; Norton et al., 1978; Kennedy, 1980). These results have been interpreted as evidence that rumen ammonia concentrations regulate the rate of urea entry into the rumen (Varady et al., 1967; Kennedy and Milligan, 1978b; Wallace et al., 1979). However, low rumen ammonia concentrations are not always associated with high rates of urea recycling to the rumen (Norton et al., 1978; Kennedy, 1980), nor do high rumen ammonia concentrations necessarily inhibit urea recycling (Norton et al., 1982a).

Urea transfer into the rumino-reticulum is low for high protein diets and at high ammonia concentrations in rumen contents (Varady et al., 1969; Thornton, 1970a; Faichney and White, 1977). However, under conditions where extensive transfer of endogenous urea to the rumen occurs (Kennedy and Milligan, 1980), ammonia from the large intestine may contribute more substantially to rumen microbial protein synthesis. Below a concentration of  $100\text{mg N L}^{-1}$  of ammonia in rumen fluid, increased entry of plasma urea-N into the rumen digesta of sheep given brome grass has been observed (Kennedy et al., 1982) during cold exposure.

Equilibration of plasma and urine urea over a longer term has been shown. Nolan and Stachiw (1979) have reported that the appearance of  $^{14}\text{CO}_2$  and  $^{15}\text{NH}_4^+$  in rumen fluid was in the same proportions as  $^{14}\text{C}$ - and  $^{15}\text{N}$ -labelled urea in blood, which indicates that all urea degraded in transfer across the rumen wall enters ruminal fluid and that there is no substantial evidence

supporting a preferential ammonia reabsorption into blood after subepithelial degradation of urea.

The rumen has been assumed to be the principal site of appearance of recycled urea in the digestive tract including salivary secretion into the rumen. This assumption has been supported by the results of experiments which demonstrated that a large quantity of urea is transferred to the rumen (Juhasz, 1965; Houtp and Houtp, 1968), and by the hypothesis that rumen microorganisms are responsible for most of the degradation of urea in the body (Waldo, 1968). However, some studies have suggested that urea recycling to the rumen accounts for only a small proportion of total decomposition most of which occurred in the lower digestive tract (Nolan and Leng, 1972; Nolan et al., 1976; MacRae et al., 1979). The amount of recycled urea that appeared in the rumen was approximately 20% of the total amount of recycled urea that appeared in the digestive tract according to (Nolan and Leng, 1972). They suggested that the lower part of the digestive tract was also an important site of decomposition of blood urea.

#### **b. SMALL INTESTINE UREA TRANSFER:**

The amount of urea entering various sections of the intestine appears to vary. The urea-N entering the gastrointestinal lumen either becomes reutilized by the microorganisms, reabsorbed as ammonia or is excreted in the feces largely in the form of microbial N. About  $6.2 \text{ g N d}^{-1}$  endogenous urea was secreted into the small intestine plus the caecum of the sheep, including urea in bile, pancreatic and intestinal fluid (Varady et al., 1979). Relatively

large amounts of endogenous N are secreted into the intestinal tract. Approximately 0.5g per day of endogenous urea is secreted into the intestinal tract with bile and pancreatic juice only in sheep of about 50kg body weight. Huntington and Prior (1983) reported that urea-N transfer to the gut in heifers was negatively correlated with PUN and positively related to increasing levels of intake of a high concentrate diet.

Urea influx across the mucosa of the small intestine is rather high, resulting in urea concentrations in digesta of the distal ileum as high as in blood (Hecker, 1971a). A total 5.7 g of urea is secreted from the blood into the small intestines with intestinal fluid according to several studies (Boda et al., 1976; Varady et al., 1979). The high urea concentration in the chyme of the small intestine (Kametaka, 1968; Hecker, 1971b) is evidence that urea is secreted from the blood into the intestine via the intestinal fluid or through the bile and the pancreatic fluid (Varady et al., 1979 is not broken down to ammonia in the small intestine.

For urea to participate in synthesis of nitrogenous substances by rumen microorganisms, it must be broken down to ammonia and  $\text{CO}_2$ ; for this, urease is needed. No urease was found either in the wall or the contents of the duodenum and the jejunum, and ureolytic activity in the contents of the ileum was low (Boda et al., 1976; Michnova et al., 1979). But, high urease activity was founded in the caecal mucosa. From this it can be concluded that endogenous urea secreted into the duodenum and the jejunum is not utilized by microorganisms in these parts of the intestine.



### c. LARGE INTESTINE UREA TRANSFER:

Although endogenous urea transfer across the wall of the large intestine was negligible, there were substantial inputs of endogenous non-urea-N (NUN) (Dixon and Nolan, 1983). The caecum and proximal colon were the major sites of the ammonia entry in the large intestine. Urea is also secreted into the rectum. Most of this ammonia was absorbed but in sheep given a high-N roughage diet this ammonia made only a small contribution (14%) to urea synthesis in the body. Approximately 0.5 g of endogenous urea-N and 0.61g endogenous non-urea-N  $\text{d}^{-1}$  penetrates through the wall of cecum and proximal colon (Boda et al., 1976; Varady et al., 1979). About 9% of caecal ammonia N was derived from blood urea. Engelhardt et al. (1978) found that from 333.1 to 935.8 mg  $\text{d}^{-1}$  urea was secreted from the blood into the large intestine in goats.

Also in the large intestine, mainly in the caecum, the degradation rate of endogenous urea is high (Hecker, 1971b; Ørskov et al., 1972; Coelho da Silva et al., 1972a,b). In cattle, degradation of urea in the post-ruminal tract was closely related to plasma urea-N concentration for all the diets used (Kennedy, 1980). Nolan et al. (1976) stated that urea transfer into the caecum was even greater than into the rumen; however, a considerable portion of this may enter the caecum with the passage of digesta from the ileum.

A considerable amount of urea hydrolysed in the gastrointestinal tract of sheep is secreted aborally of the reticulo-rumen. It is known that N metabolism in the hind gut can be substantial (Thornton et al., 1970a,b; Coelho da Silva et al. 1972a,b). Nolan et al.

(1976) have calculated that 25% of total urea hydrolysis occurs in the caecum. Ammonia used for urea synthesis may be derived from that absorbed from the digestive tract and from the catabolism of amino acids released from tissues or absorbed from the digestive tract.

A summary of the values shows that the amount of endogenous urea which passes into the intestinal lumen is considerable and that it represents a large percentage of the total daily amount of urea synthesized in the ruminant organism. Also, a considerable amount of urea-N enters the caecum with digesta from small intestine and contribution to microbial N.

#### **E. EFFECT OF DIET AND ENVIRONMENT ON UREA RECYCLING:**

Factors affecting urea recycling include plasma urea-N, rumen ammonia concentration, dietary N content, time after feeding, amount of readily fermentable carbohydrate, starvation, cold, short chain fatty acid (SCFA: butyric acid),  $\text{CO}_2$ , urease activity, OM intake, concentrate diet, sucrose and water restriction. The rate at which urea is recycled to and degraded in the digestive tract of ruminants has been shown to vary with the protein content of the diet (Cocimano and Leng, 1967; Faichney and White, 1977) and the presence of carbohydrates in the ration (Kennedy, 1980; Norton et al., 1982a).

Urea metabolism in domestic ruminants seems to vary greatly with the amount of ingested N and the serum urea level. Obara and Shimbayashi (1980) reported that in a ruminant given a low-protein ration in which the amount of N ingested was so small that the serum urea level was low, recycled urea was presumed to be utilized in the rumen. Ford and Milligan (1970) found in sheep that the quantity of

urea recycled was a linear function of the plasma urea-N concentration provided the latter was within the physiological range. In this case the method used to determine urea recycling measured the total recycling taking place within the body. It is not known what proportion of that recycling was directed to the rumen as opposed to other parts of the gastrointestinal tract. Therefore, the computed dietary N intake plus recycled N is only an approximate guide to the amount of N made available to the microorganisms.

In animals given a low-protein ration, the rumen plays an important role in the utilization of recycled urea in the whole digestive tract. The recycling of urea to the digestive tract of ruminants and its degradation to provide ammonia for microbial metabolism has been proposed as a mechanism whereby ruminants conserve dietary nitrogen during periods of low-protein diet (Haupt, 1970; Engelhardt et al., 1978). In ruminants on a low N intake up to 90% of the urea entering the pool was recycled into the digestive tract (Mugerwa and Conrad, 1971).

An inverse relationship between dietary N content and urea recycling has been observed in sheep (Cocimaro and Leng, 1967) and kangaroos (Chilcott and Hume, 1981). In a comparative study of ruminants (Emmanuel and Emady, 1978), with camels fed a medium protein (9.6%) diet, 74% of urea synthesis in the body was degraded in the gut, in contrast to 50% in sheep, 46% in goats and 47% in cattle. The percentage of urea recycled ranged from 65% to 38% on the diets containing 13% and 18% CP in ponies (Prior et al., 1974) and from 44% on a 13.1% CP diet to 86% on a 6.1% CP diet in camels.

Man and the rabbit appear to recycle slightly less urea than sheep, cattle, horses or deer (Prior et al., 1974). Houpt and Houpt (1971) reported that the horse could utilize significant quantities of endogenous urea when fed a diet low in protein. In the recent studies, Mousa et al.(1983) observed that concomitant with increasing N balance in the sheep and goats, urea recycling increased from approximately 77 - 94%. The high recycling rate in the camels on the low-N dry desert grass with water available *ad libitum* provided little opportunity for improvement during water deprivation. However, in sheep and goats water deprivation resulted in an increase in urea recycling in those species to a level very similar to the consistently high level in the camels.

There are some other factors that influence urea recycling to the rumen of ruminants. Increased urea recycling was associated with increased concentrations of butyric acid in rumen fluid and with high rates of CO<sub>2</sub> production from fermentation (Norton, 1982a). Rumen metabolites like short chain fatty acid (SCFA), particularly butyric acid, and CO<sub>2</sub> remarkably increased the permeability of the ruminal epithelium for urea (Thorlacius et al., 1971). Diets containing readily fermentable carbohydrate and fed once daily would provide the greatest potential for increased urea recycling. Varady et al. (1969) observed a change in the rate of urea recycling to the rumen of sheep with time after feeding, and low rates of recycling are usually found in starved ruminants (Engelhardt et al., 1978).

Kennedy (1980) has proposed that urea recycling to the rumen of cattle increased when digestible OM intake increased and/or ruminal

ammonia concentrations decreased. A small increase in digestible OM intake (13%) resulted in a 35% increase in the total amount of urea recycled to the rumen, and an even greater increase (93%) in the amount of urea transferred across the rumen wall (Norton et al. 1982a). Also, urea recycling increased 30% in brome pellets given sheep, but there was no difference in urea recycling for low levels of alfalfa and barley-canola meal diets given to sheep. A more likely stimulus to urea recycling in ruminants given concentrate or sucrose supplements may be the nature of the end-products of their fermentation.

The rate at which urea is recycled to and degraded in the digestive tract of ruminants is influenced by environmental temperature with more endogenous urea entering the rumen of cold-exposed sheep (Kennedy and Milligan, 1978a). Kennedy et al. (1982) and Kennedy and Hume (1978) reported that recycled urea was a major source of N for microbial growth only for the chopped and pelleted brome grass diets, where it provided 26-39% of N, made available from degradation of dietary crude protein and from recycled urea. There do not appear to have been any comprehensive studies reported in which the relationship between diet CP level and N and urea metabolism have systematically compared in different environments. Therefore, the present study was undertaken to examine the possibility of environmental and dietary CP interactions in young lambs.

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# UREA RECYCLING AND DIGESTIBILITY IN WETHERS EXPOSED TO DIFFERENT AMBIENT TEMPERATURES AT THREE DIET LEVELS OF CRUDE PROTEIN

## INTRODUCTION

The recycling of urea to the digestive tract of ruminants and its degradation to provide ammonia for microbial metabolism has been proposed as a mechanism whereby ruminants conserve dietary nitrogen during periods of protein deprivation (Houpt, 1970). In domestic ruminants fed a nitrogen deficient ration, urea excretion in the urine is suppressed with the urea being preferentially transferred to the digestive tract and converted into microbial protein (Schmidt-Nielsen et al., 1958; Cocimano and Leng, 1967). The imposition of cold stress on ruminants usually results in less efficient utilization of feedstuffs due, in part, to depressed digestibilities of dry matter (DM) and organic matter(OM). 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, 1978a,b). Therefore, animals may be better able to conserve nitrogen in a cold environment and be less constrained by a low nitrogen diet than animals in a warm environment. The present study was designed to examine this question in young lambs.

## MATERIALS AND METHODS

### ANIMALS AND THEIR MANAGEMENT

Eighteen Suffolk wethers (initially 30-45 days old with body weights ranging from 10-20 kg) were chronically exposed to temperatures of +1 to +4°C (cold) or +21 to +24°C (warm) during 10-week experimental periods. The sheep were shorn to a fleece depth of approximately 4 - 6 mm at the beginning of the experiment and after 2 weeks of each 5-week period. Animals were housed in individual metabolic crates in controlled environment rooms. Prior to the experiment all wethers were treated orally with Thiobenzol (Mark Sharp & Dohme, Ontario, Canada Ltd., one g per 10kg B.W.). For prevention of external parasites, Co-Ral (Cutter Animal Health, Bayvet Division Cutter Lab. Inc., Mississauga, Ontario) containing 25% Coumaphos was sprayed onto the animal (0.9kg of Co-Ral mixed with 45L water). During the temperature adaptation periods, sheep were accustomed to the urine collection funnels which were attached 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. One day prior to the radioisotope injection, urine collection funnels were fitted to all sheep. Both environmental chambers were lighted continuously for the duration of the experiment. Room temperatures were recorded daily with max-min mercury-in-glass thermometers (Taylor Insts., U.S.A.).

The metabolic crates were designed for sheep involved in nutrient experiments and in vivo isotope studies. A bolted steel

frame supported the sheep at a convenient working height (76cm above floor). Easily cleaned, chemically resistant and nonabsorbent molded fiberglass reinforced plastic was used for the walls and back, the feed and water containers, and the excreta collector funnels.

#### DIETS AND FEEDING

Sheep were fed pelleted diets (Table 1) containing 7, 11 or 14% crude protein (CP) *ad libitum*. Diets contained barley grain (*Hordeum vulgare*) and brome grass (*Bromus inermis* Leyss) which contained 8.4 and 4.8% CP, respectively. Soybean meal was substituted for barley grain in order to vary the crude protein content of the diet. The vitamin, mineral, and energy contents of the diets were calculated to approximate the requirements of the sheep (National Academy of Sciences - National Research Council, 1982,1985), based on body weight at the start of the experiment. The 7% crude protein diet was expected to be below the optimal CP level for growth of the lambs. Water and cobalt-iodized salt blocks (Canadian Salt Ltd., Montreal, Quebec) 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. Drinking water containers were filled at least once daily with fresh water.

#### EXPERIMENTAL DESIGN AND SCHEDULE

The experimental design consisted of 2 x 3 factorial design 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 days, after which they were transferred to the alternative temperature treatment for 35 days. During each experimental period the following schedule was adopted: day 1-21, adaptation to ambient temperature and training of sheep for urine collection procedures; days 22-28, collection of faeces and urine for measurement of apparent digestibility and N-balance; day 28,29, catheter implanted into the jugular vein of the animals; day 29,30, injection of  $^{14}\text{C}$ -urea and collection of urine and blood samples for 48 hours.

#### CATHETERIZATION AND ISOTOPE INJECTION

Animals were catheterized via one jugular vein with a PVC catheter (1.01mm ID, 1.67mm OD) inserted through a 14 gauge needle and held in place by a suture around the catheter and through the skin. The catheter was then connected by plastic tubing. Prior to the injection the radioisotope, 200ml of an isoosmotic saline solution was prepared containing 6.25  $\mu\text{Ci}$  and 0.1mg urea per ml. For practical reasons of scheduling the frequent blood and urine sample collections,  $^{14}\text{C}$ -urea was given to animals in the cold environment on day 29 and to animals in the warm environment on day 30. On day 29 or 30, each sheep within an environment treatment received a single injection of 60 - 65  $\mu\text{Ci}$  of [ $^{14}\text{C}$ ]urea (50 mCi mmole<sup>-1</sup>, >99 atom %, ICN Radiochemicals Canada Inc. Montreal,



Quebec, Canada) via the jugular catheter. After injection, the injection syringe and catheter were immediately flushed with 20 mL sterile physiological saline solution ( $9\text{g NaCl L}^{-1}$ ).

#### SAMPLING PROCEDURE

Total faeces 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 hours at  $60^{\circ}\text{C}$ . The dried samples were finely ground using a Christie-Norris grinder. Urine samples were collected in a bucket containing 25ml 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.

Following  $^{14}\text{C}$ -urea injections, blood and urine samples were collected for determining  $^{14}\text{C}$ , Urea-N, and  $\text{NH}_4^{+}\text{-N}$ . Blood samples were collected in Vacutainer tubes (10 mL, sterile, heparinized, Becton Dickinson Canada, Mississauga, Ontario) from jugular catheters just before  $^{14}\text{C}$ -urea injection and at 1, 7, 26, and 47 hours after injection. Plasma was immediately separated by centrifugation at  $1500 \times g$  for 10 minutes. Nine urine samples were collected from each wether in containers without acid surrounded by ice (to prevent microbial action) at 3, 6, 9, 12, 15, 18, 24, 36, and 48 hours after injection. Urine was directed into the collection bottles by using rubber funnels which were attached snugly to the abdomen below the prepuce of the lambs with elastic straps. 10% sub-samples were stored at  $-20^{\circ}\text{C}$ . Daily samples were combined to form a composite sample for each sheep for each digestion trial prior

to proximate analysis. In all experiments samples of the diet and any feed refusals were collected daily.

#### ANALYTICAL PROCEDURES

Nitrogen content of feed, feces and urine samples was determined by a Kjeldahl procedure (AOAC, 1980). Dry matter was determined by drying feed and feces samples in a forced-air oven at 60°C to a constant weight. OM was determined on dry samples by ignition in a combustion furnace at  $550 \pm 50^\circ\text{C}$  overnight (AOAC, 1980).

The activity of  $^{14}\text{C}$  was analyzed on a Liquid Scintillation Counter (Nuclear Chicago, Mark III, Model 6880; Searle Analytic Inc., Des Plaines, Illinois, U.S.A.). Counting efficiency was determined by the channels ratio method (Bruno and Christian, 1961) and counts for radioactivity were corrected for quenching from an external standard. Plasma was deproteinized and acidified with 6%  $\text{HClO}_4$  (1:1, v/v), which also removed any  $^{14}\text{CO}_2$  and the supernatant was saved for counting  $^{14}\text{C}$  activity. In preparation for counting, samples (1 mL urine or 400  $\mu\text{L}$  plasma supernatant) were placed in a scintillation vial (20mL, Fisher Scientific Company, Canada, Catalog No. 3-337-12) together with 15mL counting solution (Aquasol, New England Nuclear, Boston, Mass.) containing 4g per liter of 2,5-diphenyloxazole (PPO) and 100mg per liter of 1,4-bis-(2-(4-methyl-5-phenyl-oxazolyl)-benzene (POPOP). Urine samples were thoroughly mixed prior to pipetting. Samples were refrigerated for 24hr to allow for chemiluminescence decay. The samples were counted for 20min or until the counting errors were 0.25% or less. The total disintegrations per minute (DPM) were determined for each sample.

Plasma urea-N (PUN) and urine urea-N (UUN) were determined according to colorimetric assay (Fawcett and Scott, 1960). Distilled water, which was used for reagents, was a cation exchange resin distilled water in order to avoid any trace ammonia. Buffered urease solution was prepared using 200 Sumner units (-30mg) of special purified urease (70,000  $\mu\text{mol}$  Units, one  $\mu\text{mol}$  unit will liberate one  $\mu\text{mol}$  of  $\text{NH}_3$  from urea per min at pH 7.0 at  $25^\circ\text{C}$ ; 14F-7090, Sigma St. Louis MO USA) per 100mL of 1% solution of the buffer which was mixed with 6 gm of monopotassium dihydrogen phosphate and 2 gm of disodium monohydrogen phosphate in 1000mL. Plasma and urine samples were diluted by an appropriate amount (in the range of 1:100 and 1:1000, respectively) prior to analysis. One mL portions of distilled water, dilute standards, and dilute samples were measured into 15.24 cm by 2.54 cm test tubes. One mL of buffered urease solution was added to each tube and incubated in a  $37^\circ\text{C}$  water-bath for five minutes. Free ammonia was measured by the same procedure as that described, substituting water for the urease solution. After incubation, the following solutions were added immediately after each other to each tube with automatic pipettes: 2 mL of sodium phenate, 3 mL of 0.01% sodium nitroprusside, and 3 mL of sodium hypochlorite. Each solution was mixed thoroughly with a vortex mixer and placed in the dark at  $18^\circ\text{C}$  for 30 minutes to allow maximum colour development. Optical density (PC800 Colorimeter, Brinkmann Inds. Div., Rexdale, Ontario, Canada) was measured at 630  $\mu\text{m}$  and urea-N was estimated from the standard calibration curve after subtraction of the readings of free ammonia and blank.

## MATHEMATICAL COMPUTATION

Sample dpm were related to plasma urea concentrations to compute the specific activity of urea-C. The size of the urea pool (mg) was calculated as the injected dose of isotope (dpm) divided by specific activity ( $\text{dpm mg}^{-1}$ ) of the urea at zero time (Appendix A). Zero time specific activity was estimated by extrapolation of the plot of the natural logarithm of urea specific activity against to time zero time. Urea flux ( $\text{mg h}^{-1}$ ) was estimated as urea pool times the rate of decline ( $k$  fraction,  $\text{h}^{-1}$ ) of the plot of the natural logarithm of specific activity with time. Urea space (ml) was estimated by dividing the total urea pool size (mg) by the concentration of plasma urea ( $\text{mg mL}^{-1}$ ). The biological half-life of urea (h), expressed as  $t_{1/2}$ , was calculated as 0.693 divided by  $k$  ( $\text{h}^{-1}$ ). The turnover time of urea (h) was calculated as 1.44 times half-life (h). Urea space clearance ( $\text{mL d}^{-1}$ ) was estimated from urea flux ( $\text{mg d}^{-1}$ ) divided by plasma urea concentration ( $\text{mg mL}^{-1}$ ).

Urea recycled was calculated as urea flux minus urea excreted. Urea recycling was calculated as a percentage of urea flux ( $\text{mg d}^{-1}$ ) multiplied 100.

Average daily gain was calculated from weight change weekly during each 35 day period.

## STATISTICAL ANALYSIS

A  $2 \times 3$  factorial analysis of variance was used with environmental temperatures (2) and diets (3) as sources of variation. When significant treatment effects were observed, the Student-Newman-

Keuls' (SNK) test was used to test for significance of differences between means (Steel and Torrie, 1980). Linear regressions, with computation of correlation coefficients, slopes with their standard errors, and intercepts were conducted. Least-square regression analysis was used to obtain equations describing values of specific radioactivity vs time.

## RESULTS

### VOLUNTARY FEED INTAKE AND DIGESTIBILITY

There were no significant differences ( $p > 0.05$ ) in feed, DM and OM intake 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. Average feed DM intake per unit body size was 50.0, 53.1, and 57.1 g kg<sup>-1</sup> for the 7, 11, and 14% CP diet, respectively, in both environments (Table 3). On this basis there was also a positive ( $p < 0.05$ ) linear relationship with dietary CP content.

DM digestibilities for the 7, 11 and 14% CP diets were 65.1, 65.5 and 64.6% in the warm environment and 63.9, 61.4 and 62.0% in the cold environment, respectively (Table 2). In both environments, there was a small negative relationship between DM digestibility and dietary CP content. The respective values for OM digestibility were 68.4, 69.3 and 68.3% in the warm environment, and 67.1, 64.9 and 65.7% in the cold environment for the 7, 11 and 14% CP diets. Although somewhat lower DM and OM digestibilities were observed for the three diets in the cold environment compared to that of the warm environment, the effects of diet and temperature were not significant.

Apparent digestibilities of N for the 7, 11 and 14% CP diet were 51.1, 58.6 and 66.3% in the warm, and 48.2, 54.0 and 63.8% in the cold for the 7, 11, and 14% CP diets, respectively. The effect of increasing diet CP content was significant ( $p < 0.01$ ) and the highest

apparent N digestibilities were observed in the 14% CP diet in both environments.

#### BODY WEIGHT CHANGE AND FEED EFFICIENCY

Mean body weight was not different among treatments (Table 3). The average body weights were 22.9, 23.3 and 24.3 kg in the warm, and 22.1, 26.6 and 24.2 kg in the cold, respectively for the 7, 11 and 14% CP diets. Average daily gain (ADG) 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  $27 \text{ g d}^{-1}$  (Fig. 2). 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 regression equations were  $(\text{ADG}) = 157.69 + 3.51 (\text{N intake})$  ( $\text{SE}=0.43, R^2=0.85, n=18$ ) in the warm, and  $(\text{ADG}) = 81.37 + 6.65 (\text{N intake})$  ( $\text{SE}=0.62, R^2=0.75, n=18$ ) in the cold. The OM intake linearly affected ADG in the cold environment but there was a curvilinear relationship in the warm environment (Fig. 3). The regression equations describing these relationships were  $(\text{ADG}) = -206.79 + 0.70(\text{OM intake}) - 0.0002 (\text{OM intake})^2$  ( $\text{SE}=0.03, R^2=0.43, n=18$ ) in the warm, and  $(\text{ADG}) = 43.44 + 0.20 (\text{OM intake})$  ( $\text{SE}=0.05, R^2=0.66, n=18$ ) in the cold. The ADG for the warm exposed sheep plateaued at about  $290 \text{ g d}^{-1}$ .

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 (Table 3). The values for the 7, 11 and

14% CP diets were 5.53, 5.06 and 4.99 g g<sup>-1</sup> in the warm, and 5.70, 4.66 and 4.61 g g<sup>-1</sup> in the cold, respectively.

#### NITROGEN METABOLISM

The parameters of nitrogen metabolism are shown in Tables 4 and 5. Nitrogen intake, faecal N and urinary N excretion, N retention and apparent N absorption were all positively related ( $p < 0.01$ ) to diet crude protein content, but they were not significantly influenced by temperature except for faecal N excretion which was higher ( $p < 0.05$ ) in the cold environment.

Nitrogen intake substantially increased from 13.7 to 21.5 and 32.0 g d<sup>-1</sup> in the warm, and from 13.9 to 27.2 and 31.8 g d<sup>-1</sup> in the cold as diet CP content increased from 7 to 11 and 14% (Table 4). There was no significant difference in N intake between cold and warm temperature treatments. 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.

Faecal N output was significantly ( $p < 0.05$ ) lower in the warm compared to the cold environment. A significantly greater amount of faecal N (12.5 g d<sup>-1</sup>) was excreted in the C11 treatment compared to that (8.9 g d<sup>-1</sup>) of the W11 treatment. The mean faecal N per 100g of DM intake was 0.56, 0.65, 0.71 for the 7, 11, 14% CP diets, respectively, in the both environment. The relationship between faecal N output and N intake is shown in Figure 4. There was a linear relationship between faecal N and N intake in both warm and cold exposed animals. The regression equations were (Faecal N) =  $2.37 + 0.26$  (N intake) (SE=0.03,  $R^2=0.78$ ,  $n=18$ ) in the warm, and (Faecal N) =



$2.97 + 0.27(\text{N intake})(\text{SE}=0.04, R^2=0.66, n=18)$  in the cold.

Nitrogen apparently absorbed ( $\text{g d}^{-1}$ ) for the 7, 11 and 14% CP diets was 7.0, 12.6 and 21.2 in the warm, and 6.7, 14.7 and 20.3 in the cold, respectively (Table 4). The amount of N apparently absorbed was increased in response to increasing CP % in the diet, but there were no significant differences due to ambient temperature.

Urinary N excretions ( $\text{g d}^{-1}$ ) were not significantly different across environmental treatments (Table 5). 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. 5). The regression equations were (Urinary N) =  $2.04 - 0.04(\text{N intake}) + 0.006(\text{N intake})^2$  ( $\text{SE}=0.002, R^2=0.93, n=18$ ) in the cold, and (Urinary N) =  $1.75 + 0.04(\text{N intake}) + 0.005(\text{N intake})^2$  ( $\text{SE}=0.004, R^2=0.85, n=18$ ) in the warm.

The amount of N retained ( $\text{g d}^{-1}$ ) was directly related to the CP content of the diet in both environments (Table 5). 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 Figure 6. The regression equations were (N balance) =  $-0.49 + 0.41(\text{N intake})$  ( $\text{SE}=0.04, R^2=0.91, n=18$ ) in the warm and (N balance) =  $-1.39 + 0.48(\text{N intake})$  ( $\text{SE}=0.05, R^2=0.88, n=18$ ) in the cold environment, respectively. The regression coefficients did not differ between warm and cold environments.

## UREA METABOLISM

The decline in  $^{14}\text{C}$ -urea specific activity over 48 hours after injection is shown in the figure in appendix B. Urea specific activity decreased exponentially after injection and appeared to follow first order kinetics for the first 18 hours. From 18 to 48h there appeared to be a second (slower) exponential phase. Exponential equations for urea flux and kinetic parameters based on the first 18 hours after injection are shown in Table 6 and the regression lines are shown in appendix figure C. There were no significant diet nor temperature effects on the regression coefficients.

Urea pool, urea space, urea half-life, turnover time and urea space clearance are shown in Table 7. The urea pool size was positively related to the dietary CP content except for C11 treatment, which was lowest (3.1 g) among all treatments. The urea pool size was markedly increased by the 14% CP diet in both environments. There was no significant difference between the warm and cold environments.

Urea space (L) was reduced by increasing diet CP content. The values were 12.30, 7.43 and 7.99 in the warm, and 13.20, 6.41 and 9.62 in the cold for the 7, 11, and 14% diets. For the 7% CP diet, urea space appeared high in both environments. There were no significant differences due to environment treatments.

The mean biological half-lives of urea were 2.04, 2.09 and 2.17 h for the 7, 11, and 14% CP diets, respectively, in both environments (Table 7). There was no significant difference between the warm and

cold environments.

Urea turnover times were 2.94, 3.01 and 3.13 hours in both environments when sheep were fed 7, 11, and 14% CP diets. There were no significant differences due to diets or temperature treatments.

There was a significant ( $p < 0.05$ ) diet effect on urea space clearance rate. Significantly higher rates of urea space clearance were observed on the low protein diet in the both environments. However, cold exposure did not affect urea clearance rate.

Urea flux, urea excreted, urea recycled, plasma urea-N (PUN) and urinary urea-N (UUN) are shown in Table 8. There were no significant differences in the urea flux, urea excreted, and urea recycled, but there were significant differences in the percentage of urea recycled, PUN and UUN due to diet CP. Cold exposure did not significantly change urea metabolism in sheep although the highest value for urea flux ( $41.5 \text{ gN d}^{-1}$ ) appeared in the C14 treatment. The relationship between urea flux and dietary crude protein level was generally positive in both environments. The urea flux was similar between 7 and 11% CP diet in the both temperature treatment but it increased markedly in the W14 and C14 treatments. However, there was no significant difference between environments as shown by the analysis of variance.

The percentage of urea recycled was negatively ( $p < 0.01$ ) related to the N intake in both environments (Table 8). The urea recycling percentages were 94.1, 82.4 and 76.8 in the warm, and 90.0, 80.0 and 81.1 in the cold, for the 7, 11 and 14% CP diets, respectively. The diet effect was significant ( $P < 0.01$ ) but there

was no significant difference between the warm and cold environment.

Plasma urea-N ( $\text{g} \cdot 100\text{mL}^{-1}$ ) was increased ( $p < 0.01$ ) from 11.8 to 16 and 25.5 in the warm, and from 12.1 to 23.0 and 25.1 in the cold, as diet CP increased from 7 to 11 and 14% CP, respectively (Table 8). These positive ( $p < 0.01$ ) relationships were similar in both environments.

Table 1. Composition of feeds fed to lambs.

Ingredient <sup>1</sup>	DM	CP <sup>2</sup>	Ration 1	Ration 2	Ration 3
Barley grain	89	8.4	61.78	52.16	44.95
Brome grass pellets <sup>3</sup>	92	4.8	37.72	37.72	37.72
Soybean meal	89	50	-	9.62	16.83
Vit.-Min. Mix. <sup>4</sup>			0.3	0.3	0.3
Trace Min. Salts <sup>5</sup>			0.2	0.2	0.2
CP (%) <sup>2</sup>			7.0 <sub>4</sub>	11.0	14.0
DE (Mcal kg <sup>-1</sup> ) <sup>6</sup>			3.03	3.05	3.06

<sup>1</sup> Ingredients are expressed as a percentage of total diet (as-fed basis)

<sup>2</sup> Measured values with three replications

<sup>3</sup> Low CP content brome grass

<sup>4</sup> Vit.-Min. Mix : 120mg Zn, 13mg Mn, 250mg Fe, 10mg Cu, 0.11 mg Se, 5500IU Vit A, Vit D 550IU, 25IU Vit E, 13mg Riboflavin, 50mg Niacin, 30mg Calcium Pantotenat, 30µg Vit B<sub>12</sub>, 550mg Choline per kg.

<sup>5</sup> Trace Mineral Mixture (%) : 96.5 NaCl, 0.4 Zn, 0.16 Fe, 0.12 Mn, 0.0033 Cu, 0.007 I, 0.004 Co.

<sup>6</sup> Calculated value

Table 2. Voluntary feed intake and dry matter, organic matter and nitrogen digestibilities in the young lambs fed three different crude protein levels diet at the warm and cold environment.<sup>1</sup>

Diet CP(%)	Warm			Cold			SE <sup>4</sup>	Sig. <sup>3</sup>		
	7	11	14	7	11	14		D	T	DxT
Intake (g d <sup>-1</sup> )										
Feed	1114	1173	1389	1131	1472	1376	149	NS	NS	NS
Dry matter	1028	1088	1283	1044	1365	1272	141	NS	NS	NS
Organic matter	972	1027	1206	986	1289	1195	133	NS	NS	NS
Digestibility (%)										
Dry matter	65.1	65.5	64.6	63.9	61.4	62.0	1.48	NS	NS	NS
Organic matter	68.4	69.3	68.3	67.1	64.9	65.7	0.88	NS	NS	NS
Nitrogen	51.1ab	58.6c	66.3d	48.2a	54.0b	63.8d	1.24	**	NS	NS

<sup>1</sup> Means in the same row that do not have a common superscript differ ( $p < 0.05$ ).

<sup>2</sup> Mean values with 6 replications.

<sup>3</sup> Significant difference among diets(D), between temperatures(T) and interaction(DxT).

Treatment means significant at  $p < 0.01$ (\*\*), NS : not significant at  $p = 0.10$ .

<sup>4</sup> Standard error of the mean.

Table 3. Body weight, average daily gain (ADG) and feed efficiency in the young lambs fed three different crude protein levels diet in the warm and cold environment.<sup>1 2</sup>

Diet CP (%)	Warm			Cold			SE <sup>4</sup>		Sig. <sup>3</sup>		
	7	11	14	7	11	14			D	T	DxT
Body weight (kg)	22.9	23.3	24.3	22.1	26.6	24.2	2.42		NS	NS	NS
Daily Gain (g d <sup>-1</sup> )	206a	266ab	279ab	195a	311b	302b	22.8		#	NS	NS
Feed/gain (g g <sup>-1</sup> )	5.53	5.06	4.99	5.70	4.66	4.61	0.54		NS	NS	NS
Feed/B.W. (g kg <sup>-1</sup> )	48.6a	50.4a	57.2b	51.3a	55.3b	56.9b	2.12		#	NS	NS

<sup>1</sup> Means in the same row that do not have a common superscript differ ( $p < 0.05$ ).

<sup>2</sup> Mean values with 6 replications.

<sup>3</sup> Significant difference among diets(D), between temperatures(T) and interaction(DxT).

<sup>4</sup> Treatment means significant at  $p < 0.10$ (#), NS : not significant at  $p = 0.10$ .  
Standard error of the mean.

Table 4. Nitrogen intake, fecal N and N apparently digested in the young lambs fed three different crude protein levels diet in the warm and cold environment.<sup>1 2</sup>

Diet CP (%)	Warm				Cold				Sig. <sup>3</sup>	
	7	11	14	14	7	11	14	14	SE <sup>4</sup>	D T DXT
N intake (g d <sup>-1</sup> )	13.7a	21.5ab	32.0b	32.0b	13.9a	27.2b	31.8b	2.80	2.80	** NS NS
(g kg <sup>-0.75</sup> )	1.21a	1.78ab	2.67c	2.67c	1.28a	2.10bc	2.63c	0.23	0.23	** NS NS
Fecal N (g d <sup>-1</sup> )	6.7a	8.9ab	10.8abc	7.2a	12.5c	11.5bc	0.52	0.52	0.52	** * *
(g kg <sup>-0.75</sup> )	0.59a	0.74a	0.90b	0.66a	0.97b	0.95b	0.17	0.17	0.17	** # #
% of N intake	48.9d	41.4bc	33.8a	51.8d	46.0cd	36.2ab	2.57	2.57	2.57	** # #
g 100g DM <sup>-1</sup>	0.55a	0.60a	0.69b	0.57a	0.70b	0.73b	0.11	0.11	0.11	* NS NS
N apparently- (g d <sup>-1</sup> )	7.0a	12.6b	21.2c	6.7a	14.7b	20.3c	1.64	1.64	1.64	** NS NS
digested (g kg <sup>-0.75</sup> )	0.62a	1.04b	1.66c	0.62a	1.14b	1.68c	0.21	0.21	0.21	** NS NS

<sup>1</sup> Means in the same row that do not have a common superscript differ (p < 0.05).

<sup>2</sup> Mean values with 6 replications.

<sup>3</sup> Significant difference among diets(D), between temperatures(T) and interaction(DXT).

Treatment means significant at p<0.10(#), p<0.05(\*), p<0.01(\*\*); NS : not significant at p=0.10.

<sup>4</sup> Standard error of the mean.



Table 5. Total N excretion, urinary N excretion and N retained in the young lambs fed three different crude protein levels diet in the warm and cold environment.<sup>1 2</sup>

Diet CP(%)	Warm				Cold				Sig. <sup>3</sup>	
	7	11	14	7	11	14	SE <sup>4</sup>	D	T	DxT
N excretion										
Total (g d <sup>-1</sup> )	9.6a	14.8ab	20.5b	10.8a	18.9b	20.2b	1.44	**	NS	NS
(g kg <sup>-0.75</sup> )	0.85a	1.23ab	1.71c	0.99a	1.46bc	1.67c	0.14	**	NS	NS
% of N intake	70.1b	68.4b	64.1a	77.7c	69.49b	63.52a	2.06	*	NS	NS
Urinary N (g d <sup>-1</sup> )										
	2.9a	5.9ab	9.8b	3.5a	6.4ab	8.7b	1.07	**	NS	NS
(g kg <sup>-0.75</sup> )	0.26a	0.45a	0.82b	0.32a	0.49a	0.72b	0.04	*	NS	NS
% of N intake	21.2a	27.4bc	30.6c	25.2ab	23.5ab	27.4bc	1.78	#	NS	NS
N retained (g d <sup>-1</sup> )										
	4.0a	6.7ab	11.5b	3.2a	8.3ab	11.6b	1.67	**	NS	NS
% of N intake	29.2ab	31.1bc	35.9c	23.0a	30.5b	36.5c	3.26	*	NS	NS
% of N digested	57.1b	53.2ab	54.2b	47.8a	6.5b	57.1b	4.15	*	NS	NS

<sup>1</sup> Means in the same row that do not have a common superscript differ (p < 0.05).

<sup>2</sup> Mean values with 6 replications.

<sup>3</sup> Significant difference among diets(D), between temperatures(T) and interaction(DxT).

Treatment means significant at p<0.10(#), p<0.05(\*), p<0.01(\*\*); NS : not significant at p=0.10.

<sup>4</sup> Standard error of the mean.

Table 6. The relation between the natural logarithm of urine urea specific activity (Y) and time (X) after injection first 18 hours used for first order kinetics determinations.

CP(%)	Warm			Cold		
	Equation	r <sup>2</sup>	SE <sup>1</sup>	Equation	r <sup>2</sup>	SE <sup>1</sup>
7	$Y = 10.667 - 0.352X$	.756	.949	$Y = 10.620 - 0.330X$	.953	.418
11	$Y = 10.567 - 0.314X$	.700	.877	$Y = 10.721 - 0.351X$	.746	.985
14	$Y = 10.392 - 0.311X$	.825	.705	$Y = 10.189 - 0.329X$	.941	.365

<sup>1</sup> Standard error of estimate of Y-axis intercept at to

Table 7. Urea metabolism by the first order kinetics in the young lambs fed three different crude protein levels diet in the warm and cold environment.<sup>1 2</sup>

Diet CP(%)	Warm			Cold			Sig. <sup>3</sup>		
	7	11	14	7	11	14	SE <sup>4</sup>	D	T DxT
Urea Pool size (g) (g kg <sup>-0.75</sup> )	3.258a 0.289ab	3.601ab 0.298ab	4.292bc 0.358c	3.386a 0.311b	3.085a 0.238a	5.256c 0.435c	0.352 0.030	# #	NS NS NS NS
Urea Space (L) (L kg <sup>-0.75</sup> )	12.30 0.486	7.43 0.268	7.99 0.292	13.20 0.548	6.41 0.253	9.62 0.347	0.754 0.025	NS NS NS NS	NS NS NS NS
Half-life (h)	1.970	2.210	2.232	2.101	1.973	2.106	0.361	NS	NS NS
Turnover time (h)	2.837	3.182	3.211	3.025	2.843	3.033	0.218	NS	NS NS
Urea space clearance (L d <sup>-1</sup> )	233.3c	125.0a	125.6a	221.6c	113.8a	165.3b	43.7	*	NS NS

<sup>1</sup> Means in the same row that do not have a common superscript differ ( $p < 0.05$ ).

<sup>2</sup> Mean values with six replications.

<sup>3</sup> Significant difference among diets(D), between temperatures(T) and interaction(DxT).  
Treatment means significant at  $p < 0.10$ (#),  $p < 0.05$ (\*),

NS : not significant at  $p = 0.10$ .

<sup>4</sup> Standard error of the mean.

Table 8. Urea flux, urea recycling, urea excreted and plasma urea-N concentration in the young lambs fed three different crude protein levels diet in the warm and cold environment.<sup>1 2</sup>

Diet CP(%)	Warm			Cold			Sig. <sup>3</sup>		
	7	11	14	7	11	14	SE <sup>4</sup>	D	T D x T
<b>Urea metabolism<sup>5</sup></b>									
Flux (gN d <sup>-1</sup> )	27.5	27.1	32.0	26.8	26.0	41.5	3.30	NS	NS NS
(gN kg <sup>-0.75</sup> )	2.44	2.28	2.67	2.46	2.01	3.44	0.46	NS	NS NS
Excreted(gN d <sup>-1</sup> )	1.62	4.77	7.43	2.68	5.14	7.86	1.51	NS	NS NS
(gN kg <sup>-0.75</sup> )	0.14	0.40	0.62	0.25	0.39	0.65	0.11	NS	NS NS
Recycled(gN d <sup>-1</sup> ) <sup>6</sup>	25.88	22.33	24.57	24.12	20.86	33.64	2.43	NS	NS NS
(gN kg <sup>-0.75</sup> )	2.29	1.85	2.05	2.22	1.61	2.79	0.46	NS	NS NS
Urea recycling (%)	94.1c	82.4b	76.8a	90.0c	80.0b	81.1b	1.91	**	NS NS
Plasma urea-N (mg 100mL <sup>-1</sup> )	11.8a	21.6b	25.5c	12.1a	23.0bc	25.1c	0.49	**	NS NS

<sup>1</sup> Means in the same row that do not have a common superscript differ (p < 0.05).

<sup>2</sup> Mean values with six replications.

<sup>3</sup> Significant difference among diets(D), between temperatures(T) and interaction(DxT).

<sup>4</sup> Treatment means significant at p<0.01(\*\*), NS : not significant at p=0.10.

<sup>5</sup> Standard error of the mean.

<sup>6</sup> Determined on 18hrs urine after injection of <sup>14</sup>C-urea by first-order kinetics.

<sup>7</sup> Determined from urea flux minus urinary urea excreted.

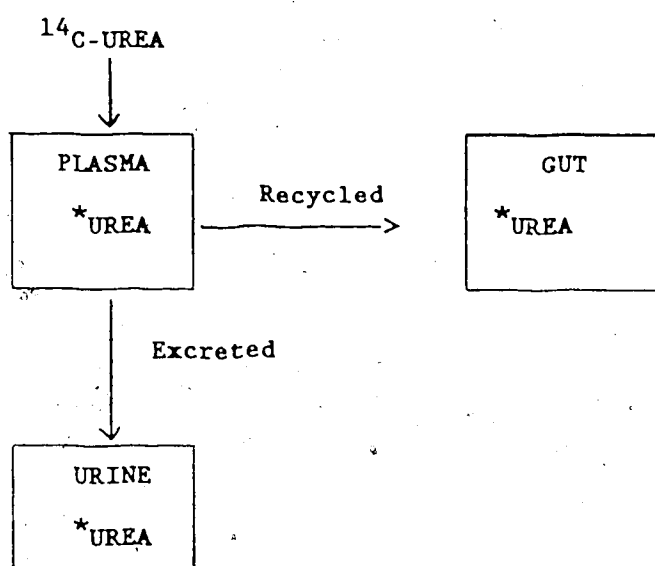


Fig. 1. Schematic representation of the urea recycling in the ruminants

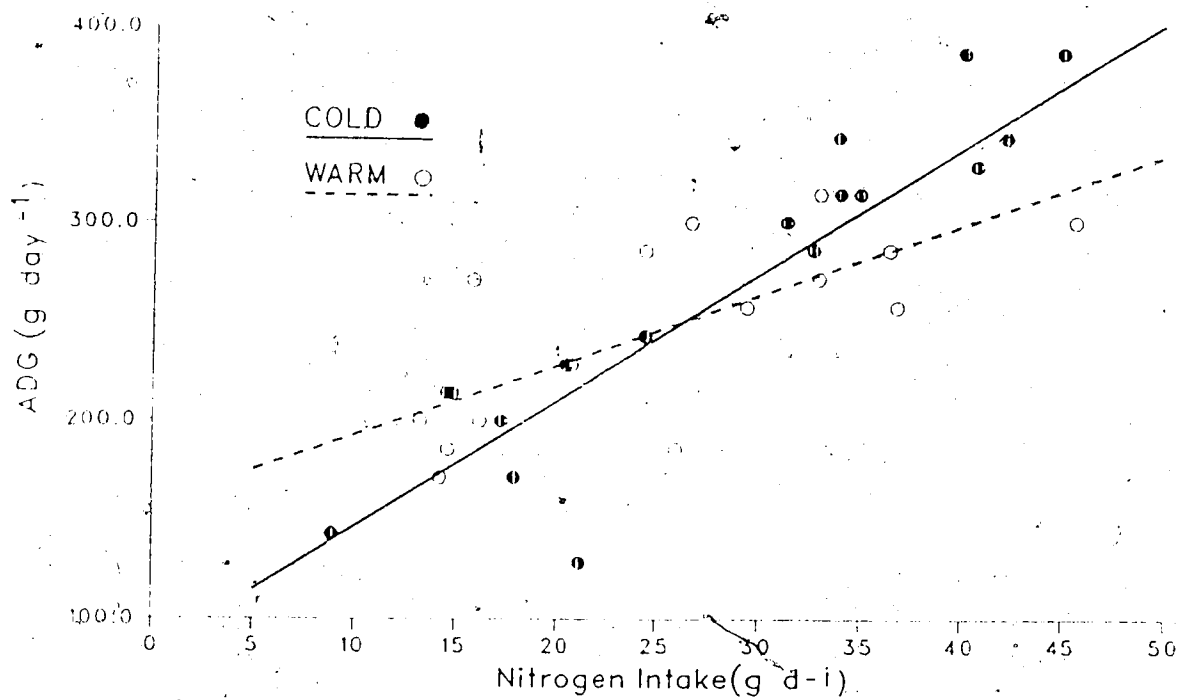


Fig. 2. The relation between average daily gain (g d<sup>-1</sup>) and nitrogen intake (g d<sup>-1</sup>) in the cold (●—● ;  $Y = 81.37 + 6.65X, SE=0.62, r^2=0.85, n=18$ ) and in the warm (○----○ ;  $Y = 157.69 + 3.51X, SE=0.43, r^2=0.75, n=18$ ) environment

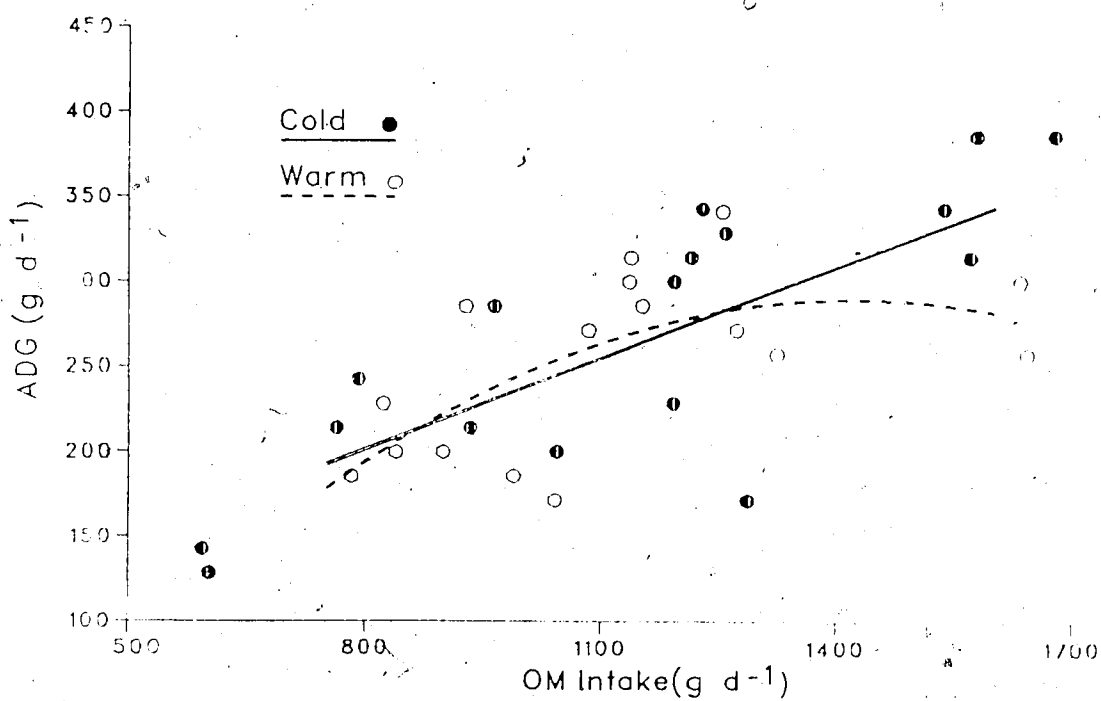


Fig. 3. The relation between average daily gain (g d<sup>-1</sup>) and organic matter intake (g d<sup>-1</sup>) in the cold (●—● ;  $Y = 43.44 + 0.20X$ ,  $SE=0.05$ ,  $r^2=0.66$ ,  $n=18$ ) and in the warm (○----○ ;  $Y = -206.79 + 0.70X - 0.0002X^2$ ,  $SE=0.03$ ,  $r^2=0.43$ ,  $n=18$ ) environment

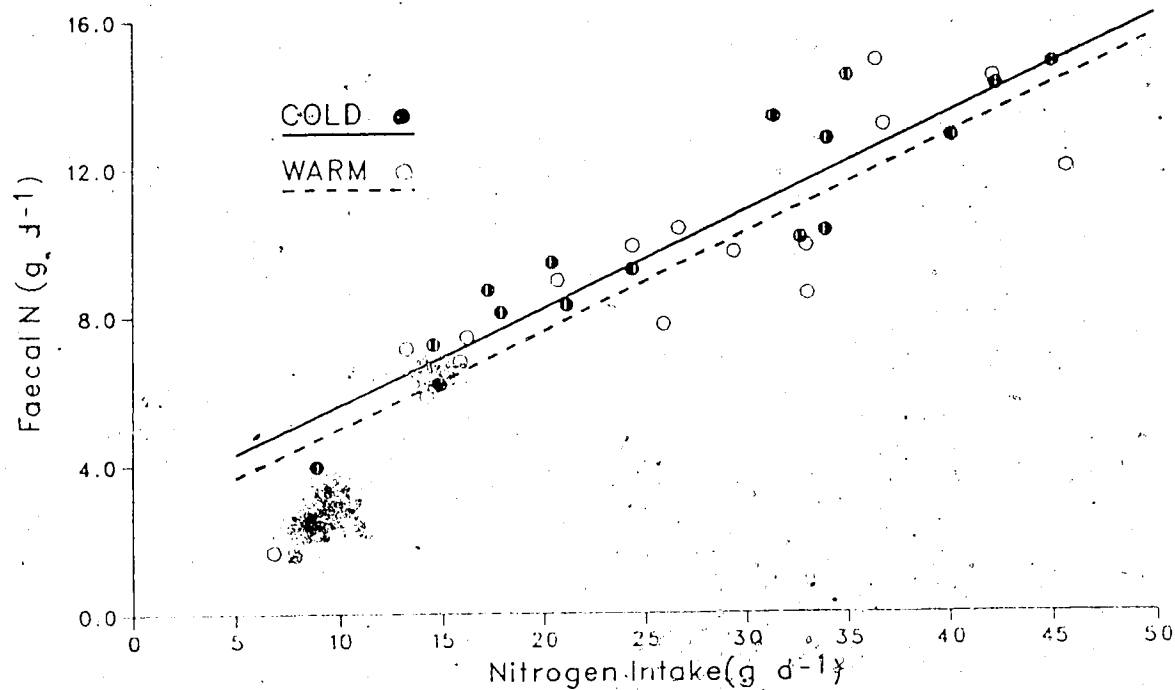


Fig. 4. The relation between faecal nitrogen excretion (g d<sup>-1</sup>) and nitrogen intake (g d<sup>-1</sup>) in the cold (●—●;  $Y = 2.97 + 0.27X$ ,  $SE=0.04$ ,  $r^2=0.66$ ,  $n=18$ ) and in the warm (○---○;  $Y = 2.37 + 0.26X$ ,  $SE=0.03$ ,  $r^2=0.78$ ,  $n=18$ ) environment.



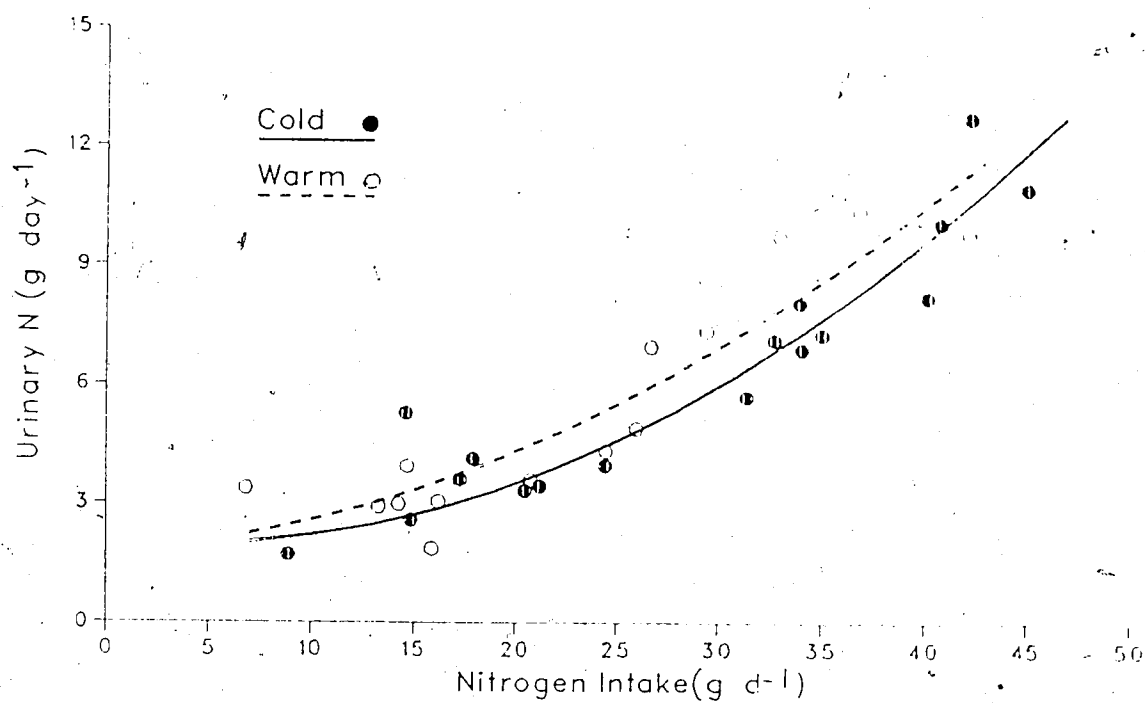


Fig. 5. The relation between urinary nitrogen excretion (g d<sup>-1</sup>) and nitrogen intake (g d<sup>-1</sup>) in the cold (●—● ;  $Y = 2.04 - 0.04X + 0.006X^2$ ,  $SE=0.002$ ,  $r^2=0.93$ ,  $n=18$ ) and in the warm (○----○ ;  $Y = 1.75 + 0.04X + 0.005X^2$ ,  $SE=0.004$ ,  $r^2=0.85$ ,  $n=18$ ) environment

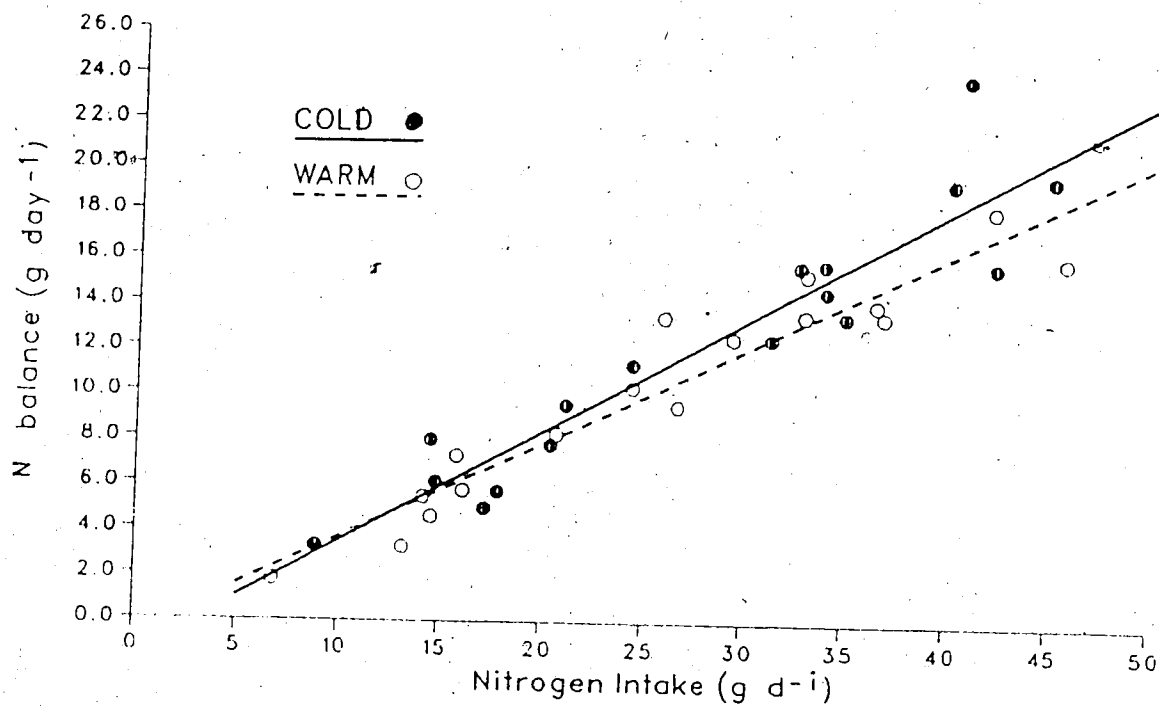


Fig. 6. The relation between nitrogen balance ( $\text{g d}^{-1}$ ) and nitrogen intake ( $\text{g d}^{-1}$ ) in the cold (●—● ;  $Y = -1.39 + 0.48X, \text{SE}=0.05, r^2=0.88, n=18$ ) and in the warm (○----○ ;  $Y = -0.49 + 0.41X, \text{SE}=0.04, r^2=0.91, n=18$ ) environment .

## DISCUSSION

### VOLUNTARY FEED INTAKE (VFI) AND DIGESTIBILITY

The voluntary feed intake was not significant in the cold, but intakes per unit body weight were 5.6 and 9.7% higher ( $p < 0.05$ ) for the 7 and 11% diets, respectively, in the cold compared to the warm environment. This result is consistent with other reports that voluntary feed intake 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 5-13% in shorn sheep fed 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).

One might expect VFI to 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. Increased feed intake when it 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 which was composed mainly of brome grass

(37.7%) and 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. (1986) 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, 1978a).

The small non-significant decreases in DM and OM digestibility 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, 1984b; Williams and Innes, 1982).

There were small overall negative relationships between feed intake and apparent OM and DM digestibilities in this study. A similar inverse relationship between intake and DM apparent digestibility has been reported in forage fed ruminants (Balch et al., 1953). This has been explained in terms of faster rates of digesta passage through the gut, particularly the rumino-reticulum, allowing less time for microbial digestion of refractory constituents

such as fiber (Balch et al., 1953).

Apparent N digestibility was increased ( $p < 0.01$ ) with increasing diet CP likely because soybean meal 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.

#### BODY WEIGHT CHANGE AND FEED EFFICIENCY

Even though there may be increased heat production and decreased digestibilities in the cold (Christopherson, 1976; Young, 1981), these may be compensated by increased intake 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 the 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. ADG 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 higher than 11% and N intake was above 27 g d<sup>-1</sup> (Fig. 2). However, below this point cold exposure depressed the growth rate. In addition, OM intake up to 1400 g d<sup>-1</sup> appeared to linearly affect animal growth in the cold environment. On the other hand, the ADG plateaued at around 290 g d<sup>-1</sup> at OM intakes

above  $1200 \text{ g d}^{-1}$  in the warm environment (Fig. 3). 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; Williams and Innes, 1982; McBride and Christopherson, 1984c). 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 head<sup>-1</sup>. Also, a high level of protein supplementation resulted in increased ADG in calves fed soybean meal (Davenport et al., 1987).

## NITROGEN METABOLISM

### FECAL NITROGEN EXCRETION:

The increase in fecal N excretions with diet CP are consistent with results of other studies (Kennedy, 1985; Kennedy and Milligan, 1978a). Kennedy and Milligan (1978a) reported that the fecal N excretion rates were  $10.7 \text{ g d}^{-1}$  in the warm and  $11.6 \text{ g d}^{-1}$  in the cold in sheep given brome grass ( $28.1 \text{ gN d}^{-1}$ ), and  $15.0 \text{ g d}^{-1}$  of fecal N excretion was observed at a high N intake ( $34.9 \text{ g d}^{-1}$ ) in sheep fed the same diet (Kennedy, 1985). However, these 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 0.73g of N per 100g DM intake when diet CP content was increased from 7 to 14%. This result agrees with Maynard and Loosli's (1962) suggestion for ruminants of a metabolic fecal excretion factor of  $0.5 \text{ gN } 100\text{gDM}^{-1}$  and for sheep ( $0.53 \text{ gN } 100\text{gDM}^{-1}$ ) (NRC, 1985).

Fecal N excretion was significantly higher ( $p < 0.05$ ) in the cold environment. This might have been due to increased escape of dietary N from the rumen to the intestine in cold-stressed sheep (Kennedy and Milligan, 1978a; Kennedy et al., 1982) but might also have been due to the higher N intake on the 11% CP diet.

#### URINARY NITROGEN EXCRETION:

The increased urinary N excretion with increased N intake in both environments agrees with result of several other studies. Urinary N output was lower ( $1.8 \text{ g d}^{-1}$ ) for a low protein ( $12 \text{ gN d}^{-1}$ ) intake than ( $6.2 \text{ g d}^{-1}$ ) for a high protein intake ( $21 \text{ gN d}^{-1}$ ) in calves (Bunting et al., 1987).

High urinary N outputs have been reported in some studies. Kinser et al. (1987) reported that urinary N excretion was  $8.1 \text{ g d}^{-1}$  at the N intake of  $13.0 \text{ g d}^{-1}$  when sheep were fed a high concentrate pelleted diet in addition to low-quality roughages. Also, urinary N output ( $\text{g d}^{-1}$ ) was 20 and 25 in roughage fed sheep at intakes of 21 and  $33 \text{ gN d}^{-1}$ , respectively (Egan and Ulyatt,

1980). Therefore, high CP diets can sometimes result in higher urinary N excretions and there is always a positive relationship between N intake and urinary N excretion within a diet type.

#### NITROGEN RETENTION:

N retention increased from 23 to 36% when the N intake increased from 49 to 57% of the apparently digested N, when diet CP increased from 7 to 14% CP. Cold exposure had a small but non-significant detrimental effect on N balance when the 7% CP diet was fed. This result supports the observation that nitrogen retention was slightly but not significantly reduced during cold exposure in calves (Christerson and Thompson, 1973). However, McAllister and Christophers (1984b) reported that N retention was significantly reduced in growing lambs during cold exposure perhaps because the stress was more severe than in the present study.

The present results agree with the report of Bunting et al. (1987) in which the N retention was 4.1 and 9.7 g d<sup>-1</sup> for intakes of 12 and 21 gN d<sup>-1</sup>, 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., 1987).

However, there was low N retention (-1.0 and 2.3 g d<sup>-1</sup>) in roughage-fed sheep consuming 21 and 33 gN d<sup>-1</sup>, 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 the high proportion of urea recycling which would be favoured by readily fermentable OM in the rumen. In addition the higher digestible energy supply to the animal would promote body growth and N retention.

#### UREA METABOLISM

##### FIRST ORDER KINETICS:

A two-compartment open model was used initially to describe the exponential decline in  $^{14}\text{C}$ -urea specific activity with the lapse of time over 48h after injection. The second compartment had very little effect on the estimation of urea kinetics. Therefore, straight lines were fitted for  $^{14}\text{C}$ -urea specific activity which had been plotted on logarithmic scales during the first 18h (Appendix-C). The sheep appeared to be in a relatively steady-state during the 18h period of estimation of urea flux, as indicated by the urinary urea excretion per unit time (see Appendix-D), even though feed was replenished once daily. This may have been because the sheep were fed ad libitum and therefore had feed available at all times.

##### UREA FLUX:

Urea flux was increased 17.2 and 57.2% in the W14 and C14 treatment compared with results for the 7 and 11% CP diets, respectively, in association with the increasing N intake. Although there was about a 30% higher urea flux and urea recycling rate in the cold compared to the warm environment for the 14% CP diet, this was not significant. This differs from the increased urea transfer to

the rumen during cold exposure reported by Kennedy and Milligan (1978a). In the latter study, endogenous urea provided 29% of N available from dietary and endogenous urea sources in sheep in the warm, compared to 41% for cold-exposed sheep given equal intakes of brome grass pellets (Kennedy and Milligan, 1978a). In the present experiment the contribution of recycled urea represented 40-64% of dietary and endogenous urea sources, although it is not clear how much of this entered the rumen.

The irreversible loss of urea was 6.7 and 10.5 g d<sup>-1</sup> in the roughage fed sheep consuming 21 and 33 gN d<sup>-1</sup>, respectively (Egan and Ulyatt, 1980). These are 30 to 45% of the flux values in the present study. Very low values of irreversible loss of urea of 0.33 and 0.78 g d<sup>-1</sup> were obtained in calves when intakes were 12 and 21 gN d<sup>-1</sup>, respectively (Bunting et al., 1987).

#### UREA EXCRETED:

The positive relationship between urea excretion and N intake is consistent with other reports in the literature (Egan and Ulyatt, 1980). However, when sheep were fed roughage diets providing 21 and 33 gN d<sup>-1</sup> intake, more than double the amount of urea was excreted (16.3 and 20.1 g d<sup>-1</sup>) in the urine compared to the present results (Egan and Ulyatt, 1980). This may have been due to a lower fermentable OM supply to the rumen microbes which limited their ability to trap recycled urea compared to the situation in the present study. Therefore, urea excretion was relatively small in the concentrate diet-fed sheep compared with roughage-fed sheep. Also urea excretion was not affected by the environment treatment but

dietary N intake had a major effect.

#### UREA RECYCLED:

The absolute amount of urea recycled was similar for all intakes of dietary N in the warm environment, however, a much higher value ( $33.64 \text{ gN d}^{-1}$ ) appeared in the C14 treatment. The present results support Kennedy and Milligan's (1980) study, in which transfer of plasma urea via the rumen wall to the rumen digesta was high ( $6.2\text{-}9.8 \text{ g d}^{-1}$ ) in sheep given brome grass pellets. Total urea transfer from blood to rumen has been reported to range from  $0.6$  to  $2.3 \text{ gN d}^{-1}$  for sheep given lucerne or low quality diets (Nolan and Stachiw, 1979) but these are considerably lower than recycling rates in sheep given high intakes of a pelleted brome-grass diet ( $7.3\text{-}9.6 \text{ gN d}^{-1}$ ) (Kennedy and Milligan, 1978b). On the other hand, somewhat lower amounts of urea were recycled ( $3.8$  and  $7.8 \text{ g d}^{-1}$ ) in calves fed low-quality roughage at intakes of  $12$  and  $21 \text{ gN d}^{-1}$ , respectively (Bunting et al., 1987). In the present study the rates of urea excretion and the amount of urea recycling were positively related to PUN since transfer of urea from blood to rumen occurs along a concentration gradient.

#### PERCENTAGE OF UREA RECYCLING:

High proportional rates of recycling (92%) were observed in the 7% CP diets in both environments. This result agrees with many other similar studies in sheep (Cocimano and Leng, 1967; Ford and Milligan, 1970; Mousa et al., 1983), and in the tarmmar wallaby (Kennedy and Hume, 1978) when low protein diets were fed. Mousa et al. (1983) observed that percent urea recycling increased from approximately 77

to 94% with decreases in N balance in sheep and goats. Kennedy and Hume (1978) found urea recycling values of 84 and 79%, respectively, on low protein diets in the tarmmar. In other study with ruminants on a low N intake, up to 90% of the urea entering the body urea pool was recycled into the digestive tract (Mugerwa and Conrad, 1971). These studies generally agreed that there is an inverse relationship between dietary N content and percent urea recycling.

#### PLASMA UREA-N (PUN):

Urea metabolism in domestic ruminants seems to vary greatly with the amount of N ingested and the serum urea level. Increased protein intake is generally associated with increased PUN concentrations in ruminants. In the present study, the plasma urea-N concentration was increased from 12.0 to 25.3 mg 100mL<sup>-1</sup> when the intake of sheep was increased from 13.8 to 31.9 gN d<sup>-1</sup>. This result is consistent with many previous studies with increased N intake in which plasma urea-N increased to reach a plateau at approximately 30 mg 100mL<sup>-1</sup> (Preston et al., 1965; Vercoe, 1969; McIntyre, 1970; Obara and Shimabayashi, 1980). Godwin and Williams (1984) reported that when N input was raised from 7.6 to 17.6 g d<sup>-1</sup>, plasma urea-N increased to approximately 30 mg 100mL<sup>-1</sup> but remained relatively constant when N intake was increased further to 23.3g. However, PUN may increase above this level when sheep are given N supplements on a low quality roughage diet (McIntyre and Williams, 1970; McIntyre, 1970). Therefore, there is a complicated regulation of PUN involving influences of dietary N content, diet type, fermentable OM, rumen ammonia concentration and urea recycling.

#### UREA POOL:

Urea pool size was positively related to the dietary CP content except for the 11% CP diet in the cold environment. This result agrees with several recent studies (Egan and Ulyatt, 1980; Bunting et al., 1987). Urea pool size was 4.9 and 6.5g in the roughage fed sheep consuming 21 and 33 gN d<sup>-1</sup>, respectively (Egan and Ulyatt, 1980). Slightly smaller urea pool sizes of 0.421 and 1.767g were observed in calves consuming 12 and 21 gN d<sup>-1</sup>, respectively (Bunting et al., 1987). On the other hand, the urea pool size markedly increased for the 14% CP diet in both environments in the present experiment. These values may have been affected not only by the high N intake and large amount of soybean meal but also by the large intake of readily fermentable carbohydrate.

#### UREA SPACE:

Urea space was negatively affected by diet CP content. Similarly Cocimano and Leng (1967) reported that urea space decreased from 55 to 25% of body weight in sheep as the protein content of the diet decreased from 27% to 4% CP. In the present experiment, the urea space was increased from 30 to 60% of body weight in young lambs when they were fed diets ranging from 7 to 14% CP. On the other hand, urea space (0.474 and 0.467 L kg<sup>-1</sup>) was not affected by level of N intake (12 and 21 g d<sup>-1</sup>) in the calves (Bunting et al., 1987). Also urea space was 21.0 and 27.4L in the roughage fed sheep (21 and 33 gN d<sup>-1</sup>), respectively (Egan and Ulyatt, 1980). There is obviously tremendous variation in estimates of urea space reported in the literature. Theoretically, urea space can never be higher than total

body water and usually is about 60-75% of body weight in regularly fed ruminants (IAEA/FAO,1984) but may differ depending on diet CP content and sources.

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## GENERAL DISCUSSION AND CONCLUSION

### VOLUNTARY FEED INTAKE AND DIGESTIBILITY

Dietary and digesta N and absorbed products of protein digestion appear to affect intake in two ways; first, there is an effect on digestion and rate of movement of digesta, thus influencing the physical controls of food intake; second, there is an effect of the protein provision to the animal which influences the metabolic response to the diet and the metabolic controls of food intake. Increased feed intake may result due to the faster rate of passage of residues through the tract and the increased energy demand of a cold environment (Baile and Forbes, 1974; Christopherson, 1976; Weston, 1982; Kennedy et al., 1982). Although in the present experiment, voluntary feed intakes were restricted by the same absolute amount in both environments they were more severely restricted relative to expected energy requirements in the moderate cold exposure than in the warm environment when sheep were fed the 7% CP diet. Therefore, growing lambs should be fed diets that are well above 7% CP regardless of temperature. In the cold environment protein restriction may have more serious consequences than in a warm environment.

In general, apparent DM digestibility of roughages is often reduced in the cold ambient environment (NAS-NRC, 1982). DM digestibility decreased about 0.1 - 0.2 units per °C decrease in temperature in the present experiment. However, this effect may differ depending on the diet (Young and Degen, 1981; Kennedy et al.,

1982; McBride and Christopherson, 1984; Williams and Innes, 1982).

#### **BODY WEIGHT CHANGE AND FEED EFFICIENCY**

The decreased ADG ( $\text{g d}^{-1}$ ) at the low N intake ( $13.9 \text{ g d}^{-1}$ ) during cold exposure suggests that a lower limitation of dietary CP exists for young lambs. Below this limit the lambs are unable to compensate for cold stress by increasing VFI. The cold-exposed animal tries to reduce heat loss and may enhance heat production in order to maintain a state of thermal equilibrium (Webster, 1974) and the maintenance requirement of the animal increases. Insufficient nutrient availability combined with an increased maintenance cost during periods of cold exposure, therefore, restricts the productivity of the animal (McBride and Christopherson, 1984). The growth rate of young ruminants exposed to cold environments can be limited by a lack of nutrients to meet both maintenance and growth requirements (Gibb and Penning, 1972; Williams and Innes, 1982). Therefore, cold exposure may reduce growth rate if nutrient availability is limited although the lambs were able to grow slightly faster in the cold when intake was high. An interesting question is; why is ADG increased at high N intake in the cold environment? Does this indicate that animals in the cold have a greater capacity for growth provided that nutrient intake is high?

#### **NITROGEN METABOLISM**

The organism may compensate for nitrogen escape from the rumen by a secretion of nitrogen from endogenous sources into the rumen. In this experiment, about 63.5 to 77.7% of N intake was excreted in the cold exposed animals. The relationship between N balance and N

intake showed the same trends as that for ADG vs N intake, but the effect of temperature was more pronounced for the latter. Nitrogen retention was slightly but not significantly reduced during cold exposure (Christopherson and Thompson, 1973; Christopherson, 1976). In addition supplying more easily fermentable material to ileal digesta in sheep increases the fecal nitrogen excretion (Thornton et al., 1970b). An interesting question in this connection is the extent to which endogenous urea used as a source of N for bacterial protein synthesis in the hindgut contributes to increased fecal N excretion.

#### UREA METABOLISM

The endogenous nitrogen circulation in ruminants includes ammonia formed from urea hydrolysis in the rumen which is then either absorbed or participates in the proteosynthetic processes of rumen microorganisms. Bacterial N-compounds are synthesized, not only directly from  $\text{NH}_3$  arising from urea hydrolysis with urease, but also from N-compounds synthesized from the absorbed  $\text{NH}_3$  which has recycled back to the rumen. In the liver urea is produced from the absorbed ammonia which can be secreted into the rumen. In the ruminant, the excretion of urea can be markedly reduced principally by its endogenous recycling and microbial assimilation.

It is known in domestic ruminants that when a nitrogen-deficient ration is ingested, urea excretion in the urine is reduced but more is transferred to the digestive tract and converted into microbial protein to be re-utilized (Schmidt-Nielsen et al., 1958). The amount of urea entering the gut and its distribution between sites are

variable and clearly affected by diet. The amount of urea N degraded in the whole tract, estimated, using isotopically labeled urea, as the difference between urea irreversible loss and urinary urea excretion, can account for 25-90% of the net urea production (Egan et al., 1985). The proportion of urea production which is degraded is increased from a low of 20% to 80-96% in sheep and goats by feeding a readily fermented CHO supplement (Cocimano and Leng, 1967; Engelhardt et al., 1984; Kennedy, 1980; Norton et al., 1982). The present results agree with previous studies in which there was a high percent urea recycling rate in N restricted animals. However, the absolute amount of recycled urea was lower in the low CP diet. In a previous study, endogenous urea provided an increased proportion of the N available from dietary and endogenous urea sources in sheep during cold exposure when given equal intakes of brome grass pellets (Kennedy and Milligan 1978a). A similar response was not seen in the present study. High urea flux and recycling rates could contribute to increased heat production and sustained animal growth in the cold when sheep are fed high N intakes. On the other hand, reducing the CP content of the diet may limit the animals' scope for adaptation and growth. The pattern of turnover observed with  $^{14}\text{C}$ -urea is assumed to indicate merely the change of urea hydrolysis. The plasma urea concentration and urinary urea-N excretion of the ruminant remained almost unchanged under the same dietary conditions in the different environments through the experiment period. This indicates that urea is in a state of dynamic equilibrium in the animal body; that is, both urea excreted into the urine plus that hydrolysed in

the digestive tract must be well balanced with the amount of urea synthesized and/or consumed.

In conclusion, diet CP content highly affects the overall nitrogen and urea metabolism in the young lambs. However, cold exposure did not change urea metabolism in the sheep even though there was likely an increased heat production and maintenance requirement. 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. Recycled urea may play a significant role in the ruminant digestive tract in order to maintain a physiological steady state. However the present study did not provide evidence for increased recycling in the cold when diet N intake was low. The results of this study do not support the hypothesis that lambs are better able to utilize a low crude protein diet in a cold environment.

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## APPENDIX

### APPENDIX A. MATHEMATICAL COMPUTATION EQUATIONS.

Parameters of urea kinetics were calculated using the following equations (FAO/IAEA, 1985; Egan and Ulyatt, 1980):

First order kinetics :  $Y = Ae^{-\kappa t}$

$$\text{Urea pool size (mg)} = \frac{\text{Dose of activity injected (dpm)}}{\text{specific radioactivity at } t_0 \text{ (dpm mg}^{-1}\text{)}}$$

$$\text{Urea flux (mg h}^{-1}\text{)} = \text{urea pool (mg)} \times \kappa \text{ (h}^{-1}\text{)}$$

$$\text{Urea recycled (mg d}^{-1}\text{)} = \text{Urea flux (mg d}^{-1}\text{)} - \text{Urea excreted (mg d}^{-1}\text{)}$$

$$\text{Urea recycling (\%)} = \frac{\text{Urea recycled (mg d}^{-1}\text{)}}{\text{Urea flux (mg d}^{-1}\text{)}} \times 100$$

$$\text{Urea space (mL)} = \frac{\text{urea pool size (mg)}}{\text{plasma urea-N (mg mL}^{-1}\text{)}}$$

$$\text{Urea half-life (T}_{1/2}\text{) (hr)} = 0.693 / \kappa \text{ (h}^{-1}\text{)}$$

$$\text{Urea turnover time (hr)} = 1.44 \text{ T}_{1/2} \text{ (hr)}$$

$$\text{Urea space clearance (mL h}^{-1}\text{)} = \frac{\text{Urea flux (mg h}^{-1}\text{)}}{\text{Plasma urea-N (mg mL}^{-1}\text{)}}$$

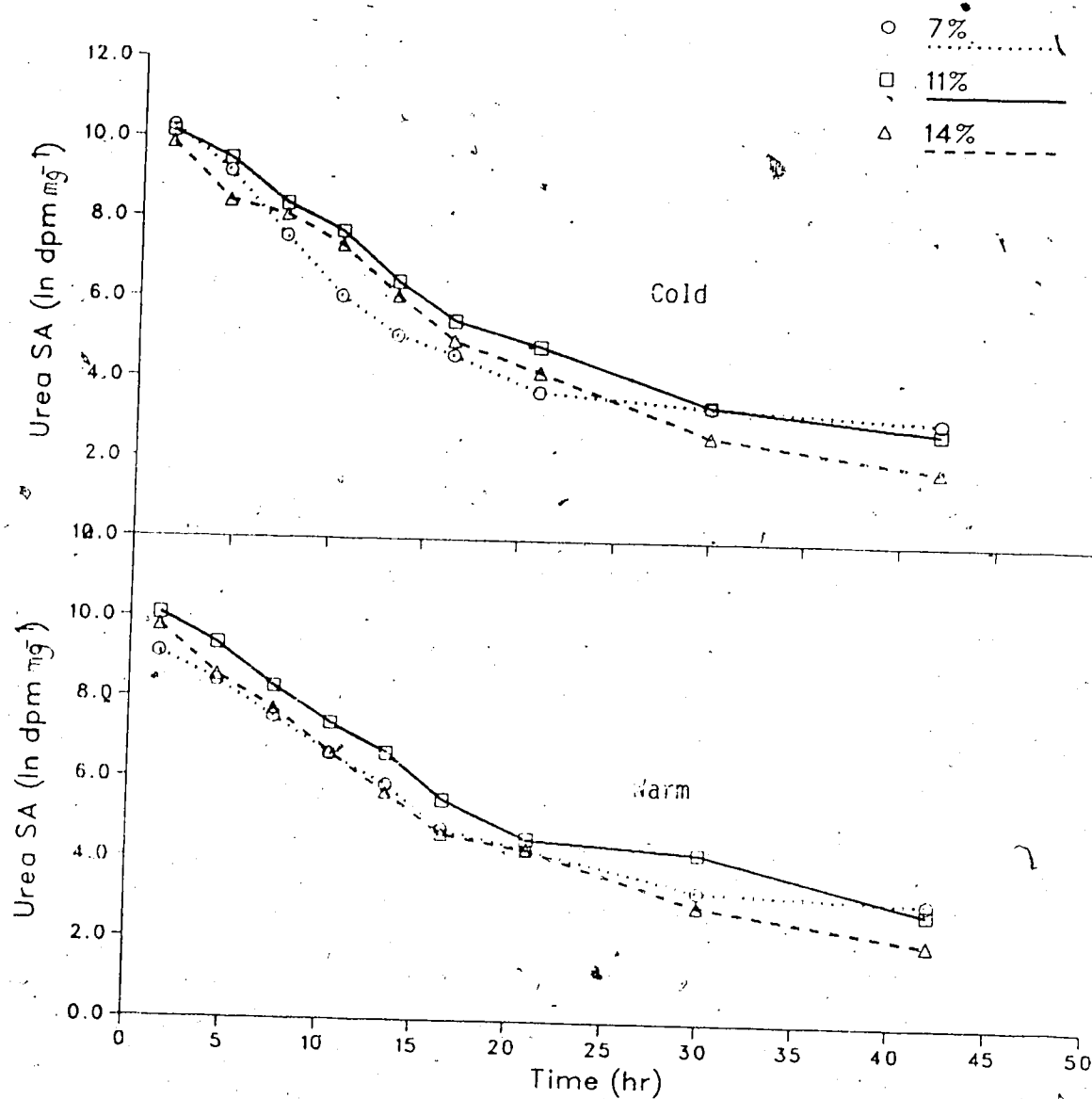
Ford and Milligan (1970) methods:

$$\text{Urea recover (\%)} = \frac{\text{excreted dose (dpm)}}{\text{injected dose (dpm)}} \times 100$$

$$\text{Urea recycling (\%)} = 100 - \text{recovered (\%)}$$

$$\text{Urea recycled (g d}^{-1}\text{)} = \frac{\text{recycled (\%)}}{\text{recovered (\%)}} \times \text{urea excreted (g d}^{-1}\text{)}$$

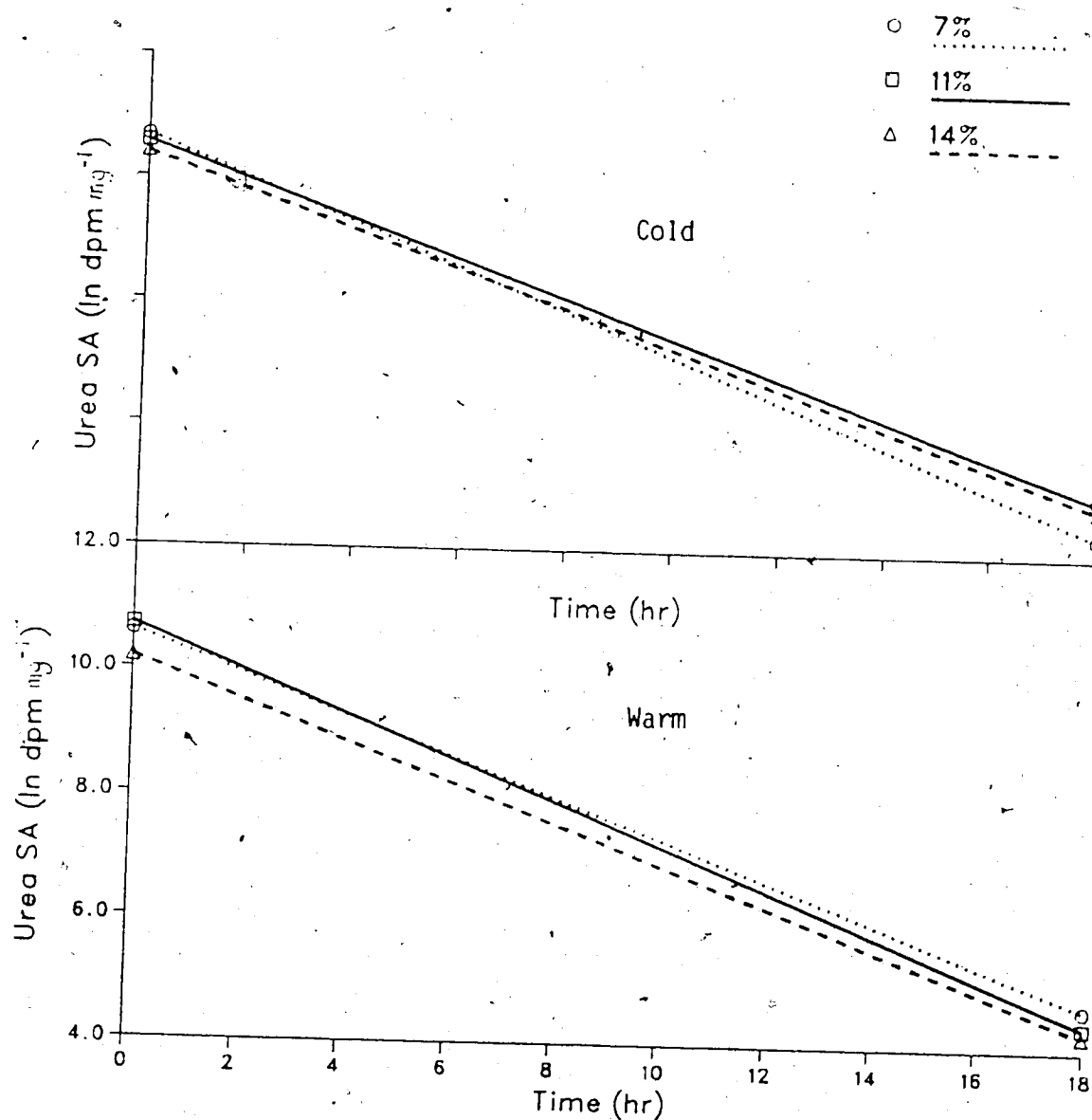
$$\text{Urea flux (g d}^{-1}\text{)} = \text{Urea recycled (g d}^{-1}\text{)} + \text{Urea excreted (g d}^{-1}\text{)}$$



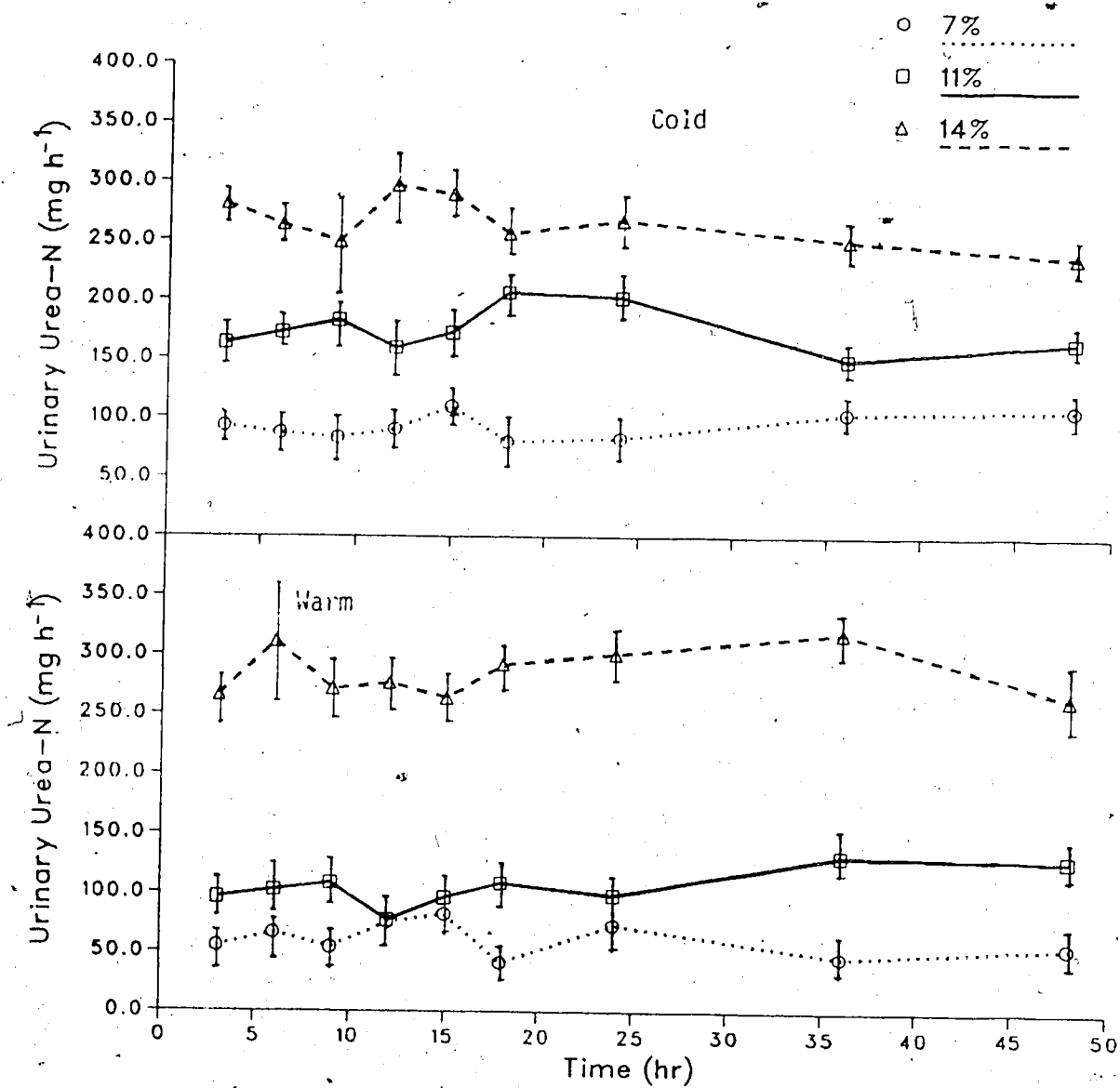
APPENDIX FIG. B. The relationship between urea spscific activity (ln dpm mg<sup>-1</sup>) and time (hrs) for 48 hours used first order kinetics in the cold and warm environment (7%.....; 11%——; 14%----)

#### TWO COMPARTMENT EQUATIONS

Temp	CP(%)	A1	$\kappa_1$	$R_1^2$	A2	$\kappa_2$	$R_1^2$
Warm	7	10.667	-.352	.756	5.280	-.055	.815
	11	10.567	-.314	.700	5.270	-.034	.999
	14	10.392	-.311	.825	5.200	-.043	.997
Cold	7	10.620	-.330	.953	4.608	-.038	.975
	11	10.721	-.351	.746	5.405	-.048	.955
	14	10.189	-.329	.941	5.070	-.043	.962



APPENDIX FIG. C. The relationship between urea specific activity (ln dpm mg<sup>-1</sup>) and time (hrs) for 18 hours following <sup>14</sup>C-urea injection in the cold and warm environment (diet CP 7%.....; 11%——; 14%----) (regression equations are shown in table 6)



APPENDIX FIG. D. Urinary urea excretion per hour bases