THE UNIVERSITY OF ALBERTA

THE APPARENT DIGESTIBILITY OF AMINO ACIDS AND OTHER NITROGEN MOIETIES IN THE SMALL INTESTINE OF SHEEP EXPOSED TO COLD ENVIRONMENTS.

by

JOHN MACLAINE KELLY

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled THE APPARENT DIGESTIBILITY OF AMINO ACIDS AND OTHER NITROGEN MOIETIES IN THE SMALL INTESTINE OF SHEEP EXPOSED TO COLD ENVIRONMENTS submitted by JOHN MACLAINE KELLY in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE in ANIMAL PHYSIOLOGY.

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Date January 22, 1987

In the first of two experiments, eight shorn yearling Suffolk wethers cannulated in the rumen, abomasum, and terminal ileum were chronically exposed to temperatures of -1 C to +1 C or +21 C to +25 C for 42 days in a crossover experiment. Animals were fed chopped brome grass at 2 hour intervals. Digesta flows through the abomasum and terminal ileum were estimated by reference to the markers ^{103}Ruthenium-Phenanthroline and ^{51}Chromium-EDTA. Dry matter (DM; $P<0.10$), organic matter (OM; $P<0.03$), non-ammonia nitrogen (NAN; $P<0.03$), amino acid nitrogen (AAN; $P<0.10$), lysine ($P<0.05$), histidine ($P<0.05$), and tyrosine ($P<0.005$) flows to the abomasum were all increased during cold exposure. The apparent digestibilities of DM ($P<0.10$) and OM ($P<0.04$) in the rumen were decreased during cold exposure. However, temperature did not significantly alter the apparent digestibilities of these components in the small intestine. The higher NAN flow to the abomasum in cold exposed animals was mainly due to an increased flow of undegraded dietary protein and was accompanied by a small but nonsignificant ($P<0.15$) increase in small intestine NAN digestibility. Disappearances of NAN and AAN in the small intestine relative to total tract OM digestibility were higher in cold exposed animals ($P<0.07$ and $P<0.08$, respectively).

In a second experiment, portal and mesenteric vein

blood flows in ewes were decreased 34% and 54%, respectively, due to cold (0 C to +2C) compared to thermoneutral 21 C to 25 C) exposure. The arterial concentrations of several essential and nonessential amino acids ($P<0.05$) were depressed in the cold, while portal and mesenteric venous concentrations were less affected. The net release of amino acids into the mesenteric circulation was slightly but not significantly reduced during cold exposure. In conclusion, apparent availability of amino acids from the small intestine per unit of total digestible OM is improved when animals are exposed to cold environments. These results together with the reduction in net released amino acids into the mesenteric blood suggests that there was increased metabolism of amino acids in the gastro-intestinal tissues.

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INTRODUCTION

Initially, digestive responses to the cold were not considered to be a physiological phenomenon (Graham et al. 1959; Blaxter and Wainman 1961). Further research at the University of Alberta has found several effects of the cold environment on ruminant animals including increased rumination activity (Kennedy 1985) and increases in the physical propulsive movements of the digesta from the rumino-reticulum (Westra and Christopherson 1976; Christopherson and Kennedy 1983; Kennedy et al. 1986a) causing more rapid transit of digesta out of the forestomach as well as a decreased rumino-reticulum volume.

Changes in the rumen environment as a consequence of cold exposure have produced differences in the products of digestion reaching the small intestine of forage fed ruminants. Decreased degradation of dietary protein as a result of decreased rumen retention time, as well as increased efficiency of microbial protein synthesis in the rumen changes the composition of digesta reaching the proximal duodenum (Kennedy and Milligan 1978; Kennedy et al. 1982).

Kennedy et al. (1986b) reported changes in the composition of amino acids flowing to the duodenum of cold exposed sheep but did not report small intestinal or post-ruminal digestibilities of the individual amino acid nitrogen or total amino acid nitrogen utilization. Also,

the post-ruminal NAN supply relative to total organic matter digestion is increased during cold exposure (Kennedy et al. 1986a). This sustained supply of protein and amino acid nitrogen from the small intestine of cold exposed ruminants may spare body tissue protein from mobilization to meet increased energy requirements (Christopherson and Young 1986). Whether there is a compensatory response occurring post-ruminally, improving the digestion of nitrogenous constituents despite decreased available digestible energy remains to be determined. Such a response could be beneficial to the ruminant particularly when they increase their intake to compensate for decreased energy digestibility.

Changes in blood flow to the gastro-intestinal tract could have marked effects on the absorption and transfer of nutrients across the small intestine (Christopherson 1985). Net amino acid release from the small intestine depends on both the flow of blood to the gastro-intestinal tract and the concentration of amino acids in both arterial and venous blood (Katz and Bergman 1969). Cold stress has been shown to affect NAN digestibility (Kennedy et al. 1986a), which may be reflected in the net release of amino acids from the small intestine.

The objectives of the studies reported herein were to examine the effects of the cold environment on the apparent digestibilities of NAN and other nitrogen moieties in the

small intestine of cold exposed sheep, and to examine the relationship between the digestion of these compounds and OM digestion in the cold environment. The flux of amino acids across the mesenteric drained viscera during cold conditions was also determined using blood flow techniques and mesenteric venous and arterial amino acid concentrations.

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LITERATURE REVIEW

Effects of Cold Exposure on Energy Requirements.

Farm animals, when exposed to changing thermal environments, undergo several metabolic adjustments in order to cope with the changing energy demands. These responses may vary from minor circulatory and postural changes to major metabolic and endocrine shifts (Kennedy et al. 1986; Sasaki and Weekes 1986).

Kleiber (1975) defined the environmental temperature below which an animals heat production increases with further decreases in environmental temperature as the lower critical temperature (LCT). The LCT is dependent on the animal's rate of heat production in thermoneutral conditions (resting heat production) as well as the thermal insulation provided by the hair coat and superficial tissues (Young 1982). The thermoneutral zone (TNZ) is a range of temperatures in which an animal maintains body temperature in the short term with little or no change in energy expenditure (Christopherson and Young 1986). The upper limit of the TNZ is called the upper critical temperature but is less defined as animals can regulate evaporative heat loss over a very wide range at negligible cost (Webster 1976)

As animals become stressed below the LCT, an immediate increase in metabolic heat production occurs in an effort to maintain a state of thermal equilibrium (Webster 1974).

When animals are chronically exposed to cold temperatures, significant alterations in the resting metabolism of the animal occur (Young 1983; Graham et al. 1980; Webster, Chlumecky and Young 1970). The metabolic changes have been associated with endocrine changes, including increases in plasma thyroxine concentrations (Westra and Christopherson 1976), increased catecholamine secretion, and cortisol metabolic clearance rate (Graham et al. 1980).

Effects of Cold on Digestion

Digestive responses to cold stress have been reported in ruminants (Graham et al. 1959; Blaxter and Wainman 1961) and have been the subject of recent reviews (see Christopherson and Kennedy 1983; Kennedy et al. 1986). Although plant species grown in moderate or cool environments tend to be more highly digestible than those grown in hot temperatures due to the lower rate of lignification (Van Soest 1982), cold stress per se reduces digestibility (Christopherson 1976).

Cold exposure of livestock results in an increase in their maintenance requirements (Blaxter and Wainman 1961; Graham et al. 1959; Young 1983). Shifts in voluntary feed consumption occur as an acute response to thermal stresses which challenge the homeothermy of the animal, or as a consequence of metabolic and digestive functional changes due to thermal acclimation (Young 1987). Voluntary feed consumption is usually increased when sheep are exposed to

cold environments (Kennedy et al. 1986a) and this results in an increase in available digestible energy despite the decrease in diet digestibility. Increased energy intake is beneficial since animal heat production is a function of the quantity and quality of food consumed (Webster 1976). Kennedy and Kelly (unpublished) observed that when an additional energy demand was placed on closely shorn lactating ewes by exposure to cold, their rate of eating chopped hay increased by 24% over corresponding values for thermoneutrally acclimated lactating sheep.

In association with the increased voluntary feed consumption, cold exposure of closely shorn sheep usually results in faster passage of digesta through the rumino-reticulum as a result of increased contraction of the reticulum (Christopherson and Kennedy 1983). Gonyou et al. (1979) also reported increased reticular motility in steers during cold exposure and even for sheep on a constant feed intake, increases in the biphasic contraction of the reticulum have been reported during chronic cold exposure (Westra and Christopherson 1976; Kennedy 1985; Kelly and Christopherson 1985). Neural and endocrine changes including increased thyroid status during cold exposure have been postulated as the major factors responsible for the increased motility (Christopherson and Kennedy 1983).

The increased rate of passage through the rumino-reticulum is associated with a decreased rumen fluid

volume and decreased retention time of digesta in the rumen in sheep (Kennedy et al. 1976; Kennedy and Milligan 1978; Kennedy et al. 1982; Kelly and Christopherson 1985) and in the digestive tract of cattle (Warren et al. 1974). A major consequence of the decreased residence time in the rumen is a depression in the time available for microbial fermentation of dietary feedstuffs. The digestibility of dry matter is directly related to the total mean retention time of digesta in the gastro-intestinal tract (Kennedy et al. 1976; Westra and Christopherson 1976).

Dry Matter and Organic Matter Digestibility and Cold Stress.

Previous studies at the University of Alberta (Christopherson 1976; Kennedy et al. 1976; Westra and Christopherson 1976; Kennedy et al. 1977; Kennedy and Milligan 1978; Gonyou et al. 1979; Kennedy et al. 1982; McBride and Christopherson 1984; Kelly and Christopherson 1985; Kennedy 1985; Chai et al. 1985) and elsewhere (Nicholson et al. 1980) have demonstrated that there is a marked effect of temperature on digestibility of forage diets, but that the response varies in different circumstances (Christopherson and Kennedy 1983). The degree of thermal stress imposed on the animals influences the extent of digestive response of the animal (Kennedy et al. 1986a). Responses to cold exposure and resulting digestibility changes are also influenced by diet. In sheep given concentrate diets, little or no digestive

responses to cold have been observed in several experiments (Young and Degen 1981; Kennedy et al. 1982; McBride and Christopherson 1984). This is likely because the rate of fermentation of concentrates is sufficiently high to avoid being influenced by a change in rumen retention time.

Depression of organic matter (OM) digestion of forage diets during cold exposure does not occur uniformly in all regions of the digestive tract. Kennedy et al. (1982) found that a 15% reduction in total gastro-intestinal tract digestibility included a 13% depression of the amount of alfalfa OM digested in the rumen with little change in postruminal digestion. Kennedy (1985) investigated the possibility that postruminal digestion of OM might possibly compensate for the decrease in the rumen but little or no quantitative compensation was observed.

Cold Stress and Nitrogen Metabolism in the Ruminant

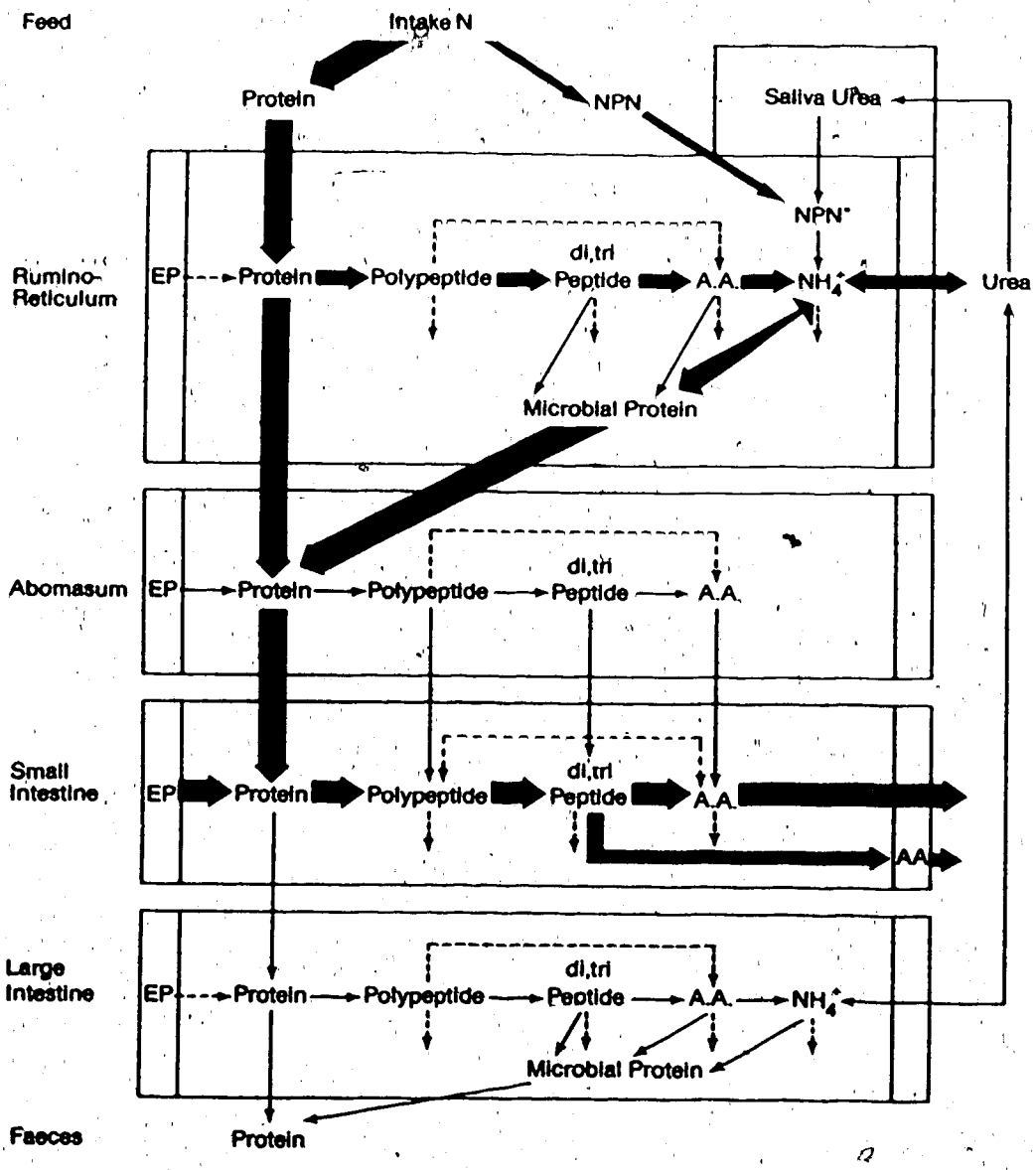
Reviews of the metabolism of nitrogen in the ruminant and its related nitrogen containing compounds have been reported in the literature (Armstrong and Hutton 1975; Thomas and Rook 1981; Buttery and Lewis 1976; Tamminga 1979; Buttery and Lewis 1982; Lindsay and Armstrong 1982; Miller 1982; Egan et al. 1986). Nitrogen and amino acid requirements are not constant but are dependent on dietary and environmental conditions as well as the physiological status and hormonal balance of the animal (Miller 1982). The purpose of this introduction is to highlight some of the basic principles of nitrogen metabolism in the ruminant

and the relevant effects of the cold environment.

Figure II-1 shows the various fates of N in the ruminant digestive tract. Entry of N into the rumen begins with the consumption of the dietary nitrogen source, coupled with the addition of endogenous N from the saliva and other sources. Once in the rumino-reticulum, these are subjected to microbial digestion and transformation (Tamminga 1979). Sources of ruminal N include endogenous inputs such as N from urea (which enters the rumen either from the saliva or directly across the rumen epithelium from the blood; Kennedy and Milligan 1980), nucleic acids from cells of either the host (which have been sloughed) or those of microbial origin, protein and amino acids of microbial origin and from the host, as well as ammonia arising from the microbial digestion of dietary and endogenous nitrogen compounds.

The rate and extent to which dietary protein is degraded in the rumen determines the ability of a diet to meet both the N requirements of the rumen microbes and the amino acid requirements of the host animal (Siddons and Paradine 1981). There are many factors affecting the digestion of N in the ruminant forestomach including the nature and solubility of the protein, rate of passage through the forestomachs (Tamminga 1979), level of feed intake (Miller 1982), quality of the diet (Christopherson and Kennedy 1983), the rate at which the protein is hydrolyzed, time spent in the rumen (Buttery and Lewis 1982), digestible energy supply, minerals (Nikolic, 1976),

Fig. II–1 Nitrogen (Protein) Flows Through the Ruminant Digestive Tract



Dotted arrows represent minor flows to following compartments of the digestive tract

NPN Non Protein Nitrogen; *Urea, NH_4^+ and other NPN sources
EP Endogenous Protein
A.A. Amino Acids

References: Salter (1973), Buttery and Lewis (1976; 1982), Bergen (1978), Egan et al. (1986)

and environmental conditions (Kennedy et al. 1986a).

Cold exposure results in an increased escape of dietary protein from degradation in the rumen (Kennedy et al. 1976; Kennedy and Milligan 1978; Kennedy et al. 1982). In addition, because of the reduced fermentation of OM in the rumen, one would expect that microbial protein synthesis would be reduced. Although this appears to be the case, there is also an increase in the efficiency of microbial synthesis (Kennedy et al. 1976; Kennedy and Milligan 1978) in the cold environment which partially compensates for the reduction in digestible OM.

Increased efficiency of microbial synthesis can be partially accounted for by the presence of endogenous N sources such as urea. Kennedy and Milligan (1980) reviewed the literature pertaining to the degradation and utilization of endogenous urea in the gastrointestinal tract of ruminants.

Figure II-1 also indicates the flows and recycling patterns of urea through the ruminant digestive tract. The recycling of N in the ruminant occurs at three major levels: protein turnover in the animal tissues; protein and amino acid secretion, desquamation, and abrasions into the gastrointestinal tract; and secretions and diffusions into the gastrointestinal tract of the end products of amino acid and nucleic acid catabolism (primarily urea) which are not directly reutilizable by the host animal (Egan et al. 1986).

Ammonia is the major source of nitrogen for microbial amino acid and protein synthesis. In the rumen, urea is rapidly hydrolyzed to ammonia which in turn is available for metabolism by rumen microbes (Van Soest 1982). Nolan and Leng (1972) found that up to 80 % of the microbial nitrogen had passed through the rumen ammonia pool. The possible sources for ammonia in the rumen include dietary, endogenous, and microbial protein N; nucleic acid N from cell sloughing, diet, and microbial origin; and supplemented or endogenous urea (Kennedy and Milligan 1980). Rumen ammonia concentrations of sheep given brome grass are reduced during cold exposure (Christopherson and Kennedy 1983), an observation which has implications for urea recycling to the rumen.

Recycling of urea to the rumen can occur via two routes; the first being transport through the saliva and the second via direct transport across the rumen epithelium (Orskov 1982).

Recycling of urea via the saliva is directly proportional to the blood urea nitrogen concentration and the quantity of saliva produced (Orskov 1982), which could potentially be an important source for animals fed long roughages. Normally, saliva urea concentrations are approximately 60 % of the plasma urea concentration (Kennedy and Milligan 1980). The transfer of urea, then, is dependent on the copious flow of saliva, which is affected by such factors as physical form of the diet (Kay

1960). There is controversy surrounding the relative importance of salivary urea with respect to the total urea transfer to the rumen. Nolan and Leng (1972) indicated that in sheep fed lucerne, salivary urea could account for most of the urea entering the rumen. More recent evidence by Kennedy and Milligan (1978) suggested salivary input only accounted for 15 % of the nitrogen entering the rumen.

Urea recycling by direct transport across the rumen epithelium is mediated by ammonia concentrations in the rumen. Increasing concentrations of ammonia in the rumen have an inhibiting effect on urea transfer to the rumen across the rumen epithelium (Kennedy and Milligan 1980) up to a maximal concentration in sheep given brome grass (Kennedy et al. 1986a). Concentration of ammonia in the rumen of sheep given brome grass declined as temperature decreased (Christopherson and Kennedy 1983) which is a consequence of decreased dietary protein degradation in the rumen (Kennedy et al. 1982). Reduced concentrations of rumen ammonia to levels below 200 mg N L^{-1} in sheep have been related to the increased transfer of plasma urea through the rumen wall directly into the rumen (Kennedy and Milligan 1980). An increase in the transfer of urea across the rumen epithelium would partially offset the decreased ammonia formation from dietary N and thereby maintain substrate for microbial protein synthesis (Kennedy et al. 1986a).

After spending some time in the forestomach, digesta

escapes via the reticulo-omasal orifice to the omasum and then the abomasum. Once in the abomasum, all protein and peptide N sources are subject to the enzymatic attack of the protease pepsin. Due to the weakly alkaline nature of pancreatic and biliary secretions, an extension of the acidic conditions in the abomasum occurs in the proximal small intestine. The slow rise in pH along the proximal intestine of ruminants may allow the actions of gastric pepsin to be lengthened, and decrease the relative time for action of pancreatic and intestinal enzymes (Ben Ghedalia et al. 1974; Armstrong and Hutton 1975).

The quantity of protein digested in the intestines and that which is subsequently available for metabolic processes will depend on both the flow and composition of NAN entering and leaving the intestines (Christopherson and Kennedy 1983). The nitrogenous materials entering the small intestine of ruminants include endogenous N secreted into the abomasum and small intestine, nitrogenous materials that result from microbial fermentation in the rumino-reticulum, those nitrogenous components of dietary or endogenous origin that have escaped ruminal fermentation and any ammonia that results from the microbial fermentation of nitrogenous materials in the rumino-reticulum but is neither utilized for microbial protein synthesis (Lindsay and Armstrong 1982) nor absorbed prior to the proximal duodenum (Armstrong and Hutton 1975). The principle forms of N entering the small intestine of

ruminants are proteins, nucleic acids and residual ammonia (Lindsay and Armstrong 1982).

Microbial protein, which usually accounts for the bulk of total amino acid N entering the small intestine (Storm and Orskov 1984), undegraded dietary protein, peptides, and possibly small amounts of free amino acids pass with the digesta from the rumino-reticulum and form the bulk of the N sources absorbed in the small intestine (Bergen 1978). Previously, it was thought that the intestinal digestibility of dietary N was dependent on the proportion of dietary N which escaped rumen fermentation (Christopherson and Kennedy 1983). Indeed it was found that the effects of the cold environment on rate of passage of digesta through the rumino-reticulum had consequent effects on protein supply to the small intestine and the proportion of digestion of carbohydrate and protein occurring in the rumen and post-ruminal sites (Kennedy and Milligan 1978). Cold exposed sheep have tended to have increased flows of NAN to the small intestine largely as a result of increased flow of undegraded dietary N (Kennedy and Milligan 1978, Kennedy et al. 1982). However, the composition of the NAN reveals that microbial contributions to this flow are influenced by diet, with a higher proportion of microbial N for alfalfa compared to bromegrass diets (Christopherson and Kennedy 1983). Recent work comparing four forage diets indicated that there were no significant effects of temperature on the absolute

amount of NAN digested in the post-ruminal tract (Kennedy et al. 1986a). On the other hand, the ratio of intestinal NAN digestion to total OM digested was increased in the cold.

Thus, despite a decline in the overall OM digestibility, there appear to be beneficial changes occurring in the gastro-intestinal tract with repect to N digestion (Christopherson 1985). It is concluded that in a cold environment, the nitrogen stability of the animal is maintained, while energy digestibility is reduced with the net result being increased total food requirements of the animal (Christopherson and Young 1986).

It appears then, that ruminants exposed to the cold environment experience a decrease in overall digestibility of the feed, but the extent of the effects on the digestibility of NAN appears to be less than that for other components of digesta. Ames and Brink (1977) concluded that lambs could be maintained on lower N containing diets during cold exposure. This suggestion is consistent with arguments presented above. However, further research is needed to verify this suggestion.

Research investigating the effects of the cold environment on the quantitative digestion of protein and amino acids in the small intestine relative to total and ruminal N transformations is lacking. The objectives of the research presented in this thesis are to examine the effects of cold stress on the relative digestibility of NAN

in various sections of the ruminant digestive tract.

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III. THE APPARENT DIGESTIBILITIES OF DRY MATTER AND ORGANIC MATTER IN THE FORESTOMACH, SMALL INTESTINE, AND LARGE INTESTINE OF WETHERS EXPOSED TO COLD ENVIRONMENTS.

INTRODUCTION

Cold environmental temperature depresses the digestibility of dry matter (DM) and organic matter (OM) of feedstuffs in sheep (Graham et al. 1959, Bailey 1964, Christopherson 1976, Kennedy et al. 1976, Westra and Christopherson 1976, Kennedy et al. 1977, Kennedy and Milligan 1978, Nicholson et al. 1980, Kennedy et al. 1982, Kennedy 1985) and cattle (Blaxter and Wainman 1961, Warren et al. 1974). In order to compensate for a reduced digestibility, the feed requirement of ruminants exposed to cold environments may be increased (Westra and Christopherson 1976).

Considerable attention has been focused on the rumino-reticulum as a major site of temperature-induced changes which result in a depression of digestibility. The rumino-reticulum kinetics and turnover of fluid and particulate matter and rumination frequency, in particular, have been investigated (Christopherson and Kennedy 1983). Studies with sheep and cattle maintained in controlled environmental chambers (Westra and Christopherson 1976, Gonyou, Christopherson and Young 1979, Kelly and Christopherson 1985, Kennedy and Kelly, unpublished) have indicated an association between cold exposure and

increased frequency of biphasic contractions of the reticulum. Retention time of digesta within the rumino-reticulum is negatively correlated with the frequency of reticulum contraction and is reduced during cold exposure (Westra and Christopherson 1976). Increased rumination activity in the cold environment (Gonyou, Christopherson and Young 1979, Kennedy 1985) has also been suggested as a further aid to the passage of digesta to the small intestine. There is, however, incomplete information on the quantitative importance of specific post-ruminal sites of digestion of DM and OM in the ruminant as influenced by cold exposure. Assessment of the effects of the cold on digestion of these components has been limited to a partitioning of the digestive processes into the forestomach and the total intestinal tract compartments (Kennedy et al. 1976, Kennedy and Milligan 1978). Only two studies have examined the partition of digestion of chopped forages between the forestomach and post-ruminal tract in the cold environment (Kennedy et al. 1982, Kennedy 1985). These studies have demonstrated that decreased ruminal digestion may be partially compensated for by increased post-ruminal digestion. Further partitioning of post-ruminal digestion into the small and large intestine with respect to the influences of the cold environment has not been investigated.

The objectives of this study were to determine in more detail the major sites of digestion of DM and OM and in particular to quantify the effects of a cold environment on the digestibility of OM and DM in the forestomach, small intestine and large intestine of the sheep.

MATERIALS AND METHODS

ANIMALS AND THEIR MANAGEMENT

Eight Suffolk yearling wethers weighing 37 to 51 Kg were fitted with ruminal, abomasal, and terminal ileal cannulae. The latter two cannulas were one piece "T" type cannulae which were made of plastisol. The animals were anaesthetized using a nitrous oxide - halothane - oxygen mixture throughout the surgical preparation of the post-ruminal cannulae, while rumen cannulae were inserted under a local anaesthetic (Lidocaine). Post-surgical care involved antibiotic treatment with Liquamycin for three days, treatment for pain with aspirin (975 mg d^{-1}) administered orally, and placement of the animal in a warm comfortable pen. All animals were given a minimum two weeks to recover from the surgery before commencement of the diet and environmental adjustment period.

Prior to being exposed to the experimental conditions, all wethers were treated for internal and external parasites by means of a drench (Thiobenzol) and spray (Co-Ral, active ingredient coumaphos - 25%), respectively.

All wethers were housed in individual metabolism crates

with floors constructed of "tenderfoot" grating. Rapid and easy collection of unused feed as well as faecal material was facilitated by placing nylon screening under the flooring of the crates.

FEEDING

All animals were fed ad libitum a diet of chopped brome hay (Bromus inermis; Table III-1) for 2 weeks prior to the start of the experiment to estimate voluntary intake. Thereafter, all animals were fed 1600 g d^{-1}, which was approximately 95% of the maximum consumption of the warm acclimated sheep. All wethers were fed this diet by means of an automatic feeder, which delivered equal portions every 2 hours twelve times daily. All feed not consumed was weighed, to acquire correct daily intakes. Water and Cobalt-Iodized salt were available ad libitum.

EXPERIMENTAL DESIGN AND ENVIRONMENTAL TREATMENTS

The experimental statistical model was a two by two crossover design, involving two periods each of 42 days duration. The first 30 days prior to experimental sampling served as an adjustment period. During period I, four animals were exposed to a cold (0 C to +2 C) environment, and four were exposed to a thermoneutral (21 C to 25 C) environment. During period II, the animals were crossed into the opposite treatment and the adjustment and experimental protocols repeated. Both environmental chambers were continuously lighted for the duration of the experiment.

SAMPLING PROCEDURE

From day 32 to day 34, faeces and digesta were sampled for the determination of digesta composition. Sixty mL of digesta was collected four times daily at 07:00, 11:00, 15:00 and 19:00 h. Samples were obtained from the rumen utilizing a solid sampling probe, which subsampled whole rumen contents for determination of DM and OM content, while separate fluid samples for the determination of volatile fatty acids (VFA) were obtained using a hollow tube the end of which was encased in a 50 um mesh which excluded particulate matter. Digesta was collected from the abomasum and terminal ileum by placing collection vials on the cannulae at each location. Because of the high fluid content of the abomasal digesta, the containers filled rapidly usually within 4 minutes. In the ileum, however, digesta has a higher dry matter content and less frequent contractions occur in this location. Therefore sample vials were left on the cannulae for up to 45 minutes to obtain sufficient sample.

ISOTOPE INFUSION AND DIGESTA COLLECTION

From day 35 to day 42, a continuous infusion (75 mL d^{-1}) of a solution containing ^{103}Ruthenium labelled Tris-(1,10-Phenanthroline)-ruthenium (II) chloride (^{103}Ru-P) complex (2.68 mCi mg^{-1} complex; 4.95 uCi ^{103}Ru-P d^{-1}); Tan, Weston, and Hogan 1971) and ^{51}Chromium (228.39 mCi mg^{-1} Cr; 60 uCi ^{51}Cr d^{-1}) complexed with ethylaminediamine tetraacetate (EDTA) was

initiated into the rumen of all sheep following a priming dose of 37.5 mL. The infusion was continued for eight days (75 ml d^{-1}) with sampling of abomasal and terminal ileal digesta occurring four times daily (07:00, 11:00, 15:00, 19:00) during the last three days of infusion. Samples were bulked in two main portions, the first six samples making up one bulk container, and the last six samples making up the second bulk container. Calculations of the flows of digesta through the abomasum and terminal ileum using the double marker method were made after corrections for absorption of ^{51}Cr-EDTA by mathematical recombination of the digesta fractions according to the techniques of Faichney (1975). On day 43, the isotope infusion was ceased and rumen samples were obtained for the determination of rumen kinetics according to the following schedule: 0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 7.0, 9.0, 11.0, 13.0, 24.0, 28.0, and 33.0 h after cessation of marker infusion. Kinetics of the particulate and fluid markers in the rumino-reticulum were determined according to the equations of Shipley and Clark (1972).

ANALYTICAL TECHNIQUES

Dry matter was determined by drying the samples in a forced air oven at 60 C to a constant weight. Organic matter was determined on these dry samples by ignition in a combustion furnace at 550 C overnight. Chromium-51 and ^{103}Ru-P were analyzed on a gamma spectrometer (Gamma 8000, Beckman Instruments Inc., Fullerton, California,

U.S.A.) with corrections for ^{103}Ru-P spillover into the ^{51}Cr window (B. V. Turner, personal communication).

Cell wall constituents (CWC), acid detergent fiber (ADF), and hemicellulose were all analyzed according to Goering and Van Soest (1970).

Volatile fatty acids were prepared utilizing a modification of the method of Chase (no date) using caproic acid as an internal standard and were analyzed on a gas chromatograph (Varian 3700, OV-351 30 m capillary column, initial temperature 130 C, temperature programmed at 10 C per minute to final temperature 185 C, Supelco Inc.).

Statistical Analysis

Data were subjected to analysis of variance using UANOVA and treatment effects were determined using sheep/groups as the error term.

RESULTS

1. Flow of Dry Matter and Organic Matter.

Dry matter and OM intake was maintained constant between treatments and no feed refusals were observed. Dry matter flow and OM flow through the abomasum were higher ($P<0.10$ and $P<0.03$ respectively) in cold exposed animals (Table III-2). Environmental temperature did not affect terminal ileal DM or OM flows. Although more faecal DM and OM tended to be excreted in the cold, the effect of temperature was not significant. Flow of CWC by the abomasum was slightly but not significantly higher in

the cold exposed animals, and faecal excretion of CWC was higher in cold exposed animals ($P<0.05$). ADF flow to the large intestine was increased in cold exposure ($P<0.01$). Faecal excretion of ADF was increased in the cold. Hemicellulose flow through the abomasum tended to be increased in the cold exposed wethers.

2. Digestion of Dry Matter and Organic Matter.

Animals exposed to the cold temperatures experienced a substantial decline in DM and OM digestibility in the forestomach ($P<0.10$ and $P<0.03$, respectively, Table III-3). There was no significant change in post-ruminal DM digestibility, however post-ruminal OM digestibility increased in the cold environment. Whole tract digestibility of DM was not altered by exposure to cold temperatures, but OM disappearance was depressed ($P<0.10$). Digestibility of DM and OM as a percentage of intake is shown in Table III-3. Whole tract disappearances of CWC and ADF were decreased, however nonsignificantly, in the cold exposed sheep. Forestomach disappearance of CWC was slightly but not significantly decreased in the cold. Hemicellulose disappearance in the forestomach was less in the cold.

3. Rumen Volatile Fatty Acid Concentrations

Concentrations of total VFA's in the rumen ranged from 830 mmol L^{-1} to 900 mmol L^{-1} and were unaffected by the temperature treatments, however individual VFA's showed significant changes due to temperature. Acetate and

propionate were decreased and increased, respectively, by the cold temperatures ($P<0.03$ and $P<0.001$). Concentrations of the other VFA's were not significantly different between treatments (Table III-4). Rumen pH was elevated ($P<0.03$) in cold exposed wethers, but temperature had no effect on abomasal or terminal ileal pH (Table III-4).

4. Rumen Turnover Time, Fluid Outflow Rate, Particulate Outflow Rate, and Rumen Volume.

Rumen fluid turnover time was non-significantly decreased ($P<0.09$) in the cold exposed animals (Table III-5) whereas the turnover time of the particulate marker (^{103}Ru-P) was decreased ($P<0.05$) in the cold. Both fluid and particulate outflow from the rumen (as labelled with ^{51}Cr-EDTA and ^{103}Ru-P, respectively) were nonsignificantly increased while rumen fluid volume was slightly less ($P<0.10$) in the cold.

DISCUSSION

In these experiments, exposure to a cold environment induced a measureable and biologically significant effect on digestibility in sheep. The overall reductions in total OM digestibility observed in this experiment were similar to other results that have been previously reported (Kennedy et al. 1976, Kennedy and Milligan 1978, Kennedy et al. 1982, Christopherson and Kennedy 1982, Kennedy 1985, Kennedy et al. 1986). Reductions in the total gastrointestinal tract digestibility of OM components (CWC, ADF, and hemicellulose) are also in agreement with

published results (Kennedy et al. 1982).

Rumen turnover times (RTT) are correlated to rumen mean retention times (MRT) and are affected by several stresses imposed on the ruminant. Faichney and White (1980) reported decreased MRT's in pregnant and lactating animals. Climatic effects on the turnover of digesta in the rumen have been observed by Faichney (1986), who reported an increase of 2.1 h in the MRT of sheep exposed to 30 C when compared to 21 C. Warren et al. (1974) also reported an increase in the MRT in cattle exposed to 32 C as opposed to thermoneutral conditions.

Conversely, cold stress has been shown to initiate a decreased MRT of the particulate phase of digesta in sheep (Kennedy et al. 1976, Westra and Christopherson 1976, Kennedy and Milligan 1978, Kennedy et al. 1982, Christopherson and Kennedy 1983, Kennedy 1985, Kennedy et al. 1986). Decreases in RTT are associated with an increased voluntary feed consumption in cold exposed sheep (Kennedy et al. 1986). In this study, feed consumption was equalized across treatments to avoid possible alterations in digestibility due to different intakes. A 9% decrease in RTT occurred in the cold exposed animals suggesting more rapid transfer of digesta to the postruminal tract even though intake was held constant. Possible explanations for these results are increased rumino-reticulum motility (Westra and Christopherson 1976, Kennedy and Kelly, unpublished, Kelly and Christopherson 1985, Lirette et al.

1987) and increased rumination activity resulting in more efficient breakdown of particulate matter to a size eligible to pass out of the rumen or reticulum (Chai et al. 1985). Lirette and Milligan (1987) suggested that the relative resistance of particles to clearance is directly related to their size. Although rumination activity was not recorded in the present experiment, a decreased RRT might have been a consequence of a relative improvement in particle breakdown due to chewing. The increased rates of passage of DM and OM to the postruminal tract were associated with a general decrease in the forestomach digestibility of OM in the cold. It is likely that the decreased residence time of digesta in the rumino-reticulum during cold stress reduced the time available for fermentation of feed by the microbial population.

The increased flow through the abomasum and decreased disappearance in the rumen of DM and OM in the cold are possibly a consequence of the thyroid status of the animals. Westra and Christopherson (1976) reported a significant correlation between plasma thyroxine (T4) and triiodothyronine (T3) and reticular motility and the mean retention time of digesta in the gastrointestinal tract in cold exposed animals. Kennedy et al. (1977) reported decreased digestibility of DM in both cold exposed animals and T3-injected sheep.

The cold induced decreases in total VFA concentrations and increases in ruminal pH environment are consistent

with results reported previously (Kennedy et al. 1976). Martz et al. (1970) reported decreased total VFA in cold exposed dairy cattle. Conversely, pH has been found to be reduced in conjunction with high lactic acid concentrations during heat stress in dairy cows (Mishra et al. 1970). Associated with this condition is decreased rumino-reticulum motility while the opposite situation is evident during cold stress (Christopherson and Kennedy 1983).

Acetate and propionate are the main endproducts of carbohydrate digestion in the rumen (Van Soest 1982). The decreased concentration of acetate and increased propionate concentration in the rumen during cold exposure indicate an alteration in the qualitative nature of energy substrates available to the tissues. A depression in the molar proportion of acetate in the rumen often accompanies a decreased turnover time of digesta in the rumen (Christopherson and Kennedy 1983). The shift observed in the molar proportions of acetate and propionate in total VFA is consistent with the reduced rumen digestion of cellulose and hemicellulose in the cold environment. An important implication of this result arises from the claim that there is an increase in the efficiency of utilization of metabolizable energy when the proportion of propionate to acetate in the rumen end products is increased (Van Soest 1982). In addition, because propionate is a major glucogenic precursor in ruminants, an important metabolic

consequence of the increased propionate/acetate ratio during cold exposure is maintenance of the availability of propionate to serve as a substrate for gluconeogenesis. This would be expected to help meet the need for increased glucose oxidation in the cold (McKay et al. 1972) thus sparing amino acids as the main alternative source of carbon for gluconeogenesis.

The reduction in total tract digestibility due to cold exposure appeared to be primarily a result of the modification of the rumino-reticulum degradation. The small intestinal disappearance of DM was unaffected by cold. However, in the small intestine a higher proportion of OM tended to be digested in the cold when expressed as a percentage of total OM disappearance (Table 6). The relative shift in the site of digestion from the forestomach to the post ruminal tract due to cold temperature is consistent with previous studies (Kennedy et al. 1976, Kennedy and Milligan 1978, Kennedy et al. 1982). The shift in proportion of OM digested in the post-ruminal tract is also consistent with previous observations, but the present results are the first to indicate the influence of the small intestine and large intestine separately.

The reduced OM digestibility in the cold environment is in agreement with the results of Kennedy et al. (1982). The apparent digestibility of OM in the rumen and consequently the whole digestive tract has been shown to be related to retention time of the particulate marker of

digesta in the rumen (Kennedy et al. 1976, Kennedy et al. 1982, Kennedy 1985), which is consistent with the results of this experiment. This is particularly true for chopped brome diets. Kennedy (1985) reported that the rate of comminution of large particles contributed significantly to retention time of digesta in the rumen and was inadequately represented by the particulate marker ^{103}Ru-P. A better prediction for the digestion of OM in the rumen would therefore be attained when both the proportion of large particles in the rumen and the ^{103}Ru-P retention time are included as independent variables.

In absolute terms, this experiment confirms the theory that the depressing effect of cold temperature on the total tract digestibility of OM is mainly a consequence of alterations in the forestomach digestion and turnover time. The proportion of digestible OM intake apparently digested in the rumino-reticulum was higher in the thermo-neutral compared to the cold environment (0.65 versus 0.58) and these values are in the range reported by Thomson and Beever (1980) and Beever and Siddons (1986), but are slightly higher than those of Ulyatt and MacRae (1974). However, when the proportion of OM digested in the small intestine relative to the total amount of OM digested in the whole gastrointestinal tract is considered, it is apparent that there was a shift in the site of digestion. Kennedy (1985) stated that although the apparent digestion of OM in the gastro-intestinal tract was reduced, the

difference was not as great as indicated by the ruminal disappearances of OM, indicating that the intestines could partially compensate for the decreased ruminal disappearance. When expressed as a percentage of total digestion, the rumen digestion of OM in the warm animals was 8% higher while the small intestinal digestion of OM was 9% higher in the animals in the cold environment. This finding could have important consequences in the energy available for microbial protein synthesis in the rumen and subsequent supply of NAN and amino acids to the small intestine. This is discussed in a subsequent paper (Kelly and Christopherson 1986).

The disappearances of CWC, ADF, and hemicellulose in the rumino-reticulum were decreased by 14%, 12%, and 14%, respectively, during exposure to cold environments. The retention time of digesta was also depressed, and these results are consistent with those reported by Kennedy (1985) who calculated that a reduction in the retention time of CWC in the forestomach from 20 h to 10 h would constitute a decrease in digestion of 9.0 g CWC/100g CWC intake. Cold exposure did not alter the rate of digestion of CWC, but decreased the time available for digestion.

Post-ruminal digestion of CWC, ADF, and hemicellulose was not affected by cold exposure, results which also agree with those of Kennedy (1985). The relatively high digestion of ADF in the small intestine as a proportion of total digestion may be due to an artifact of the analysis

for ADF. Heating probably occurred in the preparation of samples for analysis (G. W. Mathison, personal communication) which tends to generate Maillard polymers which difficult to remove or distinguish from lignin (Van Soest 1982). The Maillard reactions involves condensation of amino groups with carbonyl and dehydroreductone compounds derived from carbohydrate which are the polymerized into a lignin like complex. Polymerization results in permenantly bound and indigestible nitrogen. Other compounds which can be found resulting from this procedure are phlobaphenes (lignin like compounds formed from non-lignin precursors), tannins, and phenolic matter, which introduces uncertainty as to the true lignin content.

The proportion of the digestion of hemicellulose occurring ruminally and postruminally (Table 6) agrees with the result presented by Beever et al. (1981). The digestion of CWC in the intestines (g $100g^{-1}$ entering the small intestine) was slightly higher than the values reported by Kennedy (1985) who fed a similar diet. Beever et al. (1981) reported that pelleting roughages decreased ruminal digestion of CWC, an effect which was partially compensated for by increased intestinal digestion. In the present study, it appears that the whole gastrointestinal tract depression in CWC digestibility as well as ADF and hemicellulose digestion was a function of the decreased forestomach residence time with no compensation occurring in the intestines.

Tsuchiya et al. (1974) observed increased gastro-intestinal motility in hypothalamically cooled dogs. If the motility of the total gut is increased during cold stress and thus the turnover time of the digestive tract is decreased, the observation of a depression in forestomach digestion but not in intestinal digestion would appear to be a contradiction. This can be explained by the larger quantity of digesta arriving in the small intestine, driving an increased relative absorption in this part of the digestive tract. On the other hand, there seems to be little or no effect of temperature on post-ruminal rate of passage in sheep (Kennedy et al. 1986). The proportion of digestion occurring in the small intestine or the lower digestive tract is relatively small (28 %) compared to that occurring in the rumino-reticulum (72 %) in cattle fed a maintenance level of brome hay as described by Lirette and Milligan (1986). Values for data reported in the present study are slightly higher for intestinal digestion and lower for ruminal digestion (Table 6). The cold-induced shift of absorption site did not totally compensate for the reduced rumino-reticulum digestibility due to cold stress. Consequently, the total gut digestibility is reduced in the cold, but to a lesser extent than that caused by the reduction in the rumino-reticulum.

This study has demonstrated a shift in favour of the lower digestive tract in the location of OM digestion

occurring in cold exposed sheep. Organic matter tended to be digested to a greater degree in the small intestine relative to the total tract OM digestion during cold exposure.

Table III-1. Composition of brome hay dry matter (DM) fed to warm and cold exposed wethers.

Item	g $100g^{-1}$ DM
Dry matter (DM g $100g^{-1}$ intake)	86.25
Organic matter	92.83
Cell wall constituents	57.38
Acid detergent fibre	31.03
Hemicellulose	26.35
Protein	11.64

Table III-2. Intake; flows through the abomasum, terminal ileum; and faecal excretion (g d^{-1}) of dry matter (DM), organic matter (OM), cell wall constituents (CWC), acid detergent fibre (ADF), and hemicellulose in warm and cold exposed wethers.

	Warm	Cold	S.E.	Sig.
Intake				
DM	1380	1380	–	
OM	1281	1281	–	
CWC	791	791	–	
ADF	428	428	–	
Hemicellulose	363	363	–	
Abomasum				
DM	994	1079	20.5	*
OM	796	898	17.0	**
CWC	413	465	12.9	
ADF	277	296	6.6	
Hemicellulose	136	168	7.8	
Terminal Ileum				
DM	734	797	33.2	
OM	591	643	28.7	
CWC	375	415	7.9	**
ADF	227	264	4.6	***
Hemicellulose	148	150	6.9	
Faeces				
DM	638	712	27.3	
OM	558	628	35.2	
CWC	308	379	21.9	**
ADF	202	245	12.9	
Hemicellulose	106	134	9.8	

* Means across temperatures are different ($P<0.10$).

** Means across temperatures are different ($P<0.05$).

*** Means across temperatures are different ($P<0.01$).

Table III-3. Disappearance (g d^{-1}) of dry matter (DM), organic matter (OM), cell wall constituents (CWC), acid detergent fibre (ADF) and hemicellulose along the gastrointestinal tract of wethers exposed to warm and cold environments.

	Warm	Cold	S.E.	Sig.
Forestomach				
DM	386	301	20.5	
OM	485	383	17.0	**
CWC	378	327	12.9	
ADF	151	132	6.6	
Hemicellulose	228	195	7.8	
Small Intestine				
DM	280	282	30.8	
OM	224	256	19.0	
CWC	38	50	19.9	
ADF	50	32	10.3	
Hemicellulose	-12	18	14.2	
Large Intestine				
DM	96	88	24.0	
OM	32	16	19.0	
CWC	67	36	26.7	
ADF	25	19	13.4	
Hemicellulose	3	28	16.0	
Total Gastrointestinal Tract				
DM	762	671	24.8	
OM	745	654	20.9	
CWC	483	412	21.9	
ADF	226	183	12.9	
Hemicellulose	257	230	9.8	
Total Digestibility (%)				
DM	53.8	48.4	1.9	
OM	56.4	51.0	1.7	
CWC	61.0	52.1	2.8	
ADF	52.8	42.3	3.0	
Hemicellulose	70.7	63.1	2.7	

* Means across temperatures are different ($P<0.10$).
** Means across temperatures are different ($P<0.05$).
*** Means across temperatures are different ($P<0.01$).

Table III-4. Rumen volatile fatty acid composition, and rumen, abomasum and terminal ileum pH in warm and cold exposed wethers.

	Warm	Cold	S. E.	Sig.
Volatile Fatty Acid(mmol 1000mmol^{-1})				
Acetate	690.6	675.5	3.29	**
Propionate	192.2	210.8	1.65	***
Isobutyrate	11.2	11.1	0.38	
Butyrate	82.0	79.1	2.43	
Isovalerate	12.0	12.0	0.56	
Valerate	12.0	11.4	0.39	
Total VFA (mmol l^{-1})	900.0	830.0	18.0	
pH				
Rumen	6.72	6.96	0.04	**
Abomasum	2.87	2.70	0.10	
Terminal Ileum	8.03	7.96	0.07	

** Means across temperatures are different ($P<0.05$).

*** Means across temperatures are different ($P<0.01$).

Table III-5. Rumen fluid volumes and turnover times (using ^{51}Cr-EDTA), and rumen particulate matter turnover (using ^{103}Ru-P) in warm and cold exposed wethers.

	Warm	Cold	S.E.	Sig.
Fluid turnover time (h)	12.58	11.77	0.468	*
Rumen fluid volume (L)	6.62	5.44	0.744	*
Particulate turnover time (h)	14.47	12.92	0.429	**

** Means across temperatures are different (P<0.05).

*** Means across temperatures are different (P<0.01).

Table III-6. Disappearance of dry matter (DM), organic matter (OM), cell wall constituents (CWC), acid detergent fibre (ADF) and hemicellulose (g $100g^{-1}$ digested) along the gastrointestinal tract of warm and cold exposed wethers.

	Warm	Cold	S.E.	Sig.
Forestomach				
DM	50.7	44.9	4.6	
OM	65.1	58.5	2.8	**
CWC	79.1	80.4	3.0	
ADF	68.2	71.9	4.4	
Hemicellulose	88.9	86.2	1.9	
Small Intestine				
DM	36.7	42.0	3.3	
OM	30.1	37.1	2.8	
CWC	8.1	12.6	5.6	
ADF	21.2	22.9	8.1	
Hemicellulose	-3.5	7.6	5.6	
Large Intestine				
DM	12.7	13.1	3.4	
OM	4.3	2.5	2.9	
CWC	12.8	7.0	6.2	
ADF	10.6	5.2	8.7	
Hemicellulose	14.7	6.3	5.8	
Post Rumen				
DM	49.4	55.1	4.6	
OM	34.4	39.5	4.2	
CWC	20.9	19.6	3.0	
ADF	31.8	28.1	4.4	
Hemicellulose	11.1	13.9	1.9	

** Means across temperatures are different ($P<0.05$).

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IV. INVESTIGATIONS OF THE APPARENT DIGESTIBILITY OF AMINO ACIDS AND OTHER NITROGENOUS COMPOUNDS IN THE SMALL INTESTINE OF WETHERS EXPOSED TO COLD ENVIRONMENTS.

INTRODUCTION

Acclimatization to a cold environment induces a number of adjustments in energy and protein metabolism in ruminants (Kennedy et al. 1986a). These changes include decreased digestibility of dry matter (DM), organic matter (OM) (Kelly and Christopherson 1986), and nitrogen (N) among certain diets (Christopherson and Kennedy 1983) which are associated with physiological changes in the animal's requirements during cold exposure. However, low temperatures increased voluntary feed consumption (Kennedy et al. 1982).

Depressions in the digestion of dietary nonammonia nitrogen (NAN) in the rumino-reticulum and depressions in microbial protein synthesis have been associated with cold exposure (Kennedy et al. 1976, Kennedy and Milligan 1978, Kennedy et al. 1982). These results are likely due to changes in certain rumen characteristics during cold stress. It has been found that the rumen volume and retention time of rumen digesta are reduced while the rate of flow of digesta through the rumen is increased, an effect that is particularly apparent with roughage feeds (Kennedy et al. 1986a).

Measurement of the NAN flow through the abomasum or

duodenum is generally used to gain information on the protein available for absorption in the small intestine (Orskov et al. 1986). An increased flow of undegraded dietary protein to the small intestine is associated with the changes occurring in the rumen during cold exposure (Kennedy et al. 1986a). The increases in digestibility of protein in the post ruminal tract observed in previous studies (Kennedy et al. 1978; Kennedy et al. 1986a) may indicate that preferential absorption of individual amino acids occurs in the small intestine of sheep in cold environments (Christopherson and Kennedy 1983). However, there is no information on the effects of temperature on net digestion of proteins and amino acids in the small intestine. The objectives of this study were to determine the effect of environmental temperature on the apparent digestibilities of protein and amino acid nitrogen in various sections of the gastrointestinal tract, including the small intestine, and to determine if there is a relationship between these digestibilities and the total tract OM digestibility.

MATERIALS AND METHODS

Animals and Their Management.

Eight Suffolk yearling wethers weighing 37 to 51 kg were fitted with ruminal, abomasal, and terminal ileal cannulae. Animals were housed in either warm (21 C to 25 C) or cold (0 C to +2 C) chambers which were continually

lighted. Animal handling, feeding, experimental design and statistical analyses were described previously (Chapter III).

Sampling Procedure.

Sampling of digesta was divided into two separate trials within each period as specified in the previous chapter. Collections of digesta were used for the determination of nitrogen (N), ammonia nitrogen, NAN, and amino and non-amino acid nitrogen during the first trial of each period, and the contribution of microbial nitrogen to the total nitrogen was determined during the second trial of each period.

Flows of Nitrogenous Compounds.

Flows of digesta, and consequently the nitrogenous components, were determined using the double marker system employed by Faichney (1975). The particulate marker ^{103}Ruthenium-phenanthroline (Tan et al. 1971) and fluid marker ^{51}Chromium-EDTA were infused continuously at a rate of 75 mL day^{-1} for 8 days after a priming dose of 60 mL with sampling for the isotopes occuring during the final three days of infusion (Chapter III).

Determination of Microbial Protein.

Microbial protein contributions to total protein were determined using ^{35}S as a microbial marker (Mathers and Miller 1980). $Na_2{}^{35}SO_4$ (10.56 mCi ug^{-1} $Na_2{}^{35}SO_4$; 109 uCi ^{35}S d^{-1}) was continuously infused into the rumen of sheep for eight days along with markers for digesta flowrate. Samples of digesta for N

studies were collected as described in Chapter III.

Preparation of Microbial Fraction.

The microbial fraction of the rumen digesta was isolated using differential centrifugation techniques. Seventy-five mL of digesta was centrifuged at 1000 g for one minute at 4 C. The supernatant was decanted into a second tube and spun at 20,000 g for 20 minutes at 4 C. The supernatant of this sample was discarded and the microbial pellet resuspended in buffer (McDougall 1948). Once again, this solution was centrifuged at 20,000 g for 20 minutes at 4 C. This supernatant was then decanted and the remaining pellet was freeze dried.

Analytical Techniques.

Total N was determined by the macro-Kjeldahl method (Association of Official Analytical Chemists 1975). Ammonia N was determined using a modified colorimetric assay of Fawcett and Scott (1960).

Amino acids were analyzed in samples which were freeze dried for 6 days, and then ground in a Waring blender. This ground material was then put in a forced air drying oven for 4 hours to ensure dry samples. Samples of 50 mg were introduced into 100 mL screw top culture tubes and hydrolyzed in 5 mL 6 N HCl for 24 hours at 110 C. After hydrolysis, 1 mL of 10 mM cycloleucine was added to each sample prior to drying by rotoevaporator. Ten mL H_2O was then added and samples were frozen until further analysis. Amino acid derivatives were then prepared by using

duplicate 1.0 mL samples of the hydrolyzed material. The samples were heated under a stream of Nitrogen gas until dryness before the derivatization stage. Seventy five uL dimethyl formamide (DMF) and 25 uL N-(tert-butyldimethylsilyl)-N-methyl-triflouroacetamide (MTBSTFA) were added to the dried samples which were then warmed to 75 C in a sand bath for 40 minutes. Samples were then transferred to 100 uL vials. Amino acid analysis was performed by injecting a 1.2 uL solution into an SE 30 fused Silica capillary column in a Beckman gas chromatograph.

Sulphur-35 in abomasal and microbial samples was determined as described by Mathers and Miller (1980), and was counted using a liquid scintillation counter (Searle Mark III, Searle Analytic Inc., Des Plaines, Illinois, U.S.A.). Microbial nitrogen contributions were analyzed using the micro-Kjeldahl technique of Fleck and Munroe (1965).

Chromium-51 and ^{103}Ru-P were analyzed on a gamma counter (Gamma 8000, Beckman Instruments Inc., Fullerton, California, U.S.A.).

Statistical Analysis.

All data treatment means were tested for analysis of variance using UANOVA (University of Alberta Computing Services) using sheep within groups as the error term.

RESULTS

Ammonia Nitrogen Concentrations in the Rumen.

The mean rumen ammonia N concentration in cold exposed

animals was much lower ($P<0.003$) than that in warm acclimated sheep (Table IV-2).

Flow of Non-Ammonia Nitrogen.

Flow of NAN to the abomasum was elevated by 14.7% ($P<0.02$) for animals maintained in the cold (Table IV-1). Although cold stressed animals were passing 10.5% more NAN through the terminal ileum than those in the warm environment, this change was not statistically significant ($P>0.05$). Faecal NAN excretion was not affected by temperature.

Digestion of Non-ammonia Nitrogen.

Total gastrointestinal disappearance of NAN was not significantly affected by treatment, with mean values of 13.83 and 12.88 g d^{-1} for warm and cold exposed wethers, respectively (Table IV-1). Non-ammonia nitrogen appearance in the rumen was significantly higher ($P<0.02$) in animals exposed to cold temperatures. Post-ruminally, there was no significant difference in NAN digestibility between treatments. However, there was a tendency for NAN disappearance to be higher in the small intestine of cold exposed animals ($P<0.15$).

Although slightly more NAN was digested in the intestine relative to the amount entering the small intestine in the cold, the percent of NAN digestion occurring in the small and large intestines was not significantly affected by treatment (Table IV-2).

Feed and Microbial N Transactions.

Partitioning of the flow of NAN from the abomasum into dietary and microbial components (assuming 1.5 g N d^{-1} endogenous protein flow; Kennedy et al. 1982) revealed that the increased flow of NAN was mainly due to an increased proportion of dietary protein (Table IV-2). Feed N escaping forestomach fermentation was consequently increased ($P<0.05$) in the cold. Although total microbial protein synthesis was reduced, the efficiency of microbial protein synthesis was slightly increased in the cold ($P<0.15$).

Partitioning of Non-ammonia Nitrogen, Amino Acid Nitrogen and Non-amino Acid NAN.

Flows of amino acid nitrogen and non amino acid NAN are shown in Table IV-3. Although more NAN flowed through the abomasum ($P<0.03$), the partitioning of amino acid and non-amino acid NAN revealed non significant increases in their respective flows. Terminal ileal flows and faecal excretions of these components showed no significant differences due to temperature.

Digestion of amino acid and non amino acid NAN in the gastro-intestinal tract is shown in Table IV-4. There were increases in the appearance of amino acid and non amino acid NAN in the forestomach of cold exposed animals, but these were not significant. Disappearance of the two components in the small intestine were also slightly but non-significantly elevated in the cold. Large intestinal

disappearances of these nitrogen sources were not affected by temperature. Whole tract disappearance of amino acid and non-amino acid NAN was decreased in cold exposed animals.

Cold exposed wethers digested more NAN and amino acid NAN in the small intestine relative to the OM apparently digested in the whole gastro-intestinal tract ($P<0.07$ and $P<0.08$, respectively; Table IV-4). There was no significant change in the digestion of non-amino acid NAN relative to total OM digested in the whole gastro-intestinal tract.

Amino Acid Concentrations in the Rumen

Concentrations (mg Kg^{-1} DM) of most amino acids in the rumen were not affected by temperature (Table IV-6), although the concentration of tyrosine was elevated ($P<0.10$) and that of histidine was depressed ($P<0.10$) by the cold. Total ruminal concentration of amino acids was not affected by the temperature treatment.

Abomasal Amino Acid Nitrogen Flows and Composition

Individual amino acid nitrogen flows through the abomasum were altered by temperature (Table IV-7). The flows of all amino acids through the abomasum were generally increased, with the flows of lysine, histidine, and tyrosine being significantly increased ($P<0.03$, $P<0.06$, and $P<0.003$, respectively). However, the flow of cystine was not affected.

Amino acid composition (g N Kg^{-1} NAN) in abomasal

digesta was affected by the environmental temperature to which the animals were exposed (Table IV-8). In every case except for lysine, histidine, serine, and tyrosine, the content of amino acids relative to the amount of NAN in the digesta was elevated in the cold. The values for valine ($P<0.06$), isoleucine ($P<0.07$), and leucine ($P<0.04$) were all significantly affected by the cold environment. Total amino acid composition was also raised in the cold, however the effect was nonsignificant.

Terminal Ileal Amino Acid Flows and Composition

Flows of amino acids through the terminal ileum were unaffected by temperature (Table IV-7). For all amino acids, the individual amino acid composition in terminal ileal digesta was depressed during cold exposure except for lysine, histidine, and serine (Table IV-7). The total amino acid composition was also decreased, however nonsignificantly, in the cold environment.

Faecal Excretion of Amino Acids

The excretion of methionine was significantly lower in cold-exposed animals ($P<0.05$) while excretion of lysine and glutamate were increased ($P<0.05$ and $P<0.10$). Overall, the excretion of amino acids was higher in the cold (Table IV-7), with that of only methionine, leucine and glycine slightly lower.

Amino Acid Disappearance

Whole tract disappearance of amino acids was affected

by the cold environment (Table 9). Methionine disappearance was enhanced ($P<0.001$) while lysine ($P<0.03$) and glutamate ($P<0.10$) disappearances were not as high as those in the warm. Total amino acid disappearance was depressed by 11% but this was not statistically significant.

There was net production of most amino acids in the forestomach except for histidine, aspartate, cystine, and tyrosine. There was a higher net production of lysine ($P<0.03$) and lower net losses of histidine ($P<0.05$) and tyrosine ($P<0.005$) in cold exposed animals. Total forestomach amino acid net appearance was higher in the cold acclimated sheep ($P<0.003$).

Combining the intestinal disappearances gives the post-ruminal amino acid transactions (Table IV-9). Methionine, lysine, and tyrosine apparent digestibilities were increased in the cold ($P<0.05$, $P<0.05$, and $P<0.01$, respectively). Except for cystine, the post ruminal utilization of individual amino acids in the cold exposed animals was higher.

Disappearance of amino acids in the small intestine in every case was higher except for cystine in cold exposed animals (Table IV-10), with the disappearance of lysine, glutamate, and tyrosine being significantly affected by temperature ($P<0.05$, $P,0.09$, and $P<0.005$, respectively). Total amino acid digestion by the sheep in the small intestine was 10.9% higher ($P>0.05$) in cold exposed

animals.

The large intestine values indicate a net utilization of total amino acids, with methionine use significantly elevated ($P<0.01$) in the cold. However, for some amino acids, there was net release (histidine and glycine, and cystine in the cold), as indicated by the negative disappearance values.

Total disappearance of amino acids in the post-ruminal tract of cold exposed wethers was increased by 6.8%, although this value was not significant.

DISCUSSION

Ammonia Nitrogen Production.

Decreased residence time for digesta in the rumen has been observed in cold stressed animals. There is therefore less time for the microbial population in the rumen to ferment feed residues. Decreased digestion of dietary protein is expected to be associated with less production of ammonia nitrogen, as was the case in this experiment. Similar results have been reported by other workers in this laboratory (Kennedy et al. 1986a). The lower concentration of rumen ammonia observed during cold stress could be a consequence of the decrease of protein degradability in the forestomach (Orskov 1982).

Nitrogen Flows.

Flows of NAN to the small intestine reported in this experiment were similar to those reported for clover diets (Beever and Thomson 1981), for low levels of early and

medium cut perenial rye grass (Coelho da Silva et al. 1972), for lucerne (Hogan and Weston 1967), for alfalfa protein concentrate supplement feeds (Lu et al. 1982) and for lambs fed a lucerne-maize ground and pelleted ration (Margan et al. 1982), but higher than those reported for low intakes of rye and clover by MacRae and Ulyatt (1974) and a vetch-concentrate mixture by Ben-Ghedalia et al. (1974). For brome diets fed to cold exposed sheep, Kennedy et al. (1976) and Kennedy and Milligan (1978) observed higher flows of NAN through the abomasum than those in the present study, but it should be noted that intakes in those experiments were higher than in the present study. Kennedy et al. (1982) found slightly lower flows of NAN to the small intestine, but their animals consumed less feed than in the present study.

Increased flow of NAN to the small intestine of sheep during cold stress has been reported previously (Kennedy et al. 1976, Kennedy and Milligan 1978, Kennedy et al. 1982, Christopherson and Kennedy 1983, Kennedy et al. 1986a,b). Results from the present experiment indicate that the relative flows of both amino acid nitrogen and non amino acid NAN were increased, but not significantly, during cold exposure. Flows of NAN through the abomasum of the wethers were made up of 56% amino acid nitrogen while the contribution of amino acid nitrogen to the NAN flow at the terminal ileum was only 38%. This indicates preferential absorption of amino acids over non amino acid NAN, which

was probably comprised largely of nucleic acids, N involved in cell wall structure (such as peptidoglycans), and any nitrates and nitrites produced in the large intestine (Van Soest 1982).

The decreased N digestibility ($P<0.05$) for wethers exposed to cold is consistent with the results of Kennedy et al. (1986b) for sheep fed a chopped brome hay diet. However, the mean values are lower than those of Kennedy et al. 1976, Kennedy and Milligan (1978), and Kennedy et al. (1982).

Exposure of sheep to the cold environment increases the flow of organic matter and cell wall constituents to the small intestine, mainly due to enhancements in the ruminal rate of passage (Kennedy et al. 1986, Chapter III) associated with increased rumino-reticulum motility (Westra and Christopherson 1976). This is consistent with the results found in this study where flows of NAN to the small intestine were increased in the cold. The NAN flowing to the small intestine is composed of microbial nitrogen, undegraded feed nitrogen, and nitrogen of endogenous origin (Armstrong and Hutton 1975). In this study, less feed N was digested in the rumen in the cold stressed animals indicating increased by-pass of dietary nitrogen by the rumen. Satter and Roffler (1981) reported that high nitrogen rations containing non protein nitrogen were no better than the low N rations containing non protein nitrogen in terms of the protein flow through the abomasum

likely because of the increased contribution of endogenous N. Flows of NAN through the abomasum that are higher than the intake of N, as observed in this study, are usually expected with diets low in crude protein content (Santos et al. 1984, Spicer et al. 1986).

The enhanced passage of dietary protein to the small intestine during cold exposure is consistent with previous reports (Kennedy et al. 1976, Kennedy and Milligan 1978, Kennedy et al. 1982). The decreased residence time in the rumen (Kelly and Christopherson 1986) and the presence of slowly degradable proteins account for the depression in dietary protein degradation (Christopherson and Kennedy 1983, Kennedy et al. 1986a) and decreased rumen ammonia concentrations (Orskov 1982).

The efficiency of microbial protein synthesis was improved in the cold as more protein was synthesized in the rumen relative to the amount of energy substrate fermented. Other studies have reported this same phenomenon in response to cold exposure (Kennedy et al. 1978, Kennedy et al. 1982, Kennedy et al. 1986b). The estimates of net efficiencies of microbial synthesis were within the range of 33.6 to 53.5 g Kg^{-1}OM apparently fermented reported by Orskov (1982). McMeniman et al. (1986) recently reported a mean value of 38 g kg^{-1} OM apparently fermented in the rumen of sheep fed Mitchell grass, which is very similar to the efficiency values reported in the present study.

Partitioning of the Disappearance of Nitrogen Components.

Ruminal digestion of dietary protein is a function of the time available for the digestion by rumen microbes, microbial population, dietary constituents, and efficiency of digestion by the microbial populace. The extent of protein degradation in the rumen is an important factor in determining if the amount of protein leaving the stomach will be greater, equal to, or less than the amount of protein consumed (Santos et al. 1984).

The results from this study tend to agree with those of Kennedy et al. (1986a) which show that the level of dietary protein by-passing rumen fermentation can be substantially increased in the cold. Indeed, 54.6% of the NAN entering the small intestine of cold exposed wethers was of feed origin, whereas only 40.1% of the NAN entering the small intestine of warm acclimated wethers was of feed origin. Estimates in the literature indicate that an additional 35 to 65% of the N in brome grass pellets and 22 to 25% of the N in chopped brome grass or alfalfa diets escaped rumen fermentation upon cold acclimation (Kennedy et al. 1986).

Estimates for the disappearance of NAN in the small intestine are few. The apparent digestion of NAN in the small intestine of steers fed high concentrate diets is increased (from 64.1 % to 76.6 %) with increasing feed intake (Zinn and Owens 1983). Voluntary feed consumption is usually increased in the cold, a response which would

allow increased availability of NAN for absorption across the small intestine (Kennedy et al. 1976; Kennedy and Milligan 1978). In the present study, where feed intake was held constant, the intestinal disappearance of NAN in the cold was nonsignificantly higher than for the warm environment ($P>0.10$). This contrasts with results reported by Kennedy et al. (1976), Kennedy and Milligan (1978) and Kennedy et al. (1982) in which there was increased intestinal digestion of NAN. The higher disappearance of NAN in the small intestine during cold exposure may be even larger than reported for apparent disappearance because of evidence pointing to increased endogenous protein inputs from the pancreas in the cold (Kato and Young 1984).

The increased intestinal disappearance of NAN relative to the amount of organic matter digested in the whole tract of animals exposed to cold is consistent with previous reports by Kennedy et al. (1986a), for 12 of 14 diets. Margan et al. (1982) reported an increase in flow of NAN to the small intestine in lambs fed increased intakes of a hay:concentrate ration. Despite a reduction in the digestibility of NAN in the intestines, however, those workers found that more crude protein per unit digestible OM intake was digested in the post-ruminal tract. Although total OM digestion is depressed in the cold, it is evident that with increased flows of NAN to the small intestine and increased disappearance of NAN at this site, sheep exposed to the cold environment may be more efficient in

maintaining their nitrogen economy than sheep in a warm environment.

The digestion of NAN in the large intestine accounted for only 15% of the total intestinal NAN digestion in this study. This result indicates that the microbial fermentation process in the large intestine had a relatively minor effect on the nitrogen economy regardless of environment. Studies of Coelho da Silva et al. (1972) and MacRae et al. (1972) with sheep fed dried grass diets have also suggested a minor role for the large intestine with only 3.3 to 8.3% of the total amino acid NAN disappearing in the caecum and colon. Santos et al. (1984) observed 11.4% of the digestion of amino acid NAN occurring in the large intestine of dairy cattle fed distillers dried grains. On the other hand, Lindsay and Armstrong (1982) suggested a more substantial role for the large intestine in the disappearance of NAN. The digestion of residual NAN in the large intestine generally leads to uptake of some nitrogen from this organ, but such nitrogen probably is absorbed in the form of ammonia (Smith 1979; Lindsay and Armstrong 1982).

Amino Acid Transactions.

Total flow of amino acid N to the small intestine in the present study was slightly less than that reported by Coelho da Silva et al. (1972). If the contributions of tryptophan and arginine, which were not measured in the present work, are considered, the estimates would have been

similar. The increased flow of lysine through the abomasum could be an indication of a greater contribution of protozoa to the total NAN flow in the cold. Ulyatt et al. (1975) reported large differences in the amino acid composition of duodenal digesta with notable differences between bacterial and protozoal protein. Protozoa had consistently higher values for lysine, and lower values for alanine. Those workers suggested that the composition of duodenal amino acids could be used to indirectly assess the proportion of protozoal protein in total digesta. The amino acid composition of abomasal digesta was similar to the results of Kennedy et al. (1986) for duodenal digesta in sheep fed similar diets. Those workers reported 57% amino acid N in duodenal NAN for thermoneutrally acclimated sheep, and 61.8 % for cold exposed animals. Results from the present experiment indicate that amino acid N accounted for 55 and 57 % of NAN for thermoneutrally and cold acclimated wethers, respectively. Data presented here uses the value for arginine from Kennedy et al. (1986b) to give a similar amino acid profile. Individual amino acid compositions do vary among the studies however. In this experiment, the composition in abomasal digesta of all but four amino acids (lysine, histidine, serine and tyrosine) was increased during cold exposure. The work of Kennedy et al. (1986b) showed that the values for lysine, histidine and tyrosine were slightly decreased ($P>0.05$) in the cold in addition to the values for methionine, and

phenylalanine. Generally, most of the other duodenal amino acid composition increased during cold exposure in the study of Kennedy et al. (1986b). The increased flows of amino acid N to the duodenum and the decreased contribution of microbial N in abomasal digesta likely reflected the increased passage of undegraded dietary protein (Beever and Thomson, 1981).

Estimates of net amino acid disappearance in the small intestine of wethers exposed to cold environments are not evident in the literature. Lindsay et al. (1980) reported true digestibility estimates in the small intestine (which included estimates for endogenous amino acid inputs) to be between 70 and 80% for most amino acids with cyst(e)ine being the lowest at 52%.

Amino acids are not only precursors for proteins, but can also be a valuable glucogenic substrate or energy source to the ruminant when catabolized to produce urea and CO_2, with the release of chemical energy (MacRae and Lobley 1986). Because a decreased rate of weight gain is common in cold exposed animals, perhaps the end products of protein digestion tend to be directed to gluconeogenic functions rather than protein accretion in such circumstances (Kennedy et al. 1986b). In cold environments, the overall production and concentration of rumen volatile fatty acids (VFA) is decreased (Kennedy and Milligan 1978; Chapter III), reducing the availability of an important energy source to the sheep. In the small

intestine of the wethers exposed to cold temperatures in this experiment, the disappearance of amino acid N was increased relative to the amount of OM digested in the whole tract. The increased apparent absorption of amino acids may not, however, be reflected in a general increase in the nitrogen retention of the animal. This agrees with Kennedy et al. (1986b) who found improvement of the ratio of intestinally digested NAN:digestible OM intake for 7 out of 8 diets. As this ratio is increased in the cold, the amino acids may be utilized as an energy source especially in situations where digestible OM intake is limited, rather than serving as precursors of protein synthesis for accretion of body tissue (Weston 1973, Egan 1977, Kennedy et al. 1986b). Kennedy and Milligan (1978) reported depressions in nitrogen retention for cold exposed sheep fed pelleted brome grass. Continued availability of amino acids from the intestine will however have a protein sparing effect, reducing the need for amino acids from the gastrointestinal tract as energy precursors. Previously, it was found that when poor quality, low N diets were given, microbial fermentation resulted in an overall synthesis of NAN anterior to the small intestine (MacRae and Ulyatt 1974). Increasing the energy supply results in a reduction of protein catabolism, as well as a small increase in synthesis (Miller 1982).

In conclusion, during cold exposure increased escape of dietary protein from rumen fermentation and a more

efficient microbial protein synthesis provided the intestine with increased NAN. Hence, there was a non significant increase in the availability of amino acid nitrogen and NAN to the small intestine, and a significant increase in the digestion of specific amino acids (lysine, histidine, alanine and tyrosine) in the small intestine. There was evidence for maintenance of the amino acid supply and nitrogen economy of the ruminant species in the cold environment when energy digestibility was depressed. These adjustments may allow animals to utilize diets of poorer nitrogen content in the cold environment.

Table IV-1. Intake, flows ($g \cdot d^{-1}$) and disappearance ($g\ d^{-1}$) of non-ammonia nitrogen through the gastro-intestinal tract of warm and cold exposed wethers.

Item	Warm	Cold	S.E.	Sig.
Flows				
Intake	25.7	25.7	–	
Abomasal flow	28.4	32.0	0.67	**
Terminal ileal flow	14.3	15.8	0.62	
Faecal output	11.9	12.7	0.42	
Disappearance				
Forestomach	-2.7	-7.3	0.67	**
Small intestine	14.0	17.3	0.89	
Large intestine	2.4	2.9	0.78	
Whole tract	13.7	12.9	0.94	

** Means within a location are different ($P<0.05$).

Table IV-2. Intake and digestibility of nitrogen; rumen ammonia concentration; flow of ammonia, non-ammonia nitrogen (NAN), microbial nitrogen, and undegraded feed nitrogen from the abomasum; and intestinal digestion of NAN in sheep given brome (*Bromis inermis*) hay.

Environmental Temperature	Warm	Cold	S. E.	Sign.
N Intake (g d^{-1})	25.71	25.71	–	
N Digestibility (g g^{-1} N intake)	0.54	0.50	0.11	
Rumen ammonia Concentration (mg L^{-1})	93.13	74.48	13.18	***
Flow from abomasum (g N d^{-1})				
Ammonia	1.11	1.02	0.09	
NAN	28.13	32.97	0.67	**
Microbes	16.86	14.50	0.80	
Undegraded Feed	11.27	18.47	0.96	**
Feed N escaping fermentation in stomach (g g^{-1} intake)	0.35	0.44	0.04	*
Efficiency of Microbial Synthesis (g N kg^{-1} OM apparently fermented).	34.87	37.50	4.30	
Intestinal digestion of NAN (g d^{-1})	16.48	20.14	1.60	*
Small Intestine	14.04	17.23	1.99	
Large Intestine	2.44	2.91	1.90	
Intestinal digestion of NAN (g g^{-1} entering intestines)	0.59	0.63	0.10	
Small Intestine	0.50	0.54	0.17	
Large Intestine	0.09	0.09	0.19	
% of NAN digestion in:				
Small Intestine	84.70	85.70	10.22	
Large Intestine	15.30	14.30	1.22	

* Means across temperatures are different (P<0.10).
** Means across temperatures are different (P<0.05).
*** Means across temperatures are different (P<0.01).

Table IV-3. Intake, and flows by the abomasum, and terminal ileum and faecal excretion (g d^{-1}) of non-ammonia nitrogen (NAN), amino acid nitrogen, and non-amino acid NAN of wethers exposed to warm and cold environments.

Item	Non-Ammonia Nitrogen			Amino Acid Nitrogen[1]			Non-amino Acid NAN		
	Warm	Cold	S.E.	Warm	Cold	S.E.	Warm	Cold	S.E.
Intake	25.71	25.71	–	14.91	14.91	–	10.80	10.80	–
Abomasum	28.36	32.97	0.67**	16.12	18.14	0.41	12.24	14.83	11.59
Terminal Ileum	14.82	15.74	0.62	5.37	6.21	0.30	8.96	9.53	8.40
Faecal Excretion	11.88	12.83	0.42	4.12	5.32	0.96	7.76	7.51	0.35

* Means across temperatures are different ($P<0.10$).
** Means across temperatures are different ($P<0.05$).

[1]Amino acid nitrogen does not include tryptophan or arginine

Table IV-4. Digestion of non-ammonia nitrogen (NAN), amino acid nitrogen, and non-amino acid NAN (g d^{-1}), and digestion relative to total tract OM digestion in the small intestine of wethers exposed to warm and cold environents.

Item	Warm	Cold	S.E.	Sig.
Non-ammonia nitrogen	14.04	17.22	1.06	
Amino Acid nitrogen	10.76	11.93	0.358	
Non-amino acid NAN	3.28	5.29	0.92	
Digestion relative to total OM digestion				
Non-ammonia nitrogen	0.0188	0.0263	0.0017	**
Amino acid nitrogen	0.0144	0.0182	0.0014	**
Non-amino acid NAN	0.0044	0.0081	0.0013	

** Means across temperatures are different ($P<0.08$).

Table IV-5. Digestion of non-ammonia nitrogen (NAN), amino acid nitrogen, and non-amino acid NAN in the forestomach, small intestine, large intestine and total gastro-intestinal tract of wethers exposed to warm and cold environments.

Item	Non-Ammonia Nitrogen			Amino Acid Nitrogen			Non-amino Acid NAN		
	Warm	Cold	S.E.	Warm	Cold	S.E.	Warm	Cold	S.E.
Forestomach	-2.65	-7.26	0.67**	-1.21	-3.22	0.41*	-1.44	-4.04	11.59
Small Intestine	14.04	17.23	0.89	10.76	11.93	0.64	3.28	5.30	0.92
Large Intestine	2.44	2.91	0.78	1.24	0.89	6.53	1.20	2.02	7.83
Post Ruminal	16.48	20.14	1.32	12.00	12.82	0.42	4.48	7.32	11.49
Whole Tract	13.83	12.88	0.94	10.97	9.75	0.09*	3.04	3.28	0.35
Whole Tract Digestion (Percentage of Intake)	53.70	50.10	1.35	73.59	65.38	3.53	28.15	30.42	1.11

* Means across temperatures are different (P<0.10).
** Means across temperatures are different (P<0.05).

Table IV-6. Amino acid nitrogen concentration (mg kg $^{-1}$ dry matter) of rumen contents in warm and cold exposed sheep given brome (Bromus inermis).

Amino Acids	Warm	Cold	S.E.	Sig.
Essential Amino Acids				
Threonine	0.74	0.86	1.76	
Valine	0.74	0.81	1.90	
Methionine	0.10	0.13	0.38	
Isoleucine	0.63	0.68	1.17	
Leucine	0.92	1.04	2.41	
Phenylalanine	0.55	0.65	1.42	
Lysine	1.25	1.55	2.91	
Histidine	0.21	0.12	0.43	*
Non-essential Amino Acids				
Aspartate	1.07	1.21	0.261	
Serine	0.65	0.80	0.151	
Glutamate	0.97	1.11	0.242	
Proline	0.48	0.53	0.12	
Glycine	2.94	3.42	0.11	
Alanine	0.84	0.98	0.20	
Cystine	0.41	0.14	0.63	
Tyrosine	0.26	0.35	0.07	*

* Means across temperatures are different ($P<0.10$).
** Means across temperatures are different ($P<0.05$).

Table VI-7. Intake; flows through the abomasum, terminal ileum; and faecal excretion (g d^{-1}) of amino acid nitrogen in warm and cold exposed sheep given brome (Bromus inermis) hay.

Amino acid	Intake	Abomasum			Terminal Ileum			Faeces		
		Warm	Cold	S.E.	Warm	Cold	S.E.	Warm	Cold	S.E.
Essential Amino Acids										
Threonine	0.649	1.263	1.351	0.035	0.381	0.410	0.022	0.111	0.163	0.050
Valine	1.015	1.211	1.270	0.025	0.433	0.436	0.016	0.307	0.351	0.043
Methionine	0.100	0.183	0.202	0.005	0.064	0.073	0.003	0.051	0.044	0.002**
Isoleucine	0.622	1.024	1.062	0.021	0.315	0.330	0.012	0.283	0.317	0.043
Leucine	1.007	1.490	1.562	0.025	0.472	0.506	0.019	0.404	0.386	0.340
Phenylalanine	0.548	0.856	0.938	0.023	0.221	0.245	0.013	0.115	0.155	0.035
Lysine	1.460	1.744	2.267	0.071**	0.571	0.762	0.035	0.446	0.613	0.043**
Histidine	0.687	0.208	0.383	0.035**	0.055	0.122	0.035	0.135	0.286	0.089
Non-essential Amino Acids										
Aspartate	2.093	1.534	1.782	0.050	0.481	0.580	0.022	0.388	0.388	0.075
Serine	0.706	1.147	1.332	0.050	0.340	0.411	0.021	0.076	0.145	0.043
Glutamate	1.287	1.412	1.512	0.025	0.502	0.530	0.025	0.443	0.528	0.079
Proline	1.199	0.783	0.833	0.017	0.300	0.307	0.012	0.180	0.219	0.025
Glycine	1.240	1.136	1.320	0.035	0.461	0.562	0.016	0.576	0.611	0.083
Alanine	1.239	1.403	1.572	0.035	0.588	0.644	0.015	0.565	0.629	0.056
Cystine	0.497	0.322	0.207	0.057	0.009	0.008	0.016	0.047	0.099	0.029
Tyrosine	1.115	0.407	0.543	0.011***	0.172	0.194	0.017	0.133	0.168	0.035
TOTAL	14.913	16.123	18.136	0.409	5.365	6.210	0.202	4.122	5.317	0.686

** Means across temperatures are different ($P<0.05$).
*** Means across temperatures are different ($P<0.005$).

Table IV-8. Amino acid composition (g N kg^{-1} total non-ammonia nitrogen) in abomasal contents in warm and cold exposed sheep given brome (*Bromus inermis*).

Amino Acids	Warm	Cold	S.E.	Sign.
Essential Amino Acids				
Threonine	35.96	37.91	1.37	
Valine	33.04	36.66	0.78	*
Methionine	5.24	5.61	0.22	
Isoleucine	27.78	30.97	0.62	**
Leucine	41.13	45.16	0.92	
Phenylalanine	24.73	25.79	0.90	
Lysine	58.97	53.32	2.41	
Histidine	8.85	6.45	0.93	*
Non-essential Amino Acids				
Aspartate	46.46	46.54	1.59	
Serine	35.21	34.66	1.78	
Glutamate	38.98	42.98	1.42	
Proline	21.74	23.75	0.58	*
Glycine	33.18	35.84	1.06	
Alanine	40.83	42.95	1.14	
Cystine	2.60	3.83	1.96	
Tyrosine	14.31	13.12	0.47	
TOTAL	470.01	485.51	12	
Arginine[1]	62.40	61.78		
TOTAL	532.41	547.28		

[1]Values estimated from Kennedy *et al.* (1986b).

* Means across temperatures are different (P<0.10).
** Means across temperatures are different (P<0.05).

Table VI-9. Disappearance of amino acid nitrogen (g d^{-1}) in the forestomach and post ruminal and whole gastro-intestinal tract of warm and cold exposed sheep given brome (Bromus inermis) hay.

Amino acids	Forestomach			Post-ruminal			Whole tract		
	Warm	Cold	S.E.	Warm	Cold	S.E	Warm	Cold	S.E.
ESSENTIAL AMINO ACIDS									
Threonine	-0.614	-0.702	0.035	1.152	1.188	0.035	0.538	0.486	0.018
Valine	-0.196	-0.255	0.025	0.904	0.915	0.025	0.708	0.660	0.021
Methionine	-0.083	-0.102	0.007	0.132	0.158	0.021	0.049	0.056	0.002***
Isoleucine	-0.362	-0.400	0.022	0.741	0.745	0.061	0.379	0.345	0.005
Leucine	-0.483	-0.555	0.025	1.086	1.176	0.083	0.603	0.621	0.009
Phenylalanine	-0.308	-0.390	0.023	0.741	0.783	0.061	0.433	0.393	0.033
Lysine	-0.284	-1.260	0.071**	1.298	1.654	0.083	1.014	0.847	0.009**
Histidine	0.479	0.304	0.035**	0.073	0.097	0.035	0.552	0.401	0.019
NON-ESSENTIAL AMINO ACIDS									
Aspartate	0.559	0.311	0.050	1.146	1.276	0.100	1.705	1.587	0.008
Serine	-0.441	-0.626	0.130	1.071	1.187	0.083	0.630	0.561	0.0058
Glutamate	-0.125	-0.225	0.024	0.989	0.984	0.071	0.844	0.759	0.008*
Proline	-0.204	-0.373	0.071	0.603	0.614	0.035	1.019	0.980	0.003
Glycine	0.104	-0.008	0.035	0.560	0.709	0.043	0.664	0.629	0.015
Alanine	-0.164	-0.333	0.035	0.838	0.943	0.050	0.674	0.610	0.009
Cystine	0.175	0.290	0.056	0.275	0.108	0.056	0.450	0.398	0.015
Tyrosine	0.708	0.572	0.011***	0.274	0.375	0.014	0.982	0.947	0.003
TOTAL	-1.210	-3.223	0.740	12.001	12.819	0.415	10.791	9.596	0.532

* Means across temperatures are different (P<0.10)
** Means across temperatures are different (P<0.05)
*** Means across temperatures are different (P<0.01)

Table IV-10. Disappearance of amino acid nitrogen (g d^{-1}) in the small intestine and large intestine of warm and cold exposed sheep given brome (Bromus inermis) hay.

Amino acid	Small Intestine			Large Intestine		
	Warm	Cold	S.E.	Warm	Cold	S.E.
ESSENTIAL AMINO ACIDS						
Threonine	0.882	0.941	0.090	0.270	0.247	0.018
Valine	0.778	0.834	0.195	0.126	0.081	0.156
Methionine	0.119	0.129	0.009	0.013	0.029	0.002***
Isoleucine	0.709	0.732	0.061	0.032	0.013	0.008
Leucine	1.018	1.056	0.083	0.068	0.120	0.050
Phenylalanine	0.635	0.693	0.061	0.106	0.090	0.009
Lysine	1.173	1.505	0.083**	0.125	0.149	0.035
Histidine	0.153	0.261	0.035*	-0.080	-0.164	0.020
NON-ESSENTIAL AMINO ACIDS						
Aspartate	1.050	1.212	0.100	0.093	0.074	0.018
Serine	0.807	0.921	0.083	0.264	0.266	0.019
Glutamate	0.910	0.982	0.071	0.059	0.002	0.025
Proline	0.483	0.526	0.035	0.120	0.088	0.010
Glycine	0.675	0.758	0.043	-0.115	-0.015	0.010
Alanine	0.815	0.928	0.050*	0.023	0.015	0.009
Cystine	0.313	0.199	0.056	0.069	-0.064	0.025
Tyrosine	0.235	0.349	0.014***	0.039	0.026	0.006
TOTAL	10.758	11.926	0.348	1.212	0.920	0.252

* Means across temperatures are different (P<0.10).
** Means across temperatures are different (P<0.05).
*** Means across temperatures are different (P<0.01).

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V. THE EFFECTS OF THE COLD ENVIRONMENT ON THE PORTAL AND MESENTERIC BLOOD FLOW AND AMINO ACID FLUX ACROSS THE SMALL INTESTINE OF ADULT EWES.

INTRODUCTION

Exposure of animals to different thermal environments can result in substantial changes in the circulatory system (Hales 1974). These shifts in circulation can lead to metabolic and thermal redistribution among tissues in environmentally stressed animals (Schaeffer et al. 1982). In heat stress, blood will tend to be shunted from the deep body tissues to the periphery to facilitate efficient exchange of heat with the environment. Essentially, a redirection of blood flow occurs which may result in more, or less, blood flowing to the gastrointestinal tract. Hales (1973) and Von Englehardt and Hales (1977) observed a reduced capillary flow in the gut using labelled microspheres in sheep exposed to hot environments, possibly with a consequential decrease in the efficiency of nutrient uptake by the gut.

Exposure to cold environments has had less well defined effects on the blood flow and nutrient uptake by the gut. In acute cold stress, Alexander et al. (1973) observed depressed blood flow to the gut in newborn lambs, which might be expected to influence colostrum availability. Decreases in relative blood flow to the rumino-reticulum have been observed (Schaeffer et al. 1982), which may be

important in nutrient transfer and consequent availability to the animal.

Effects of exposure to cold temperatures on the apparent digestibility of amino acids in the small intestine of sheep have been reported in Chapter IV. The possibility that these effects have implications in the arterio-venous flux of amino acids across the small intestine has been investigated.

The objectives of this experiment were to investigate the effect of cold exposure on blood flow through the gastrointestinal tract and to determine if there was an influence of temperature on the net flux of amino acids across the mesenteric drained viscera in adult ewes.

MATERIALS AND METHODS

Animals and their management

Six mature, dry, Suffolk ewes weighing 47 to 53 kg were housed individually in metabolic crates. Each was fed 1600 g d^{-1} cut brome hay (Bromus inermis) by means of an automatic feeder 12 times daily. Water and Cobalt Iodized salt were available ad libitum.

Environmental Conditions

Three of the ewes were acclimated to a cold environment (0 C to +2 C) while the other three were acclimated to a thermoneutral environment (22 C to 25 C) for a minimum of twenty-five days prior to experimental measurements. Each

animal was closely shorn prior to entering their respective environment as well as two weeks prior to the time of sampling.

Surgical Preparation

Ten days prior to experimentation, anaesthesia was induced with Nembutal, and the ewes were maintained under general anaesthesia using Halothane. Sterile silastic catheters were implanted in a mesenteric artery, the portal vein, and two sites in the mesenteric venous drainage. The first mesenteric vein catheter was located three cm posterior to the portal vein and was designated as the mesenteric sampling catheter. The second was located 15 cm upstream from the first mesenteric vein catheter, and was designated the mesenteric para-aminohippuric acid (PAH) infusion catheter.

Post-surgical care involved immediate placement of the animal under a heat lamp in individual holding pens bedded with wood shavings. Injections of antibiotic (Liquamycin; 100 mg mL^{-1}) were given for three days post surgery. Animals were returned to their respective environments three days post surgery and a further seven days was given as a recovery period before any sampling of blood occurred.

Measurement of Portal and Mesenteric Blood Flow

Flow of blood through the portal and mesenteric drained viscera was determined using the PAH dilution technique of Katz and Bergman (1969).

A 2% PAH solution was continuously infused into a small

branch of the mesenteric vein utilizing a continuous infusion pump (Ismatec M-13) at a rate of 65 mL h^{-1} (Katz and Bergman 1969) following a priming dose of 18 mL PAH solution. The infusion was continued for 45 minutes before blood sampling was performed. To verify the infusion rate, the infusate container was weighed before and after the timed infusions.

After PAH equilibrium (after 45 minutes infusion), three sets of blood samples were withdrawn from each vessel, at 10 minute intervals. This series of sampling occurred four times daily for 3 days for a total of twelve samples for each ewe.

Samples were immediately separated into whole blood and plasma fractions, the whole blood being used for PAH analysis and hematocrit determination; and plasma for amino acid analysis.

Blood flows through the portal and mesenteric drained viscera were calculated by:

$$F_{pv} = I / (C_{pv} - C_a)$$

where F_{pv} is the rate of blood flow (mL min^{-1}) in the portal vein; I is the infusion rate of PAH (mg min^{-1}); and C_{pv} and C_a are the concentrations of PAH (mg mL^{-1}) in the portal venous and arterial blood, respectively (Katz and Bergman 1969).

Plasma flow may be determined using a slight modification of this technique. Wolff et al. (1972) calculated plasma flow by multiplying the flow of whole

blood by the percent plasma. This was calculated as follows:

Plasma flow = blood flow x ((100-PCV)/100)

where PCV represents the packed cell volume or hematocrit. Determination of gastrosplenic vein flow was calculated as the difference between mesenteric and portal vein flows (Webster and White 1973).

Amino Acid Analysis

Amino acids were analyzed using the ortho-phthaldialdehyde precolumn derivatization and reversed-phase high-performance liquid chromatography (HPLC) technique of Jones and Gilligan (1983). Amino acids were separated and quantified using a Varian 5000 high performance liquid chromatograph and a Varian Fluorichrom detector. Prior to injection, samples were mixed using a modified Technicon autosampler and a Chemlab perstaltic pump with a stainless steel mixing tee. Samples were mixed with flouraldehyde reagent and injected using a Valco autoinjector valve equipped with a 20 uL loop. The column was a Supelcosil 3 um LC-18 reverse phase column (4.6 x 150 mm; Supelco) and a guard column (4.6 x 50 mm) packed with Supelco LC-18 reverse phase packing (20 - 40 um) Chromatograph peaks were recorded using a Fisher recorder and integration was accomplished using a Hewlitt Packard 3353 data system with a Hewlett Packard 18652a A/D converter.

Net release of amino acids across the mesenteric

viscera was calculated as the venous-arterial plasma amino acid concentration differences times the mesenteric plasma flow.

Statistical Analysis

Treatment means were compared by means of an unpaired T-Test (Steel and Torrie 1980).

RESULTS

Because of difficulties encountered during infusion of PAH and blood sampling, an inadequate number of samples was acquired from the portal catheters. The values reported for portal flow are presented only as a reference and must therefore be judged accordingly.

Flows of plasma through the portal, mesenteric and gastro-splenic veins are shown in Table V-1. In the cold environment, portal flow was decreased by 34% while mesenteric flow was decreased by 27% compared to the warm environment. Gastro-splenic flow, which was calculated by the difference between portal and mesenteric flow, was also decreased by 54% in the cold temperatures.

Hematocrit values of 27.2%, 27.7%, and 28.0% in the warm and 26.6%, 26.5%, and 26.3% in the cold temperature from the portal venous, mesenteric venous, and arterial vessels, respectively, were not influenced by temperature treatment ($P>0.05$).

Concentrations of amino acids in arterial plasma were affected by temperature (Table V-2). Isoleucine, serine, and asparagine concentrations were all slightly depressed

($P<0.10$) during cold exposure. Threonine, valine, phenylalanine, tryptophan, and glutamate were all decreased ($P<0.05$), and lysine and tyrosine were the most significantly decreased ($P<0.01$) in the cold environment.

Net release of amino acids across the mesenteric drained viscera is shown in Table V-3. There were some differences which occurred in response to cold temperatures, although they were nonsignificant changes. There was a lower net release ($P>0.05$) of seven of the nine essential amino acids measured in this study.

DISCUSSION

Exposure of animals to stressfully cold environments can lead to major shifts in the circulatory system (Sasaki and Weekes 1986). Blood tends to be shifted away from the non-thermogenic tissues to tissues which will aid in the maintenance of homeothermy (Christopherson 1985)

Estimates of portal blood flow in the literature range from 1440 mL min^{-1} (Wolff et al. 1972; Thompson et al. 1978) to as high as 3425 mL min^{-1} (Katz and Bergman 1969) in normally fed sheep which is in agreement with the estimates reported here.

It has been shown that various physiological states can alter portal blood flow. Fasting has decreased the flow substantially in both pregnant and non-pregnant ewes, whereas the pregnancy elevated the portal flow in ewes by 23% (Katz and Bergman 1969). Barnes et al. (1986) reported a 19% increase in hepatic portal flow in response to

feeding, the majority of the difference being due to a larger increase in the forestomach fractional blood flow. However, those authors also reported a decrease in the proportional contribution to blood flow from the abomasum, small intestine, pancreas, and gut fat. Gregory and Christopherson (1986) reported increases in capillary blood flow in fed versus fasted sheep in all stomach compartments and the duodenum.

Cold exposure increases cardiac output (Sasaki and Weekes 1986) although Schaefer et al. (1982) reported a significant decrease in the proportion of cardiac output flowing to the digestive tract of cold exposed sheep.

Even though the effects of cold exposure and consequent blood flow to the gut were non-significant in the current experiment, the number of animals utilized and the wide variation in blood flow values can explain the lack of a significant effect of the cold environment. There is some disagreement in the literature on the effects of cold exposure on blood flow to the gastro-intestinal tract. Acute cold stress tended to increase flow, although nonsignificantly, in fed and fasted rams (Thompson et al. 1978). Thompson et al. (1975) did report an increased portal flow of blood in acute cold exposure, but when expressed as a percent of cardiac output, the flow did not differ from that in the thermoneutral environment. In contrast to the latter studies are the reports by Hales et al. (1976) and Schaefer et al. (1982) who observed no

significant change in absolute capillary flow to the gut in chronic or moderate acute cold stress. The latter authors, though, did report decreased flow to the reticulo-rumen, omasum, and large intestine; and an increased flow to the abomasum and the small intestine in acute cold exposure as a percentage of cardiac output. Alexander et al. (1973) reported a decreased blood flow to the gut of severely stressed newborn lambs. Presumably, a decreased flow to the intestines would not be beneficial to the cold exposed animal because of a potential limitation of nutrient absorption (Christopherson 1985). Von Englehardt and Hales (1977) reported that flow of blood to the reticulum and dorsal rumen was depressed in mild heat stress conditions but did not observe any change in cardiac output. This indicates a redistribution of blood within the body.

Amino acids in arterial plasma originate either from hepatic drained viscera or from extra-hepatic tissues due to metabolism of tissue proteins. The decreased arterial concentrations of several of the essential amino acids during cold exposure might be due either to a decrease in the supply from non-hepatic tissues due to increases in net uptake or decreases in net release, or to decreased amino acid entry into the hepatic vein circulation. The latter could reflect changes in net absorption from the gut, net metabolism by gut tissues, and net utilization of amino acids by the liver. Millward et al. (1983) as cited by Sasaki and Weekes (1986), reported increased rates of

protein degradation and synthesis in tissues of chronically cold exposed rats. Muscle protein degradation in cold exposed cattle, as estimated using 3-methyl histidine excretion, is increased (J. R. Thompson, personal communication). There are no estimates in the literature of muscle protein synthesis during cold exposure in ruminants. Since the arterial concentration of circulating amino acids of sheep is decreased during cold exposure, it is speculated that the supply of amino acids from the hepatic drained viscera is decreased during cold exposure. Increased metabolism of amino acids in the liver via gluconeogenesis could account for some of the increased energy demand placed on cold exposed ruminants.

The net release of amino acids across the mesenteric drained viscera indicates that cold exposed ewes decrease the quantity of amino acids available from the gut for metabolism in tissues other than the intestine. However, there was a net utilization of some amino acids by the gut in the warm which did not occur in cold exposed animals (ie. histidine, glutamine, and glutamate). The values for net amino acid release in this study does had very large standard errors, probably due partly to a large individual animal variation and to the small number of animals in each treatment. In thermoneutrally acclimated fed sheep, Wolff et al. (1972) reported net utilization of glutamine by the portal drained viscera, which is in agreement with the work reported here. The net release of essential amino acids

was affected more by cold exposure than that of the non-essential amino acids, indicating preferential utilization of essential amino acids by the intestine. In Chapter IV, increased disappearance of amino acid nitrogen relative to the total organic matter disappearance in the small intestine of wethers exposed to cold environments was reported. That study also found increases in the disappearance of histidine, lysine, alanine and tyrosine.

There are other factors which play their parts during cold exposure, such as increased gut motility and rate of passage of digesta with the consequent effects on fermentation in the forestomachs (Kennedy et al. 1986). The decreases in plasma arterial amino acid concentrations in sheep exposed to cold environments could be a result of increased hepatic utilization of amino acids as gluconeogenic precursors, but further studies on the hepatic utilization of amino acids during cold exposure are required before any definitive conclusions can be made. Decreased blood flow to the gut would not be expected to enhance the transfer of nutrients from the intestines. Indeed, the tendency for reduced release of amino acids into the mesenteric vein in the present experiment could have contributed to the lower arterial amino acid concentrations. Because of the discrepancy of values for blood flow to the gut reported in the present study and in the literature and consequent uptake of nutrients across the intestines, it is not possible at present to suggest a

conclusive role on the effects of cold exposure on gut absorption.

Table V-1. Blood flow (mL min^{-1}) through the portal, mesenteric and gastro-splenic drained viscera in warm and cold exposed ewes

Location of Flow	Warm	Cold	S. E.
Portal[1]	3350	2204	758
Mesenteric[2]	1780	1297	190
Gastro-splenic	1990	914	774

[1]Portal blood flows determined on 5 samples per treatment.

[2]Mesenteric blood flows determined on 16 and 11 samples per treatment, respectively.

Table V-2. Concentrations of amino acids (nmol mL^{-1}) in arterial, mesenteric venous, and portal venous plasma of warm and cold exposed ewes given brome (Bromus inermis) hay.

Amino acid	Arterial Concentration				Portal Concentration				Mesenteric Concentration			
	Warm	Cold	S.E.		Warm	Cold	S.E.		Warm	Cold	S.E.	
Essential Amino Acids												
Threonine	184.87	106.58	14.14	**	141.66	152.36	16.21		203.05	129.25	26.29	
Valine	288.47	195.78	21.33	**	241.65	364.88	30.92		316.67	256.43	33.46	
Methionine	18.60	16.56	0.95		22.77	22.86	2.86		34.61	23.24	4.18	*
Isoleucine	113.40	84.98	7.67	*	110.37	135.58	16.20		167.32	133.66	19.64	
Leucine	173.27	140.03	10.52		189.03	193.65	20.13		249.32	195.34	25.56	
Phenylalanine	64.33	43.69	3.72	**	82.41	66.67	10.22		100.44	66.10	9.74	
Lysine	156.44	101.24	13	***	171.12	148.29	18.08		221.83	161.92	24.80	
Histidine	44.95	42.92	6.28		46.59	51.88	12.00		45.66	61.96	18.39	
Tryptophan	53.04	33.44	7.42	**	58.52	54.27	8.30		64.55	58.35	10.72	
Non-essential Amino Acids												
Aspartate	12.09	10.60	0.96		7.95	13.55	1.61	**	18.14	14.45	1.01	
Serine	89.16	64.96	6.42	*	107.56	106.17	13.57		152.47	103.33	19.64	
Glutamate	383.24	253.62	29.43	**	338.78	399.49	45.30		372.36	320.34	48.16	
Glycine	314.75	257.37	25.07		386.20	430.08	74.50		403.12	521.33	106.67	
Alanine	200.23	173.67	13.93		216.61	275.49	34.90		310.06	310.71	60.27	
Tyrosine	71.82	42.61	3.92	***	81.21	69.38	10.74		105.28	71.07	10.36	*
Asparagine	36.67	25.30	3.13	*	44.29	40.23	6.27		66.93	47.03	8.98	
Glutamine	41.32	8.66	10.48		52.85	13.63	6.79	***	26.33	23.02	9.17	*
Citrulline	167.50	172.55	14.98		166.76	137.30	24.13		206.49	216.92	23.89	
Arginine	172.33	126.93	15.83		137.24	213.05	27.97	*	208.88	220.11	42.58	
Taurine	67.27	58.05	8.63		79.87	60.22	11.19		69.47	45.70	9.35	*

* *Means within the same sampling point are different ($P<0.10$).*
** *Means within the same sampling point are different ($P<0.05$).*
*** *Means within the same sampling point are different ($P<0.01$).*

Table V-3. Net release of amino acids (umol min^{-1}) from the mesenteric drained viscera of warm and cold exposed ewes given brome (Bromus inermis) hay.

Amino acid	Warm	Cold	S.E.
Essential amino acids			
Threonine	44.2	8.7	86.6
Valine	65.3	62.5	109.7
Methionine	31.2	5.2	17.2
Isoleucine	98.8	48.2	66.3
Leucine	139.4	56.2	95.2
Phenylalanine	71.1	25.4	42.8
Lysine	125.1	61.8	86.0
Histidine	-17.3	26.6	42.2
Tryptophan	19.8	29.7	32.2
Total	575.6	324.1	381.1
Non-essential amino acids			
Aspartate	13.6	5.8	15.9
Serine	119.9	40.3	61.1
Glutamate	-3.8	71.5	124.6
Glycine	162.6	267.1	290.4
Alanine	221.9	166.3	211.4
Tyrosine	66.8	28.4	43.8
Asparagine	56.1	22.0	27.6
Glutamine	-38.9	-15.5	40.3
Citrulline	87.1	74.9	88.1
Arginine	78.2	113.4	113.0
Taurine	0.5	-16.5	38.8
Total	764.0	788.7	990.0

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VI. GENERAL DISCUSSION AND CONCLUSIONS

The experiments conducted on the apparent digestion of nitrogen and organic matter during cold exposure of ruminants confirm previously reported results in the literature as well as provide new information on the specific sites of digestion.

Many of the effects of cold exposure on the physical changes on rumen metabolism have been confirmed by these experiments and in addition some new information has been obtained.

Increased rate of passage of digesta through the abomasum with a corresponding decrease in the mean retention time (MRT) and the physical volume of the rumino-reticulum found in this experiment agree with the results of several studies reviewed by Christopherson and Kennedy (1983) and Kennedy et al. (1986a).

In absolute terms, the effects of the cold environment on the digestion of dry matter and organic matter in the small intestine appear to be inconsequential. There does appear to be a shift in the digestion of OM from the forestomach to the post-ruminal tract when the relative proportion of digestion is considered. The proportion of digestion occurring post-ruminally was similar to estimates obtained in previous studies (Thomson and Beever 1980; Beever and Siddons 1986).

The digestion of the cell wall constituents, acid

detergent fiber, and hemicellulose in the whole tract is decreased by cold exposure, due mainly to a decreased forestomach disappearance, which is in agreement with the results of Christopherson and Kennedy (1983) and Kennedy (1985).

The flows of NAN through the abomasum of sheep were elevated due to cold exposure. Increased flows of dietary protein relative to the total flow of NAN appear to be responsible for the difference (Kennedy and Milligan 1978; Kennedy et al. 1982; Christopherson and Kennedy 1983; Kennedy et al. 1986a). Decreased residence time of digesta in the rumen decreased the total digestion by the microbial population and the proportion on NAN derived from microbial cells. Consequently, a different amino acid profile was presented to the small intestine (Kennedy et al. 1986b).

The relationship between NAN digestion in the small intestine and total OM digestion suggests that there is a repartitioning of the efficiency of digestion when sheep are exposed to cold environments. Relative to the amount of total OM digested, it is evident that NAN and amino acid NAN are digested to a greater extent in the small intestine. Specifically, the sheep appear to be able to maintain a high utilization of the NAN and AAN components of the diet despite of the total OM digestion occurring in the gastro-intestinal tract.

Kennedy and Milligan (1980) suggested that there was an improvement in the recycling of urea in ruminants exposed to cold environments. With the decreased total OM digestion and increased flow of NAN to the small intestine, there appears to be an effort to maintain the nitrogen economy of the ruminant despite the reduced energy digestion in the whole tract (Christopherson and Young 1986). The net result of reduced OM digestion is an increase in the feed requirement of the animal to meet the increased energy demand in a cold environment.

The results from the experiment on the amino acid release indicate there is increased utilization of amino acids by the intestinal tract itself during cold exposure. The major effects of cold on the amino acid net release from the mesentary is a decrease in the concentration of circulating arterial amino acids. There is a consistent decrease in both the essential and nonessential amino acids. Associated with this, there was a consequent decline in the net release across the mesenteric drained viscera.

Christopherson (1985) indicated that it is difficult to ascribe a clear understanding of the effects of cold on the blood flow to the gut. Several conflicting reports in the literature on the effects of cold exposure on portal blood flow exist (see Alexander et al 1973; Hales et al. 1976; Schaeffer et al. 1982). The benefits of decreased blood flow to the gut are not clearly evident. Presumably,

increased flow would facilitate the more efficient transfer of nutrients to the blood and consequent utilization (Christopherson, 1985) and shunting of the blood to the thermogenic tissues would aid in the maintenance of homeothermy (Sasaki and Weekes 1986).

From the experiments conducted, it is evident that there are changes in the disappearance and efficiency of utilization of protein and amino acids along the intestinal tract of cold exposed sheep. Although it is possible that sheep exposed to cold temperatures could utilize their feed more efficiently, the results of the experiments on amino acid uptake indicate that the increased disappearance of amino acids in the small intestine seems to be augmented by increased metabolism by the gastro-intestinal tract itself. Further studies on the turnover of amino acids arising from the intestinal tract are required to isolate and quantify the relative contributions of gut turnover to the amino acid economy of the ruminant during cold exposure.

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