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Digestibility, nitrogen balance, and blood metabolites in llama (*Lama glama*) and alpaca (*lama pacos*) fed barley or barley alfalfa diets

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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) and 20% alfalfa/80% barley (BA) hay. Animals were housed in metabolism crates and diets were fed for a 7 days adjustment period followed by a 5 days collection period. Feed, feed refusal, feces and urine were collected, dried and N content determined by combustion analysis. 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), 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 similar between species, and were unaffected by diet. 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 g N/day and (BA) 1636 g N/day for llamas, and for alpacas (B) 835 g N/day and (BA) 1034 g N/day, respectively. Nitrogen intake followed the same pattern, (B) 21.4 g N/day and (BA) 33.9 g N/day, respectively for llamas, and (B) 13.6 g N/day and (BA) 20.6 g N/day, respectively for alpacas (diet, $P < 0.002$). Diet affects were significant for urine N excretion ($P < 0.02$), (B) 11.2 g/day and (BA) 18.2 g/day for llamas, and (B) 6.8 and (BA) 10.8 g N/day for alpacas. Fecal N excretion was different for diet ($P < 0.03$), with fecal excreted N of 9.0 g N/day and 11.9 g N/day for B and BA in llamas, and 5.9 g N/day and 9.1 g N/day for B and BA respectively in 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 lower N requirement to meet metabolic needs than llamas, which are likely related to the smaller body size of the alpaca. When examining the biological value of N from the respective diets, 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 requirement and balance are not valid.

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

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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, 1989; 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 3500 to over 4000 m above sea level. The literature indicates that camelid digestive efficiency increases at higher altitudes (San Martin and Bryant, 1989; López and Raggi, 1992), a factor that further complicates interpretation and application of available nutritional information for alpacas and llamas. Due to the positive altitudinal influence on camelid digestive efficiency, López and Raggi (1992) indicated that digestible protein values are a more suitable basis for reporting the 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; Carmago and Cardozo, 1971; Hintz et al., 1973; Vernet et al., 1997; Genin and Tichit, 1997; Lemosquet et al., 1996; San Martin, 1987; Dulphy et al., 1994, 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 and Novoa (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 when 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 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 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, since the majority of their feed is consumed during the day.

2.2. Treatments

The experimental design administered dietary treatments in random order to two repetitions of animals. Treatments consisted of two forages: 100% barley (*Hordeum vulgare*) hay (B) and 80% barley (*H. vulgare*) /20% alfalfa (*Medicago sativa*) hay (BA), each diet was chopped to 3–4 cm length. Forage chemical composition was determined at a certified forage lab (DHI Forage Testing Laboratories, Dairy One Inc., Ithaca, NY) using wet chemical procedures (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 in the urine as it was collected to prevent volatilization of ammonia. On days 8–12, feed intake was measured, refused feed, fecal output, and urine quantity determined, 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 dry feed samples, feed refusal, and fecal samples were

Table 1
Diet composition

Component	Diet (% 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	63.0	64.2

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 at the BYU Soil and Plant Analysis Laboratory (Provo, UT) with values expressed as a percent of dry matter (Table 1).

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 × *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 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 data (intake and excretion) were analyzed 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 daylight 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, (B 1272, and BA 1636 g N/day for llamas, versus B 835 g N/day and BA 1034 g N/day for alpacas). The effect of the barley diets on N balance is shown in Fig. 1. Nitrogen intake followed a similar pattern as dry matter intake: (B) 20.6 g N/day

Table 2
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		S.E.M.	$P < 0.05$	
	Alpaca	Llama	Alpaca	Llama		Diet	Species
DM intake (g/day)	835	1272	1034	1636	114	0.0289	0.0006
N intake (g/day)	13.6	20.6	21.4	33.9	2.1	0.0003	0.0006
Fecal N (g/day)	5.9	9.0	9.1	11.9	1.0	0.0122	0.02
Urine N (g/day)	6.8	10.8	11.2	18.2	1.6	0.0033	0.005
Total N excreted (g/day)	12.8	19.8	20.3	30.1	2.3	0.0021	0.003
UN%TN* (%)	53.1	54.5	55.2	60.5	2.6	0.0033	0.005
N retained (g/day)	0.9	0.79	1.1	3.8	1.6	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.

and (BA) 33.9 g N/day for llamas, and (B) 13.6 g N/day and (BA) 21.4 g N/day for alpacas (diet, $P < 0.002$). Diet affects were significant for urine N excretion ($P < 0.02$), measured at (B) 10.8 g N/day and (BA) 18.2 g N/day for llamas, and (B) 6.8 g N/day and (BA) 11.2 g N/day for alpacas. Fecal N excretion was also different for diet ($P < 0.03$), determined to be (B) 9.0 g N/day and (BA) 11.9 g N/day for llamas, and (B) 5.9 g N/day and (BA) 9.1 g N/day for alpacas. When calculated on a metabolic weight basis, only diet was significant for N intake, urinary and fecal N excretion, and daily total N excretion (data not shown). Nitrogen retention was unaffected by diet or species. Nitrogen digestibility, although unaffected by diet or species, did show a positive trend in llamas consuming BA, increasing from 57% to 65%.

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 g/dl and (BA) 6.3 g/dl versus (B) 5.9 g/dl and (BA) 6.0 g/dl for alpacas and llamas, respectively. Phosphorus levels were significant by species ($P < 0.03$) with llama values higher than those for alpaca fed each diet, (B) 2.2 mmol/l and (BA) 2.4 mmol/l compared to (B) 2.0 mmol/l and (BA) 1.9 mmol/l in llamas and alpacas, respectively. No statistical significance was evident by diet or by diet × species interaction.

4. Discussion

The barley diets 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. (2005), 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 for 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., 2005). 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, which was attributed to the excess protein provided by alfalfa in the BA forage.

Available N, expressed as the difference between N intake and fecal N as a percent of N intake, representing the portion of crude protein that was digestible by the animal, resulted in a higher amount of N being absorbed from the gut of llamas (available N, 65% BA compared to B 56%). However, it was not different between B and BA diets, both providing 57% available N to alpacas. Urine N excretion was significantly higher with the BA diets

Table 3
Effects of two barley diets of differing protein content on plasma metabolite and electrolyte concentrations in mature gelded llamas and alpacas

	Barley		Barley alfalfa		S.E.M.	$P < 0.05$		
	Alpaca	Llama	Alpaca	Llama		Diet	Species	D × S
Glucose (mmol/l)	8.2	7.6	7.7	7.9	0.4	SEM	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.

for both species ($P < 0.02$). Although there was no significant difference in plasma urea N or creatinine between diets 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 alpacas and llamas, 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, the biological value of the digestible protein N available, was approximately 56% for alpacas and llamas when consuming the barley hay. The BA diet had a higher biological value of 65% in llamas compared to 57% in alpacas. The biological value of the digestible protein 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 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 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 (MEm) required by these alpacas and llamas was calculated using the equation derived by Carmean et al. (1992), ME (Mcal) = (84.5 × body weight$^{0.75}$ [BW;kg]/1000), gave the alpacas in this study a value of 1.59 Mcal ME/day and the llamas 2.41 Mcal ME/day, respectively. Engelhardt et al. (1977) published a ME value of 61 Mcal/$W^{0.75}$ as the ME requirement for llamas. The equation cited in the literature to calculate ME to DE, DE (Mcal) = ME × 1.22 resulted in a DE requirement of 1.94 for alpacas and 2.94 Mcal for llamas (Carmalt, 2000). Maintenance crude protein was determined by the equation CP (g) = 31 g × DE (Mcal) for both llamas and alpacas (Carmalt, 2000). Using the maintenance value of 0.752 g N (see calculations in Robinson et al., 2005), the llamas in this study had an estimated daily maintenance requirement of 133.8 g CP/day. These values are considerably higher than those determined from previous studies conducted at higher 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 (Robinson et al., 2005; 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 character-

ized 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 diet, llamas displayed a much higher increase in N retention compared to alpacas. The llamas in this study had an estimated daily maintenance requirement of 133.8 g CP/day. When examining the biological value of N from the respective diets, alpacas experienced a increase from the barley treatment value of 56.2–57.4% with barley alfalfa, while llamas showed an increase between the two diets of 56.2 (B) to 65.0% (BA), 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.

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