2005-04-28

Digestibility, Nitrogen Balance, and Blood Metabolites in Llama and Alpaca Fed Barley and Barley Alfalfa Forages.

Heather Loree Davies
Brigham Young University - Provo

DIGESTIBILITY, NITROGEN BALANCE, AND BLOOD METABOLITES IN LLAMA (*Lama glama*) AND ALPACA (*Lama pacos*) FED BARLEY AND BARLEY ALFALFA DIETS

by

Heather L. Davies

A thesis submitted to the faculty of

Brigham Young University

in partial fulfillment of the requirements for the degree of

Masters of Science

Department of Integrative Biology

Brigham Young University

April 2005

BRIGHAM YOUNG UNIVERSITY

GRADUATE COMMITTEE APPROVAL

of a thesis submitted by

Heather L. Davies

This thesis has been read by each member of the following graduate committee and by majority vote has been found to be satisfactory.

_____	_____
Date	Beverly L. Roeder, Committee Chair
_____	_____
Date	Todd F. Robinson, Committee Member
_____	_____
Date	N. Paul Johnston, Committee Member

BRIGHAM YOUNG UNIVERSITY

As chair of the candidate's graduate committee, I have read the dissertation of Heather L. Davies in its final form and have found that (1) its format, citations, and bibliographical style are consistent and acceptable and fulfill university and department style requirements; (2) its illustrative materials including figures, tables, and charts are in place; and (3) the final manuscript is satisfactory to the graduate committee and is ready for submission to the university library.

_____ _____

Date

Beverly L. Roeder
Chair, Graduate Committee

Accepted for the Department

_____

Larry St. Clair
Chair, Department of Integrative Biology

Accepted for the College

_____

Dr. Kent Crookston
Dean, College of Biology and Agriculture

ABSTRACT

DIGESTIBILITY, NITROGEN BALANCE, AND BLOOD METABOLITES IN LLAMA (*Lama glama*) AND ALPACA (*Lama pacos*) FED BARLEY AND BARLEY ALFALFA FORAGES.

Heather L. Davies

Department of Integrative Biology

Masters of Science

These projects were conducted to determine the digestibility of forage diets with differing CP levels in llamas and alpacas. The Utah study was designed to compare llama and alpaca nutritional parameters to determine if nutritional recommendations for llamas can be directly extrapolated to alpacas. The first study evaluated the effects of forage quality on blood metabolites and nitrogen balance in mature, intact male llamas (n = 4, 36 ± 4.4 months, 87 ± 17 kg) at high altitude in Letanias, Bolivia (4,267 m = aprox.14,000 ft above sea level). A second experiment was conducted with eight adult gelded camelids (n = 8; 4 llamas, 24-36 months, 90 ± 10.7 kg; 4 alpacas, 24-36 months, 50 ± 4 kg) at Brigham Young University, Provo, UT (altitude 1370 m). Animals were randomly fed barley hay (B) and 80% barley/20% alfalfa hay (BA). A fresh cut grass pasture (P) was included as the third forage for Bolivian llamas. Animals were housed in metabolism crates and diets were fed for a 7 d adjustment period followed by a 5 d collection period. Feed, feed

refusal, feces and urine were collected, dried and N content determined by combustion analysis. Venous blood samples were collected on d 12 at 30 min intervals over a 6 h period. Plasma was harvested and analyzed for electrolytes (Na, K, Cl, Ca, Ca^{++}, P, Mg) and metabolites (glucose, non-esterified fatty acids (NEFAs), urea N, creatinine, albumin, total protein (TPP), osmolality (Osm)). Llamas and alpacas demonstrated differences with respect to nitrogen metabolism when consuming forage diets with differing protein concentration. Llamas showed a N maintenance requirement of 0.75 g crude N/ $W^{0.75}$. Using the standard CP to digestible protein (DP) conversion factor of 0.8, llamas required 0.60 digestible N/$W^{0.75}$. When consuming the same high protein barley alfalfa diet, llamas had a much greater increase in N retention than alpacas. These species differences indicate that alpacas have a higher N requirement to meet metabolic needs, and extrapolations with respect to nitrogen requirements and balance are not valid between llamas and alpacas. In the Bolivian llama trial, locally grown and harvested hycrested and Siberian wheat grass pasture (P), barley (B), and barley80%/alfalfa20% (BA) hays were fed. The Bolivian llamas were in negative N balance when fed the B and P diets. Dry matter digestibility was greater with the B and BA than P forage, and N digestibility was significantly higher with BA than either the B or P forages. Nitrogen maintenance requirement for Bolivian llamas at 4,267 m was 0.58 compared to 0.75 g crude N/$W^{0.75}$ for Utah llamas, an increased digestive efficiency and a lower N maintenance requirement at higher altitude.

ACKNOWLEDGEMENTS

I owe my appreciation to many people for making this thesis possible. First of all, I would like to thank my major professor and advisor, Dr. Beverly Roeder, who helped direct me in the writing and data analysis process; who also shared with me her vast knowledge and expertise, which made the completion of this project possible.

I would like to thank Dr. Paul Johnston for his guidance; I thank Dr. Allen Christensen, BAFI Director, for his advice and helpful suggestions for thesis revision; and Dr. Todd Robinson for his help in setting up the project.

I am also indebted to the Ezra Taft Benson Agriculture and Food Institute (BAFI) for their assistance in funding this project as well providing the use of their facilities in Letanias, Bolivia, for the completion of one of the studies. I also thank Elizabeth Garcia for her assistance with language translation and coordination of BAFI personnel in Bolivia who assisted with this research effort, and José Pocuaca, Jacinto Callisaya for their help with animal care in this study.

The assistance of Micah Sharp and Christina Horey in the project was very much appreciated. I especially thank Micah for all her help through many months of working side by side as well as living together in Bolivia. She has made this experience very memorable, and I am grateful for the friendship we developed along the way.

Finally, I would like to thank my family and friends who supported and encouraged me along the way; I would never have gotten through this without them.

TABLE OF CONTENTS

PLASMA METABOLITES AND NITROGEN BALANCE IN *Lama glama* ASSOCIATED WITH FORAGE QUALITY AT ALTITUDE.

DIGESTIBILITY, NITROGEN BALANCE, AND BLOOD METABOLITES IN LLAMA (*Lama glama*) AND ALPACA (*Lama pacos*) FED BARLEY OR BARLEY ALFALFA DIETS.

LIST OF TABLES

Page

LIST OF FIGURES

Page

INTRODUCTION

South American Camelids commonly reside in the harsh climate of the Andes Mountains at altitudes greater than 3,000 m. South American Camelids (SACs) include the llama, alpaca, vicuna and guanaco. Of the four species of SACs, llamas and alpacas are the only domesticated and economically important species. They may be the oldest domesticated animals in the world due to thousands of years of selective breeding by the Inca people (Birutta, 1997). In the high altitude (> 3000 m) region of Bolivia and Peru, known as the altiplano, approximately 350,000 families rely solely on camelid herding for their survival (Sumar, 1988). Llamas and alpacas have adapted to thrive in the harsh altiplano environment. Low atmospheric oxygen availability, drought, and daily temperature extremes are among the challenges to their survival. Low camelid fertility (40-60%) and high embryonic death (50% within the first 30 days), greatly effect the production of alpacas (as cited by Raggi, 1994). South American camelid productivity is not only limited by the harsh climate, stark environment, and overgrazing, but also due to lack of knowledge about these animals behavioral and disease problems (Sumar, 1988).

Since 80% of all South American llamas and alpacas are owned by private pastoralists, very little is known about their nutritional requirements (San Martin, 1989). The pastoralists tend to own small, mixed herds of camelids and other ruminants which include llamas, alpacas, sheep and cows. Research examining the differences between the digestive abilities of these high altitude pseudoruminants compared to domesticated ruminants has been published (Dulphy, 1994 and 1998, Vernet, 1997, Lemosquet, 1996, Genin, 1997). The nutritional requirements for

SACs are little understood. Current camelid recommendations are usually extrapolated from requirements for sheep, goats and cattle (Carmalt, 2000). It has been inferred from previous studies that the digestive ability of camelids is superior to true (pecoran) ruminants, when consuming poor quality forages (Dulphy, 1994 and 1998, Vernet, 1997, Lemosquet, 1996, Genin, 1997). Although the llama and alpaca are closely related, little is known about the digestive and nutrient requirement differences between the two species.

History of South American Camelids

Llamas and alpacas are members of the Camelidae family in the order Artiodactyla (ungulates). This family is divided into two major groups: the Old World camels which live in Africa and Asia (the one humped Dromedary and two humped Bactrian camels) and the New World camelids (llama, alpaca, guanaco and vicuna, commonly referred to as South American Camelids or SACs) indigenous to South America. One theory explaining the division of the two groups suggests a common ancestor inhabited North America, but was forced into South America or over the Bering Sea land bridge into Asia due to the encroaching ice age. This theory is supported through fossil evidence of these ancestors found in North America that have been dated back to 16 million years ago (Sumar, 1988). Their dentition is unique, having incisors that grow throughout their entire life, enabling them to eat tough plants that grow close to the ground.

Llamas have been imported into the USA since the 1800's but were owned primarily by zoos and a few private breeders. Llama import was popular in the late 1970-1980's as the demand for their fiber, use as pack animals (can carry 25-30 kg

cargo load 15-20 km per day), and consumer use of llama manure in the fertilizer market (high nitrogen content, rivaling that of bat guano) has increased (Sumar, 1988, Birutta, 1997). Presently, there are 95,000 llamas in the USA today.

Fig 1. A vicuña in a herd of llama at the University of Oruru, Bolivia.

Appearance and Growth

Llamas are the largest of the SACs, reaching up to 1.2 m in height (Table 1). (Sumar, 1988). They have coarse wool which ranges in color from: black, white, gray-brown or multi- colored. They can be distinguished from alpaca by their size and their large, banana-shaped ears (Ordonez, 1994).

Alpacas are gregarious and have a smaller stature with shorter ears than llamas. There are two different breeds in the alpaca family: the Huacaya and the Suri. These breeds are readily distinguished by their characteristic wool. The Huacaya can

be distinguished from the Suri by its hairy face and short, more crimped looking fiber. The Suri, however has a hairless face with long, straight fiber that resembles dreadlocks. The Huacaya fiber is more valuable and therefore is the more prevalent breed. Due to years of breeding they are typically solid colored, ranging from white, fawn, brown, black or gray.

The guanaco greatly resembles the llama in shape and size but they have a very shaggy fleece that is typically solid chestnut brown that is highly desirable like the vicuña. It has not been domesticated and its natural range is limited to the southern tip of South America. It was once hunted for its meat, but is now protected by the government.

The vicuña is the smallest SAC (Fig. 1). It is very slender and delicate compared to its relatives. Like the guanaco it is also wild and is currently protected by the government. They have a distinctive herd structure in which they defend a separate sleeping and year-round feeding ground (Sumar, 1988). The vicuña is desirable due to its extremely high wool quality that is comparable to the Angora rabbit and of higher quality than cashmere. The high popularity of their fiber resulted in them being hunted almost to extinction. They are currently protected in Peru, Bolivia and Chile to avoid extinction.

Camels and SACs can interbreed. Interbreeding SACs with the vicuna is very desirable as it greatly increases the fiber value of the offspring. Crossbreeding is most commonly conducted between the vicuna and the alpaca (Sumar, 1988).

Table 1. Comparison of height and weight of South American Camelids.

Species	Withers height (cm)	Head Height (cm)	Mature Weight (kg)
Llama	90-125	175-225	75-115
Alpaca	80-90	150-175	50-100
Guanaco	90-110	125-210	75-100
Vicuña	70-90	120-130	35-60

Adapted from Ordoñez (1994)

Water Requirement and Body Water

Average water consumption is approximately 8-11 L (2-3 gallons) a day for a clinically normal 136 kg (300 lb) llama (Table 3). Water intake may increase in extreme weather conditions, and an increased intake of water is associated with the consumption of dry hay (Purdy, 2000). Llamas have a body water content of 67% compared to 60% in goats, but have been shown to have similar water requirements. A study determining water turnover (water intake by drinking, plus food and oxidative water) rates in llamas and goats found that llamas had a water turnover rate of 62.1 ml/$BW_{kg}^{0.82}$/24 hr compared to goats 59 ml/$BW_{kg}^{0.82}$/24 hr (Rubsamen and Engelhardt, 1979). Compared to other ruminants, this relatively low water turnover rate increased when llamas were allowed to forage outside for food, increasing by nearly two times in the llama and 3 times in the goat. Llamas demonstrated their superior water ability to conserve body water over the goat when water restriction caused the goats to dramatically decrease food intake, but food intake in llamas was largely unaffected. Camelid water requirement is met by drinking, eating and metabolic water. Llamas absorb ingested water and ions from food and GIT

secretions. The absorption rate of water, Na, Cl and VFAs in the forestomach of llamas are 65.2, 18.6, 16.2, and 96.3 mmol/h, respectively (Rubsamen and Engelhardt, 1979).

Unlike their camel cousins, SACs are unable to adapt to elevated ambient temperatures by increasing body temperature and avoiding evaporative cooling, while dissipating the heat during the cold, desert nights. SACs therefore have an increased water requirement during high ambient temperatures. SACs are similar to the camel in that they produce a very concentrated urine and relatively dry, pelleted feces. The water turnover rate in llamas suggest that llamas are better adapted than goats or other ruminants to reduced water intake, during which they are able to eat comparatively more feed, thus increasing their metabolic water supply (Engelhardt, 1974). Although an increased metabolic rate would predict that llama would increase extra-renal water loss due to increased oxygen demand, the llama is able to overcome this barrier by concentrating their urine to a much greater degree, 3,190 mosm/L maximum concentration compared to the goat 2841 mosm/L. Water turnover rate and energy metabolism are both decreased with a reduction in food intake (Engelhardt, 1974).

It is unknown if SACs are equally adapted to dehydration as Old World camels, but their similar erythrocyte structure and often desert-like habitats would suggest that they may be able to compensate to some extent. The dromedary have specialized blood erythrocytes which aid their ability to withstand high levels of dehydration and rapid rehydration. Camels can drink large quantities of water in one session to reverse a water deficit by slowing absorption from the gut, and through their specialized erythrocytes which are able to swell up to 240% of their normal

volume without rupturing, thereby facilitating conservation this process (Fowler, 1989). The dromedary can tolerate losses of up to 40% of its body water and still survive, whereas domestic livestock and companion animals can only survive up to 10-12% dehydration. Camelids have small elliptical erythrocytes that are able to continue circulating even during times of increased blood viscosity associated with dehydration (Fowler, 1989).

The body fluid is divided into two main compartments, the extra-cellular fluid (ECF) and intracellular fluid (ICF). The ICF accounts for 2/3 of the total body water whereas the ECF is 1/3. The extracellular fluid is separated into two compartments separated by the capillary wall: the interstitial fluid which surrounds the cells in body tissues making up 75% of the ECF and plasma volume accounting for the other 25%. Na^+ is the major cation while Cl^- and HCO_3^- are the major anions in the ECF. The major difference between ISF and plasma is that plasma contains significantly more protein, whereas ionic composition is similar. Na concentrations can be used to give a rough estimate for ECF by doubling the [Na]. Normal plasma osmolality ranges from 285 – 295 mOsm/kg H_2O. In the ICF, the major cation is K^+ and major anions are phosphates, organic anions and proteins unlike Cl^- and HCO_3^- of ECF. The ICF and ECF are at osmotic equilibrium. Therefore all exchanges of water with the external environment occur through the ECF (IV fluids, drinking, etc) which eventually equilibrates with the ICF.

Table 2.Water requirements for llamas and alpacas

Body weight (kg/Ib)	Body weight ($kg^{0.82}$)	Water requirements: 2x turnover in controlled environment (Approx. water consumption in a moderate environment) (ml/kg /24 hr)	Water turnover on pasture ($125\ ml/kg^{0.82}$/24 hr) (L or qt)	Water requirements: 2x turnover Water turnover (Approx. water consumption in a moderate environment including water in forage) (ml/kg /24 hr)
10/22	6.61	1.0[6]	NA	NA
20/44	11.66	2.0[6]	NA	NA
40/88	20.59	0.88	44	2.57
50/110	24.73	1.05	42	3.09
75/165	34.48	1.47	39	4.31
100/220	43.65	1.86	37	5.46
125/275	52.42	2.23	36	6.55
150/330	60.87	2.59	35	7.61
175/385	69.07	2.94	34	8.63
200/440	77.06	3.28	33	9.63

Adapted from Grace (1994)

Nutrition and Feeding

Data indicate that the camelid digestive tract is significantly different from true ruminants, making it difficult to compare these animals, thus necessitating focused nutritional requirement research on camelids (Dulphy, 1994 and 1998, Vernet, 1997, Lemosquet, 1996, Genin, 1997). Extrapolation of information from ruminants, estimates for camelid requirements, and comparisons to ruminants have been published (Tables 3, 4, 5; Figure 2).

Table 3. Estimated basic nutrient requirements for llamas and alpacas

Body Weight (kg/lb)	Metabolic weight ($W_{kg}^{0.75}$)	Metaboliz. Energy Maintenance (Mcal)	Crude Protein Mainten. (g)	Ca (g)	Phos. (g)	Dry matter consumed at 2.5 Mcal ME/kg of forage		Feed consumption % of body weight	
						kg	lb	100% DM	As fed
10/22	5.62	0.47	15	5	3	0.19	0.42	1.9	2.1
20/44	9.50	0.80	25	6	4	0.32	0.73	1.6	1.8
40/88	15.91	1.34	42	7	5	0.52	1.115	1.3	1.4
50/110	18.80	1.59	51	8	6	0.65	1.43	1.3	1.4
75/165	25.50	2.15	68	9	7	0.87	1.92	1.2	1.3
100/220	31.60	2.67	84	11	9	1.08	2.38	1.1	1.2
125/275	37.40	3.16	99	13	10	1.28	2.82	1.0	1.1
150/330	42.90	3.63	114	16	12	1.47	3.24	1.0	1.1
175/385	47.50	4.01	126	18	12	1.63	3.59	0.9	1.0
200/440	53.20	4.50	141	20	13	1.82	4.01	0.9	1.0
225/495	58.10	4.91	184	21	14	1.99	4.39	0.9	1.0
250/550	62.90	5.32	167	23	17	2.15	4.70	0.8	1.0

Note: Metabolizable energy, maintenance - ME = 84.5 x $W_{kg}^{0.75}$. Protein requirement = 31g/Mcal energy. Adapted from Fowler (1989) p.18, DM = dry matter

Some feeding regimens have been suggested by veterinarians and implemented successfully among llama breeders. Just as in all animals, llamas and alpacas require different nutritional supplementation depending on their age, weight and reproductive status (Table 3). A Colorado study suggested that protein intake for maintenance in llamas is 10% of a 100% dry matter diet whereas 16% protein is recommended in growing, lactating and gestating llamas (Johnson, 1994, Johnson, 1989).

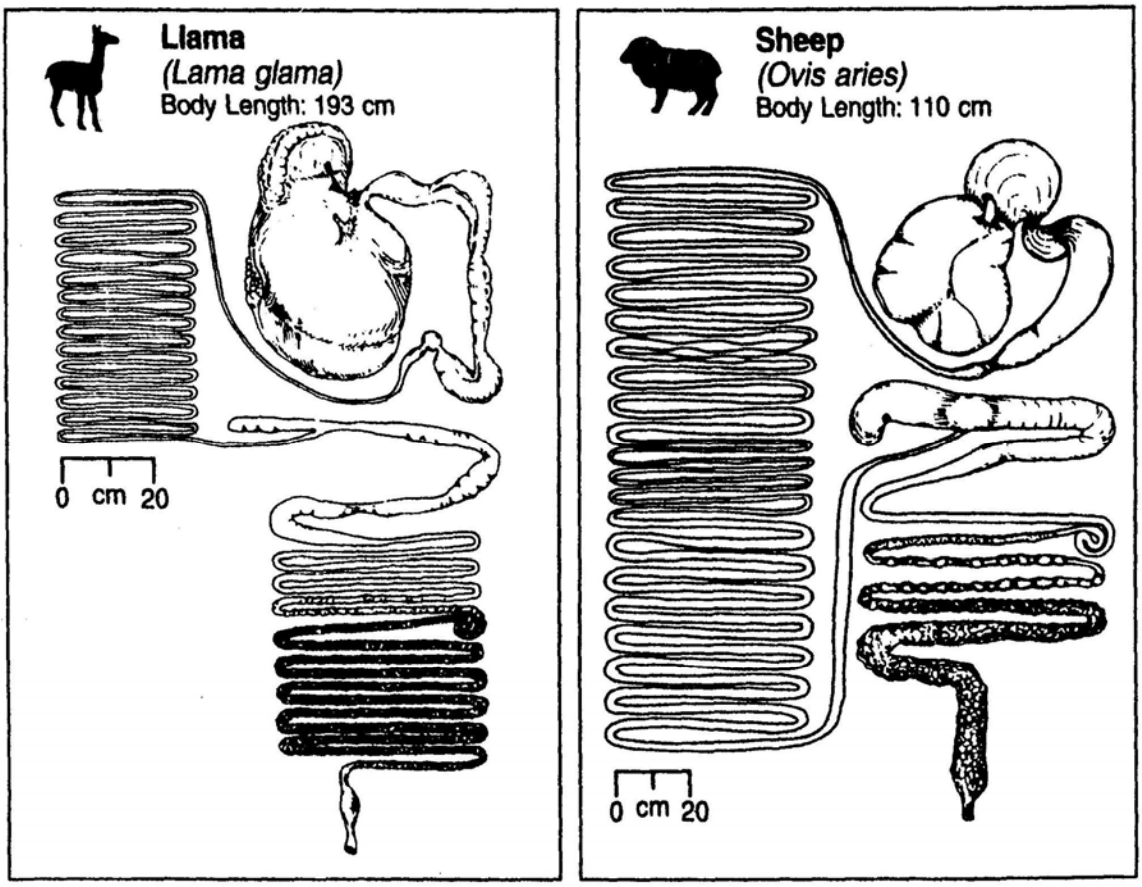
Table 4. Comparison between Camelids and Ruminants

South American Camelids	Ruminants
Evolution	
Evolutionary pathways diverged 40 million years ago	Evolutionary pathways diverged 40 million years ago
Blood	
Red blood cells elliptical and small (6.5 p); predominant white blood cell is neutrophil; leukocytes up to 22,000	RBCs round and larger (10 p); predominant WBC is lymphocyte; leukocytes up to 12,000
Foot	
Foot has toenails and soft pad. Second and third phalanges are horizontal	Foot has hooves and sole. Second and third phalanges are nearly vertical
Digestive system	
Foregut fermenter, with regurgitation, rechewing and reswallowing	Same (parallel evolution)
Stomach-3 compartments, resistant to bloat	Stomach-4 compartments, susceptible to bloat
Dental formula-I 1/3, C 1/1, PM 1-2/1-2, M 3/3 x 2 =28-32[a]	Dental formula-I 0/3, C 0/1, PM 3/3, M 3/3 x 2 = 32
Reproduction	
Induced ovulator	Spontaneous ovulation
No estrous cycle	Estrous cycle
Follicular wave cycle	No follicular wave cycle
Copulation in the prone position	Copulation in standing position
Placenta diffuse	Placenta cotyledonary
Epidermal membrane surrounding fetus	No epidermal membrane on fetus
Cartilaginous projection on tip of penis	No cartilaginous projection on tip of penis
Ejaculation prolonged	Ejaculation short and intense
Respiratory system	
Soft palate elongated; primarily a nasal breather	Soft palate short; nasal or oral breather
Urinary system	
Kidney smooth and elliptical	Kidney smooth or lobed
Suburethral diverticulum in female at external urethral orifice	No suburethral diverticulum
Dorsal urethral recess in male at junction of pelvic and penile urethra	Dorsal urethral recess in some species
Parasites	
Unique lice and coccidia; share some gastrointestinal nematodes with cattle, sheep, and goats	Unique lice and coccidia; share GI nematodes
Infectious diseases	
Minimally susceptible to tuberculosis; no known natural bovine brucellosis; mild susceptibility to foot-and-mouth disease; rare clinical disease with other bovine and ovine viral diseases	Highly susceptible to tuberculosis, bovine brucellosis, and foot-and-mouth disease

[a]I = incisors, C = canines, PM = premolars, M = molars

Adapted from Fowler (1989) pg. 3

Fig.2. Comparison between llama and sheep GIT



Adapted from Stevens and Hume (2004) pg. 80

According to Fig. 2, it is apparent that the digestive tract of the sheep and llama are quite different. These anatomical differences demonstrate the necessity of further research to define SAC nutritional requirements as common ruminant information should not apply. The anatomy of ruminants and camelids clearly defines a different number of forestomach compartments, and significantly different small and large intestinal lengths. The llama has the longest large intestine of all mammals (Stevens and Hume, 2004). Table 5 compares the similarities and differences between the domestic ruminant and camelid digestive systems.

Table 5. Comparison of GIT between Ruminants and Camelids.

Ruminant	Camelid
4 compartments	3 compartments
Esophagus enters between rumen and reticulum	Esophagus enters C-1 only
Pillars pronounced, horizontal	Pillars minimal, horizontal
Glands in abomasum only	All compartments glandular
Esophageal groove double lipped	Esophageal groove single lipped
Rumen papillated	C-1 nonpapillated
Motility pattern 2-4/min	Motility pattern 3-5/min
Rumen-epithelium keratinized, stratified squamous	C-1 epithelium nonkeratinized, squamous
Abomasum-entirely covered with enzyme and acid-secreting epithelium	C-3- only distal one-fifth covered with enzyme and acid-secreting epithelium

Adapted from Bohlken (1960)

Camelid Digestive Tract

Similar to ruminants, camelids have a maxillary dental pad and mandibular incisors. They use incisors to shear off food by pressing it against their upper dental pad. In general, camelids tend to move around and selectively eat bites in all directions, selecting food close to the ground, in contrast to cattle that consume as much as they can in one area. The upper lip of camelids is split into two independently manipulated labia, allowing them to select certain foods and avoid foreign objects, which trait is absent in cattle and less developed in Old World camels (Fowler, 1989). Camelids do not use their tongue to manipulate or grab food. In fact their tongue rarely protrudes from their mouth, making them unlikely to lick themselves, or their young, or even salt licks (Fowler, 1989).

Saliva production is significant in mastication and ingestion of food, allowing food to be swallowed and digested. The flow of saliva in an alpaca has been reported to be 140 ml/hr pre-feeding, 202 ml/hr during feeding and 150ml/hr post feeding. Reported saliva composition in the pre-fed state: 121 mEq/L HCO_3, 33.5 mEq/L

HPO_4, 164.8 mEq/L Na and 13.7 mEq/L K. Alpacas had a higher HCO_3 level of 127.8 mEq/L, while the pH remained constant at ~ 8.6 during feeding (Fowler, 1989).

The camelid stomach (Figs. 2 & 3) is divided into 3 main compartments: compartments 1, 2 and 3 (C-1, C-2, C-3). C-1 is the largest of the three, comprising 83% of total stomach volume (Johnson, 1983). C-1 is divided into a cranial and caudal portion by an internal transverse pillar. The ventral sections of both the cranial and caudal portions of C-1 are lined with secretory saccules which absorb Cl in exchange for HCO_3 secretion (Eckerlin and Stevens, 1973, Vallenas et al., 1971). Though it is impossible to draw exact anatomical homologues between the camelid and ruminant stomach, some similarities may be noted. C-1 corresponds to the rumen in pecoran ruminants. C-2 is similar to the reticulum, comprising 6% of total stomach volume (Johnson, 1983). This compartment is also made up of glandular mucosa secretory saccules, but also contains absorptive cells. The first part of this compartment is made up of muscular folds which form the ventricular groove allowing milk from the suckling cria to bypass C-1 and C-2 altogether and pass directly into the third compartment. C-3 is divided anatomically into 2 different sections. The proximal 4/5 being lined with glandular mucosa secreting cells whereas the distal 1/5 is lined by gastric and pyloric glands similar to those found in simple stomach, monogastric animals (Vallenas et al., 1971). This final portion, making up 11% of the total stomach volume is analogous to the ruminant abomasum (Johnson, 1983.) Contents in C-3 are relatively dry due to water absorption that occurs in the cranial 4/5 of this compartment (Fowler, 1989). C-1 and C-2 act as fermentation vats

which house the microbial flora and fauna that aid in anaerobic fermentation and cellulolytic degradation.

Little research has been done on the function of the llamoid hindgut, which consists of a large cecum and extremely long, spiral colon, which diminishes in diameter by 2/3 about halfway through the spiral and produces a relatively dry, pelleted feces. This lengthy spiral colon enables camelids to excel in water retention and absorption of electrolytes.

The main anatomical difference noted between the camelid and ruminant stomach is the presence of extensive glandular mucosal arrangements that have no counterpart in the ruminant. This glandular mucosa covers about 50% of the llama forestomach (Rubsamen and Engelhardt, 1979). Since this is such a significant difference between camelids and ruminants, Rubsamen and Engelhardt (1979) analyzed chemically the glandular portion of the camelid stomach and discovered that the glandular epithelium was more comparable to highly absorptive cells found in the intestine. Also, the mucous layer and mucus production may greatly enhance microbial activity in the camelid forestomach as sloughed mucous was digested by the microorganisms (Rubsamen and Engelhardt, 1979). Recent studies attribute this glandular epithelium with the ability to rapidly absorb volatile fatty acids (VFAs), water and solutes which may partially explain the greater digestive capacity of new world camelids. The glandular surface is even greater in the new world camelids than their old world cousins. C-1 differs greatly from the rumen as parts of it are lined by stratified squamous epithelium and non-papillated mucosa (Vallenas et al., 1971). Also, true rumen contents are stratified into gaseous, solid and liquid layers whereas

the camelid stomach tends to have a drier, more homologous food distribution (Fowler, 1989). This, along with the fact that fermentation results in little gas production in the forestomach of camelids, may explain why bloat is uncommon in pseudoruminants.

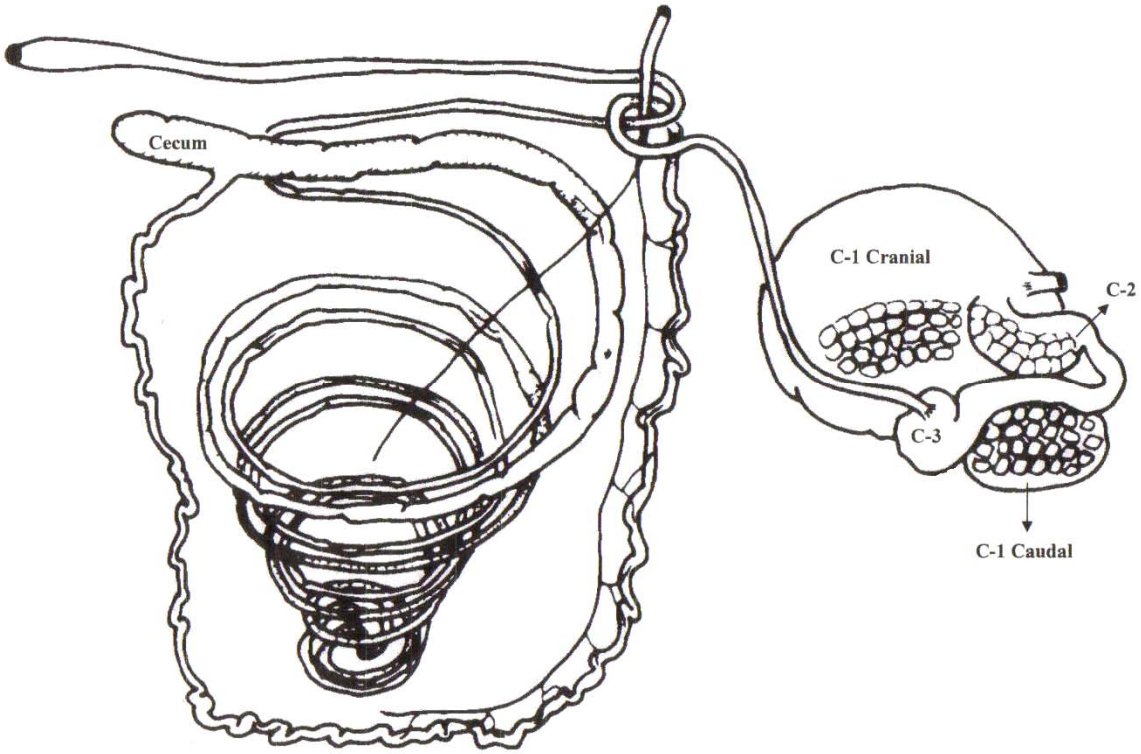


Fig.3. Lamoid gastrointestinal tract. Adapted from Fowler (1989) pg. 320
C-1 = Compartment 1, C-2 = Compartment 2, C-3 = compartment 3. The circular globules you see in C-1 and C-2 represent the glandular mucosa of these compartments.

Motility and contractility

Stomach contraction and motility patterns differ between camelids and common ruminants. Differences in motility may contribute to an increased ability

to digest low quality feed by SACs. A contraction cycle begins in C-2, followed by 6-7 contractions in the caudal sac of C-1 followed by contraction in the cranial sacs of C-1. Each contraction causes the glandular pouches in the corresponding section to evert and spill their contents. Contraction of the cranial sac of C-1 during camelid rumination causes regurgitation. Eructation occurs at the height of the caudal sac contraction. The cyclic contraction of the forestomach of camelids is more frequent than that of ruminants (Heller, 1984) and occurs at a rate of 0.6±0.1/min, increasing slightly during feeding and decreasing during rumination (Vallenas and Stevens, 1971).

Retention time

Retention time or the length of time feed remains in the digestive tract affects digestive efficiency of feedstuffs (Foose, 1982, Heller, 1986, San Martin, 1987, Silanikove et al., 1993, Silanikove, 2000, Van Soest, 1994, Sponheimer et al., 2003). The mean retention time (MRT) in SACs has been demonstrated to be longer than in sheep and goats (Florez, 1973, Sponheimer, 2003; San Martin, 1987). The MRT for llamas, alpacas, goats, horses and rabbits were 72±14, 71±5, 54±1, 27±5 and 7±2h, respectively (Sponheimer et al., 2003). The amount of time a feed is retained within the GIT depends on its quality and digestibility. Lower quality, high fibrous feeds are usually retained longer than more readily digestible feeds. It is interesting to note that llamas and alpacas have always been assumed to have the same nutritional requirements when adjusted for size (San Martin, 1987, Fowler, 1998). Sponheimer et al. (2003) discovered that alpacas, which

were 33% smaller than the llamas in their study, had a similar MRT, indicating a comparatively longer MRT in alpacas. This caused the alpaca to have a much lower adjusted intake (AI) 29±5 g/$BW^{0.75}$/day compared to 53±5 g/$BW^{0.75}$/day for llama. This study showed a much higher DDM/MW for llama than alpaca, indicating that llamas can perform better on lower quality feed than alpaca (Sponheimer et al., 2003).

The retention time of fluids and different particle sizes were compared between llamas and ruminants by Heller et al. (1986). They discovered that selective retention of larger food particles in the forestomach of ruminants led to better microbial digestion and utilization. Llamas were less selective about particle size, and permitting up to 40% of the dry matter in C3 to be composed of particles larger than 1mm (Heller et al., 1986). The passage of larger particle sizes from the llama forestomach, however, still allowed the feed to be retained for large periods of time. Mean retention time for fluid in the forestomach was 15.3 ± 3.1h, averaging 27.0 ± 4.4h for small particles 0.2-1.0cm and 32.5 ± 5.7h for larger particles. The MRT for the total digestive tract was 36.2 ± 6.0h fluids, 52.0 ± 5.3 h small particles and 59.9 ± 6.2h large particles (Heller, 1986). Differing feed quality may also explain the disparity between MRT values in different studies. The short retention time of fluid compared to feed particles causes a high dilution rate which should improve microbial growth, thus improving the digestive capacity of llamas compared to pecoran ruminants (Heller, 1986).

Energy Metabolism-VFA production

Carbohydrates make up the major portion of food fed to camelids and ruminants. These carbohydrates can be divided primarily into two categories: readily available or nonstructural carbohydrates (NSCs) including: sugars and starches, and fibrous structural carbohydrates which is made up of hemicellulose, cellulose and xylans, which are typically more difficult to digest. Rumen microbes ferment carbohydrates into volatile fatty acids (VFAs). Almost 100% of the NSCs fed to ruminants and pseudoruminants are fermented by ruminant microorganisms to produce: VFAs (acetate, propionate and butyrate), carbon dioxide, methane and heat (Pond, 1995). VFAs are absorbed and incorporated into the Kreb's Cycle to produce energy. An increase in NSCs or reduction in pH below 5.0 will kill rumen microbes and reduce acetate/propionate levels in preference for lactate production (Stevens and Hume, 2004). The forestomach contents of camels were studied and compared to that of cows and sheep and showed no morphological differences between the rumen microbial population between camelids and ruminants (Williams, 1963). A lack of protozoa, which contribute little to carbohydrate fermentation, in camelid forestomach was noted. The proportions of propionate, butyrate and other VFAs were also similar to cows and sheep (Williams, 1963). The animal then absorbs the propionate and transports it to the liver to be used as substrate for gluconeogenesis and consequent energy production.

Rumen microorganisms, primarily anaerobic bacteria, degrade protein and other nitrogenous compounds such as urea and ammonia to make their own microbial

protein. Urea is the principal endogenous source of nitrogen used for microbial protein synthesis. It is produced in the liver from excess ammonia and enters the rumen through diffusion through the rumen wall or saliva secretions (Stevens and Hume, 2004).

Carbohydrate and protein metabolism are interdependent. When dietary carbohydrates are limited, microbial protein production is reduced. Inadequate dietary nitrogen also limits microbial protein production. Therefore animals begin to utilize proportionally more endogenous urea for the purpose of maintaining the microbial population when energy is limited, the end result being a reduction in VFA production (Stevens and Hume, 2004).

VFA Production

The rapid rate of VFA absorption is significant, because it stabilizes the pH of the stomach, allowing it to remain at higher levels for longer periods of time, which is favorable to further microbial fermentation, thereby increasing VFA production and availability. VFA absorption has been shown to affect luminal pH and to be directly correlated with HCO_3^- secretion during H^+ accumulation (Rubsamen and Engelhardt, 1979). Rubsamen and Engelhardt (1979) found the average HCO_3^- secretion rate in the llama forestomach to be 4.5 mmol/hr. Increased VFA absorption and therefore production may be the defining factor that makes camelids more adapted than are ruminants to low-quality diets.

Short chain fatty acids (SCFA), also known as volatile fatty acids (VFAs), are found in low levels in food and are rapidly absorbed from the GIT, making their

presence in digesta an indicator of microbial fermentation. VFAs are rapidly absorbed in C-1 and the proximal 4/5 of C-3 at a rate 2 to 3 times that of the rumen in sheep and goats (Fowler, 1989). Englehardt and Sallmann (1972) found that the absorption rates in the forestomach of the llama are higher than in the rumen and are similar to that of the intestine of other animals (Engelhardt and Sallman, 1972). VFA concentrations found in the forestomach of cows and sheep are 60-120 mmol/l (Huntgate, 1966) compared to 94-186 mmol/l in llamas (Stevens and Hume, 2004, Vallenas and Stevens, 1971, Engelhardt and Sallman, 1972). However, the VFA quantity produced and absorbed varies with gut capacity and retention time.

A study by Vallenas et al. (1971) showed the presence of VFAs all along the digestive tract of camelids; however, when compared to the concentrations found in studies of sheep, cattle and deer, the concentrations of VFAs caudal to the stomach were lower. Another study conducted by the same authors, (Vallenas et al., 1971) from which they obtained their VFA data for comparison, stated that they were unable to compare VFA data between studies due to differences in experimental design.

Vallenas et al. (1971) noted several differences in feed selection as well as fermentation between sheep and alpacas. They discovered that independent of altitude or feed, within the first hour of digestion, sheep exhibit a more rapid fermentation than that of alpacas, marked by a quicker rise in VFA levels. Sillan et al. (1973) also compared alpacas and sheep. They demonstrated greater fermentation activity after an hour's incubation in the stomach fluid of the sheep when compared to the llama. One reason for this may be that the metabolic products of fermentation during the

first hour come from the microbial attack on the soluble or easily fermentable parts of the diet such as soluble carbohydrates and simple nitrogenous compounds. This would suggest that microbial fermentation of the fibrous component of feed (cellulose, hemicellulose, etc.) come later (Huntgate, 1968). Flores (1973) and Riera Y Carsozo (1970) show that under the same conditions, sheep consumed more feed than alpacas. Sheep also seem to have greater selectivity or the ability to select for less fibrous parts of the feed, preferring leaves and soft parts of the plants, which does not occur in alpacas. This could explain the greater fermentation velocity in sheep due to the fact that sheep will have a relatively greater quantity of substrate which is readily digestible as well as selecting for a greater quality of feed compared to the alpaca. Sheep attained a VFA peak concentration 2 hrs post feeding, which was unaffected by the type of feed (green alfalfa or hay) or change in altitude. The VFA concentration began to fall and reached pre-feed values 10-11 hrs later at sea level and 13-14 hrs later at altitude with a relatively constant decrease in concentration.

Alpaca's maximum VFA concentration is reached 2 hrs post-prandially at sea level and 6 hours after eating at altitude, independent of feed. It also maintains it s peak between 5-6 hours, and reaches initial values 16-18 hrs after initiation of feeding. Sheep reached their maximum VFA concentration more rapidly, which also declined more rapidly, than the alpaca, which maintained high concentrations of VFAs over a longer period of time. Since passage rate influences the amount of time a feed is open to microbial fermentation, increased passage rate would likely result in reduced overall VFA production. These facts could explain, in part, results which indicate that feed retained for a longer time in the stomach of alpaca provides

additional time for microbial attack and fermentation and, thereby, increases levels of VFAs over a longer time period (Sponheimer et al., 2003).

VFA levels are also influenced by pH as microbes tend to function better at higher pH (less acidic levels). When the regression lines of pH and VFA levels were compared between alpacas and sheep, a similar regression coefficient was noted but the lines are parallel, with the sheep always being more acidic per VFA concentration. The ability of the alpaca to maintain a higher pH could therefore improve the VFA production as well. Alpacas have a mechanism of bicarbonate secretion by the glandular saccules in C-1 and C-2 that provides for more effective buffering power in the presence of VFAs than do sheep. As suggested by Ortiz, it may also be due to large amounts of bicarbonate secreted in the saliva by alpacas (Ortiz et al., 1974).

Another factor that determines the concentration of VFA in the ingesta is the velocity of absorption by the stomach. Previous studies (Vallenas et al., 1971, Vallenas and Stevens, 1971, Cummings et al., 1972, Engelhardt and Sallmann, 1972) show that the stomach morphology in SACs has peculiarities that should enable greater absorptive capacity. Although it is possible that sheep are capable of more rapid VFA absorbance under certain circumstances, causing a more rapid decrease in concentrations, there are no studies indicating sheep are better at VFA absorption than alpacas.

Altitude apparently plays a significant role in microbial fermentation as both the alpaca and sheep showed greater magnitude of fermentation at higher altitudes. A study by Vallenas and Stevens (1971) showed similar results where peak VFA levels

were reached 2-2.5 hrs at sea level in llamas and guanacos. Chinn and Hannon (1969) suggested that greater fermentation and VFA levels reached at higher altitude may be related to more efficient energy costs. Both animals showed a better digestive ability at altitude that could be attributed to more active stomach and saliva secretions of neutralizing agents at altitude. It is interesting to note that the alpaca showed an initial fermentation pattern similar to that of the sheep at sea level in which VFA peak was reached at about 2 hrs. Although VFA levels reached their peak concentration more rapidly at sea level, the magnitude of VFA production was not as intense as it was at altitude. Thus, the alpaca appears to be better adapted to increased fermentation and food utilization ability at higher altitudes than sheep. Further studies are needed to determine the exact physiological effect which altitude has on VFA production in tylopod pseudoruminants and pecoran ruminants.

Vallenas et al. (1971) found that in the camelid stomach, as food progressed along the GIT, VFA levels were reduced with the lowest concentrations being found in the distal third of C-3 where an acidic pH is present, with continued low VFA concentrations in the duodenum/jejunum and ileum. There was a considerable increase in VFAs in the cecum and proximal colon. This implies that the major place of fermentation is in C-1, where the conditions are most favorable for microbial action. The following compartments (with exception to the distal 1/3 of C-3) appear to have fermentation occurring, but to a lesser intensity. The presence of VFAs in the 2^{nd} and 3^{rd} compartment may have several explanations: 1. VFAs are found due to the passage of digesta from the anterior portion of the stomach. 2. There is also active fermentation occurring in these compartments. Acid secretion is the presumed reason

for the decrease in VFA concentration observed in the middle and distal portions of C-3. The low pH levels in C-3 appear to reduce VFA production by killing microbes. This information, along with other reported data on SAC, suggest that the main reason for the digestive efficiency of SACs is due to a more efficient production and absorption of VFAs (Vallenas, 1971). This theory compared with data from ruminants favors the hypothesis that SACs have a more efficient absorption of VFAs in the forestomach as well as the rest of the GIT.

VFA concentrations in the cecum and proximal colon are relatively high, showing that significantly active fermentation occurs there. The bacteria inhabiting the large intestine is well described in several mammals and are generally similar to that found in the rumen in both total numbers and species (Wolin, 1981, Allison, 1984). Also VFA concentrations in the hindgut of mammalian carnivores, omnivores and herbivores are similar to those seen in the rumen. Absorption of VFAs from the hindgut provides an additional source of energy for other body functions, as well as providing a substantial contribution to the absorption of water (Stevens and Hume, 2004), and a large part of the maintenance energy of hindgut fermenting herbivores. Measurements of 70-90 mmole of VFA/l (Argenzio, 1974) and 90 mmole/l (Hoover and Heitmann, 1972) have been found in the hindgut of ponys or rabbits, respectively. Camelids have the longest hindgut of all artiodactyls, have a longer mean retention time (MRT), and recycle nitrogen better than most pecoran ruminants (Sponheimer et al., 2003). The presence of a large cecum and highly specialized large intestine in camelids could allow more efficient absorption of VFAs produced not only by the forestomach but also by their hindgut. Formation of VFAs in the hindgut, along with

an increased rate of VFA concentration formed in the forestomachs, may enable camelids to better digest poorer quality forages with a lower nitrogen availability compared to ruminants and hindgut fermenting herbivores.

Nitrogen Acquisition and Metabolism

Nitrogen is an element essential to maintaining normal body structure and function. It is a necessary component of nucleic acids and structural and enzymatic proteins necessary for cell function and digestion. The body acquires nitrogen through the intake and breakdown of food with varying protein, nucleic acid and amino acid ratios, as well as from the recycling of nitrogen from endogenous sources.

Protein Metabolism and Excretion

Dietary protein is stored in muscle; excess protein is deaminated and the carbon skeletons converted into carbohydrates primarily in the liver via gluconeogenesis and stored as glycogen. During gluconeogenesis, the α-amino acid is released as metabolic waste nitrogen. The three major end-products of amino acid catabolism are: ammonia, urea and uric acid, the latter predominant in avian and reptilian species. A less common form of N excretion is as guanine. In camelids, nitrogen is excreted as urea and is highly correlated with water availability and the animal's nutritional and physiological state. Ammonia is the primary waste product for aquatic animals because it is highly soluble in water, is readily excreted across body surfaces and has a high diffusion coefficient due to its low molecular weight; also biological membranes are typically permeable to NH_3 but not NH_4^+. Large

concentrations of ammonia from amino acid deamination, is toxic to the animal's system. Therefore, non-aquatic animals needing to conserve water will excrete either urea or uric acid, which are less toxic and require less water to excrete.

In living organisms, nonprotein nitrogen is primarily in the form of ammonia- which is defined as the sum of ammonia [NH_3] and ammonium [NH_4^+], a major constituent of protein and nucleic acid pools. In mammals, at least 20 metabolic reactions generate ammonia: glutaminase, glutamate dehydrogenase and the purine nucleotide cycle are the major contributors. Animals with high protein intake will deaminate 10-15% of dietary protein for energy resulting in ammonia and urea production. Therefore high protein diets may also increase blood ammonia and urea concentrations (Withers, 1992).

Ammonia Metabolism

Ammonia and ammonium ions are very toxic so blood levels must be kept low, ranging from 0.03-0.08 μM in mammals. Ammonia levels of 0.5 - 5 μM can become toxic and even fatal when they begin to interfere with the nervous system, membrane permeability, effecting iono- and osmoregulation. Ammonia inhibits Na^+/ NH_4^+ exchange pump, effects O_2 transportation ability of hemocyanin, effects carbohydrate metabolism, acid/base balance and the blood/brain barrier (Dimski, 1994, Withers, 1992). Although hyperammonemia can affect many bodily functions it most readily affects central nervous system (CNS). To counteract hyperammonemia, mammals increase ureagenesis and have developed a system that uses glucose to convert

ammonia to glutamine. The brain, however, has a limited capability to convert ammonia to glutamine and therefore is easily over-ridden (Berne, 2004).

The negative logarithm of the acid ionization constant (pKa) of ammonia-ammonium is 9.2, therefore, under physiological conditions with a blood pH = 7.4, approximately 99% of the ammonia pool is in the ionized NH_4^+/ ammonium form (Dimski, 1994). In mammalian urine at pH 5.6, 99.98% of ammonia is in the NH_4^+ form (Withers, 1992). It is essential for mammalians to maintain a physiologic pH, therefore ammonia disposal plays a large role in the acid/base balance of the body. Both the liver and kidneys are vital to the detoxification and excretion of ammonia and therefore, the acid/base balance of the body.

The body utilizes several mechanisms to detoxify ammonia. Besides urea synthesis, restricted areas around the centrolobular veins of the liver act as scavengers of excess ammonia that haven't been converted to urea by periportal hepatocytes. Glutamine synthesis by the body is responsible for 1/3 of total body ammonia detoxification. This mechanism is highly sensitive to the acid/base status of the body. Glutamine synthetase has a high-affinity for ammonia to aid in ammonia detoxification, but has a much lower capacity than urea production. (Lobley, 2003).

Liver Function: Its role in protein catabolism and urea synthesis

The liver has the ability to metabolize or convert amino acids, lipids and carbohydrates into glucose through gluconeogenesis when blood glucose levels are low. Hepatic metabolism converts excess nutrients (except fatty acids) into glycogen for storage, a readily available source of glucose to help maintain a constant blood

glucose level. In addition to blood flow, muscle activity and subsequent branched-chain amino acid catabolism help supply amino acids, especially alanine and glutamine, to the liver. Alanine is especially gluconeogenic in the liver.

Lobley (2003) noted that only 1-20% of amino acids that enter the liver are extracted per pass, and that this fractional extraction remained similar even during high rates of amino acid infusion, indicating that the hepatic transport and catabolism are not influenced under normal rates of protein intake. When low rates of amino acid removal versus blood flow occur, a rise in arterial amino acid concentrations occur, allowing the majority of amino acids to be utilized by peripheral tissues. Research indicates that the main role of the liver is not to regulate amino acid extraction by catabolism, but rather to respond to immediate needs and metabolic activity of peripheral tissues, which are probably the largest determinant of amino acid extraction and catabolism by the liver. Rather than regulating outflow of amino acids, the liver seems to respond to inflow in the removal and processing of excess amino acids.

The liver is a major site of amino acid catabolism, containing the necessary oxidative enzymes for essential and non-essential amino acids. The liver receives amino acids freshly absorbed from the small intestine via the portal vein as well as re-circulated amino acids from tissue breakdown. In pigs and sheep, re-circulated amino acids account for 72-98% of hepatic amino acid supply (Lobley, 2003). Amino acids are first transaminated to α-ketoglutarate in the hepatic cytosol to form glutamate which is taken into the mitochondria and deaminated or transaminated. Therefore, cytosolic glutamate represents a major pool of waste nitrogen from amino acids

broken down in the liver. In mammals, much of this nitrogen is returned to the liver from extrahepatic tissues as the amide function of glutamine where it is released intra-mitochondrially as ammonia to be converted into urea.

Liver extraction of amino acids and hepatic protein synthesis account for all major plasma proteins including: plasma lipoproteins, albumins, globulins, fibrinogens and other blood clotting proteins. The liver also plays a vital role in metabolism, detoxification and excretion of many substances, such as hormones, drugs, and toxic substances such as ammonia. Hepatic smooth endoplasmic reticulum has enzymes and cofactors responsible for the chemical transformation of these substances, typically increasing their solubility water, and facilitating excretion by the kidneys. Excess protein is deaminated by the liver. The resulting ammonia is detoxified by attaching two ammonia molecules to a carbonyl C=O group, to form urea, which is a highly water soluble substance. Urea is transported to the kidney for excretion or it is recycled. The amount of urea excreted is dependent, in part on hydration, as well as hormone and physiological status.

Liver synthesis of urea plays a physiological role in acid/base balance via. Mammals convert NH_3 and NH_4^+ to urea to prevent toxicity. Urea is utilized by the kidney in countercurrent exchange in the renal medulla, helping produce concentrated urine when excess amounts are excreted. Urea is synthesized from ammonia in the ornithine cycle (Krebs-Henseleit cycle), and also from aspartate via the urea cycle in which 2 ammonia molecules are combined to form1 urea molecule. The total energy cost is 4 ATP. The enzymes required for the urea cycle are found between the hepatic cytoplasm and the mitochondrial matrix. Ureagenesis helps maintain the acid base

balance because it utilizes equal amounts of NH_4^+ and HCO_3^- produced by the catabolism of amino acids such as alanine. The net balance for urea synthesis is:

$$2NH_4^+ + 2HCO_3^- \rightarrow CO(NH_2)_2 + CO_2 + 3\ H_2O$$

It transfers a H^+ from $NH_4^+$to HCO_3^- allowing CO_2 excretion by lungs rather than HCO_3^- excretion by the kidneys. It is metabolically expensive costing 2 ATP per H^+ transfer (Withers, 1992). Some of the energy costs of ureagenesis are negated by the formation of useful byproducts of the urea cycle. Urea synthesis has significant acid-base implications with equivalent amounts of bicarbonate produced for every amino acid metabolized to NH_3/NH_4^+ (Withers, 1992). Urea is very soluble (1190 g/L) and once produced, it enters the blood stream and is eliminated via renal excretion or is recycled.

Diet influences the urea cycle since it is dependent on certain amino acids and enzymes to function properly. In herbivorous mammals, the urea cycle is controlled by the activities of enzymes whose formation is contingent upon their substrate availability. This allows them to adjust to high or low protein diets, since at normal intake the enzymes only operate at 20-50% capacity. This mechanism is important for nitrogen conservation at times of deprivation, but it also reduces reaction and detoxification time after high protein meals. Non-carnivorous mammals are able to use alternative pathways to synthesize some of the amino acids utilized in the urea cycle, instead of relying on the cycle itself to produce these amino acids from the diet. Carnivores, however, are diet dependant for these amino acids needed in the urea

cycle, making herbivorous mammals better adapted to low protein, poorer quality diets that may not contain all the amino acids necessary for the urea cycle to function (Lobley, 2003).

Endogenous urea turnover is related to protein and non-protein urea availability through diet and the amount of hydrolysis in the GIT. When fed ad lib, llamas and goats tended to have a higher endogenous urea turnover rate than animals with reduced food intake, thereby correlating an increased energy availability with and increased permeability of the gastrointestinal tract to urea (Engelhardt, 1974). The rate of urea metabolism in relation to plasma urea concentrations, are similar in sheep, goats and llamas. In a study conducted by Engelhardt et al. (1974), a correlation was suggested between blood ammonia and urea levels in llamas and goats. Upon feeding, urea blood concentrations fell as urea was shunted to the GIT. Just prior to the subsequent feeding times, plasma urea concentrations were at their maximum. To identify the permeability of the mucosa to urea, endogenous urea clearance was measured by determining endogenous urea turnover divided by the plasma urea concentration. An increase in dietary metabolizable energy per unit of metabolic body weight resulted in an increase in endogenous urea clearance (Engelhardt, 1974). Thoracius (1971) suggested that carbon dioxide may result in an increase of the influx of body urea across the rumen wall. Feeding a diet low in protein but high in energy (carbohydrate-rich) to sheep caused an increase in ruminal urea as compared to feeding a high protein diet (as cited in Engelhardt, 1974). The high protein feed failed to elicit the same response, even when fed with increased levels of metabolizable energy. A high protein diet results in increased nitrogen

production, and therefore, increased plasma urea concentration. When this occurs, urea permeability in the GIT is significantly decreased. It is suggested that this decrease is due to a subsequently higher level of ammonia in the rumen or its epithelium which deter this transport (Engelhardt, 1974). Although plasma urea concentrations increased from 6.0 ± 0.6 mmol/l when llamas were fed hay *ad libitum* to 7.3 ± 0.8 mmol/l during a 40% reduction, the urea turnover rate decreased. (Engelhardt, 1974). Nitrogen balance is a very complicated process within the body. It is dependent on diet, and the body's ability to reabsorb endogenous N from proteins from sloughed cells and body secretions.

Urea concentration in the medullary interstitium greatly affects the body's ability to concentrate urine. When ADH is present, the withdrawal of water significantly concentrates the amount of urea found in the lumen, causing passive diffusion into the medullary interstitium and into the descending and ascending loop of Henle. Urea improves urine concentration ability because it increases the osmotic concentration gradient available in the medulla to concentrate urine. The addition of urea causes more withdrawal of water from the thin loop of Henle, and, may then cause further diffusion of Na or Cl from the lumen due to greater concentrations (Berne, 2004).

Feed restrictions of 20-40% decreased renal excretion of urea by 30% (Engelhardt, 1974). This result was not significant however, due to high daily variability. A reduction of 60% did not result in any noticeable decrease in renal urea excretion. Feed restriction caused urea concentration in the plasma and urine to increase, with renal urea clearance reduced by 20-30% from ad lib feeding (24ml/h/

kg of body weight). Surprisingly, increased tubular reabsorption of urea was not present as was expected, based on this adaptation being present in camels. Therefore, the main mechanism through which llamas retain urea in the kidney is through a reduction in glomerular filtration rate by 20-40 % during feed restriction (Engelhardt, 1974). Part of this reduction in GFR may be facilitated by a subsequent reduction in water intake upon food restriction.

Renal Function and Nitrogen Metabolism

The kidney plays a critical role in the acid/base balance of the body. The kidney utilizes ammonia to act like a base to trap hydrogen, forming ammonium ions, thereby helping regulate systemic pH. This reaction occurs in the renal tubules where ammonia diffuses into the lumen, reacts with excess H^+ to form ammonium ($NH_3 + H^+ \leftrightarrow NH_4^+$) which is trapped within the tubule due to its charge, and therefore must be excreted. The kidney also attempts to combat metabolic acidosis by reducing ammonia concentration through glutamine synthesis, conserving bicarbonate while ridding the body of excess ammonia. Glutamine synthesis reduces serum ammonia levels and safely transports nitrogenous waste products to the kidney. Glutamine hydrolysis then releases ammonia into the tubule for excretion. Catabolism of proteins produces CO_2, NH_4^+, and HCO_3^-. CO_2 is eliminated through respiration in the lungs, a bicarbonate buildup resulting in a severe alkalosis if CO_2 is not eliminated from the body. The urea cycle helps prevent alkalosis by removing a proton from ammonium during the formation of urea, which titrates the bicarbonate. Finally, hepatic glutaminase activity, that increases intra-mitochondrial ammonia's ability to

enter the urea cycle, is a mechanism to release protons, thus preventing metabolic alkalosis (Lobley, 2003). Water and Na reabsorption are also functions shared by the kidney, liver and GIT. These two substances are necessary to maintain vascular or tubular pressures necessary for reabsorption and excretion of certain materials.

The kidney's ability to filter and concentrate urine is related to N metabolism. The ascending limb of Henle's loop and the first part of the distal convoluted tubule in the nephron are impermeable to water and urea but are highly active in Na reabsorption and K secretion, which is an important factor in concentrating the urine. The renal ultrafiltrate has concentrations of salts, organic molecules (glucose, amino acids) similar to plasma. Fenestrations in the endothelium are freely permeable to water and small solutes such as: Na, urea, glucose and some small proteins but not cells (Berne, 2004). The process of urine formation in mammals is ultrafiltration, followed by reabsorption, secretion and osmoconcentration. Almost all of the filtered primary urine is reabsorbed in the PCT and DCT. The kidney reabsorbs 99.7% of the filtered water. The kidney has a remarkable ability to clear certain vital ions such as Na, K, Cl and urea.

Clearance and Fractional Excretion

Creatinine is an anhydride of creatine, which is most commonly found stored in muscles in the form of creatine phosphate, a high energy reservoir for conversion into ATP. Creatinine is a waste product formed by dehydration in the kidney and is an important indicator of kidney function as the amount of creatinine clearance is approximately equal with the amount filtered in the glomerulus. Serum

concentration of creatinine is inversely correlated with glomerular filtration rate (GFR) in most species. A knowledge of GFR and creatinine excretion are useful in the determination of the fractional excretion (FE) rates of other substances in relation to excretion of creatinine can be assessed as a percentage of the excretion of creatinine over a certain period of time. The FE represents the proportion of a substance excreted in the urine compared with that filtered through the glomerulus.

Calculations to determine clearance, fractional excretion, and total excretion (as cited by Lackey et al., 1995):

$$CL_a = (U_a \times U_{vol}/S_a)/\text{kg of BW}$$

Where CL_a = endogenous clearance of a given electrolyte (a) or creatinine at a certain time, U_a and S_a = urine and serum concentrations of the electrolyte, U_{vol} = urine flow rate (ml/min) and kg of BW = body weight in kilograms.

Fractional excretion is represented by:

$$FE_a = (U_a/S_a)/(U_{Cr}/S_{Cr}) \times 100$$

FE_a is fractional excretion of a substance expressed as a percentage, U_{Cr} and S_{Cr} are urine and serum concentrations of creatinine.

Total excretion (TE) of a given electrolyte can be determined as follows:

$$TE_a = (U_a \times U_{vol})/\text{kg of BW}$$

Lackey et al. (1995), used these equations in a study with llamas fed two different diets, a mixed alfalfa/grass hay diet and 100% grass hay diet with water and feed provided ad lib. Urine production was higher in the mixed hay diet ranging from 628-1,760 ml/24 hr compared to 620-1,380 ml/24 hr 100% grass hay

diet. The osmolality was also higher in the mixed diet with a median of 1,906 mOsm/kg of body weight compared to 1,666 mOsm/kg. Creatinine and electrolyte clearance was not significantly different between diets, although some variation was present within time periods, the overall difference was not significant.

The kidney is important in the electrolyte balance in the animal. Extracellular or plasma electrolytes are maintained within very narrow limits relying on renal excretion of excess or reabsorption of needed amounts of electrolytes in the nephron. Clearance represents a volume of plasma from which all the substances have been removed and excreted into the urine per unit of time (mg/min). The percentage of ion reabsorption by the mammalian kidney is as follows: 99.6% Na and Cl, 88% K, 97% osmolytes and 72% urea. The llama, is able to concentrate its urine up to 3,190 mosm/L compared to the goat, which can only attain a maximum concentration of 2,841 mosm/L (Engelhardt, 1974).

Feed Profiles

LEGUMES

Alfalfa is the most common legume forage used as a feedstuff in the US. Legumes, in general, contain greater than 20% crude protein (CP), which on average is substantially higher than in grasses. Legumes tend to have higher concentrations of Ca, P, Mg and Cu and lower concentrations of Mn and Zn than grasses (Pond, 1995). The nutritive value of alfalfa is determined by the form and stage of maturity in which it is harvested or fed. As the plant matures and grows

CP, Ca, K, P and trace mineral levels decline, while lignin and crude fiber levels increase. This may be a reflection of a decreased ratio of leaves to stems in mature plants. Leaves provide the nutritionally richer part of the plant in respect to protein and other nutrients, while the stems become increasingly lignified and crude fiber dense with maturity. The effect of maturity on the CP levels of alfalfa is shown as follows (as given on a % dry matter basis with second cutting alfalfa): immature = 21.5, pre-bloom = 19.4, early bloom = 18.4, mid-bloom = 17.1, full bloom = 15.9, and mature = 13.6 (Pond, 1995).

Feeding alfalfa to camelids has been highly debated, but it has been considered an acceptable dietary component as long as caloric intake is monitored to avoid obesity. Alfalfa and other legumes can fix atmospheric N. This is accomplished with N-fixing bacteria, primarily rhizobia, which inhabit symbiotic nodules on the leguminous roots. Alfalfa is an excellent N source for camelids. However, its N ratios may exceed the amount required by camelids, and even be detrimental to these pseudoruminant animals adapted to environments with low quality protein feedstuffs. A recent study conducted by Robinson et al. (2004) suggested that alfalfa is not a satisfactory feed for camelids because of its excessive protein content. This study suggested that the protein levels fed to camelids should not exceed 12% CP. When feed protein levels become too high, it reduces camelid fiber quality, resulting in a more coarse fleece (Robinson, personal communication, 2005). Alfalfa also contains a significant amount of calcium. It has been suggested that the Ca:P ratio in alfalfa is too high to be fed to camelids, but if indigestible calcium oxalate crystals are present that tie up 50-

70% of the Ca, bringing the Ca:P ratio to 1.7:1, it would be acceptable (Fowler, 1988). A larger amount of Ca is available in the leaves than stems in alfalfa, therefore, an effort should be made to feed the animals a mixture, and not allow selective feeding or sorting such that animals can eat mainly leafy parts of the hay, leaving the more fibrous stems. Although alfalfa is known to cause bloat in cattle and various other ruminants, it is not known to cause bloat in camelids (Pond, 1995). This may be due to their increased ability to buffer acids and other products produced by VFA formation with the high quantities of bicarbonate secreted by the glandular saccules.

GRASS

Grass is a low, non-woody plant, typically with round, hollow stems and blade-like leaves ("Feed", 2005). Grasses are typically adequate in Ca, Mg and K but may be borderline adequate to deficient in P (Pond 1995). Grass is similar to legumes in the total digestible nutrient percentage (TDN) as well as crude fiber ("Feed", 2005). Digestible energy is nearly 70% in young plants but rapidly declines with maturity (Pond, 1995). Immature grasses have high levels of water and supply protein in excess of total protein necessary for ruminants that may cause diarrhea. Grasses that are grown as pasture may become weather-leached when they reach maturity resulting in reduced digestible energy, protein as well as soluble carbohydrates, carotene and other minerals.

The type of grass and location or season in which it is grown, may also affect nutritional quality. There are two main types of grasses: cool and warm

season varieties. The cool season grasses tend to mature at slower rates, and therefore, their overall quality also tends to deteriorate less rapidly (Pond, 1995).

PASTURE

Grasses are often grown as a free-ranging pasture for ruminants. In North America, some cereals such as barley, oats and rye are also used as pasture during the early spring stage of growth. These cereals can be used as pasture at this time because grazing does not greatly impede grain yield at maturity. Barley is a highly nutritious feed that is high in readily available carbohydrates (>50%) and high CP levels. Pasture follows the same trend as alfalfa and grasses described above in respect that as the plant matures the lignin (fiber) content increases therefore reducing CP levels and energy availability.

HAY

Hay is usually made from grasses and legumes. It is generally harvested when the crop is at its optimum stage of maturity to ensure the maximum yield of nutrients without damaging the next crop. The manner in which hay is harvested or treated can greatly affect its nutrient value. Some crops may have a very high water content (65-85%), in order to prevent crop damage from molding and fermenting, the water content must be reduced to about 15% or less before baling. Baling can result in the loss some nutritional value. To maximize the nutrient content of hay, the following things can be done: (1) cut at an early stage of maturity consistent with total yield of nutrients. (For alfalfa, this is

frequently1/10 to ¼ bloom.) (2) utilize recommended methods of handling, (3) bale hay when moisture levels are conducive to prevent mold formation - usually 14% moisture or less, (4) bale at the time of day when leaf shatter is minimized, ideally late evening when dew is beginning to add light moisture to the leaves. Rain or sun may cause leaching or bleaching of nutrients causing a lower quality feed that may reduce the feed quality up to 33% (Pond, 1995). Rapid drying should also occur in order to reduce oxidation of carbohydrates by the plant's cells. If done properly, however hay should have similar values to the forage and will tend to maintain these values for years if maintained under proper conditions. Carotene levels that are important for vitamin A levels, however, drop during extended times of storage.

STRAW

Straw is made up of the stems with varying amount of leaves of various plants that remain after the removal of seeds and is often produced from wheat, barley, rye, rice and oats and occasionally legumes. Straws are very low in digestible protein, very high in fiber and lignin and are usually a poor feed. Straw should typically be supplemented with other feedstuffs to ensure proper nutrient balance. Straw may be a sufficient diet for animals that have a low productive requirement. (Pond, 1995) Oat and barley straws are decidedly better than wheat, rye and rice straws (Christensen, personal communication 2005).

Analysis of feedstuffs

Dry Matter (DM): The amount of water may vary significantly between feedstuffs, making the comparison between different feed or feed components very difficult. A representative feed sample is dried in a 100 ˚C drying oven to remove all moisture. The difference in weight before and after drying determines the percent of moisture present in the feedstuff. Results are expressed on an as fed or dry-matter basis. Occasionally some feeds have high ratios of volatile substances and alternative methods of drying are utilized (Pond, 1995).

Nitrogen Combustion Analysis

The amount of N present in a feed can be determined by weighing a pulverized sample, then burning it in the presence of oxygen in an oven chamber. The N in the sample is converted into either nitrogen gas or NO gaseous forms. The gas is then carried through the system via helium gas through absorbance tubes to remove moisture, and then through a copper column which reduces the N into nitrogen gas. It is then passed through a thermocouple detector which measures the amount of heat produced from the N gas. The heat is converted into the amount of N present and compared to the original sample to determine the % nitrogen (Leco FP-2000, St. Joseph, MO).

Nitrogen Kjeldahl Analysis

Crude Protein (CP): the Kjeldahl method is used to determine the amount of nitrogen in a feed so CP can be calculated. The procedure includes digesting the feed in concentrated sulfuric acid which converts the N to $(NH_4)_2SO_4$, it is

then cooled, diluted with water and neutralized with NaOH to change the N into ionized ammonium. The sample is distilled and the distillate which contains the ammonium is titrated with acid to determine the amount of N. The percentage of nitrogen found in the original sample can be calculated by: % nitrogen = (gm nitrogen /gm sample) x 100. Although there are differences between different samples, the amount of "crude protein" (CP) is determined by multiplying the percent nitrogen by a factor (usually 6.25) with this equation CP = %N x 6.25 (www.brooklyn.cuny.edu). Although once common practice, the Kjedahl method is very time-consuming and is now being replaced by spectrophotometric methods that don't require distillation after feed digestion. This information is important in ruminant and pseudoruminant nutrition because their microbes are able to utilize almost all forms of N, including nonprotein nitrogen such as urea and biuret to produce microbial protein. Crude protein only defines the quantity of N present in a feed sample, but is unable to determine the source of N.

ASH

The ash or mineral component is what remains after all other combustible organic material has been burned away in an oven reaching 500-600˚C. Typically these values are low and of lesser importance in feed analysis. If the values are high, it indicates that the feed has been contaminated with either dirt or supplemented with substances such as salt and limestone.

Crude Fiber (CF)

This method uses ether exaction to isolate lipids, fats, fatty acids and fat-soluble vitamins or provitamins in a sample to determine the fraction of the diet that is of high-caloric value. The ether extract is then boiled in dilute acid followed by base and then filtered, dried and burned in a furnace. The difference in weight before and after burning is the crude fiber fraction. This method is an attempt to simulate digestion. The crude fiber is made up primarily of cellulose and hemicellulose, as well as lignin and some highly indigestible material.

Neutral Detergent Fiber (NDF)

The sample is boiled for one hour in the presence of sodium laurel sulfate to extract lipids, sugars, organic acids and other water soluble material, pectin, nonprotein N compounds, soluble protein and some of the silica and tannin. The remaining residue is known as the neutral detergent fiber (NDF). It is made up of the cell wall components: cellulose, hemicellulose and lignin as well as some minor components associated with the cell wall including: protein, bound N, minerals and cuticle. The soluble portion is known as cell wall component (CWC) and is highly digestible by all species. The NDF portion is most important to ruminants and pseudoruminants, since microbial fermentation can make the potential energy and fiber more available to them.

Acid Detergent Fiber (ADF)

A sample is extracted by boiling the sample in sulfuric acid in the presence of cetyl trimethylammonium bromide for 1 hr. Hemicelluloses and cell wall proteins are soluble in this solution. The insoluble portion, or acid-detergent fiber (ADF), includes: cellulose, lignin, lignified N (indigestible N), cutin, silica and some pectins. This fraction is much less digestible by animals.

Nitrogen Free Extract (NFE)

This is the portion which remains when the water, ether extract, CP, CF, lignin and ASH have been subtracted from the original weight of the feed sample. The remaining fraction essentially contains no N and is composed primarily of readily available and digestible carbohydrates including sugars and starches. This portion of the feed is readily available through digestion and absorption by all species.

Digestible Energy (DE)

The amount of digestible energy (DE = gross energy – fecal energy) present in a feed is determined by burning the feed in a bomb calorimeter. This instrument measures the heat released when dried organic substances are combusted to ash in an atmosphere of oxygen. The amount of energy present in a substance is determined by the rise in temperature of a surrounding water jacket. This raise in

temperature is converted into calories by the following equation: Calorie = amount of heat to raise 1 gm of water 1 degree Celsius (1 calorie = 4.18 joules).

Mineral Analysis

Mineral analysis is done by digesting 0.5 g of sample in nitric and perchloric acid. The sample is then diluted with distilled water to a volume of 50 ml and analyzed using inductively coupled plasma (ICP) spectrometry. In this method the atoms from the sample are excited by the energy of ionized argon plasma in a torch at high temperatures ranging from 1100-1400˚C. When the atoms are excited they emit light at a specific wavelength unique to each element present. The amount of the element present is correlated to the amount of light given off and is read in ppm. This method is very effective and allows simultaneous determination of all elements present in a sample (Webb, personal communication, 2005).

Volatile Fatty Acids (VFAs)

These important nutrient components in the rumen are separated using gas-liquid chromatography. The sample is placed in the instrument and moved by gas, through heated chromatographic columns. This determines the quantitative fractionation of closely related substances like VFAs.

Digestion Trials

Apparent Digestibility can be defined as the disappearance of food from the GI tract, accounting for both absorption and digestion. Digestion trials

determine the ratio of nutrients in a feed that are absorbed from the GI tract by measuring the amount of food intake of a diet with known composition and analyzing the amount of the specific substance present in the feces, urine and blood. This may be difficult to determine in feces due to the presence of variable amounts of endogenous waste in the feces. Apparent digestibility takes into consideration the amount of undigested feed in feces as well as endogenous sources to determine digestibility using the following equation (Pond, 1995):

$$\text{Apparent digestibility (\%)} = \frac{\text{Nutrient intake - Nutrient in feces}}{\text{Nutrient intake}} \times 100$$

True digestibility of a nutrient is the amount of nutrient absorbed from the GI tract excluding endogenous contributions.

True digestibility = dietary intake – endogenous contributions

CONCLUSION

There are many characteristics that make South American Camelids (SACs) different from pecoran ruminants. SACs have the longest large intestine of all mammals (Stevens and Hume, 2004). It has been shown in many studies that both llamas and alpacas have increased digestive efficiencies when fed the same forage when compared to sheep, goats and cattle. These differences are exacerbated when fed poor quality diets. Studies have noted increased mucosal surface area of the stomach, glandular saccule bicarbonate secretions, increased motility, increased retention time, and increased buffering ability in the SAC stomach compartments. Further research on the effect of each of these adaptations on digestive efficiency of SACs needs to be done. It has also been shown that some VFA fermentation occurs in the large intestine. Further research needs to be conducted to quantify the contribution of short chain fatty acids to the total VFA production from hind gut fermentation. This information is needed to help determine when to supplement indigenous feedstuffs to add the appropriate levels of nutrients needed by these animals, thereby increasing their health and maximizing production.

LITERATURE CITED

Allison, .J., 1984. Microbiology of the rumen and small and large intestines. In Duke's physiology of Domestic Animals, 10th ed. M.J. Swenson, pg. 304-350. Ithaca:Cornell Univ. Press.

Argenzio, R.A., Southworth, M., 1974. Sites of organic acid production and absorption in the gastrointestinal tract of the pig. Am. J. Physiol. 228, 1043-1050.

Berne, R.M., Levy, M.N., Koeppen, B.M., Stanton, B.A., 2004. Physiology 5th Edition. Mosby, St. Louis, MO, pg. 426-429.

Birutta, G., 1997. Raising Llamas,Versa Press. Pownal, VT.

Bohlken, H., 1960. Remarks on the stomach and the systematic position of the Tulopoda. Proc. Zool. Soc. Lond. 134, 207-215.

Campbell, J.W., 1994. Excretory nitrogen metabolism and gluconeogenesis during development of higher vertebrates. Israel J. Zool. 40, 363-381.

Carmalt, J.L., 2000. Protein-energy malnutrition in alpacas. Compendium. 22, 1118-1123.

Christensen, A., 2005. Personal communication. Benson Institute of Agriculture and Food, Provo, UT 84602.

Christensen, H.N., 1995. Hepatic amino acid transport primary to the urea cycle in regulation of biologic neutrality. Nutr. Revs. 53, 74-76.

Dimski, D.S., 1994. Ammonia metabolism and the urea cycle: function and clinical implications. J. Vet. Int. Med. 8, 73-78.

Dulphy J.P., Dardillat, C., Jailler, M., Jouany, J.P. 1994. Comparison of the intake and digestibility of different diets in llamas and sheep: a preliminary study. Annales de Zootechnology. 43, 379-387.

Dulphy J.P., Dardillat, C., Jailler, M., Jouany, J.P. 1998. Intake and digestibility of different forages in llamas compared to sheep. Annales de Zootechnology. 47, 75-81.

Eckerlin, R.H., Stevens, C.E., 1973. Bicarbonate secretion by the glandular saccules of the llama stomach. Cornell Vet. 63, 436-445.

Emmanuel, B., Howard, B.R., Emady, M., 1976. Urea degradation in the camel. Can. J. Anim. Sci. 56, 595-601.

Emmanuel, B., Emady, M., 1978. A comparative study of endogenous urea degradation in ruminants. Comp. Biochem. Physiol. 59(A), 215-217.

Engelhardt, W.v., Becker, G., Engelhardt, W., Hauffe, R., Hinderer, S., Rubsamen, K. Schneiderer, W., 1974. Energy, water and urea metabolism in llamas. Tracer studies on non-protein nitrogen for ruminants II: Proceedings of Joint FAO-IAEA Division of Atomic Energy in Food and Agriculture, Vienna, Austria, Feb. 18-22, 1974, pg. 111-122.

Engelhardt, W.v., Sallmann, H.P., 1972. Resorption und sekretion im pasen des guanakos (*Llama guanicoe*). Zbl. Vet. Med. A, 18, 117-132.

Engelhardt, W.v. Schneider, W., 1977. Energy and nitrogen metabolism in the llama. Anim. Res. Develop. 5, 68-72.

"Feed", 2005. Britannica Concise Encyclopedia. 2005. Encyclopædia Britannica Premium Service <http://www.britannica.com/ebc/article?tocId=9364292.

Florez, L.A., 1973. Velocidad de pasaje de la ingesta y digestibilidad en alpacas y ovinos. Tesis. Acad. Prog. Med. Vet., Univ. Nac. Mayor de San Marcos. Lima, Peru, pg. 46.

Foose, T., 1982. Trophic strategies of ruminant versus nonruminant ungulates. Ph.D.

Dissertation, Pritzger School of Medicine, Chicago, IL.

Fowler, M. E., 1989. Medicine and Surgery of South American Camelids: llama, alpaca, vicuna, guanaco. 2nd Ed. Iowa State University Press. Ames: Iowa.

Genin, D., Villca, Z., Abasto, P., 1994. Diet selection and utilization by llama and sheep in a high altitude-arid rangeland of Bolivia. J. Range Mgt. 47, 245-248.

Genin, D., Tichit, M., 1997. Degradability of Andean range forages in llamas and sheep. J. Range Mgt. 50, 381-385.

Grace, N.D., Hill, F.I., Death, A.F., Wyeth, T.K., 1994. A preliminary study of copper supplementation in alpacas (*Lama pacos*). N. Z. Vet. J. 42, 114-116.

Heller, R., Gregory, P.C., Engelhardt, W.v., 1984. Pattern of motility and flow of digesta in the forestomach of the llama (*Lama guanacoe f. glama*). J. Comp. Phys. B. 154, 529-533.

Heller, R, Cercasov, V., Engelhardt, W.v., 1986. Retention of fluid and particles in the digestive tract of the llama (*Llama guanacoe f. glama*) J. Comp. Biochem, Physiol. 4, 687-691.

Hinderer, S., Engelhardt. W.v., 1974. Urea metabolism in the llama. Comp. Biochem.

Physiol. 52(A), 619-622.

Hinderer, S., Engelhardt, W.v., 1976. Entry of blood urea into the rumen of the llama. Proceedings of a research coordination meeting Call # SF98.N5R3, pg. 59-60.

Hintz, H.F., Schryver, H.F., Halbert, M., 1973. A note on the comparison of digestion by new-world camels, sheep and ponies. Anim. Prod. 16, 30-35.

Hoover, W.H., Heitmann, R.N., 1972. Cecal nitrogen metabolism and amino acid absorption in the rabbit. J. Nutr. 105, 245-252.

Huntgate, R.E., 1966. The Rumen and its Microbes. New York: Academic Press.

Johnson, L.W., 1983. The llama stomach: structure and function. Llama World. Winter pg. 12-13.

Lackey, M.N. Belknap, W.B., Salman, D, Tinguelly, L, Johnson, L.W., 1995. Urinary indices in llamas fed different diets. Am. J. Vet. Res. 56, 859-865.

Lemosquet, S., Dardillat, C., Jailler, M., Dulphy, J.P., 1996. Voluntary intake and gastric digestion of two hays by llamas and sheep: influence of concentrate supplementation. J. Agr. Sci. 127, 539-548.

Lobley, G.E., 2003. Protein turnover-what does it mean for animal production? Can. J. Anim. Sci. 83, 327-340.

Nelson, D.L. Cox, M.M. Lehninger Principles of Biochemistry: 3rd ed., 2000. Worth Publishers, New York: New York, pg. 485-721.

Ordonez, T.H., 1994. Llamas, llama production and llama nutrition in the Ecuador highlands. J. Arid Environs. 26, 67-71.

Ortiz, C., Cavero, J., Sillau, H., Cueva, S., 1974. The parotid saliva of the alpaca (*Lama pacos*). Res. Vet. Sci. 16, 54-56.

Purdy, S., 2004. Common Camelid Diseases. www.purdyvet.com

Raggi. L.A., Jiliberto, E., Urquieta, B., 1994. Feeding and foraging behavior of alpaca in northern Chile. J. Arid Environs. 26, 73-77.

Riera, S., Cardozo, A., 1970. Consumo de ensilaje de alfalfa y agua en llamas y ovinos. ALPA Mems. 5, 49-54.

Robinson, T.F., Roeder, B.L, Schaalje, G.B., Hammer, J.D., Burton, S., Christensen, M., 2004. Nitrogen balance and blood metabolites in alpaca (*Lama pacos*) fed three forages of different protein content. Small. Rumin. Res. (in Press)

Rubsamen, K., Engelhardt. W.v., 1979. Morphological and functional peculiarities of the llama forestomach. Ann. Rech. Vet., 10, 473-475.

San Martin, F., 1987. Comparative forage selectivity and nutrition of South American camelids and sheep. Ph.D. Dissertation . Texas Tech University, Lubbock, TX.

San Martin, F., Bryant, F.C., 1989. Nutrition of domesticated South American llamas and alpacas. Small Rumin. Res. 2, 191-216.

Sponheimer, M., Robinson, T., Roeder, B., Hammer, J., Ayliffe, L., Passey, B., Cerling, T., Dearing, D., Ehleringer, J., 2003. Digestion and passage rates of grass hays by llamas, alpacas, goats, rabbits and horses. Small Rumin. Res. 48, 149-154.

Silanikove, N., Tagari, H., Shkolnik, A., 1993. A comparison of rate of passage, fermentation rate and efficiency of digestion of high fiber diet in desert Bedouin goats as compares to Swiss Saanen goats. Small Rumin. Res. 14, 45-60.

Silanikove, N.L., 2000. The physiological basis of adaptation in goats to harsh environments. Small Rumin. Res. 35, 181-193.

Sillan, H., Chauca, D., Antonieta, B., 1973. Evaluacion de la actividad microbiana del fluido ruminal del ovino y la alpaca. Rev. Invest. Pec. (IVITA) Univ. N.M.S.M. 2, 15-21.

Stevens, E.C., Hume, I.D., 2004. Comparative Physiology of the Vertebrate Digestive System. 2nd ed. Cambrige Univ. Press., New York:New York.

Stipanuk, M.H., 2000. Biochemical and Physiological Aspects of Human Nutrition. W.B. Saunders Company. Philadelphia, PA.

Sumar, J., 1988. Present and potential role of South American camelids in the High Andes. Outlook Agr. 17, 23-29.

Thorlacius, S.O., Dobson, A., Sellers, A., 1971. Effect of carbon dioxide on urea diffusion through bovine ruminal epithelium. Am. J. Physiol. 220, 162-170.

Vallenas, A.P and C.E. Stevens. 1971. Motility of the llama and guanaco stomach. Am. J. Physiol. 220, 275-282.

Vallenas, A. P., Cummings, J.F, and J.F. Munnell. 1971. A gross study of the compartmentalized stomach of two New World camelids, the llama and guanaco. J. Morphol. 134, 399-423.

Van Soest, P.J., 1994. Nutritional Ecology of the Ruminant, 2nd ed. Comstock, Ithaca, New York.

Vernet, J., Vermorel, M., Jouany, J.P., 1997. Digestibility and energy utilization of three diets by llamas and sheep. Ann. Zootech. 46, 127-137.

Webb, B., 2005. Personal communication. Brigham Young University, Dept. Plant and Animal Sciences, Provo, UT 84602.

Williams, V.J., 1963. Rumen function in the camel. Nature. 197, 1221.

Withers, P.C., 1992. Excretion. In Comparative Animal Physiology. Brooks/Cole Publishing Co., New York, pgs.211-305, 841-891.

Wolin, M.J., 1981. Fermentataion in the rumen and human large intestine. Science 213, 1463-1468.

PLASMA METABOLITES AND NITROGEN BALANCE IN *Lama glama* ASSOCIATED WITH FORAGE QUALITY AT ALTITUDE.

H.L. Davies, T.F. Robinson, B.L. Roeder[*], M.E. Sharp, N.P. Johnston[†], A.C. Christensen[§]

Department of Integrative Biology, Brigham Young University, 386 WIDB, Provo, UT 84602, USA

*Corresponding author: Tel.: +1 801 422 6873; fax: +1 801 422 0090.

E-mail address: beverly_roeder@byu.edu (B.L. Roeder).

[†] *Department of Nutrition, Dietetics and Food Science, Brigham Young University, Provo, UT 84602, USA*

[§]*Ezra Taft Benson Institute, Provo, UT 84602, USA*

Abstract

This study evaluated the effects of forage quality on blood metabolites and nitrogen balance in mature, intact male llamas (n = 4, 36 ± 4.4 months, 87 ± 17 kg) at high altitude (4,267 m Letanias, Bolivia). Llamas were randomly fed barley hay (B), 80% barley/20% alfalfa hay (BA), and fresh cut grass pasture (P). Animals were housed in metabolism crates and diets were fed for a 7 d adjustment period followed by a 5 d collection period. Feed, feed refusal, feces and urine were collected, dried and N

content determined by combustion analysis. Venous blood samples were collected on d 12 at 30 min intervals over a 6 h period. Plasma was harvested and analyzed for electrolytes (Na, K, Cl, Ca, Ca^{++}, P, Mg) and metabolites (glucose, NEFA, urea N, creatinine, albumin, total protein (TPP), osmolality (Osm)). Plasma electrolytes (Na, K, Mg, P, Cl) and metabolites (glucose, Osm, albumin, creatinine, TPP) were unaffected by forage treatment. Dry matter digestibility was greater for the B and BA than P forage, and N digestibility was significantly higher for BA than either the B or P forages. Nitrogen balance varied significantly between diets. N intake was significantly different between each diet ($P<0.0001$), with B having the least N (7.1 g/d), followed by P (14.4 g/d), and BA (19.0 g/d), which provided the most N. Urine N excretion was similar between P (7.7 g/d) and BA (10.6 g/d), similar between P (7.7 g/d) and B (6.2 g/d), but was different ($P<0.04$) between B (6.2 g/d) and BA (10.6 g/d). Fecal N excretion was similar between BA (7.4 g/d) and P (8.9 g/d). Both of these treatments produced significantly higher quantities of fecal N than B (4.1 g/d; $P<0.0004$). Nitrogen excretion followed the same trend as N intake. Total N excretion was highest in BA followed by P and B forages. Llamas were in negative N balance on the B and P diets. Llamas had an estimated daily maintenance requirement value of 0.58 g N and a daily maintenance requirement of 3.63 g crude protein/$W^{0.75}$ or 106.2 g CP/day. Mineral intake varied significantly between diets. Overall, pasture provided higher amounts of minerals than the barley forages, except for copper, phosphorus, and zinc. These date demonstrate the effects of feeding forages of varying quality on whole-body N utilization, blood metabolite and electrolyte patterns in llamas at altitude.

Keywords: Llamas; Bolivia; Nitrogen balance; Plasma metabolites

1. Introduction

South American camelids (SACs) play a vital role in the economy and culture of South American countries, including Argentina, Bolivia, Chile, Ecuador, and Peru. In the high altitude zone (> 3000 m) of these countries, many families rely solely on camelid herding for their survival (Sumar, 1988). The four species of South American camelids include llama, alpaca, vicuña and guanaco. Llamas and alpacas are the domesticated and economically important species. Llamas are used for pack, fiber, meat, and to guard other livestock such as sheep. Their productivity is limited by factors such as harsh climate and environment, overgrazing of rangelands, and lack of knowledge concerning their reproductive, behavioral, nutritional, and disease problems (Sumar, 1988).

Current camelid nutritional recommendations are usually extrapolated from requirements for domesticated sheep, goats and cattle (Carmalt, 2000). Very little is known about camelid nutritional requirements consuming locally grown forages (San Martin, 1989). South American pastoralists tend to own small, mixed herds of camelids and ruminants, including llamas, alpacas, sheep and cattle. Research examining the differences between the digestive abilities of these high altitude tylopod pseudoruminants compared to domesticated pecoran ruminants has been

published (Dulphy, 1994 and 1998; Genin, 1997; Lemosquet, 1996; Riera and Cardozo, 1970; Vernet, 1997). These studies suggested that llamas are better adapted to digest poor quality forages than their ruminant counterparts under the same conditions. Llamas have a higher dry matter, organic matter, and NDF digestibility than do sheep, and these differences were greatest with poorer quality diets. However, the nutritional requirements for SACs at high altitude consuming locally raised forages of varying quality and protein levels are not well understood. The literature indicates that camelid digestive efficiency increases at higher altitudes (San Martin and Bryant, 1989; Lopéz and Raggi, 1992). That fact further complicated interpretation and application of available nutritional information as it relates to alpacas and llamas. Due to the positive altitudinal influence on camelid digestive efficiency, Lopéz and Raggi (1992) indicated that digestible protein values are more suitable to report than protein requirement for these species at a particular altitude.

Information concerning the nutritional status of indigenous llamas consuming locally raised forages at high altitude is needed to better understand local forage digestibility and the protein levels needed to meet energy requirements and maintain nitrogen balance to help improve health and productivity. The digestibility of local forages needs to be further investigated to know the approximate levels of nutrient supplementation necessary. The purpose of this study was to determine the digestibility of three different forages and the effect on blood metabolites and nitrogen balance in llamas living on the Bolivian Altiplano at an altitude of 4,267 m (14,000 ft) above sea level.

2. Materials and Methods

2.1 Animals

Four intact adult llamas (36±4 months, 87.7 ± 17 kg) were included in this study conducted at Letanias, Bolivia (altitude 4,267 m). Animals were housed in metabolism crates with expanded metal flooring (Fig.1a), with skylights to provide natural lighting. All llamas were fed 100% barley hay (B) prior to onset of the study. During the first week of the study, llamas were adapted to the metabolism crates and first treatment. The second week, llamas consumed the treatment diet which was fed during the 5 d collection period. The animals were removed from the metabolism crates and exercised for 30 min twice daily in a paddock during the acclimation period. The animals were provided with water *ad libitum* and they were fed twice daily at 12 h intervals with the majority of the forage given in the morning. This was done to accommodate camelid diurnal eating patterns. The majority of their feed is consumed during the day.

2.2 Treatments

The experimental design administered forage (grown locally) treatments in random order to three repetitions of animals. Treatments consisted of three forages: 100% barley (*Hordeum vulgare*) (B), 80% barley/20% alfalfa (*Medicago sativa*) hay (BA), both B and BA hay were chopped to 3-4 cm length to avoid selectivity, and

grass pasture made up of hycrest crested wheatgrass (*Agropyron cristatum*) and siberian wheatgrass (*Agropyron sibirium*), locally grown and cut fresh daily. Forage chemical composition was determined at the BYU Soil and Plant Analysis Laboratory (Provo, UT) using wet chemistry procedures with values expressed as a percent of dry matter (Table 1). Treatment periods were 12 days, with days 1-7 for diet adjustment and days 8-12 for data collection. A harness system with a fecal collection bag and urine funnel was placed on the animals on day 7 prior to starting the collection period. Each metabolism crate had a slanted receptacle tray for urine collection by gravity flow into a container (Fig. 1a & 1b) with 50 mL 50/50 HCl acid added to fix N to prevent volatilization of ammonia. On days 8-12, feed intake was measured, refused feed, fecal output, and urine quantity were determined, and saved for later analysis. Feed refusal and feces were dried at 100 ^{o}C, composited by animal, and stored for later analysis. Urine volume was recorded, composited by animal, and an aliquot was frozen for later analysis. Composite dry feed samples, feed refusal, and fecal samples were ground using a Wiley Mill (Author A. Thomas Co., Philadelphia, PA) with a 1 mm screen. Nitrogen content was determined for feed, feed refusal, fecal and urine samples by combustion analysis (Leco, 2005).

2.3 Blood profile

On day 12, blood samples were collected every 30 min for six hours via indwelling jugular catheters (Micro-Renathane, Braintree Scientific, Braintree, MA). The time 0 sample was taken prior to the 08:00 feeding. Fresh feed was immediately

offered post sampling. Plasma was obtained by centrifugation at 2400 x *g* for 20 min, aliquotted and frozen at –20 ^{o}C within 60 min of collection for later analysis. Plasma samples were analyzed for glucose, urea N, creatinine, sodium, potassium, and chloride using a NOVA 16 blood chemistry analyzer (Nova Biomedical, Waltham, MA). Non-esterified fatty acids (NEFA) were determined using a NEFA-C kit (#990-75401, Wako Chemical USA Inc., VA). Plasma ionized calcium (Ca) was determined using a Chiron 860 analyzer (Bayer Diagnostics, Indianapolis, IN). Albumin, total plasma protein (TPP), total calcium, phosphorus, and magnesium (Mg) were analyzed using colorimetric methods (TECO Diagnostics, Anaheim, CA). Vapor pressure osmolality was measured with a 5500 Vapor Pressure Osmometer (Wescor, Logan, UT).

2.4 Statistics

Statistical analysis of blood chemistry values and nitrogen balance (intake and excretion) data were analyzed using a general linear model with forage as the main effects. Data are presented as LS means $\pm$ standard error. The SAS (SAS, Inst., Cary, NC) PROC GLM was used for all calculations. Least squares means were used to determine statistical difference between forages, and the SAS means procedure for T TEST used to detect statistical difference within groups using unadjusted *t* tests, with $P \leq 0.05$ as the accepted level of significance. Regression analyses were performed to determine N requirement. The response variable was intake and the predictor variable was N retention. The model allowed separate slopes for each feed

group but a common intercept. The intercept was used as the estimate of N requirement.

3. Results

Similar to previous metabolism studies with llamas and alpacas, these animals consumed most of their feed allocation during the daylight hours, between 08:00 and 16:00 feeding times, and they ate very little during the nighttime (personal observation). Dry matter intake was lowest with B (917 g/d; $P<0.006$) but similar between P (1392 g/d) and BA (1284 g/d). Dry matter digestibility averaged 47-63% (Table 2), was similar between B and BA forages, but was different between pasture (46.9%) and the two barley diets (B, 62.3% and BA, 62.9%; $P<0.0002$).

3.1 Nitrogen utilization

Whole-body N utilization is presented in Table 2. The effect of B, BA, and P forages on N balance and whole-body N utilization is illustrated in Figs. 2 and 3. However, N intake was significantly different between each diet ($P<0.0001$), with B having the least N (7.1 g N /d), followed by P (14.4 g N/d), and BA (19.0 g/d) which provided the most N. Urine N excretion was similar between P (7.7 g N/d) and BA (10.6 g N/d), similar between P (7.7 g N/d) and B (6.2 g N/d), but was different ($P<0.04$) between B (6.2 g N/d) and BA (10.6 g N/d). Fecal N excretion was similar

between BA (7.4 g N/d) and P (8.9 g N/d), both of these forages produced significantly higher quantities of fecal N than B (4.1 g N/d). Total N excretion followed the same trend as N intake, with the quantity excreted by BA (18.0 g N/d) being similar to P (16.6 g N/d), both of which were significantly higher ($P<0.004$) than B (10.3 g N/d). Overall N balance was unaffected by forage type. Llamas exhibited a negative trend for N balance when consuming B and P forages (-3.2 and -2.2 g N/d, respectively), however these values were not significantly different than N retained with consumption of BA (1.1 g N/d). N digestibility was similar between P and B forages (38.3 and 41.9%, respectively). Both of these treatments had significantly less digestible N than that provided by BA (60.9 %; $P<0.0009$). The lower digestibility found with the P forage is likely related to its higher NDF (64.2%) and ADF (42.7%) levels compared to BA (NDF 53.4%, ADF 34.3%) and B (NDF 57.5%, ADF 35.3%).

3.2 Blood metabolites and electrolytes

The blood metabolite and electrolyte data are presented in Table 3 as means of all the samples across the 6 h sampling period. Blood electrolytes (Na, K, Cl, total and ionized Ca, P, Mg) and metabolites (glucose, NEFA, urea N, creatinine, albumin, TPP, Osm) were unaffected by forage treatment. When data was compared on a metabolic body weight basis, TPP became significant between P and the barley treatments, BA = $P<0.03$ and B = $P<0.05$ (data not shown).

3.3 Mineral intake

Mineral intake varied significantly between diets as shown in Table 4, and mirrored the forage composition analyses (Table 1). In general, P forage provided an overall higher amount of minerals than B, except for Cu (B, 0.011 g/d; BA, 0.006 g/d; P 0.003 g/d; $P<0.0003$), P (B, 2.512 g/d; BA, 2.675 g/d; P 1.205 g/d; $P<0.002$) and Zn (B, 0.026 g/d; BA, 0.030 g/d; P 0.013 g/d; $P<0.003$), which were higher in the B and BA diets. Pasture had similar levels of Ca, Fe, K, Mg, and S as did the BA diet. The B diet provided the highest daily intake of Cu (0.011 g/d; $P<0.0003$) and the lowest daily intake of Na (0.191 g/d; $P<0.001$) of all the test diets. P provided the highest level of intake for Mn (0.399 g/d; $P<0.0001$) and Na (0.550 g/d; $P<0.001$). When data was compared on a metabolic weight basis, mineral intake was similarly different between the three forages as presented above.

3.4 Mineral balance

Mineral balance, expressed as the difference between mineral intake and mineral excretion in feces and urine, is presented in Figs. 4 and 5. Cu and Na were the only minerals that were affected by diet (Cu: B 4.4, BA –5.1, and P –6.4 g/d, respectively, with BA similar to P; $P<0.0058$) and (Na: B -82, BA -159, and P 297 g/d, respectively, BA was different from P, but B was similar to both BA and P; $P<0.004$). Total excretion trends for Na did not follow intake trends, as evidenced by higher levels of Na excreted with B and BA treatments, while Na intake was highest

with P consumption. Ca and Cu balance followed intake trends, such that the diets with the highest concentrations of these two minerals correlated with a positive trend for balance of the mineral. Ca balance was 1.03, 0.43 and -.030 g/day for P, BA and B, respectively, while Cu balance was 0.004, -0.005 and -0.006 g/day for B, BA and P, respectively. Trends for Fe, K, Mg and Mn mineral balance were inversely related to intake trends. Phosphorus and sulfur balance did not correspond with intake trends; however, both minerals retained the highest concentrations in B followed by BA and P, respectively (phosphorus: B -0.80, BA -0.61 and P -0.22 g/d) and (sulfur: B -0.031, BA -0.028 and P 0.099 g/d). The trend for Zn balance indicated that P had the highest retention levels followed by BA and B (-0.24, -0.013 and -0.007 g/d, respectively).

4. Discussion

The barley forages used in this experiment had differing CP concentrations (B, 6.6% and BA, 10.6%). Alfalfa, was added with more nitrogen, higher CP levels, and less fiber than grass hays (Minson, 1990) to increase the protein level of the barley forage in this study while minimally effecting other forage parameters. The CP concentration of the barley forages partitioned around the wheat grass pasture, such that B < P < BA, permitting comparison of protein digestibility and whole-body nitrogen utilization in mature, intact male llamas consuming these dry hays versus fresh grass pasture. Though the pasture appeared green and lush, this experiment was conducted during the harsh, dry Bolivian winter. Pasture yielding plants were higher in fiber and more lignified than we anticipated, as evidenced by the high NDF

(64.2%) and ADF (42.7 %) values. Palatability was the apparent reason for significantly lower DM intake of B forage ($P<0.006$). In the Robinson et al. (2004), it was noted that DM intake in alpacas fed barley straw or barley hay was lower when compared to grass hay. The CP content of the barley forage was 6.6%, equivalent to the CP level of 6.6% cited in the previous study (Robinson, et al., 2004) to approximate the level of forage protein found where camelids are indigenous in South America (Sponheimer, et al., 2003). It was concluded by Robinson et al. (2004) that grass hay with a CP of 11.8% was sufficient to meet metabolic needs at any geographical location whereas an alfalfa diet providing 16.0% CP is in excess of nutritional needs (Robinson et al., 2004). Overall, our P diet provided 8.6% CP which had a slightly negative trend below N maintenance requirement. The BA diet, however, with a CP of 10.6 % exhibited a positive trend to provide N in excess of requirement (Fig.2). We conclude that CP maintenance requirements for llamas at altitude lie between 8.6% and 10.6% CP.

Although dry matter digestibility was similar between the B (62.3%) and BA (62.9%) forages, the higher CP level, afforded by the addition of alfalfa, increased N intake to a level of 19.0 g N/d in BA ($P<0.0001$). Pasture provided significantly less N intake (14.4 g N/d) than BA, but it had twice as much N as B (7.1 g N/d; $P<0.0001$). Thus, fecal N, urine N, and total N excretion were similar between BA and P forages, and produced higher N excretion levels than B forage as a result of the low N intake it provided, even though P (38.3%) and B (41.9%) forages had similar N digestibility.

Although all four llamas in this study were clinically normal, intact males of similar age, a significant size difference existed between them (Fig. 1b). Two llamas were robust, weighing 101.1 and 99.5 kg, respectively, while the smaller two weighed 83.8 kg and 77.2 kg. Data analyses were performed on a metabolic weight basis to determine any differences which may have been related to frame size, in as much as smaller sized animals had significantly lower dietary intake values than the larger llamas. The portion of crude protein that was digestible by the animal (available N) expressed as the difference between N intake and fecal N as a percent of N intake, resulted in the highest amount of N being absorbed by llamas when BA was consumed (available N: BA 61.1%, compared to B 42.3%, and P 38.2%). As expected, less urinary N was excreted with consumption of B (6.2 g/d) compared to BA (10.6 g/d; $P<0.04$). However, urinary N excretion was similar between BA and P (7.7 g/d) and comparable between B and P. Even though there was no significant difference in plasma urea N or creatinine between treatments, the high plasma urea N and low plasma creatinine concentrations found with both BA and P forages indicated catabolism of feed protein and excretion of excess N. Llamas showed a trend toward increased plasma creatinine (215 mmol/l) with the lowest N intake levels provided by B (N intake, 7.1 g/d) with a corresponding trend of negative N balance (-3.2 g/d) as described in Table 2 and illustrated in Fig. 2, suggesting animals may have been to catabolizing body protein reserves to meet energy requirements when eating the B forage.

Although not significantly different between treatments, the lowest mean creatinine concentration (162 mmol/l) was found with the highest intake of N

associated with BA (N intake, 19.0 g/d). A trend for a higher percentage of the total N to be excreted in urine was seen with B and BA (B 60.2%, BA 58.9%), with P having only 46.4% of total N excreted via urine, as shown in Table 2 as UN%TN. The increased fecal N excreted ($P<0.0004$) and the increase in total N excreted ($P<0.004$) with BA and P treatments compared to the B diet was attributed to the excess N intake beyond requirement, associated with increased palatability of the former diets compared to the barley forage. When examining N absorbed (N intake - fecal N) as a percent of N intake (biological value), compared to the BA value of 61.1%, B had a value of 42.3%, while P was 38.2%. The llamas in this study increased their N intake by 62.6% when they were fed the BA treatment compared to B, resulting in a 134.4% increase in N retention with the addition of 20% alfalfa hay. Barley alfalfa forage increased N intake by 24.2% and produced a 150% increase in N retention compared to the native grass P. Native grass P provided llamas with a 50.7% increase in N intake compared to B diet, which produced a 31.3% increase in N retention over barley.

The nitrogen requirement was determined for llamas by regressing N retained against N intake per unit of metabolic body weight (kg $W^{0.75}$; Preston, 1966). Maintenance requirement was determined to be the zero intercept. The N maintenance requirement for llamas calculated from our study using a regression model that allowed separate slopes for each diet but a common intercept was 0.52 g crude $N/W^{0.75}$. However, the lower consumption of the B diet, believed to be due to poor palatability, caused the slope of the regression line for B to be significantly different from that of BA and P. Calculating the N maintenance requirement for

llamas using a regression model that included only BA and P, whose slopes were not significantly different, gives a common intercept value of 0.58 g crude $N/W^{0.75}$, which may be a more reliable estimate. Using the standard CP to digestible protein (DP) conversion factor of 0.8, our values range from 0.42 for all three diets to 0.46 g digestible $N/W^{0.75}$ for llamas consuming only the BA and P diets harvested on the Bolivian Altiplano. A study conducted by Huasasquiche (1974) on the Peruvian Altiplano with alpacas determined maintenance digestible N requirement to be 0.38 g/ $W^{0.75}$. In a summary, San Martin and Bryant (1989) noted that with similar species of camelids demonstrated higher feed efficiency and improved digestibility at the high altitudes of the Altiplano compared to sea level. Robinson et al. (2004) determined maintenance digestible N requirement to be 0.60 g crude $N/W^{0.75}$ for alpacas fed three forages of differing protein content at an altitude of 1370 m (4500 ft) above sea level. That study's disparate value, from that reported by Huasasquiche's data that indicated a maintenance digestible N requirement of 0.38 $g/W^{0.75}$ or 2.38 g crude protein per unit of metabolic weight (kg $W^{0.75}$) for alpacas, was attributed to this efficiency phenomenon associated with the difference in altitude. The low maintenance digestible N requirement found with llamas in the current study conducted on the Bolivian Altiplano at an altitude of 4,267 m (14,000 ft) above sea level appear to support this theory.

Engelhardt and Schneider (1977) estimated the metabolizable energy (ME) maintenance requirement of llamas to be 61 $Mcal/W^{0.75}$. Using measurements of oxygen consumption and carbon dioxide and methane production in an open-circuit indirect respiration calorimeter, Carmean et al. (1992) derived the value of 84.5 Mcal/

$W^{0.75}$ for ME required by llamas. Equations derived by Carmalt (2000) define daily digestible energy DE (Mcal) = ME x 1.22 and maintenance crude protein CP (g) = 31 g x DE (Mcal). The mean metabolic weight for llamas in this study was 29.3 kg, giving an estimated daily ME maintenance requirement of 2.48 Mcal DE/day and estimated DE = 3.02 Mcal/day. Using the daily maintenance requirement of 0.58 g N gives the llamas in this study an estimated daily maintenance requirement of 3.63 g crude protein/day. Multiplying this CP value by the llamas' $W^{0.75}$ of 29.3 kg, derives a value of 106.2 g CP/day. As indicated by López and Raggi (1992), digestible protein (DP) is a better estimate of daily requirement, and used a conversion of 0.68 CP per unit DP, thereby increasing the 0.38 g N/ $W^{0.75}$ to 0.56 g DP N/ $W^{0.75}$. However, the positive altitudinal effect associated with an increase in feed efficiency must be taken into account when determining the energy and protein maintenance requirements for llamas.

Mineral intake varied significantly between the three treatments, and mirrored the dietary composition analyses. Overall, pasture provided higher amounts of minerals than the barley forages, except for copper, phosphorus, and zinc. Copper and sodium were the only minerals whose balance was different by diet (Cu: B 4.4, BA –5.1, and P –6.4 g/d, with BA similar to P; $P<0.0058$) and (Na: B -82, BA -159, and P 297 g/d; BA was different from P, but B was similar to both BA and P; $P<0.004$). The addition of alfalfa to the barley forage increased calcium, iron, potassium, magnesium, and sulfur intake to levels comparable to the wheat grass pasture. Mineral balance differed from mineral intake on a dry matter basis for the three diets. The trend for mineral balance was toward negative for all except sodium,

calcium, sulfur, and iron in llamas consuming wheat grass pasture may be indicative of plant stage of maturity and mineral availability as related to mid-winter seasonal changes. The barley hay, harvested in the late Bolivian summer, provided adequate amounts of iron, and with the addition of alfalfa provided adequate calcium levels, but otherwise resulted in a negative trend for the mineral balance of all other elements' in this study. It can be inferred from these data that these diets provided borderline concentrations of minerals to meet llama mineral requirements. In order to determine what mineral supplementation is needed for llamas consuming wheat grass pastures, dry hay forages further research in needed; taking into account the minerals supplied by drinking water sources and forage in relation to season.

5.1 Conclusions

This study determined the digestibility of three different diets and the effect on blood metabolites and nitrogen balance in llamas living on the Bolivian Altiplano at an altitude of 4,267 m (14,000 ft) above sea level. The crude protein concentration of the barley forages partitioned around the wheat grass pasture, such that barley < grass pasture < barley alfalfa, permitting comparison of protein digestibility and whole-body nitrogen utilization in mature, intact male llamas consuming dry hays versus fresh grass pasture. Blood electrolytes and metabolites were unaffected by forage treatment. Llamas had high plasma urea N and low plasma creatinine concentrations indicating feed protein catabolism and excretion of excess N with both barley alfalfa and wheat grass pasture forages. Llamas showed a trend of increased

plasma creatinine with the lowest N intake levels provided by barley hay with a corresponding trend of negative N balance, suggesting animals may have begun to catabolize body protein reserves to meet energy requirements when eating this forage. The positive nitrogen balance in the llamas on the BA diet indicated that the treatment provided N in excess of requirements. We conclude that CP maintenance requirements for llamas at altitude lie between the BA value of 10.6% and 8.6% CP as provided by the P diet. In future studies, lower percentages of alfalfa may be tested to determine requirement and lower feeding costs of these animals. The N maintenance requirement for llamas calculated from our study was 0.58 g crude $N/W^{0.75}$. The low maintenance digestible N requirement found with llamas in the current study conducted on the Bolivian Altiplano at an altitude of 4,267 m (14,000 ft) above sea level supports the hypothesis that there is an efficiency phenomenon associated with the difference in altitude, whereby camelids are more efficient at feed utilization with improved digestibility at the high altitudes. The forages, consumed as fresh cut grass pasture or hay harvested in the late Bolivian summer provided borderline concentrations of minerals to meet llama mineral requirements. Further study is needed to determine what mineral supplementation is needed for llamas consuming wheat grass pastures, dry hay forages, and drinking water sources in relation to season.

Acknowledgements

This work was supported by the Ezra Taft Benson Agriculture and Food Institute (BAFI), Provo, UT. We would also like to thank Elizabeth García for her assistance with translation and coordination of BAFI personnel in Bolivia who assisted with this research effort, and José Pocuaca and Jacinto Callisaya for their help with the animals in this study.

References

AOAC, 1990. Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists, Arlington, VA.

Baca F., Novoa, C., 1966. Estudio comparativo de la digestibilidad de los forrajes en ovinos y alpacas. (Comparative study of forage digestibility in sheep and alpacas.) Rev. Fac. Med. Vet., Univ. Nac . Mayor de San Marcos, Lima. pp 18-29; 88-96.

Carmalt, J.L., 2000. Protein-Energy Malnutrition in Alpacas. Compendium. 22, 1118-1123.

Carmago, R., Cardozo, A., 1971. Comparative digestion trials between llama and ewe. III. Reunion Lat. Prod Anim. Bogota, R. 18:46.

Carmean, B.R., Johnson, K.A., Johnson, D.E., Johnson, L.W., 1992. Maintenance energy requirement of llamas. Am. J. Vet. Res. 53, 1696-1698.

Dimski, D.S., 1994. Ammonia metabolism and the urea cycle: function and clinical implications. J. Vet. Int. Med. 8, 73-78.

Dulphy J.P., Dardillat, C., Jailler, M., Jouany, J.P., 1994. Comparison of the intake

and digestibility of different diets in llamas and sheep: a preliminary study. Annales Zootech. 43, 379-387.

Dulphy J.P., Dardillat, C., Jailler, M., Jouany, J.P., 1998. Intake and digestibility of different forages in llamas compared to sheep. Annales Zootech. 47, 75-81.

Ellis, J., 1982. The hematology of South American Camelidae and their role in adaptation to altitude. Vet. Med./Small An. Clin. 77, 1796-1802.

Engelhardt, Wv.. Schneider, W., 1977. Energy and nitrogen metabolism in the llama. Anim. Res. Dev. 5, 68-72.

Engelhardt, Wv.. Becker, G., Engelhardt, W., Hauffe, R., Hinderer, S., Rübsamen, K., Schneider, W., 1974. Energy, water, and urea metabolism in the llama. In: Tracer studies on Nonprotein Nitrogen for Ruminants II. IAEA, Vienna.

Florez, L.A., 1973. Velocidad de pasaje de la ingesta y digestibilidad en alpacas y ovinos. Tesis. Acad. Prog. Med. Vet., Univ. Nac. Mayor de San Marcos. Lima, Peru. pp. 46.

Fowler, M.E., 1989. Medicine and Surgery of South American Camelids, 2nd ed. Iowa State University Press, Ames, Iowa. pp. 18.

Genin, D., Tichit, M., 1997. Degradability of Andean range forages in llamas and sheep. J. Range Mgt. 50, 381-385.

Grace, N.D., F.I. Hill, A.F. Death, T.K. Wyeth. 1994. A preliminary study of copper supplementation in alpacas (Lamos pacos). N.Z. Vet. J. 42(3):114-116.

Huasasquiche, A., 1974. Balance del nitrogeno y digestibilidad en alpacas y ovinos. (Nitrogen balance and digestibility in alpacas and sheep.) Prog. Acad. Med. Vet., Univ. Nac. Mayor de San Marcos, Lima (B.S. Thesis), pp. 49.

Heller, R, Cercasov, V., Engelhardt, W.V., 1986. Retention of fluid and particles in the digestive tract of the Llama (*Llama guanacoe f. glama*) J. Comp. Biochem, Physiol. 4, 687-691.

Hintz, H. F., Schryver, H.F., Halbert, M., 1973. A note on the comparison of digestion by new-world camels, sheep and ponies. Anim. Prod. 16, 30-35.

Johnson, L.W. 1989. Nutrition. Vet. Clin. North Am. Food Anim. Pract. 5:37-54.

Johnson, L.W. 1994. Llama nutrition. In L.W. Johnson, ed. Update on llama medicine. Vet. Clin. North Am. Food Animal Pract. 10:187-200.

Lemosquet, S., Dardillat, C., Jailler, M., Dulphy, J.P., 1996. Voluntary intake and

gastric digestion of two hays by llamas and sheep: influence of concentrate supplementation. J. Agric. Sci. 127, 539-548.

López, A., Raggi, L.A., 1992. Requerimeintos nutritivos de camélidos sudamericanos: llamas (*Lama glama*) y alpacas (*Lama pacos*). Arch. Med. Vet. 24, 121-130.

Minson, D.J., 1990. Forage in Ruminant Nutrition, Academic Press, San Diego.

Preston, R.L., 1966. Protein requirements of growing-finishing cattle and lambs. J. Nutr. 90, 157-160.

Raggi. L.A., Jiliberto, E., Urquieta, B., 1994. Feeding and foraging behavior of alpaca in northern Chile. J. Arid Environments 26, 73-77.

Riera, S., Cardozo, A., 1970. Consumo de ensilaje de alfalfa y agua en llamas y ovinos. ALPA Mems. 5, 49-54.

Robinson, T., Roeder, B.L, Schaalje, G.B., Hammer, J.D., Burton, S., Christensen, M., 2004. Nitrogen balance and blood metabolites in alpaca (*Lama pacos*) fed three forages of different protein content. Small. Rumin. Res. (in Press)

San Martin, F., Huasaquiche, A., Farfan, R., Del Valle, O., Holfado, D., Arbaiza, T.,

Nava, M., Villarroel, C.. 1982. Consumo y digestibilidad camparativa de pastos cultivados entre alpacas y ovinos. (Comparative intake and digestibility for alpacas and sheep of cultivated grasses during two seasons.) Resumenes Proyectos Investigacion. Univ. Nac. Mayor de San Marcos, Lima. pp 1,255.

San Martin, F., 1987. Comparative forage selectivity and nutrition of South American camelids and sheep. PhD Dissertation, Texas Tech University, Lubbock, TX.

San Martin, F., Bryant, F.C., 1989. Nutrition of domesticated South American llamas and alpacas. Small Rumin. Res. 2, 191-216.

Sponheimer, M., Robinson, T., Roeder, B., Hammer, J., Ayliffe, L., Passey, B., Cerling, T., Dearing, D., Ehleringer, J., 2003. Digestion and passage rates of grass hays by llamas, alpacas, goats, rabbits and horses. Small Rumin. Res. 48, 149-154.

Sumar, J., 1988. Present and Potential Role of South American Camelids in the High Andes. Outlook Agric. 17, 23-29.

Van Soest, P.J., 1982. Nutritional Ecology of the Ruminant. O&B Books, Corvallis, OR. pp. 374.

Vernet, J., Vermorel, M., Jouany, J.P., 1997. Digestibility and energy utilization of three diets by llamas and sheep. Ann. Zootech. 46,127-137.

Table 6. Diet composition

Component	Diet % DM		
	Barley	BA	Pasture
Dry Matter	92.7	92.8	93.3
Crude Protein	6.6	10.6	8.6
NDF	57.5	53.4	64.2
ADF	35.3	34.3	42.7
Fat	3.21	3.06	4.88
Ash	6.32	7.67	12.25
Available Protein	6.6	10.6	8.4
Dig. Protein Est.	5.3	7.9	6.0
Total Digestible N (TDN)	62.3	63.4	53.8
Phosphorus %	0.20	0.21	0.11
Calcium %	0.17	0.49	0.53
Potassium %	1.22	1.44	1.93
Magnesium %	0.10	0.16	0.19
Sulfur %	0.09	0.13	0.17
Copper %	0.0008	0.0005	0.0004
Manganese %	0.0009	0.0018	0.0336
Sodium %	0.0155	0.0293	0.0483
Iron %	0.0179	0.0225	0.0399
Zinc %	0.0021	0.0024	0.0014

NDF = neutral detergent fiber, ADF = acid detergent fiber.

Table 7. Effects of feeding diet treatments of varying quality and CP concentration on whole-body N utilization in mature, intact male llamas on the Bolivian Altiplano.

	Diet			SEM	P<0.05
	Barley	Barley/Alfalfa	Pasture		
DM intake, g/d	917[a]	1284[b]	1392[b]	80	0.006
N intake, g/d	7.1[a]	19.0[b]	14.4[c]	0.9	0.0001
Fecal N excreted, g/d	4.1[a]	7.4[b]	8.9[b]	0.5	0.0004
Urine N excreted, g/d	6.2[a]	10.6[b]	7.7[ab]	1.3	0.04
Total N excreted, g/d	10.3[a]	18.0[b]	16.6[b]	1.2	0.004
UN%TN[†]	60.2	58.9	46.4	7.6	NS
N retained, g/d	– 3.2	1.1	– 2.2	1.7	NS
DM digestibility, %	62.3[a]	62.9[a]	46.9[b]	1.8	0.0002
N digestibility, %	41.9[a]	60.9[b]	38.3[a]	3.0	0.0009

DM= dry matter. [a, b, c] Means in the same row with different superscripts differ significantly between diets (P<0.05). [†]Urine N excreted as a percentage of total N excreted.

Table 8. Effects of three diet treatments of differing protein content on plasma metabolite and electrolyte concentrations in mature, intact male llamas on the Bolivian Altiplano.

	Diet			SEM	P< 0.05
	Barley	Barley/Alfalfa	Pasture		
Glucose (mmol/l)	8.9	8.2	7.9	0.4	NS
NEFA (μmol/L)	264	186	273	51.6	NS
Urea N (mmol/l)	8.6	10.6	7.6	1.1	NS
Creatinine (mmol/l)	215	162	180	32.4	NS
Albumin (g/dl)	4.0	4.1	4.3	0.3	NS
TPP (g/dl)	6.4	5.9	6.5	0.3	NS
Sodium (mmol/l)	165	157	159	5.5	NS
Potassium (mmol/l)	4.4	4.1	4.4	0.2	NS
Chloride (mmol/l)	118	119	121	7.4	NS
Total Ca (mmol/l)	9.3	9.3	8.6	0.6	NS
Ionized Ca (mmol/l)	1.3	1.3	1.3	0.1	NS
P (mmol/l)	2.2	2.5	2.3	0.2	NS
Mg (mmol/l)	2.12	2.11	2.06	0.2	NS
Osm (mOsm/kg)	329	315	317	10.8	NS

NEFA = non-esterified fatty acids, TPP = total plasma protein, Osm = osmolality

Table 9. Mineral intake in mature, intact male llamas fed three diets.

Mineral	Diet			SEM	P<0 .05
g/day	Barley	BA	Pasture		
Ca	2.08[a]	6.15[b]	6.05[b]	0.550	0.0007
Cu	0.011[a]	0.006[b]	0.003[c]	0.008	0.0003
Fe	0.208[a]	0.267[ab]	0.420[b]	0.051	0.04
K	15.33[a]	18.56[ab]	22.08[b]	2.062	0.04
Mg	1.204[a]	2.023[b]	2.096[b]	0.208	0.03
Mn	0.010[a]	0.022[a]	0.399[b]	0.027	0.0001
Na	0.191[a]	0.375[b]	0.550[c]	0.047	0.001
P	2.512[a]	2.675[a]	1.205[b]	0.212	0.002
S	1.123[a]	1.651[ab]	1.954[b]	0.180	0.03
Zn	0.026[a]	0.030[a]	0.013[b]	0.003	0.003

[a, b, c] Means in the same row with different superscripts differ significantly between diets (P<0.05). Intake= fed-refused feed.

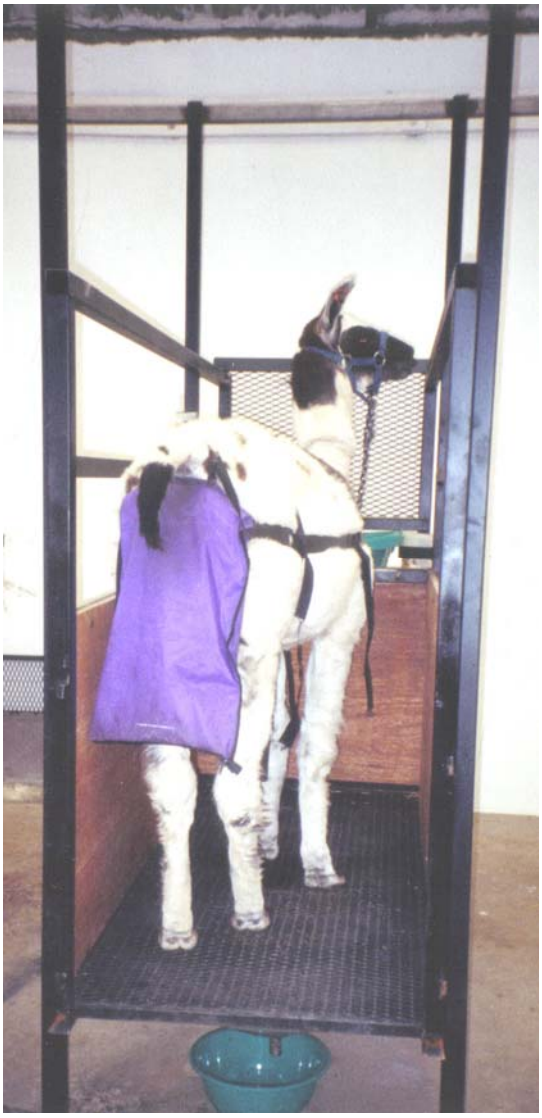


Fig 5a. Llama housed in metabolism crate with fecal collection bag and urine collection tray with attached funnel.

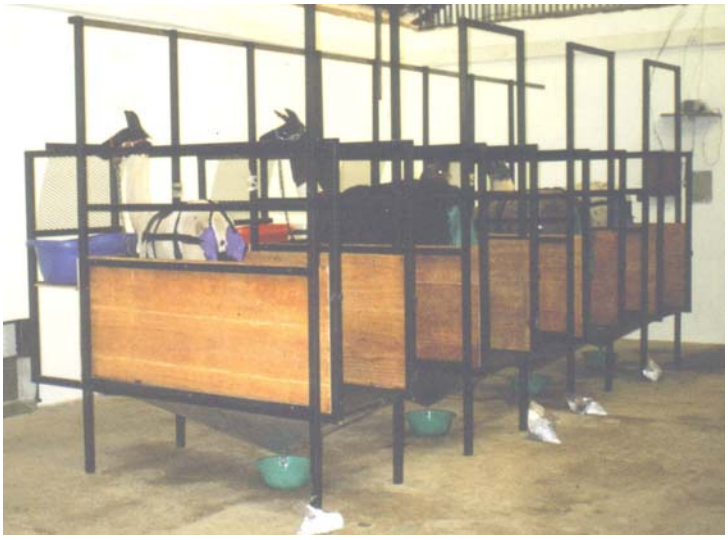


Fig 5.b. Llamas housed in metabolism crates with noticeable size difference between the first and second llama.

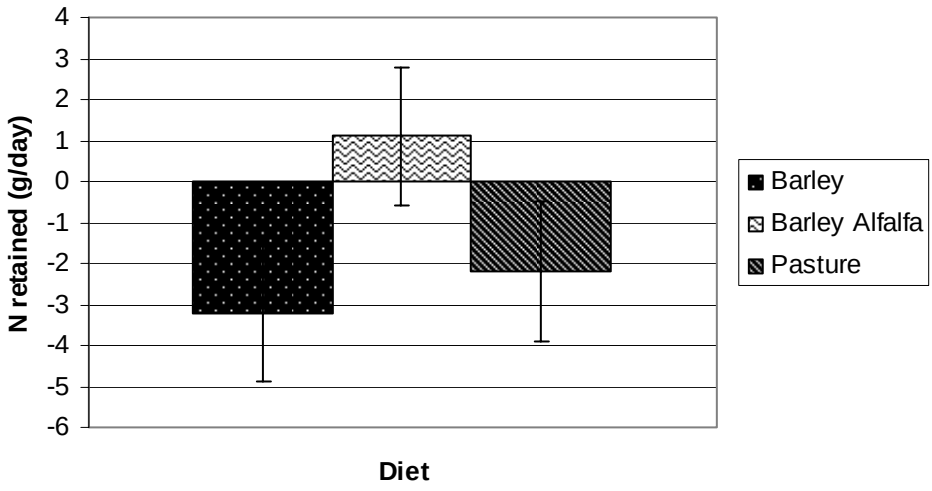


Fig 6. Effects of three diet treatments of differing protein content on N retention in mature, intact male llamas.

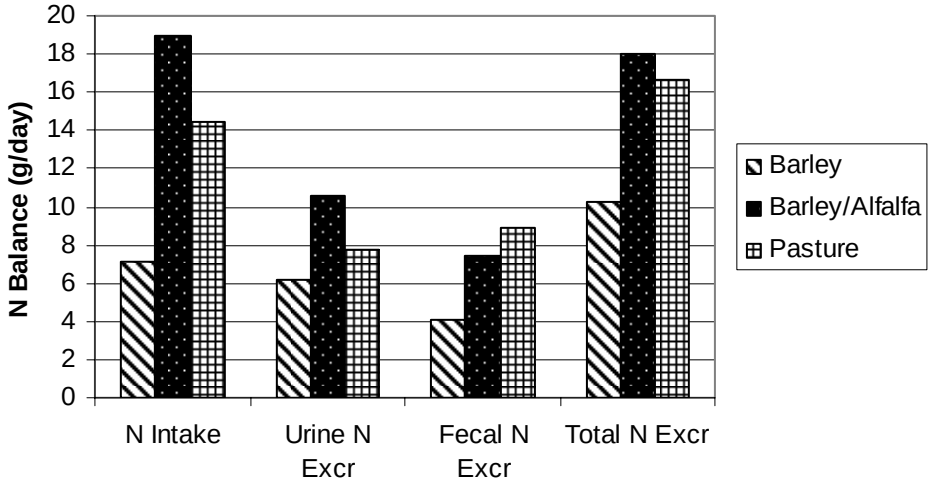


Fig 7. Effects of three diet treatments of differing protein content on N balance in mature, intact male llamas.

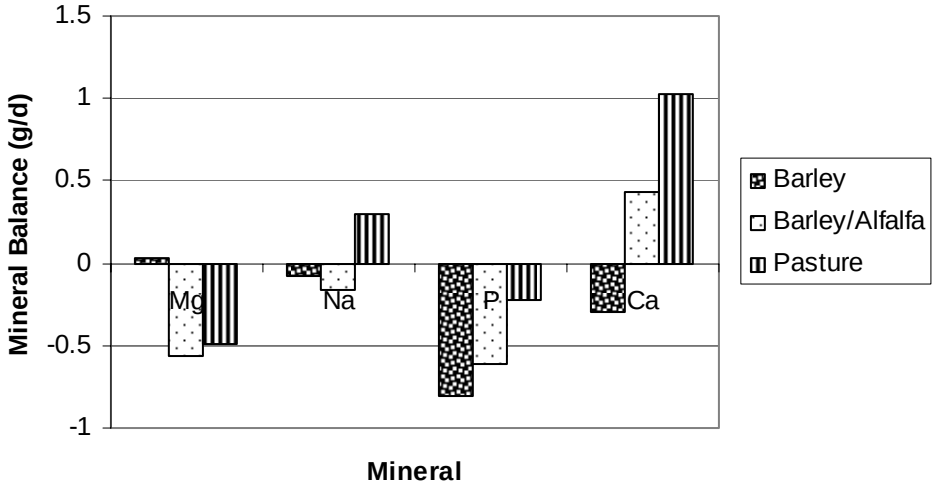


Fig 8. Effect of three diet treatments on magnesium, sodium, phosphorus, and calcium balance in mature, intact male llamas.

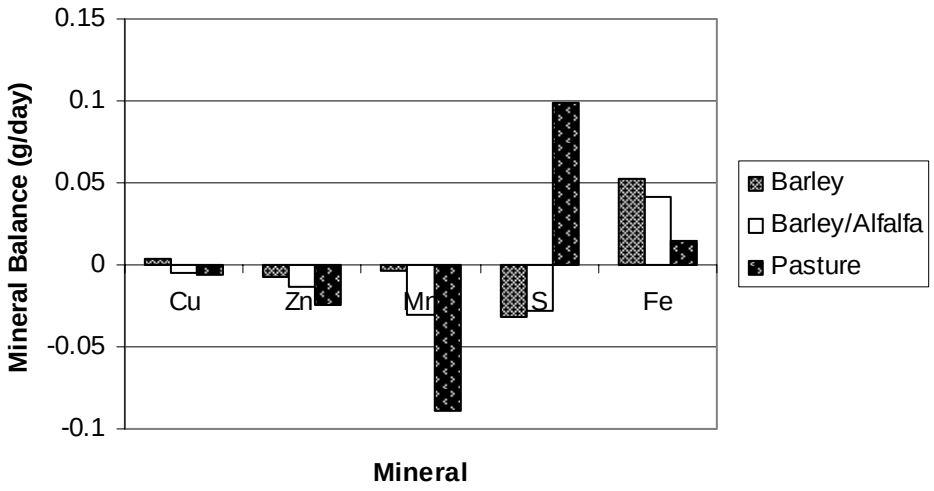


Fig 9. Effect of three diet treatments on copper, zinc, manganese, sulfur, and iron balance in mature, intact male llamas.

DIGESTIBILITY, NITROGEN BALANCE, AND BLOOD METABOLITES IN LLAMA (*Lama glama*) AND ALPACA (*Lama pacos*) FED BARLEY OR BARLEY ALFALFA DIETS.

H.L. Davies, T.F. Robinson, B.L. Roeder[*], M.E. Sharp, N.P. Johnston[†], A.C. Christensen[§]., G.B. Schaalje[¶]

Department of Integrative Biology, Brigham Young University, 386 WIDB, Provo, UT 84602, USA

*Corresponding author: Tel.: +1 801 422 6873; fax: +1 801 422 0090.

E-mail address: beverly_roeder@byu.edu (B.L. Roeder).

[†] *Department of Nutrition, Dietetics and Food Science, Brigham Young University, Provo, UT 84602, USA*

[§]*Ezra Taft Benson Institute, Provo, UT 84602, USA*

[¶]*Department of Statistics, Brigham Young University, Provo, UT 84602, USA*

Abstract

To determine the effect of barley diets on digestibility, nitrogen balance, and blood metabolites, mature gelded llamas and alpacas (n = 8; 4 llamas, 36±4 months, 90 ± 10.7 kg; 4 alpacas, 24-36 months, 50 ± 4 kg) were randomly fed 100% barley (B) hay

and 20% alfalfa/80% barley (BA) hay. Animals were housed in metabolism crates and diets were fed for a 7 d adjustment period followed by a 5 d collection period. Feed, feed refusal, feces and urine were collected, dried and N content determined by combustion analysis. Blood samples were collected on d 12 at 30 min intervals over a 6 h period. Plasma was harvested and analyzed for electrolytes (Na, K, Cl, Ca, Ca^{++}, P, Mg), metabolites (glucose, non-esterified fatty acids (NEFAs), urea N, creatinine, albumin, total protein (TPP), osmolality (Osm)). Plasma glucose, urea N, albumin, osmolality, electrolyte and metabolite levels were affected by species, all significantly higher ($P<0.05$) in alpacas than in llamas. On a metabolic weight basis, only diet was significant for N intake, urinary and fecal N, and total N excreted. Dry matter intake was not significantly different; however, BA consumption was greater than B, (B) 1272 and (BA) 1636 g N/d for llamas, and (B) 835 and (BA) 1034 g N/d for alpacas. Nitrogen intake followed the same pattern, (B) 21.4 and (BA) 33.9 g N/d for llamas and (B) 13.6 and (BA) 20.6 g N/d for alpacas (diet, $P<0.002$). Diet affects were significant for urine N excretion ($P<0.02$), (B) 11.2 and (BA) 18.2 g/d for llamas and (B) 6.8 and (BA) 10.8 g N/d for alpacas. Fecal N excretion was different for diet ($P<0.03$), with (B) 9.0 and (BA) 11.9 g N/d for llamas, and (B) 5.9 and (BA) 9.1 g N/d for alpacas, respectively. Nitrogen retention, DM digestibility and N digestibility were unaffected by diet or species. However, the llamas in this study displayed an increase in nitrogen intake of 64.6% between the B and BA diets with a 381% increase in N retention. Alpacas increased their N intake by 57.4% when they consumed the BA forage, which only increased N retention by 22.2%. These species differences indicate that alpacas have a higher N requirement to meet metabolic needs

which are not related to body size. When examining the biological value of N for the two species from the respective forages, alpacas and llamas had a value of 56.2% when consuming barley. The BA diet had a higher biological value of 65.0% in llamas compared to 57.4% in alpacas. Therefore, on the basis of this study, extrapolations between llamas and alpacas with respect to nitrogen requirements and balance do not seem valid.

Keywords: Llama; Alpaca; Nitrogen balance; Blood electrolytes; Nitrogen requirements

1. Introduction

Camelid nutritional requirements generally have been based on information extrapolated from requirements for domesticated goats, sheep and cattle, and from limited data reported from studies performed at various altitudes (Carmalt, 2000; Fowler, 1998; San Martin and Bryant, 1989). Protein requirements cited by Carmalt (2000) were from a study with alpacas on the Peruvian altiplano, a high plateau ranging in altitude from 3,500 to over 4,000 m above sea level. The literature indicates that camelid digestive efficiency increases at higher altitudes (San Martin and Bryant, 1989; Lopéz and Raggi, 1992), which factor could further complicate interpretation and application of available nutritional information as such pertains to alpacas and llamas. Due to the positive altitudinal influence on camelid digestive

efficiency, Lopéz and Raggi (1992) indicated that digestible protein values are more suitable to report than protein requirement for these species at a particular altitude.

There have been several studies comparing the digestive performance of sheep and llamas (Riera and Cardozo, 1970; Carmargo and Cardozo, 1971; Hintz et al., 1973 and 1976; Vernet, 1977; Genin, 1997; Lemosquet, 1996; San Martin, 1987; Dulphy, et al., 1994 and 1998). These studies indicate that llamas have a higher dry matter, organic matter and NDF digestibility than do sheep, and that these differences are greatest when fed a poor quality diet. Baca (1966) similarly reported that alpacas have higher digestion coefficients than sheep. San Martin et al. (1982) and Van Soest (1982) reported greater dietary selectivity by sheep than alpacas, in which the less lignified portions of forage were preferred. Since alpacas are not as selective concerning forage quality, caution should be taken when comparing them to sheep, because apparent digestibility may be skewed to give the more selective feeder a higher digestibility coefficient. Florez (1973) also noted an increased digestive capacity in alpacas compared to sheep, and suggested that it may be due to increased retention time in alpacas.

There is a paucity of comparative camelid nutritional data, with nutrient requirements usually extrapolated from the llama to the alpaca (Fowler, 1989). Heller, et al. (1986) described prolonged retention times for fluid and particulate matter in the digestive tract of llamas with increased digestibility of feedstuffs compared to domestic ruminants. A comparative study by Sponheimer et al. (2003) indicated that although both tylopod species had increased mean retention times, higher digestive efficiencies than pecoran ruminants and hindgut fermenters only

existed when camelids consumed forages with highly-vascularized bundle sheath cells that made the plant protein less digestible. This study demonstrated that llamas had a higher digestible dry matter relative to metabolic weight than alpacas, suggesting that llamas perform better on low-quality forages. Our study was conducted to define protein digestibility, nitrogen balance, and differences in blood metabolites between llamas and alpacas fed barley or barley alfalfa forages at an altitude of 1370 m (4500 ft) above sea level.

2. Materials and methods

2.1 *Animals*

Eight adult gelded camelids (n = 8; 4 llamas, 36±4 months, 90 ± 10.7 kg; 4 alpacas, 24-36 months, 50 ± 4 kg) were included in this study which was conducted at Brigham Young University, Provo, UT (altitude 1370 m). Animals were housed in metabolism crates with tenderfoot flooring in an environment of 20˚C with 12:12 h on:off lighting cycle. Prior to the study, the animals were fed grass hay (late-bloom Tall Fescue, *Festuca arundinacea*). During the first week of the metabolism crate adjustment period, the llamas and alpacas were fed their first treatment diet. The animals were removed from the metabolism crates and exercised for 30 min twice daily in a paddock during the acclimation period. The animals were provided with water *ad libitum* and they were fed twice daily at 12 h intervals, providing 2/3 of the daily feed at the 08:00 h feeding and the remaining 1/3 at the 20:00 h. This was done

to accommodate camelid diurnal eating patterns, for the majority of their feed is consumed during the day.

2.2 *Treatments*

The experimental design administered forage treatments in random order to the animals. Treatments consisted of two forages: 100% barley (*Hordeum vulgare*) hay (B) and 80% barley/20% alfalfa (*Medicago sativa*) hay (BA), each barley diet was chopped to 3-4 cm length. Forage chemical composition was determined by Dairy One Inc. forage lab (DHI Forage Testing Laboratory, Ithaca, NY) (Table 1). Treatment periods were for 12 days, with days 1-7 for diet adjustment and days 8-12 for data collection. A harness system with a fecal collection bag and a urine funnel was placed on the animals on day 7 prior to starting the collection period. Urine was collected under continuous vacuum into a bottle containing 50 ml of 50/50 HCl to fix N as urine was collected to prevent volatilization of ammonia. On days 8-12, feed intake was measured, refused feed, fecal output, and urine quantity were determined, and saved for later analysis. Feed refusal and feces were dried at 100 ^{o}C, composited by animal, and stored for later analysis. Urine volume was recorded, composited by animal, and an aliquot was frozen for later analysis. Composite dry feed samples, feed refusal, and fecal samples were ground using a Wiley Mill (Author A. Thomas Co., Philadelphia, PA) with a 1 mm screen. Nitrogen content was determined for feed, feed refusal, fecal and urine samples by combustion analysis (Leco, 2005).

2.3 *Blood profile*

On day 12, blood samples were collected every 30 min for 6 h via indwelling jugular venous catheters (Micro-Renathane®, Braintree Scientific, Braintree, MA). The time 0 sample was taken prior to the 08:00 feeding. Fresh feed was immediately offered post sampling. Plasma was obtained by centrifugation at 2400 x *g* for 20 min, aliquotted and frozen at –20 ^{o}C within 60 min of collection for later analysis. Plasma samples were analyzed for glucose, urea N, creatinine, sodium, potassium, and chloride using a NOVA 16 blood chemistry analyzer (Nova Biomedical, Waltham, MA). Non-esterified fatty acids (NEFA) were determined using a NEFA-C kit (#990-75401, Wako Chemical USA Inc., VA). Plasma ionized calcium (Ca) was determined using a Chiron 860 analyzer (Bayer Diagnostics, Indianapolis, IN). Albumin, total plasma protein (TPP), total calcium, phosphorus, and magnesium (Mg) were analyzed using colorimetric assays (TECO Diagnostics, Anaheim, CA). Vapor pressure osmolality was measured with a 5500 Vapor Pressure Osmometer (Wescor, Logan, UT).

2.4 *Statistics*

Statistical analysis of blood chemistry values and nitrogen balance (intake and excretion) data were analyzed calculated using a linear model with diet, species, and diet by species interaction as fixed effects. The SAS (SAS, Inst., Cary, NC) PROC GLM was used for all calculations. Level of significance was set at $P \leq 0.05$. Least

squares means for diet and species were determined using unadjusted *t* tests. Regression analyses were performed to determine N requirement for each species. The response variable was intake and the predictor variable was N retention. The model allowed separate slopes for each feed group but a common intercept. The intercept was used as the estimate of N requirement.

3. Results

Similar to previous metabolism studies with llamas and alpacas, these animals consumed most of their feed allocation during the day light hours, between 08:00 and 16:00 feeding times, and they ate very little during the nighttime. Dry matter digestibility was unaffected by either forage or species and averaged 50-59%. Dry matter intake was affected by both diet and species (Table 2).

3.1 Nitrogen utilization

Nitrogen utilization and whole-body N are reported in Table 2. Dry matter intake relative to body size was higher in llamas for both barley diets, with BA intake greater than B, (B) 1272 and (BA) 1636 g N/d for llamas, and (B) 835 and (BA) 1034 g N/d for alpacas. The effect of feeding the barley diets on N balance is shown in (Fig.1). Nitrogen intake followed a similar pattern as dry matter intake, (B) 20.6 and (BA) 33.9 g N/d for llamas and (B) 13.6 and (BA) 21.4 g N/d for alpacas (diet, $P<0.002$). Diet affects were significant for urine N excretion ($P<0.02$), (B) 10.8 and (BA) 18.2 g N/d for llamas and (B) 6.8 and (BA) 11.2 g N/d for alpacas. Fecal N

excretion was also different for diet ($P<0.03$) with (B) 9.0 and (BA) 11.9 g N/d for llamas, and (B) 5.9 and (BA) 9.1 g N/d for alpacas. On a metabolic weight basis, only diet was significant for N intake, urinary and fecal N excretion, and daily total N excretion. Nitrogen retention was unaffected by diet or species. Nitrogen digestibility, although unaffected by diet or species, did show a positive trend with BA from 57% to 65% in llamas.

3.2 Blood metabolites and electrolytes

The blood metabolite and electrolyte data are presented in Table 3 as means of all the samples across the 6 h sampling period. Plasma glucose, NEFA, urea N, creatinine, albumin, Na, K, Cl, total and ionized Ca, Mg, and osmolality were unaffected by diet or species. Total plasma protein was significantly higher in alpacas than llamas for each forage ($P<0.006$), (B) 6.8 and (BA) 6.3 g/dl versus (B) 5.9 and (BA) 6.0 g/dl for alpacas and llamas, respectively. Phosphorus was also significant by species ($P<0.03$) with llama values higher than those with alpaca for each diet, (B) 2.2 and (BA) 2.4 mmol/l compared to (B) 2.0 and (BA) 1.9 mmol/l in llamas and alpacas, respectively. No statistical significance was in evidence by diet or by diet x species interaction.

4. Discussion

The barley forages used in this experiment were supplemented with alfalfa to increase CP concentrations to permit comparison of protein digestibility and whole-body nitrogen utilization in mature llamas and alpacas. Palatability was assumed to be the reason for the lower DM intake of B forage as cited in a previous study conducted by Robinson et al. (2004), in which it was noted that DM intake in alpacas fed barley straw or barley hay decreased in comparison to grass hay. The CP content of the barley diet was 9.9%, 3.3 percentage points higher, an actual 50% increase in CP than the CP level of 6.6% used in the previous study to approximate the level of forage protein found in areas where camelids are indigenous (Robinson, et al., 2004). Alfalfa, a legume with more nitrogen, higher CP levels, and less fiber than grass hays (Minson, 1990) was added to raise the protein level of the barley forage in this study while minimally affecting the other diet parameters. Even though DM intake was not significantly different between the B and BA forages on a metabolic weight basis, N intake, fecal and urine N excretion, and total N excreted were significantly increased by diet, attributed to the excess protein provided by alfalfa in the BA forage.

The portion of crude protein that was digestible by the animal, available N expressed as the difference between N intake and fecal N as a percent of N intake, resulted in a higher amount of N being absorbed from the gut with llamas (available N, 65% BA compared to B 56%). However, it was not different between B and BA diets, both 57% available N, in alpacas. Urine N excretion was significantly higher with the BA diets for both species ($P<0.02$). Although there was no significant

difference in plasma urea N or creatinine between forages or species, the high plasma urea N and low plasma creatinine concentrations in both species indicated feed protein catabolism and excretion of excess N with both the B and BA treatments. A higher percentage of the total N excreted for B and BA was in urine (B 53.1%, 54.5% compared to BA 55.2%, 60.5% in alpaca and llama, respectively) reported as UN%TN in Table 2. The increased urinary N excretion ($P<0.02$) and the increase in total N excreted ($P<0.02$) seen with the BA diet was attributed to the excess N intake beyond requirement for both species provided by the addition of alfalfa to the barley diet. When examining N absorbed (N intake- fecal N) as a percent of N intake, on a metabolic weight basis, alpacas and llamas had a biological value of 56.2% when consuming barley. The BA diet had a higher biological value of 65.0% in llamas compared to 57.4% in alpacas. The biological value of N increased by 7.6% in llamas compared to alpacas fed the BA diet, indicating that the llamas utilized the increased dietary N more efficiently than the alpacas. The llamas in this study displayed an increase in nitrogen intake of 64.6% between the B and BA diets with a 381% increase in N retention. Alpacas increased their N intake by 57.4% when they consumed the BA forage, which only increased N retention by 22.2%. These species differences may be related to body size, with alpacas having a lower N requirement to meet their metabolic needs, while llamas require more N to maintain body functions related to their comparatively larger body size.

Nitrogen requirement was determined for each species by regressing N retained against N intake per unit of metabolic body weight (kg $W^{0.75}$; Preston, 1966). Maintenance requirement was determined to be the common zero intercept between

diets for each species. This regression between diets was valid for llamas in this study, since the difference between intercepts was not significantly different. However, using this model with the alpaca data (even without one animal's data which appeared to be outliers), allowing for separate slopes for each feed group, calculated a significant difference ($P<0.029$) between intercepts. This difference was attributed to variable and reduced consumption of the barley forage compared to the BA diet in alpacas. Future comparative studies between llamas and alpacas should utilize feedstuffs of similar palatability to both species to determine a more reliable N maintenance value. The N maintenance requirement calculated in this study, under these conditions, was 0.83 ± 0.097 g crude $N/W^{0.75}$ for llamas, but could not be determined for the alpacas. The calculated maintenance requirement for alpacas living at an altitude of 1370 m (4500 ft) above sea level was previously reported to be 0.60 g crude $N/W^{0.75}$ (Robinson, et al., 2004). Using the standard CP to digestible protein (DP) conversion factor of 0.8, determined a value of 0.60 g digestible $N/W^{0.75}$ for llamas. Using the equation derived by Carmalt (2000), the estimated daily maintenance metabolizable energy (ME) required by these alpacas and llamas was calculated to be ME (Mcal) = (84.5 x Body $Weight^{0.75}$ [BW;kg]/1000), as established by Carmean, et al. (1992), giving the alpacas in this study a value of 1.59 and the llamas 2.41 Mcal using $W^{0.75}$, respectively. Engelhardt and Schneider (1977) published a ME value of 61 $Mcal/W^{0.75}$ for llamas. The equation cited in the literature to calculate ME to DE, DE (Mcal) = ME x 1.22 resulting in a DE requirement of 1.94 for alpacas and 2.93 $g/W^{0.75}$ for llamas (Carmalt, 2000). Maintenance crude protein is determined by the equation CP (g) = 31 g x DE (Mcal)

for both llamas and alpacas (Carmalt, 2000). Using the maintenance value of 0.752 g N (see calculations in Robinson et al., 2004), the llamas in this study had an estimated daily maintenance requirement of 4.67 g crude protein or 133.8 g CP/$W^{0.75}$ day. These values are considerably higher than those determined from previous studies conducted at high altitudes of > 3000 m (Huasasquiche, 1974; López and Raggi, 1992; San Martin and Bryant, 1989). These differences were attributed to an efficiency phenomenon associated with the difference in altitude, whereby camelids in previous studies were noted to be more efficient at feed utilization with improved digestibility at the high altitudes of the Altiplano compared to sea level as described previously (Robinson et al., 2004; López and Raggi, 1992; San Martin and Bryant, 1989).

Plasma metabolite and electrolyte concentrations were similar to those reported previously in the literature for llamas and alpacas (Fowler, 1989). Plasma glucose, NEFA, urea N, creatinine, albumin, Na, K, Cl, total and ionized Ca, Mg, and osmolality were similar between species and were unaffected by diet. Total plasma protein was significantly higher in alpacas than llamas ($P<0.006$), but was unaffected by dietary treatment. Since plasma albumin concentration was not different between species, the significantly higher TPP concentration in alpacas compared to llamas was attributed to increased globulin levels, which is a species difference reported previously in the literature (Ellis, 1982). Similar to other species, camelids experience a general age-related increase in total protein which is characterized by reduced albumin and increased globulins with advancing age. The animals in this study had similar reference range values for their age group. Phosphorus was also

significantly different by species ($P<0.03$), but was unaffected by diet type, with llama values higher than those of alpacas. However, we are not able to explain the significance of this from the findings in this study.

5. Conclusions

This study determined the effects of feeding two barley diets with differing CP concentrations on digestibility, whole-body nitrogen utilization, blood metabolites and electrolytes in mature llamas and alpacas. Llamas and alpacas demonstrated differences with respect to nitrogen metabolism as related to forage protein variations. This study demonstrated a difference in protein and energy requirements between llamas and alpacas on a metabolic weight basis. When consuming the same high protein barley-alfalfa forage, llamas displayed a much higher increase in N retention compared to alpacas. The llamas in this study had an estimated daily maintenance requirement of 4.67 g crude protein and 133.8 g $CP/W^{0.75}$ day. When examining the biological value of N to the two species from the respective forages, alpacas showed a decrease from the barley treatment value of 6.6% to 5.1% with barley alfalfa, while llamas showed an increase between the two diets of 3.8% to 11.2%, indicating that the llamas utilized the increased dietary N more efficiently than the alpacas. These species differences indicate that alpacas have a higher N requirement to meet metabolic needs which is not related to body size. Therefore, extrapolations with respect to nitrogen requirements and balance between llamas and alpacas do not seem valid.

Acknowledgements

This work was supported by the Ezra Taft Benson Agriculture and Food Institute, Provo, UT. The authors also thank Christina Horey for help with the camelids and technical support.

References

AOAC, 1990. Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists, Arlington, VA.

Baca F., Novoa, C., 1966. Estudio coomparativo de la digestibilidad de los forrajes en ovinos y alpacas. (Comparative study of forage digestibility in sheep and alpacas.) Rev. Fac. Med. Vet., Univ. Nac . Mayor de San Marcos, Lima. pp 18-29; 88-96.

Carmalt, J.L., 2000. Protein-Energy Malnutrition in Alpacas. Compendium. 22, 1118- 1123.

Carmago, R., Cardozo, A., 1971. Comparative digestion trials between llama and ewe. III. Reunion Lat. Prod Anim. Bogota, R. 18:46.

Carmean, B.R., Johnson, K.A., Johnson, D.E., Johnson, L.W., 1992. Maintenance energy requirement of llamas. Am. J. Vet. Res. 53, 1696-1698.

Dimski, D.S., 1994. Ammonia metabolism and the urea cycle: function and clinical implications. J. Vet. Int. Med. 8, 73-78.

Dulphy J.P., Dardillat, C., Jailler, M., Jouany, J.P., 1994. Comparison of the intake

and digestibility of different diets in llamas and sheep: a preliminary study. Annales Zootech. 43, 379-387.

Dulphy J.P., Dardillat, C., Jailler, M., Jouany, J.P., 1998. Intake and digestibility of different forages in llamas compared to sheep. Annales Zootech. 47, 75-81.

Ellis, J., 1982. The hematology of South American Camelidae and their role in adaptation to altitude. Vet. Med./Small An. Clin. 77, 1796-1802.

Engelhardt, Wv.. Schneider, W., 1977. Energy and nitrogen metabolism in the llama. Anim. Res. Dev. 5, 68-72.

Engelhardt, Wv.. Becker, G., Engelhardt, W., Hauffe, R., Hinderer, S., Rübsamen, K., Schneider, W., 1974. Energy, water, and urea metabolism in the llama. In: Tracer studies on Nonprotein Nitrogen for Ruminants II. IAEA, Vienna.

Florez, L.A., 1973. Velocidad de pasaje de la ingesta y digestibilidad en alpacas y ovinos. Tesis. Acad. Prog. Med. Vet., Univ. Nac. Mayor de San Marcos. Lima, Peru. pp. 46.

Fowler, M.E., 1989. Medicine and Surgery of South American Camelids, 2nd ed. Iowa State University Press, Ames, Iowa. pp. 18.

Genin, D., Tichit, M., 1997. Degradability of Andean range forages in llamas and sheep. J. Range Mgt. 50, 381-385.

Heller, R, Cercasov, V., Engelhardt, W.V., 1986. Retention of fluid and particles in the digestive tract of the Llama (*Llama guanacoe f. glama*) J. Comp. Biochem, Physiol. 4, 687-691.

Hintz, H. F., Schryver, H.F., Halbert, M., 1973. A note on the comparison of digestion by new-world camels, sheep and ponies. Anim. Prod. 16, 30-35.

Lemosquet, S., Dardillat, C., Jailler, M., Dulphy, J.P., 1996. Voluntary intake and gastric digestion of two hays by llamas and sheep: influence of concentrate supplementation. J. Agric. Sci. 127, 539-548.

López, A., Raggi, L.A., 1992. Requerimeintos nutritivos de camélidos sudamericanos: llamas (*Lama glama*) y alpacas (*Lama pacos*). Arch. Med. Vet. 24, 121-130.

Minson, D.J., 1990. Forage in Ruminant Nutrition, Academic Press, San Diego.

Preston, R.L., 1966. Protein requirements of growing-finishing cattle and lambs. J. Nutr. 90, 157-160.

Raggi. L.A., Jiliberto, E., Urquieta, B., 1994. Feeding and foraging behavior of alpaca in northern Chile. J. Arid Environments 26, 73-77.

Riera, S., Cardozo, A., 1970. Consumo de ensilaje de alfalfa y agua en llamas y ovinos. ALPA Mems. 5, 49-54.

Robinson, T., Roeder, B.L, Schaalje, G.B., Hammer, J.D., Burton, S., Christensen, M., 2004. Nitrogen balance and blood metabolites in alpaca (*Lama pacos*) fed three forages of different protein content. Small. Rumin. Res. (in Press)

San Martin, F., Huasaquiche, A., Farfan, R., Del Valle, O., Holfado, D., Arbaiza, T., Nava, M., Villarroel, C.. 1982. Consumo y digestibilidad camparativa de pastos cultivados entre alpacas y ovinos. (Comparative intake and digestibility for alpacas and sheep of cultivated grasses during two seasons.) Resumenes Proyectos Investigacion. Univ. Nac. Mayor de San Marcos, Lima. pp. 1,255.

San Martin, F., 1987. Comparative forage selectivity and nutrition of South American camelids and sheep. PhD Dissertation, Texas Tech University, Lubbock, TX.

San Martin, F., Bryant, F.C., 1989. Nutrition of domesticated South American llamas and alpacas. Small Rumin. Res. 2, 191-216.

Sponheimer, M., Robinson, T., Roeder, B., Hammer, J., Ayliffe, L., Passey, B., Cerling, T., Dearing, D., Ehleringer, J., 2003. Digestion and passage rates of grass hays by llamas, alpacas, goats, rabbits and horses. Small Rumin. Res. 48, 149-154.

Sumar, J., 1988. Present and Potential Role of South American Camelids in the High Andes. Outlook Agric. 17, 23-29.

Van Soest, P.J., 1982. Nutritional Ecology of the Ruminant. O&B Books, Corvallis, OR. pp. 374.

Vernet, J., Vermorel, M., Jouany, J.P., 1997. Digestibility and energy utilization of three diets by llamas and sheep. Ann. Zootech. 46,127-137.

Table 10. Diet composition

Component	Forage (% DM)	
	B	BA
Dry Matter	90.2	90.6
Crude Protein	9.9	12.0
Available Protein	9.1	10.6
Acid Detergent Fiber	32.1	33.0
Neutral Detergent Fiber	54.9	53.0
% Lignin	5.7	5.9
Total Digestible N	63	64.2

Table 11. Effects of feeding barley or barley alfalfa diets with different CP concentrations on whole-body nitrogen utilization in mature gelded llamas and alpacas.

	Barley				Barley Alfalfa				SEM		P<0.05	
	Alpaca		Llama		Alpaca		Llama				Diet	Species
DM intake (g/day)	835	(44.9)	1272	(44.8)	1034	(55.4)	1636	(58.3)	114	(6.1)	0.0289	0.0006
N intake (g/day)	13.6	(0.73)	20.6	(0.73)	21.4	(1.15)	33.9	(1.2)	2.1	(0.12)	0.0003 (0.002)	0.0006
Fecal N (g/day)	5.9	(0.32)	9.0	(0.32)	9.1	(0.49)	11.9	(0.42)	1.0	(0.05)	0.0122 (0.03)	0.02
Urine N (g/day)	6.8	(0.37)	10.8	(0.39)	11.2	(0.6)	18.2	(0.65)	1.6	(0.09)	0.0033 (0.02)	0.005
Total N excreted (g/day)	12.8	(0.69)	19.8	(0.71)	20.3	(1.09)	30.1	(1.07)	2.3	(0.14)	0.0021 (0.02)	0.003
UN%TN* (%)	53.1	(53.6)	54.5	(54.9)	55.2	(55.0)	60.5	(60.7)	2.6	(3.2)	0.0033 (0.02)	0.005
N retained (g/day)	0.9	(0.04)	0.79	(0.01)	1.1	(0.05)	3.8	(0.14)	1.6	(0.06)	NS	NS
DM digestibility (%)	50.4		54.8		56.7		59.3		4.0		NS	NS
N digestibility (%)	56.3		55.8		57.2		64.9		3.8		NS	NS

DM = dry matter. Diet by species interaction was not significant. *Urine N excreted as a percentage of total N excreted. Values in parenthesis are presented as metabolic weight values ($g/d/kg^{.75}$).

Table 12. Effects of two barley diets of differing protein content on plasma metabolite and electrolyte concentrations in mature gelded llamas and alpacas.

	Barley		Barley Alfalfa		SEM	P< 0.05		
	Alpaca	Llama	Alpaca	Llama		Diet	Species	D x S
Glucose (mmol/l)	8.2	7.6	7.7	7.9	0.4	NS	NS	NS
NEFA (μmol/L)	354	437	309	381	57	NS	NS	NS
Urea N (mmol/l)	13.9	13.9	12.5	13.3	1.2	NS	NS	NS
Creatinine (mmol/l)	135	159	108	146	15	NS	NS	NS
Albumin (mmol/l)	3.8	4.2	3.6	4.1	0.25	NS	NS	NS
TPP (mmol/l)	6.3	5.9	6.8	6.0	0.17	NS	0.006	NS
Sodium (mmol/l)	163	160	160	165	3.7	NS	NS	NS
Potassium (mmol/l)	4.6	4.5	4.5	4.7	0.18	NS	NS	NS
Chloride (mmol/l)	124	124	122	125	2.2	NS	NS	NS
Total Ca (mmol/l)	2.1	1.9	2.0	2.1	0.1	NS	NS	NS
Ionized Ca (mmol/l)	0.97	0.75	0.93	0.82	0.11	NS	NS	NS
P (mmol/l)	2.0	2.2	1.9	2.4	0.13	NS	0.03	NS
Mg (mmol/l)	2.2	2.6	2.7	2.6	0.2	NS	NS	NS
Osm (mOsm/kg)	327	322	320	331	7	NS	NS	NS

NEFA = non-esterified fatty acids, TPP = total plasma protein, Osm = osmolality

Fig.10. Effect of feeding two diets of differing protein content on N balance in mature gelded alpacas and llamas.