Available online at www.sciencedirect.com
ScienceDirect
Small Ruminant Research 69 (2007) 1–9
Small Ruminant Research
www.elsevier.com/locate/smallrumres

Plasma metabolites and nitrogen balance in *Lama glama* associated with forage quality at altitude

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

[a] *Department of Integrative Biology, Brigham Young University, 386 WIDB, Provo, UT 84602, USA*
[b] *Department of Nutrition, Dietetics and Food Science, Brigham Young University, Provo, UT 84602, USA*
[c] *Ezra Taft Benson Institute, Brigham Young University, Provo, UT 84602, USA*

Received 4 May 2005; received in revised form 10 November 2005; accepted 29 November 2005
Available online 18 January 2006

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 (4267 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-day adjustment period followed by a 5-day collection period. Feed, feed refusal, feces and urine were collected, dried and N content determined by combustion analysis. Venous blood samples were collected on day 12 at 30 min intervals over a 6 h period. Plasma was harvested and analyzed for electrolytes (Na, K, Cl, Ca, Ca^{2+}, 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/day), followed by P (14.4 g/day) and BA (19.0 g/day), which provided the most N. Urine N excretion was similar between P (7.7 g/day) and BA (10.6 g/day), similar between P (7.7 g/day) and B (6.2 g/day), but was different ($P < 0.04$) between B (6.2 g/day) and BA (10.6 g/day). Fecal N excretion was similar between BA (7.4 g/day) and P (8.9 g/day). Both of these treatments produced significantly higher quantities of fecal N than B (4.1 g/day; $P < 0.0004$). 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 crude N/$W^{0.75}$ and a daily maintenance requirement of 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 data demonstrate the effects of feeding forages of varying quality on whole-body N utilization, and trends in 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.

* Corresponding author. Tel.: +1 801 422 6873; fax: +1 801 422 0090.
E-mail address: beverly_roeder@byu.edu (B.L. Roeder).

0921-4488/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.smallrumres.2005.11.016

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, vicuÒa 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 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 and Bryant, 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 et al., 1994, 1998; Genin and Tichit, 1997; Lemosquet et al., 1996; Riera and Cardozo, 1970; Vernet et al., 1997). These studies suggest that llamas are better adapted to digest poor quality forages than their ruminant counterparts under the same conditions. Llamas had 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; López and Raggi, 1992). That fact further complicates interpretation and application of available nutritional information as it relates to alpacas and llamas. Due to the positive altitudinal influence on camelid digestive efficiency, López 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 llamas consuming locally raised forages at high altitude is needed to better understand local forage digestibility and protein levels needed to meet energy requirements, to maintain nitrogen balance and to improve health and productivity. The digestibility of 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 4267 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 4267 m). Animals were housed in metabolism crates with expanded metal flooring (Fig. 1), with skylights to provide approximately 12 h of 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 continued consuming the treatment diet which was fed during the 5-day 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 diet given in the morning. This was done to accommodate camelid diurnal eating patterns with the majority of their feed consumed during the day.

2.2. Treatments

The experimental design administered dietary treatments in random order to three repetitions of animals. Treatments consisted of three diets: barley hay (*Hordeum vulgare*) (B), 80% barley hay (*H. vulgare*)/20% alfalfa hay (*Medicago sativa*; (BA)) and grass pasture (P) made up of hycrested wheatgrass (*Agropyron cristatum*) and Siberian wheatgrass (*Agropyron sibirium).* The B and BA hay were harvested in late summer (March in Bolivia), then chopped to 3–4 cm length to avoid selectivity. The pasture was locally grown and cut fresh daily. Forage 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 each animal 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. 1) with 50 ml 50/50 HCl acid added to fix N to prevent volatilization of ammonia. On days 8–12, feed intake, refused feed, fecal output

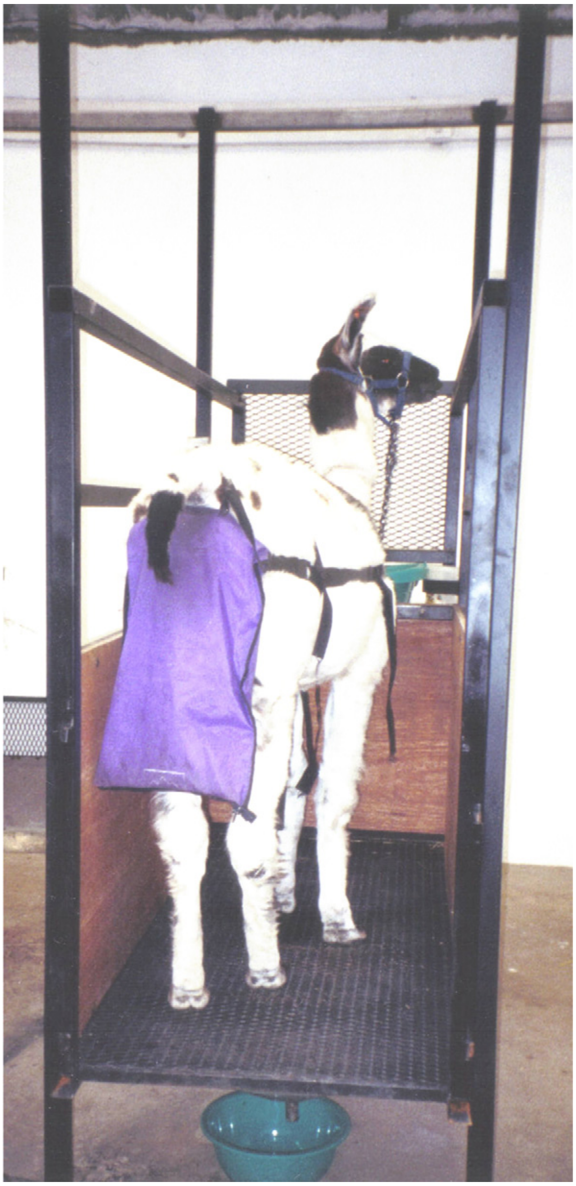

Fig. 1. Llama housed in metabolism crate with fecal collection bag and urine collection tray with attached funnel.

and urine quantity were measured, and saved for later analysis. Feed refusal and feces were dried at 100 °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 dried feed samples, feed refusal and fecal samples were ground using a Wiley Mill (Arthur 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. Mineral balance was determined as the difference between mineral intake and mineral excretion. Daily, the total feces output was ground, a 10% representative fecal sample frozen at −80 °C, and from the 24 h urine volume, a thoroughly mixed aliquot of urine was frozen at −80 °C. Fecal and urine minerals were determined using combustion analysis with a Leco analyzer (Figs. 3 and 4).

Table 1
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.

2.3. Blood profile

On day 12, blood samples were collected every 30 min for 6 h 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 × g for 20 min, aliquotted and frozen at −20 °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^{2+}) 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 diet as the main effects. Data are presented as LS means ± standard error of the mean (S.E.M.). The SAS (SAS, Inst., Cary, NC) PROC GLM was used for all calculations. Least squares means were used to determine statistical difference between diets, 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/day; $P < 0.006$) but similar between P (1392 g/day) and BA (1284 g/day). 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%; $P < 0.0002$).

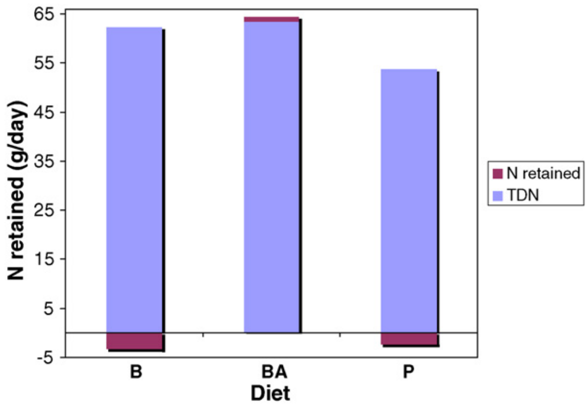

Fig. 2. Effects of three diet treatments with differing total digestible N on N retention in mature, intact male llamas.

3.1. Nitrogen utilization

Whole-body N utilization is presented in Table 2. The effect of B, BA and P diets on N balance and whole-body N utilization is illustrated in Fig. 2. N intake was significantly different between each diet ($P < 0.0001$), with B having the least N (7.1 g N/day), followed by P (14.4 g N/day) and BA (19.0 g/day) which provided the most N. Urine N excretion was similar between P (7.7 g N/day) and BA (10.6 g N/day), similar between P (7.7 g N/day) and B (6.2 g N/day), but was different ($P < 0.04$) between B (6.2 g N/day) and BA (10.6 g N/day). Fecal N excretion was similar between BA (7.4 g N/day) and P (8.9 g N/day), both of these forages produced significantly higher quantities of fecal N than B (4.1 g N/day). Total N excretion followed the same trend as N intake, with the quantity excreted by BA (18.0 g N/day) being similar to P (16.6 g N/day), both of which were significantly higher ($P < 0.004$) than B (10.3 g N/day). Overall

Table 2
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			S.E.M.	$P < 0.05$
	Barley	Barley/alfalfa	Pasture		
DM intake (g/day)	917[a]	1284[b]	1392[b]	80	0.006
N intake (g/day)	7.1[a]	19.0[b]	14.4[c]	0.9	0.0001
Fecal N excreted (g/day)	4.1[a]	7.4[b]	8.9[b]	0.5	0.0004
Urine N excreted (g/day)	6.2[a]	10.6[b]	7.7[ab]	1.3	0.04
Total N excreted (g/day)	10.3[a]	18.0[b]	16.6[b]	1.2	0.004
UN%TN[a]	60.2	58.9	46.4	7.6	NS
N retained (g/day)	−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. Means in the same row with different letters (a–c) differ significantly between diets ($P < 0.05$).
[a] Urine N excreted as a percentage of total N excreted.

Table 3
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			S.E.M.	$P<0.05$
	Barley	Barley/alfalfa	Pasture		
Glucose (mmol/l)	8.9	8.2	7.9	0.4	NS
NEFA (μmol/l)	264.0	186.0	273.0	51.6	NS
Urea N (mmol/l)	8.6	10.6	7.6	1.1	NS
Creatinine (mmol/l)	215.0	162.0	180.0	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)	165.0	157.0	159.0	5.5	NS
Potassium (mmol/l)	4.4	4.1	4.4	0.2	NS
Chloride (mmol/l)	118.0	119.0	121.0	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)	329.0	315.0	317.0	10.8	NS

NEFA: non-esterified fatty acids; TPP: total plasma protein; Osm: osmolality.

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/day, respectively), however these values were not significantly different than N retained with consumption of BA (1.1 g N/day). 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 as means of all the samples across the 6-h sampling period (Table 3). 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, pasture provided an overall higher amount of minerals than B, except for Cu (B, 0.011 g/day; BA, 0.006 g/day; P 0.003 g/day; $P<0.0003$), P (B, 2.512 g/day; BA, 2.675 g/day; P, 1.205 g/day; $P<0.002$) and Zn (B, 0.026 g/day; BA, 0.030 g/day; P, 0.013 g/day; $P<0.003$), 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/day; $P<0.0003$) and the lowest daily intake of Na (0.191 g/day; $P<0.001$) of all the test diets. P provided the highest level of intake for Mn (0.399 g/day; $P<0.0001$) and Na (0.550 g/day; $P<0.001$). When data was compared on a metabolic weight basis, mineral intake differed between the three forages as presented above.

Table 4
Mineral intake in mature, intact male llamas fed three diets

Mineral (g/day)	Diet			S.E.M.	$P<0.05$
	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

Means in the same row with different letters (a–c) differ significantly between diets ($P<0.05$). Intake = fed-refused feed.

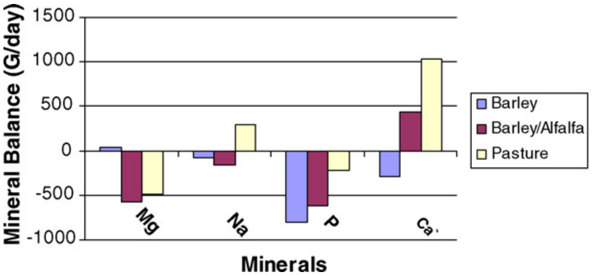

Fig. 3. Effects of three diet treatments on magnesium, sodium, phosphorus and calcium balance in mature, intact male llamas.

3.4. Mineral balance

Mineral balance, expressed as the difference between mineral intake and mineral excretion in feces and urine, is presented in Figs. 3 and 4. Cu and Na were the only minerals that were affected by diet (Cu: B 4.4 g/day, BA −5.1 g/day and P −6.4 g/day, respectively, with BA similar to P; $P < 0.0058$) and (Na: B −82 g/day, BA −159 g/day and P 297 g/day, 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 resulted in the highest retention of the mineral and correlated with a positive trend for balance of the mineral. Ca balance was 1026.9, 431.4 and −294.7 g/day for P, BA and B, respectively, while Cu balance was 4.4, −5.1 and −6.4 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 g/day, BA −0.61 g/day and P −0.22 g/day, respectively) and (sulfur: B −0.031 g/day, BA −0.028 g/day and P 0.099 g/day, respectively). The trend for Zn balance indicated that P had the lowest retention levels followed by BA and B (−0.24, −0.013 and −0.007 g/day, respectively).

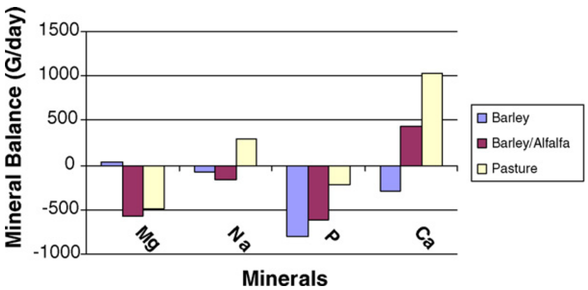

Fig. 4. Effects of three diet treatments on copper, zinc, manganese, sulfur and iron balance in mature, intact male llamas.

4. Discussion

The barley forages used in this experiment had differing CP concentrations (B, 6.6% and BA, 10.6%). Alfalfa, a legume, with more nitrogen, higher CP levels and less fiber than grass hays (Minson, 1990) was added to increase the protein level of the barley in this study while minimally affecting other forage parameters. The CP concentration of the barley diets 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 cut 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 anticipated, as evidenced by the high NDF (64.2%) and ADF (42.7%) values. Palatability was the reason attributed to the significantly lower DM intake of B forage ($P < 0.006$), as cited by Robinson et al. (2005), DM intake in alpacas fed barley straw or barley hay was lower when compared to grass hay consumption. The CP content of the barley forage was 6.6%, equivalent to the CP level of 6.6% cited in a previous study (Robinson et al., 2005) 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. (2005) that grass hay with a CP of 11.8% was sufficient to meet camelid metabolic needs at any geographical location, whereas an alfalfa diet providing 16.0% CP is in excess of nutritional needs. 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 providing 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%) diets, the higher CP level, afforded by the addition of alfalfa, increased N intake to a level of 19.0 g N/day in BA ($P < 0.0001$). Pasture provided significantly less N intake (14.4 g N/day) than BA, but it had twice as much N as B (7.1 g N/day; $P < 0.0001$). Thus, fecal N, urine N and total N excretion were similar between BA and P diets, 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. Two llamas were robust, weighing 101.1 and 99.5 kg, respectively, while the smaller two weighed 83.8 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, inasmuch 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/day) compared to BA (10.6 g/day; $P<0.04$). However, urinary N excretion was similar between BA and P (7.7 g/day) 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/day) with a corresponding trend of negative N balance (−3.2 g/day; see Table 2 and Fig. 2), suggesting animals may have begun to catabolize 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/day). 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 biological values for the diets used in this study are those meeting the classical criteria in which protein is a limiting nutrient (Robbins et al., 2005). 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. The barley alfalfa diet increased N intake by 24.2% and produced a 150% increase in N retention compared to consumption of wheat grass P. Wheat grass P provided llamas with a 50.7% increase in N intake compared to the B diet, producing 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 daily 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, gave 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 by San Martin and Bryant (1989), it was noted that 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. (2005) 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 indicating 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 4267 m (14,000 ft) above sea level appeared 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 kcal of ME/BW$^{0.75}$ for ME required by llamas. Equations derived by Carmalt (2000) define daily digestible energy DE (Mcal) = ME × 1.22 and maintenance crude protein CP (g) = 31 g × 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 a daily maintenance requirement of 0.58 g N (see calculations in Robinson et al., 2005), gave the llamas in this study an estimated daily maintenance requirement of 106.2 g CP/day. López and Raggi (1992) indicated 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 diets, except for copper, phosphorus and zinc. Copper and sodium were the only minerals whose balance was different by diet (Cu: B 4.4 g/day, BA −5.1 g/day and P −6.4 g/day, respectively, with BA similar to P; $P < 0.0058$) and (Na: B −82 g/day, BA −159 g/day and P 297 g/day, respectively; 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 toward negative for mineral balance 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 that these diets provided borderline concentrations of minerals needed to meet llama mineral requirements. In order to determine what mineral supplementation is required for llamas consuming grass pasture, or dry hay diets, further research is needed, taking into account minerals supplied by drinking water sources and seasonal effect on forages.

5. 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 4267 m (14,000 ft) above sea level. Blood electrolytes and metabolites were unaffected by forage treatment. Llamas catabolized feed protein and excreted excess N with both barley alfalfa and wheat grass pasture, but may have begun to catabolize body protein reserves to meet energy requirements when eating barley forage. The N maintenance requirement for llamas calculated from our study was 0.58 g crude N/W$^{0.75}$. Crude protein maintenance requirement for llamas at this altitude in this study appear to lie between 8.6 and 10.6% CP on a dry matter basis. In future studies, lower percentages of alfalfa should be tested to better determine requirement and to lower feeding costs for these animals. The low maintenance digestible N requirement found with llamas in this study conducted on the Bolivian Altiplano (altitude 4267 m) supported the hypothesis that there is an efficiency phenomenon associated with an increase in altitude, whereby camelids are more efficient at feed utilization with improved digestibility at high altitudes. Feeding mid-winter 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 required by llamas consuming grass pasture or dry forages along with drinking water sources, in relation to season.

Acknowledgements

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

References

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

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.

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. Ann. 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. Ann. Zootech. 47, 75–81.

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

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

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

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, CA.

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

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

Robbins, C.T., Felicetti, L.A., Sponheimer, M., 2005. The effect of dietary protein quality on nitrogen isotope discrimination in mammals and birds. Oecologia 144, 534–540.

Robinson, T., Roeder, B.L, Schaalje, G.B., Hammer, J.D., Burton, S., Christensen, M. 2005. Nitrogen balance and blood metabolites in alpaca (*Lama pacos*) fed three forages of different protein content. Small Rumin. Res. 58, 123–133.

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.

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