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THE EFFECT OF AN OMEGA-3 AND VITAMIN E-ENHANCED DIET ON NUTRITIONAL STATUS OF ALPACA

E. Koutsos[1], C. Kuball[2], M. Griffin[3], S. Tornquist[4], K. Flegel[5] and N. Evans[6]

Mazuri Exotic Animal Nutrition/Purina Mills LLC, 100 Danforth Dr, Gray Summit, MO 63039,[1,2 and 3] Currently: Omega Protein, Inc, 2105 City West Blvd., Houston, TX, 77042,[3] Department of Biomedical Sciences, College of Veterinary Medicine, Oregon State University, Corvallis, OR 97331,[4] Dos Doñas Alpaca Farm, LLC, 4515 Dieckmann Lane, Florissant, MO 63034,[5,] 14413 Sinks Road, Florissant, MO 63034,[6]

ABSTRACT

The effect of omega 3 polyunsaturated fatty acid (n3 PUFA) and vitamin E supplementation on blood fatty acids and vitamin E in alpaca were studied, and fatty acid profiles of managed alpaca were compared to Peruvian alpaca consuming native forage. In Experiment 1, 16 adult female alpaca, blocked by phenotype (n=8 Huacaya, 8 Suri), were offered either a control diet or supplemented diet through breeding, gestation and lactation. Cria remained with their dams and had access to the assigned diets until weaning at 6 months. In Experiment 2, 12 female alpaca (Huacaya) at maintenance were transitioned from their normal dietary ration to the supplemented diet for 5 months. In both experiments, blood nutrient profiles were examined. In experiment 3, the fatty acid profiles of blood samples from Peruvian alpaca (n=4) consuming native forage were analysed. Minor differences between phenotypes existed, but in general, supplemented diet consumption was associated with higher serum vitamin E concentrations compared to control or pre-supplemented diet periods ($p<0.05$ for each). Plasma fatty acids were changed by feeding supplemented diet, with increases in n3 PUFA concentrations ($p<0.05$). Peruvian alpaca had higher concentrations of n3 PUFA (particularly 18:3n3) and saturated fatty acids than US alpaca in these trials. These data demonstrate that plasma and serum fatty acids and vitamin E can be modulated by diet in alpaca and that further dietary modulation may be warranted based on values from Peruvian alpaca.

Key words: Alpaca, nutrition, omega-3, vitamin E

Alpaca can be divided into two distinct phenotypes based on their fibre types, the Huacaya and the Suri alpaca (Frank *et al,* 2006). These animals are kept in the United States as a source of pleasure and for fibre production (Gegner, 2000). A number of research reports have examined alpaca nutrition (e.g., Russel and Redden, 1997; San Martin and Bryant, 1989; Van Saun, 2006 and 2009), although examination of modulation of fatty acid and vitamin E status of alpaca has not been conducted.

Increases in circulating vitamin E in ruminants is associated with improved growth rates and reduced morbidity and mortality, likely due to modulation of immune responses (McDowell *et al,* 1996). Improvement in omega-3 polyunsaturated fatty acid (n3 PUFA) status may impact immune function and reproductive status (Mattos *et al,* 2004). It is likely that the fatty acid status of domestically raised animals is unlike their native-foraging counterparts, given the marked differences in fatty acid composition of wild-type green forages available at each location, and the fatty acid contribution of the grain products and hays that would be encountered in domestic feedstuffs (Davidson, 1998; Grant *et al,* 2002). Therefore, a supplemented diet enriched in the n3 PUFA linolenic acid (18:3n3) as well as vitamin E was designed. The purpose of this work was to examine the effect of this supplemented diet on the plasma and serum fatty acid and vitamin E status of alpaca, and to compare fatty acid profiles from these studies to those of alpaca consuming native forage. These data were collected from alpaca farms, thus representing typical conditions experienced by US alpaca.

Materials and Methods

Experiment 1: Effect of fatty acid and vitamin E supplementation on breeding females and their cria

Sixteen adult female alpaca (ages 1-10 years of age at the onset of the trial) were tested in a completely randomised block design starting in April 2008 and ending in September 2009 (location: Florissant, MO – USA). Alpaca were blocked by

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type (n=8 Huacaya, 8 Suri), then randomly assigned to one of two dietary treatments. Alpaca in each blocked group were offered either a control diet or supplemented diet (Table 1), along with *ad libitum* access to grass hay and pasture. Alpacas were given access to electrolyte water as well as fresh water. Each group was housed in a 4.5 x 12 m three-sided shelter, with access to approximately 8,100-12,100 m^2 of pasture. Animals had routine health checks and necessary vaccinations conducted, as well as routine faecal examinations with anthelmentic care as individually needed.

All females in the trial were bred and fed their assigned diets for the duration of the pregnancy and lactation. Alpaca were offered approximately 0.34 kg of feed/45 kg body weight per day during pregnancy and 0.43 kg feed/45 kg body weight per day during lactation. Cria remained with their dam and were also offered access to the assigned diets until weaning, at around 6 months after parturition.

A blood sample was collected via jugular venipuncture at the onset of the trial for a biochemical profile (serum, Oregon State University Veterinary Diagnostic Lab, Corvallis, OR) and a complete blood count (CBC, whole blood in EDTA tubes, Oregon State University Veterinary Diagnostic Lab, Corvallis, OR). Assays were also conducted for serum minerals (Co, Cu, Fe, Mn, Mo, Zn and Se) and vitamin E profile (Michigan State University Diagnostic Center for Population and Animal Health, Lansing, MI). At 24 h post-parturition and at weaning, blood sample were collected from dams and cria and analysed for the same parameters. Additionally, at weaning, blood was collected for plasma fatty acid profile (NP Analytical Labs, St. Louis, MO). Cria serum and dam milk were analysed for IgG at 24 h post-parturition (Camelid Radial Immunodiffusion Test Kit, Kent Laboratories in Bellingham, WA).

Experiment 2: Effect of fatty acid and vitamin E supplementation on adult non-breeding alpaca

Twelve female weanling and yearling Huacaya alpacas housed in a 10,000 m^2 pasture with a 6 x 9 m protected shed, were transitioned over a period of 2 weeks from their normal dietary ration (All-Stock sweet feed) to the supplemented diet (Table 1), then fed the supplemented diet for 5 months (Mantua, OH; October 2008-March 2009). Animals were fed 0.36 kg supplemented diet per day along with *ad libitum* access to grass hay and pasture. Two jugular blood samples were collected, just prior to diet transition and at the end of the trial. All samples were submitted

Table 1. Diet composition offered to alpaca (Experiment 1 and 2).

Ingredients	Control	Supplemented
	Wheat 1midds, soybean hulls, cracked corn, oats, processed molasses, alfalfa, mono-dicalcium phosphate, molasses, distillers dried grains and solubles, soybean meal, beet pulp, calcium carbonate, molasses, salt, brewers dried yeast, salt, apple flavouring, vitamin/ mineral mix.	**Wheat midds, soybean hulls, Fibre Enhancer® (extruded flaxseed, brewers dried yeast, wheat flour, vitamins), processed molasses, oats, beet pulp, mono-dicalcium phosphate, molasses, soybean meal, calcium carbonate, brewers dried yeast, salt, apple flavouring, vitamin/mineral mix.**
Crude protein, %[1]	12.0	12.5
Crude fat, %[1]	3.8	6.5
n6 fatty acids, %[1]	47.6	42.9
n3 fatty acids, %[1]	4.9	16.3
n3:n6 ratio[2]	0.10	0.38
Crude Fibre, %[1]	12.0	14.0
Neutral detergent fibre, %[1]	30.2	31.7
Acid detergent fibre %[1]	13.9	16.2
Calcium, %[1]	1.6	2.0
Phosphorus, %[1]	1.6	1.6
Sodium, %[2]	0.32	0.37
Magnesium, %[2]	0.47	0.49
Potassium, %[2]	1.12	1.15
Iron, ppm[1]	878	875
Zinc, ppm[1]	1305	863
Manganese, ppm[1]	409	263
Copper, ppm[1]	36	39
Selenium, ppm[1]	1.1	1.2
Vit A, IU/kg[2]	52,122	51,935
Vit D_3, IU/kg[2]	13,539	15,136
Vit E, IU/kg[2]	873	1,199
Choline, ppm[2]	337	337
Niacin, ppm[2]	436	436
Thiamin, ppm[2]	531	531
Biotin, ppm[2]	7.9	7.9
Camelid DE kcal/kg[2]	3,100	3,083

[1] Analysed value [2] Calculated value

for analysis of serum minerals, vitamin E, and plasma fatty acids as described above.

Experiment 3: Examination of plasma fatty acid profile of alpaca grazing native pasture in Peru

Blood samples were obtained from four adult female alpacas (phenotype unknown) housed at La Raya Research Station, Peru in October 2008. These alpacas were pastured on native forage and were not fed complete feeds or supplements. One blood sample from each animal was taken by venipuncture from the jugular vein, separated, then stored at -20° C. Samples were analysed for plasma fatty acids as described above.

Statistical analysis:

For Experiment 1, dam data were analysed by 2 way ANOVA (JMP, SAS, Cary NC) for main effect of dietary treatment, phenotype and their interactions, using initial measurements as a covariate and animal as the experimental unit. Dam and cria fatty acids, as well as the remainder of cria data were analysed by 2 way ANOVA for main effect of dietary treatment, alpaca breed, and their interactions. If an interaction was significant, means were compared using Students-test. For Experiment 2, data were analysed by repeated measures ANOVA using the initial and final time points. For Experiment 3, data were subjectively compared to data gathered in Experiment 1 and 2. For all statistics, significance was achieved at $p \leq 0.05$ and trends examined at $p \leq 0.10$. In tables, text and Figs, data are presented as means ± SEM.

Results

Experiment 1:

Summary clinical chemistry, haematology and nutrient analysis results in dams and cria are presented in Table 2. Differences between Huacaya and Suri alpaca phenotypes were significant for several haematologic parameters in dams and cria, including dam leukocyte counts at parturition ($p = 0.05$; Huacaya = 13.98 ± 1.21, Suri = 9.79±0.95

Table 2. Mean clinical chemistry and CBC in alpaca (Experiment 1 and 2).

Parameter	Experiment 1		Experiment 2	Reference Range
	Dam	Cria		
BUN (mg/dl)[b]	20.94 ± 0.51	17.23 ± 1.07	ND	13-28
Creatinine (mg/dl)	1.39 ± 0.03	1.39 ± 0.07	ND	0.9-1.7
Glucose (mg/dl)	114.09 ± 1.73	132.39 ± 5.06	ND	88-151
Cholesterol (mg/dl)	39.68 ± 1.64	54.26 ± 2.94	ND	75-150[c]
Triglycerides (mg/dl)	21.63 ± 1.37	47.36 ± 4.38	ND	
Total Protein (g/dl)	6.13 ± 0.06	5.46 ± 0.11	ND	5.1-6.9
Albumin (g/dl)	3.75 ± 0.04	3.42 ± 0.08	ND	3.5-4.9
Total Bilirubin (mg/dl)	<0.1	0.11 ± 0.02	ND	0.005-0.42[d,e,f]
Creatine Kinase (U/L)	177.15 ± 35.90	98.48 ± 9.42	ND	43-750
GGT (U/L)	23.64 ± 1.20	56.13 ± 13.06	ND	10-37
AST (U/L)	192.49 ± 7.81	196.23 ± 12.68	ND	127-298
tCO_2 (mEq/L)	24.34 ± 0.32	24.07 ± 0.77	21.75 ± 0.50	23-33
SDH (U/L)	3.03 ± 0.23	5.73 ± 0.70	ND	1.5-15.7
Anion gap (mEq/L)	17.70 ± 0.29	19.91 ± 0.79	ND	15-27
b-hydroxybutryate (mg/dl)	0.62 ± 0.06	0.56 ± 0.13	ND	0-2.5 f
NEFA (mEq/L)	0.21 ± 0.02	0.39 ± 0.04	ND	<0.6 (Van Saun)
Haemoglobin (g/dl)	12.35 ± 0.20	12.89 ± 0.29	ND	12-18
Haematocrit spun (%)	27.57 ± 0.42	30.45 ± 0.73	ND	27-45
MCHC calc	45.71 ± 0.36	42.36 ± 0.65	ND	36-49[c]
WBC (x 10^3/µl)	12.01 ± 0.52	10.57 ± 0.55	ND	8-21.4
Seg Neut (x 10^3/µl)	6.29 ± 0.35	5.83 ± 0.53	ND	4.7-14.7
Band Neut (x 10^3/µl)	ND	0.92 ± 0.89	ND	
Lympho (x 10^3/µl)	4.19 ± 0.31	4.34 ± 0.39	ND	0.7-4.8

Parameter	Experiment 1		Experiment 2	Reference Range
	Dam	Cria		
Eos (x 10^3/µl)	1.22 ± 0.16	0.60 ± 0.28	ND	0.7-4.7
Baso (x 10^3/µl)	0.04 ± 0.01	0.04 ± 0.02	ND	
Na (mEq/L)	151.25 ± 0.27	150.74 ± 0.69	150.00 ± 0.47	142-154
K (mEq/L)	4.43 ± 0.07	4.80 ± 0.09	6.93 ± 0.23	4.1-6.3
Cl (mEq/L)	114.94 ± 0.44	111.23 ± 0.58	111.86 ± 0.63	100-115
Ca (mg/dl)	8.95 ± 0.08	10.20 ± 0.12	8.90 ± 0.08	8.4-10.4
P (mg/dl)	4.83 ± 0.20	8.07 ± 0.26	7.61 ± 0.42	5.1-11.5
Mg (mg/dl)	2.20 ± 0.02	2.43 ± 0.06	2.40 ± 0.06	1.4-3.6[c,d]
Cu (µg/ml)	0.51 ± 0.01	0.41 ± 0.03	0.50 ± 0.02	0.25-0.85[f]
Fe (µg/dl)	130.11 ± 4.07	175.70 ± 17.01	128.61 ± 7.17	120-140[c]
Mn (ng/ml)	5.23 ± 0.77	6.13 ± 0.84	1.99 ± 0.12	
Zn (µg/dl)	0.74 ± 0.05	0.75 ± 0.07	0.54 ± 0.09	0.5-0.6[c]
Se (ng/ml)	228.76 ± 3.16	147.43 ± 5.48	158.00 ± 4.22	160-230c (60-140)[c]
Vitamin E (µg/ml)	3.53 ± 0.20	2.43 ± 0.27	1.61 ± 0.10	0.69-5.98[f]

[a] Reference ranges for adult animals, cria in parentheses if values differ. If no reference provided, reference range is from Oregon State University Veterinary Diagnostic Lab http://oregonstate.edu/vetmed/sites/default/files/CP_Biochemistry_Reference_Ranges_04_09.pdf.

[b] Abbreviations: BUN, blood urea nitrogen; ND, not determined; GGT, Gamma glutamyl-transferase; AST, Aspartate amino-transferase; tCO_2, total carbon dioxide; SDH, Sorbitol dehydrogenase; NEFA, non esterified fatty acids; MCHC, mean corpuscular haemoglobin concentration; WBC, white blood cell; Seg Neut, segmented neutrophil; Band Neut, banded neutrophill; ND = not detected; Lympho, lymphocyte; Mono, monocyte; Eos, eosinophil; Baso, basophil; Na, sodium; K, potassium, Cl, chloride; Ca, calcium; P, phosphorus; Mg, magnesium; Cu, copper; Fe, iron; Mn, manganese; Zn, zinc; Se, selenium.

[c] From (Evans, 2003) [d] From (Simons *et al,* 1993)

[e] From (Andreasen *et al,* 1998) [f] From (Foster *et al,* 2009)

total leukocytesx10^3/µl), which was due in part to differences in eosinophil count (p=0.04; Huacaya = 1.96 ± 0.51, Suri = 0.46 ± 0.17 eosinophilsx10^3/µl). Similarly, cria leukocyte numbers at parturition were affected and were greater in Huacaya cria compared to Suri cria (p<0.01; Huacaya = 11.39 ± 0.80, Suri = 7.27 ± 0.75 total leukocytes x10^3/µl), which was due in part to differences in the number of segmented neutrophils (p<0.001; Huacaya = 8.82 ± 1.08, Suri = 3.87 ± 0.95 segmented neutrophils x10^3/µl). There was no phenotype affect on total leukocyte counts at weaning for dams or cria (p>0.15 for each). There was no phenotype or diet effect on colostrum or cria serum IgG at birth (mean = 19714 ± 2214, 1814 ± 292, respectively, p>0.20 for each).

Phenotype also affected dam serum calcium (Ca) levels at parturition and weaning (p=0.03, 0.05, respectively), in which Huacaya had lower mean serum Ca than Suri dams at parturition (8.69 ± 0.13 vs 9.5 ± 0.24 mg/dl) and weaning (8.49 ± 0.14 vs 9.13 ± 0.12 mg/dl), but this effect was not seen in cria. However, mean cria serum phosphorus (P) was greater in Huacaya than in Suri cria at parturition (p=0.03; 8.98 ± 0.34 vs 7.48 ± 0.44 mg/dl, respectively). In dams, mean serum selenium (Se) concentration was lower in Huacaya than Suri dams at parturition (p=0.01; 212.25 ± 5.08 vs 236.13 ± 8.83 ng/ml) and at weaning (p=0.04; 219.71 ± 2.04 vs 246.88 ± 8.00 ng/ml), but there was no effect of phenotype on cria Se at either time point (p>0.20 for each). Mean cria serum bilirubin concentration was greater in Huacaya cria at parturition as compared to Suri cria (p=0.01; 0.2 ± 0.05 vs 0.11 ± 0.02, respectively).

Diet also affected several haematologic parameters. In dams, total leukocytes were different at weaning (p=0.05; Control Diet = 10.36 ± 1.03x 10^3/µl, Supplemented Diet = 13.96 ± 1.09 x10^3/µl), and eosinophils were greater in animals fed supplemented diet as compared to control diet (parturition p=0.02; Control Diet = 0.91 ± 0.17 x10^3/µl, supplemented diet = 2.03 ± 0.55 x10^3/µl; weaning p=0.05; Control Diet = 1.05 ± 0.24 x10^3/µl, supplemented diet = 2.27 ± 0.60 x10^3/µl). There were no significant effects of diet on cria blood leukocyte numbers.

Mean serum glucose was also different due to diet at parturition in cria, but not dams; glucose was greater in cria from dams on control diet (163.50 ± 12.49 mg/dl) as compared to those from dams on

supplemented diet (119.38 ± 7.25 mg/dl), but there was no difference in mean serum glucose due to diet in cria at weaning (p>0.20). Mean serum copper (Cu) was affected by a diet x phenotype interaction in cria at parturition and weaning (p=0.02 for each; Fig 1). At parturition, Suri cria from dams fed supplemented diets had higher mean serum Cu than Huacaya cria from dams fed supplemented diets (p<0.05). At weaning, a similar response was observed and additionally, Suri cria fed control diets had lower mean serum Cu than Suri cria fed supplemented diets (p<0.05). Mean serum vitamin E concentration was greater in dams fed supplemented diet than those fed control diet (p=0.05, 0.03, respectively, Fig 2A). There was a trend for a similar response in cria at parturition and weaning (p=0.08, 0.09, respectively, Fig 2B).

Diet also affected blood lipid profiles. Mean plasma cholesterol and triacylglycerols (TAG) in dams were affected by the interaction of phenotype x diet at parturition (p=0.04, 0.02, respectively, Fig 3), and TAG were similarly affected at weaning in dams (p=0.01). In general, Huacaya dams fed supplemented diet had lower concentrations of plasma cholesterol as compared to Huacaya dams fed control diet, and Suri fed supplemented diet had higher TAG as compared other animals. This difference was not observed in cria.

Mean plasma fatty acid concentrations in dams and cria are shown in Table 3. There was no effect of breed on blood fatty acids in dams or cria (p>0.20 for all). Diet affected certain blood fatty acids, primarily for dams (Table 4). Dams fed supplemented diet tended to have lower concentrations of total fatty acids, trans fatty acids, and PUFA compared to dams fed control diet (p<0.10 for each). The reduction in total PUFA was associated with lower mean concentrations of n9 PUFA but higher mean concentrations of n3 PUFA compared to dams fed control diets (p<0.05 for each). Specific fatty acids changed in response to dietary treatment: supplemented diet-fed dams had greater mean concentrations of 16:0 (palmitic acid), 17:1n7 (margaroleic acid), 18:0 (stearic acid) and 18:3n3 (linolenic acid) and lower mean concentrations of 18:1n9T (elaidic acid) (p<0.05 for each). Cria from dams fed supplemented diet had lower mean concentrations of 16:1n7 (palmitoleic acid) and 18:1n7 (vaccenic acid) and greater mean concentrations of 17:1n7 (margaroleic) and 18:0 (stearic acid) compared to those from dams fed control diet (p<0.05 for each).

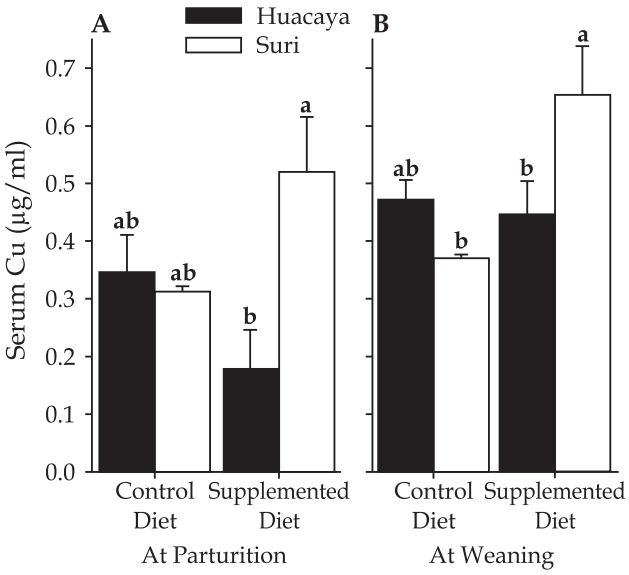

Fig 1. Effect of dietary supplementation and breed type of alpaca cria on serum Cu at parturition (A) and weaning (B) (Experiment 1). a-b Within a graph, means with different superscripts are significantly different (p<0.05).

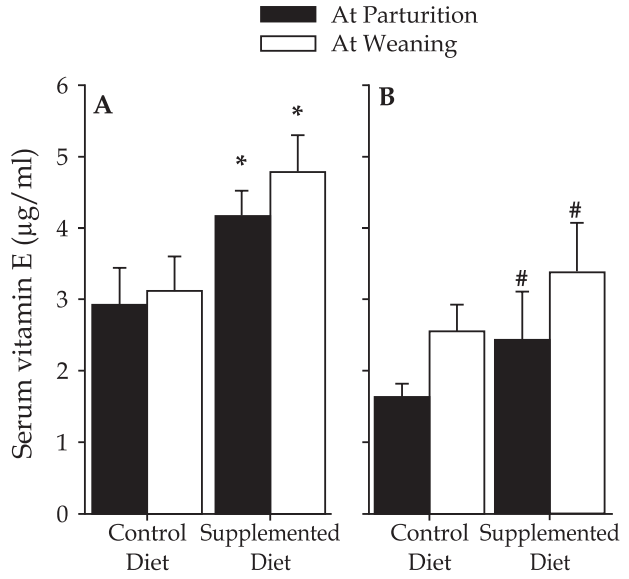

Fig 2. Effect of dietary supplementation on serum vitamin E in alpaca A) dams and B) cria at parturition and weaning (Experiment 1). * Significantly different from control diet within an age and time point (p<0.05). # Tendency to be different from control diet within an age and time point (p<0.10).

Experiment 2

Mean biochemical analytes and nutrients from alpaca in Trial 2 are presented in Table 2. Several minerals were increased in serum at the end of the dietary treatment as compared to prior to feeding, including Mg (p<0.01; 2.47 ± 0.07 vs 2.28 ± 0.07 mg/dl respectively), Mn (p=0.05; 2.26 ± 0.20 vs 1.75 ± 0.08 ng/ml, respectively), Se (p<0.01; 172.69 ± 4.51 vs

Table 3. Mean blood fatty acids in alpaca (Experiment 1 to 3).

Fatty acid	Experiment 1		Experiment 2	Experiment 3
	Dam	Cria		
Total Fat (mg/g)	0.69 ± 0.11	0.74 ± 0.16	0.49 ± 0.02	0.55 ± 0.06
MUFA (mg/g)[1]	0.06 ± 0.01	0.10 ± 0.02	0.07 ± 0.01	0.08 ± 0.02
PUFA (mg/g)	0.16 ± 0.01	0.17 ± 0.02	0.12 ± 0.01	0.11 ± 0.02
n9 PUFA	27.07 ± 5.46	24.69 ± 4.36	14.78 ± 0.39	13.32 ± 1.07
n6 PUFA	20.25 ± 2.58	20.60 ± 1.65	18.90 ± 1.22	15.88 ± 1.48
n3 PUFA	5.88 ± 0.68	5.82 ± 0.52	5.21 ± 0.48	5.96 ± 0.96
SFA (mg/g)	0.18 ± 0.01	0.22 ± 0.02	0.21 ± 0.01	0.26 ± 0.02
tFA (mg/g)	0.19 ± 0.08	0.17 ± 0.09	ND	0.01 ± 0.01
12:0 (%)	0.22 ± 0.08	0.20 ± 0.04	0.16 ± 0.01	0.31 ± 0.04
14:0 (%)	1.09 ± 0.13	1.46 ± 0.15	1.29 ± 0.05	2.27 ± 0.14
14:1n5 (%)	0.26 ± 0.11	0.15 ± 0.08	ND	ND
15:0 (%)	0.84 ± 0.10	0.73 ± 0.09	1.23 ± 0.04	1.94 ± 0.08
16:0 (%)	12.78 ± 1.30	15.68 ± 1.06	20.49 ± 0.62	22.43 ± 0.70
16:1n7 (%)	1.17 ± 0.20	2.37 ± 0.45	2.63 ± 0.21	2.69 ± 0.32
17:0 (%)	0.70 ± 0.13	0.49 ± 0.09	0.64 ± 0.02	1.18 ± 0.02
17:1n7 (%)	0.31 ± 0.11	0.27 ± 0.08	0.02 ± 0.01	ND
18:0 (%)	13.32 ± 1.29	13.65 ± 1.47	19.37 ± 0.63	19.1 ± 1.43
18:1n9T (%)	19.58 ± 5.99	14.45 ± 4.76	4.53 ± 0.32	2.58 ± 0.26
18:1n9C (%)	7.20 ± 0.72	9.96 ± 1.17	10.44 ± 0.36	10.32 ± 1.34
18:1n7C (%)	0.36 ± 0.04	0.54 ± 0.08	0.92 ± 0.12	0.68 ± 0.12
18:2n6 (%)	17.39 ± 2.43	17.70 ± 1.46	17.11 ± 0.88	14.38 ± 1.40
18:3n6 (%)	0.22 ± 0.02	0.19 ± 0.03	0.33 ± 0.05	0.03 ± 0.04
18:3n3 (%)	3.04 ± 0.34	2.92 ± 0.30	2.29 ± 0.21	3.73 ± 0.60
18:4n3 (%)	0.11 ± 0.04	0.08 ± 0.03	0.07 ± 0.02	ND
19:0 (%)	0.14 ± 0.04	0.12 ± 0.04	0.23 ± 0.02	0.12 ± 0.10
20:0 (%)	0.15 ± 0.03	0.17 ± 0.02	0.26 ± 0.03	0.54 ± 0.05
20:2n6 (%)	0.19 ± 0.08	0.12 ± 0.07	ND	ND
20:3n6 (%)	0.40 ± 0.06	0.38 ± 0.07	0.43 ± 0.05	0.22 ± 0.03
20:4n6 (%)	2.00 ± 0.26	2.20 ± 0.22	2.16 ± 0.16	1.26 ± 0.10
20:5n3 (%)	1.77 ± 0.25	1.80 ± 0.18	2.13 ± 0.17	1.54 ± 0.22
22:0 (%)	0.29 ± 0.06	0.29 ± 0.07	0.44 ± 0.04	0.74 ± 0.20
23:0 (%)	0.21 ± 0.09	0.14 ± 0.06	0.03 ± 0.02	0.25 ± 0.35
22:5n3 (%)	0.49 ± 0.07	0.57 ± 0.06	0.57 ± 0.08	0.64 ± 0.19
22:6n3 (%)	0.39 ± 0.19	0.42 ± 0.13	0.26 ± 0.04	0.06 ± 0.08
24:0 (%)	0.24 ± 0.10	0.34 ± 0.12	0.24 ± 0.07	1.06 ± 0.30
24:1n9 (%)	0.21 ± 0.03	0.22 ± 0.04	0.35 ± 0.05	0.42 ± 0.06

1 Abbreviations: MUFA – monounsaturated fatty acid; PUFA – polyunsaturated fatty acid; SFA – saturated fatty acid; tFA – trans fatty acid, ND – not detected.

144.15 ± 5.40 ng/ml, respectively), and Zn ($p<0.01$; 0.83 ± 0.11 vs 0.18 ± 0.01 ug/dl, respectively). In contrast, mean serum P was reduced at the end of the diet treatment as compared to prior to feeding Supplemented Diet ($p<0.01$; 6.54 ± 0.70 vs 7.97 ± 0.41 mg/dl, respectively). Mean serum vitamin E concentration was elevated at the end of the dietary treatment ($p<0.01$; 1.94 ± 0.15 vs 1.31 ± 0.08 µg/ml, respectively).

Mean plasma fatty acid concentrations are shown in Table 3. Feeding supplemented diet affected certain plasma fatty acids (Table 5). Mean PUFA, n6

Table 4. Effect of diet treatment on blood fatty acids of alpaca dams and cria at weaning (Experiment 1). Absence of values reflects no significant differences or trends between treatments (p>0.10).

Fatty acid	Dam			Cria		
	Control	Supplemented	p	Control	Supplemented	p
Total Fat (mg/g)	0.87 ± 0.17	0.51 ± 0.06	0.09			
PUFA (mg/g)	0.18 ± 0.01	0.14 ± 0.02	0.08			
n9 PUFA	37.51 ± 7.24	16.63 ± 5.19	0.05			
n3 PUFA	4.33 ± 0.76	7.42 ± 0.54	0.01			
tFA (mg/g)	0.33 ± 0.14	0.06± 0.04	0.09			
16:0 (%)	10.25 ± 1.46	15.30 ± 1.53	0.04			
16:1n7 (%)				3.39 ± 0.59	1.35 ± 0.27	0.01
17:1n7 (%)	0.08 ± 0.06	0.53 ± 0.18	0.04	0.12 ± 0.05	0.42 ± 0.13	0.05
18:0 (%)	10.99 ± 1.47	15.66 ± 1.61	0.05	11.06 ± 1.16	16.23 ± 2.24	0.04
18:1n9T (%)	30.72 ± 8.41	8.43 ± 5.33	0.05			
18:1n7C (%)				0.69 ± 0.13	0.38 ± 0.02	0.05
18:3n3 (%)	2.26 ± 0.35	3.83 ± 0.29	<0.01			

Table 5. Effect of diet treatment on blood fatty acids of alpaca (Experiment 2).

Fatty acid	Before	Supplemented	p
PUFA (mg/g)	0.10 ± 0.01	0.13 ± 0.01	0.04
n9 PUFA	16.32 ± 0.58	13.45 ± 1.09	0.03
n6 PUFA	16.92 ± 1.52	20.61 ± 1.78	0.02
n3 PUFA	4.29 ± 0.70	6.01 ± 0.61	0.01
16:0 (%)	21.51 ± 0.97	19.6 ± 0.73	0.06
17:0 (%)	0.69 ± 0.03	0.60 ± 0.02	0.03
18:0 (%)	21.09 ± 0.89	17.63 ± 0.70	0.01
18:1n9T (%)	5.50 ± 0.47	3.54 ± 0.30	0.01
18:2n6 (%)	14.80 ± 1.29	18.82 ± 0.93	0.03
18:3n6 (%)	0.24 ± 0.05	0.42 ± 0.07	0.04
18:4n3 (%)	0.05 ± 0.02	0.10 ± 0.02	0.05
20:0 (%)	0.35 ± 0.05	0.19 ± 0.03	0.03
20:5 n3 (%)	1.67 ± 0.23	2.58 ± 0.18	0.03
22:6n3 (%)	0.17 ± 0.04	0.35 ± 0.05	0.03

PUFA, n3 PUFA, 18:2n6 (linoleic acid), 18:3n6 (gamma linoleic acid), 18:4n3 (octadecatetraenoic acid), 20:5n3 (eicosapentaenoic acid) and 22:6n3 (docosahexaenoic acid) concentrations were significantly increased by feeding Supplemented Diet. In contrast, mean n9 PUFA, 16:0 (palmitic acid), 17:0 (margaric), 18:0 (stearic acid), 18:1n9T (elaidic acid), 20:0 (arachidic acid) concentrations were reduced by feeding supplemented diet.

Experiment 3

Mean plasma fatty acids are shown in Table 3. In comparison to data from adult alpaca from Experiment 1 and 2, mean plasma n9 PUFA were 35% lower in Peruvian alpaca, and mean trans fatty acids were 89% lower in Peruvian alpaca. Specific plasma fatty acids that had lower mean plasma concentration in Peruvian alpaca were 18:1n9T (elaidic acid, 78% lower), 18:3n6 (linoleic, 89% lower), 19:0 (nonadecanenoic, 35% lower), 20:3n6 (gamma linolenic, 46% lower), 20:4n6 (arachidonic, 39% lower), and 22:6n3 (docosahexaenoic, 81% lower). Mean plasma saturated fatty acids (SFA) concentrations were generally higher in Peruvian alpaca (33% higher), as was 12:0 (lauric acid, 63% higher), 14:0 (myristic, 90% higher), 15:0 (pentadecaenoic, 87% higher), 16:1n7 (palmitoleic, 42% higher), 17:0 (margaric, 76% higher), 18:3n3 (linolenic, 40% higher), 20:0 (arachidic, 163% higher), 22:0 (behenic, 102% higher), 23:0 (tricosaenoic, 108% higher), 24:0 (lignoceric, 340% higher) and 24:1n9 (nervonic, 50% higher). Several fatty acids were not detected in plasma of Peruvian alpaca, but were detected in US animals, including 14:1n5 (myristoleic acid), 17:1n7 (margaroleic acid), 18:4n3 (octadecatetraenoic) and 20:2n6 (eicosadienoic).

Discussion

These studies examined the effect of a supplemented diet on the blood nutrient parameters of alpaca. In Experiment 1, feeding the supplemented diet, which contained higher levels of vitamin E than control diet, increased the serum vitamin E concentration of dams, and tended to enhance serum vitamin E concentrations of cria. These data are supported by many reports of supplemental vitamin E in ruminants resulting in higher blood and liver vitamin E levels (reviewed by

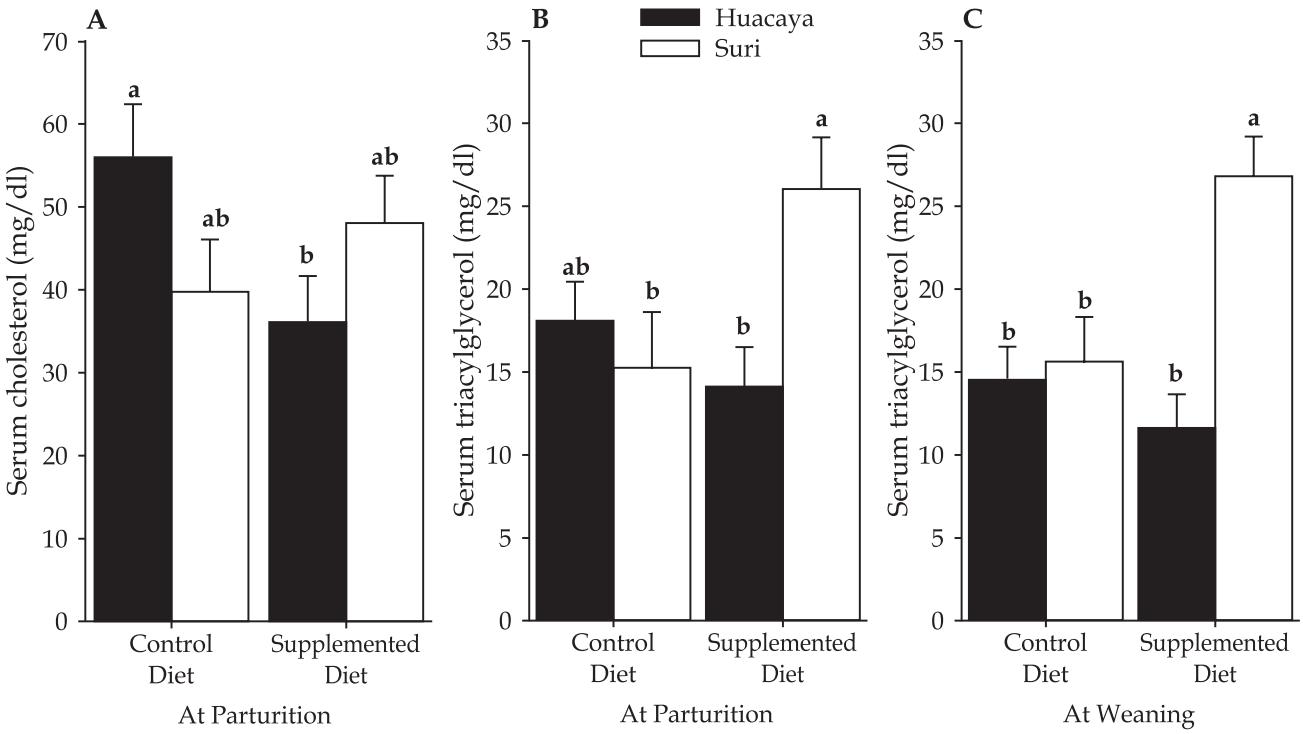

Fig 3. Effect of dietary supplementation and breed type of alpaca dams on blood cholesterol at parturition (A), and blood triacylglycerol at parturition (B) and weaning (C) (Experiment 1). a-b Within a graph, means with different superscripts are significantly different ($p<0.05$).

McDowell *et al,* 1996). Additionally, since placental transport of vitamin E is minimal in ruminants (Van Saun *et al,* 1989), these data support the idea that milk vitamin E concentration was likely enhanced in supplemented dams, thus allowing for higher vitamin E intake by suckling cria. A similar improvement in serum vitamin E concentrations were noted in Experiment 2, suggesting that the supplemented diet provided higher vitamin E than animals were receiving prior to this diet transition. Supplementation with vitamin E has been shown to reduce morbidity due to a number of diseases (reviewed by McDowell *et al,* 1996), thus enhanced serum vitamin E in alpaca and their cria fed supplemented diet is expected to improve the health status of these animals. Published findings of vitamin E for grazing alpacas found castrated male in Australian pastures had plasma concentrations of 2.2 µg/ml and those with dark coats had 1.6 µg/ml (Judson *et al,* 2011). The mean values found in dam and cria (3.53 and 2.43 ug/ml, respectively) in Experiment 1 suggest levels of the vitamin were adequate. Mean values of 1.6 ug/ml in Experiment 2 also fall in line with those findings.

The optimal fatty acid profile for alpaca (either dietary origin or derived from microbial synthesis in the forestomach) is unclear. The diet of alpaca eating native forage has not been examined for fatty acid content, but other free-ranging ruminant species have been shown to have higher concentrations of serum n3 fatty acids, as compared with captive counterparts (e.g., Clauss *et al,* 2007; Schmidt *et al,* 2007). This is presumably due to a wild-type diet being rich in linolenic acid (Davidson, 1998), as n3 PUFA predominate in green forages such as grasses or browse, whereas grain products generally are greater in n6 PUFA (e.g., Grant *et al,* 2002). Experiment 3 examined the differences between plasma fatty acid profiles of Peruvian alpaca grazing native forage and US alpaca on several feed regimes. Higher concentrations of n3 linolenic acid and saturated fatty acids were found in plasma from Peruvian alpaca grazing native forage. They generally had lower concentrations of n9 and n6 fatty acids (20:4 n6), and higher concentrations of saturated fatty acids (12;0, 14:0, 15:0, 20:0, 23:0, 24:0) and shorter chain n3 fatty acids (18:3 n3) than the US alpacas examined in these trials.

The ability of supplemented diet containing an extruded form of flaxseed, a source of 18:3n3, to modulate plasma fatty acids was examined in experiment 1 and 2. In experiment 1, feeding the supplemented reduced n9 concentrations and increased 18:3 n3, although not to the levels seen in

Peruvian animals. Interestingly, in Experiment 2, the increase in mean plasma n3 PUFA was associated with increases in other n3 PUFA including 20:5 n3 (eicosapentaenoic acid) and 22:6n3 (docosahexaenoic acid), which were not supplemented in the diet. Improvements in SFA concentration were not noted, and in Experiment 2, SFA were generally reduced by feeding supplemented diet. Feeding of PUFA's in both forage and fish oil forms has been shown to modify the microbiota diversity the rumen (Huws *et al,* 2010). This change in the bacterial environment can subsequently alter the amount of SFA's produced by the microbes. These data indicate the following: 1) native diets of alpaca vary in lipid composition compared to those fed to alpaca in the US; 2) blood fatty acids can be modulated by dietary fatty acids; and 3) due to the presence of longer chain n3 PUFA that were not supplied in the diet, either rumen microflora or the animals themselves were capable of some fatty acid elongation and desaturation. Unlike grazing ruminants, browsing ruminants do not appear to have complete biohydrogenation of diet-derived fatty acids, potentially due to shorter rumen retention times (Rowell-Schafer *et al,* 2001), allowing for dietary manipulation of blood and tissue fatty acid profiles (Cordain *et al,* 2002). It is possible that the forestomach fermentation of the alpaca and other camelid species allows for bypass, similar to that seen in browsing ruminant species. Dietary supplementation of serum n3 PUFA may impact immune function and other physiological variables (Chapkin *et al,* 2008), although the impact on ruminants has not been well-documented. In dairy cattle, a n3:n6 ratio of 0.31 (achieved by feeding fish oil) did not affect systemic inflammation (Ballou *et al,* 2009), but higher intakes reduced PGF2α post-partum (Mattos *et al,* 2004). In the current trial, a n3:n6 ratio of 0.27 was achieved in plasma of alpaca in Experiment 1 and 2, compared to 0.37 in Peruvian alpaca. Therefore, the impact of n3 supplementation when higher ratios are achieved remains to be determined. Similarly, the modulation of SFA in alpaca warrants further investigation.

Cria plasma fatty acid concentrations were not generally affected by the supplemented diet suggesting that milk fatty acid composition was not modulated to the same extent as plasma fatty acids by dietary manipulation. Limited data on milk fatty acid composition of alpaca shows higher mean plasma concentrations of 16:0 and 16:1 in milk and no detectable 18:3 (Glass *et al,* 1971), and this was potentially reflected in higher levels of 16:0 and 16:1 and lower levels of 18:3 in the blood of cria on this trial.

Overall plasma lipid concentrations did vary due to dietary treatment in Experiment 1, and there were also phenotypic effects observed. In general, dams fed supplemented diet had lower plasma cholesterol and higher plasma TAG than dams fed control diet. However, all of the values observed fell within reference intervals and did not approach concentrations indicating hyperlipidemia (Waitt and Cebra, 2008). These data support the conclusion that the level of dietary lipid (6.5%) provided in the supplemented diet did not cause any negative metabolic alterations in these animals, and all animals appeared to be maintained in good energetic status throughout the trial on the Supplemented Diet.

In contrast to changes in serum vitamin E and plasma fatty acids, there were no major change in blood mineral concentrations in Experiment 1 (despite lower levels of dietary Mn and Zn), and a slight elevation of blood Zn and Se in Experiment 2. In the latter, the initial serum Zn and Se concentrations were relatively low, so the increase found after feeding supplemented diet brought animals into a more typical reference interval (Evans, 2003). Serum electrolyte concentrations (Na, K, Mg) appeared to vary due to dietary treatment in Experiment 1 (Na, K) and 2 (Mg), but these were not statistically analysed as electrolyte-enriched water had been provided to the animals. All levels were within normal reference intervals (Evans, 2003; Simons *et al,* 1993).

Overall, these data suggest that feeding the supplemented diet did not have major impacts on mineral nutrition except to improve circulating serum Se and Zn concentration in animals with low initial status. Though it is unclear if observed changes in serum nutrient profiles are biologically relevant, differences found between Huacaya and Suri alpaca may be related to different needs for fibre growth of the two distinct alpaca phenotypes (Frank *et al,* 2006).

In summary, supplementation of alpaca with diets containing higher levels of linolenic acid and vitamin E improved the n3 fatty acid and vitamin E profile of the blood. However, more n3 supplementation would be needed to achieve the concentrations seen in Peruvian alpaca, and modulation of SFA was not achieved in this trial. Further research should examine the effect of modulation of fatty acid and vitamin E profiles on immune and fibre characteristics.

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