

Relationship between the nutritional state before the breeding period and the reproductive success in alpacas (*Vicugna pacos*) from the Chilean Puna

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ABSTRACT. A poor nutritional state may explain the low fertility rates of domestic camelids from Puna herds. The objective of this study was to determine the relationship between the nutritional state of alpacas before the breeding period and their reproductive success. The body live weight (BLW) and body condition score (BCS; scale from 1-5) of 35 non-pregnant non-lactating alpacas from the Caquena bofedal in Chile were registered previous to the breeding period. In addition, blood samples were collected (heparinised tubes) to determine the plasma concentration of cholesterol (CHOD-PAP method), triglycerides (GPO-PAP method) and NEFA (enzymatic colorimetric method). Pregnancy was determined by trans-rectal ultrasonography (7.5 MHz, Samsung Madison, Korea) at the end of the breeding period. Data from Pregnant and Non-Pregnant groups were compared using Mann-Whitney U test (SPSS programme; $P < 0.05$). Pregnant ($n=20$) and Non-pregnant groups ($n=15$) had similar BLW (47.1 ± 8.8 compared to 52.8 ± 11.1 kg, respectively; $P = 0.9$), BCS (3.0 ± 0.4 compared to 3.1 ± 0.4 , respectively; $P = 0.5$), plasma cholesterol (1.0 ± 0.4 compared to 0.9 ± 0.3 mmol/l, respectively; $P=0.7$), triglycerides (0.3 ± 0.1 compared to 0.4 ± 0.1 mmol/l, respectively; $P = 0.3$), and NEFA (0.6 ± 0.4 compared to 0.8 ± 0.6 mmol/l, respectively; $P = 0.1$) before the breeding period. NEFA values of both groups showed evidence of negative energy balance. The nutritional state of alpacas before the breeding period did not explain the differences obtained in their reproductive success.

Key words: nutrition, fertility, camelids.

INTRODUCTION

South American camelids (Family Camelidae) live above 3,000 masl in an ecosystem called the Puna, foraging on plant formations such as tolar, bofedal, and pajonal. The Puna is characterised by a wet season in which 95% of the annual precipitations are concentrated (December to March), when the grazing reaches the highest levels of quality and quantity. During the dry season (April to November) the dry matter produced per Ha is close to half the amount produced during the wet season (Castellaro *et al* 2004).

Domestic South American camelids (llama and alpacas) are both, induced ovulators and uniparous species. In the Puna, their breeding and parturition are restricted to the wet season, while gestation and lactation last 11.5, and nine months, respectively (San Martín 1996, Vaughan 2011). Several studies have reported low fertility of Puna herds (less than 50%). A birth rate of 40-60% has been described in Chile (FAO 2005), and an embryonic mortality rate of 45-56% has been determined in herds from Peru (Fernández-Baca *et al* 1970, Bravo and Sumar 1985, Ratto *et al* 2011). Low fertility rates are partly attributable to high embryonic mortality and reproductive mismanagements, and especially to the poor nutritional state of the livestock: the high nutritional demand for fetal growth

coincides with a critical period of nutrient deficiency (Van Saun 2008, San Martín and Van Saun 2014). In Chile, the recommendations to breed wild vicuñas include having a body live weight over 40 kg to reduce the risk of winter abortion (Ellmen 2004, Raggi and Parraguez 2005).

Energy imbalance in ruminant species can cause a sub-fertility syndrome with abnormal oestral behaviour, prolonged *post-partum* anoestrus, low conception rate, increase in embryo mortality and retarded involution of the uterus (Diskin *et al* 2003, Patton *et al* 2007). Thus, reproduction is sensitive to changes in the availability of nutrients and energy through direct and complex interactions of different hormones, metabolites and neuropeptides such as leptin, ghrelin, insulin-like growth factor-1, insulin, glucagon, growth hormone, glucose and neuropeptide Y (Schneider 2004, Webb *et al* 2004). It has been described in camelids that llamas under chronic nutritional restriction had small dominant follicles and corpus luteum, and lower progesterone secretion (Norambuena *et al* 2013).

The aim of this study was to determine the relationship between the nutritional state of alpacas before the breeding period with their reproductive success.

MATERIAL AND METHODS

The study was authorised by the bioethics committee of the Universidad Católica de Temuco. Thirty-five non-pregnant, non-lactating female alpacas from a herd located in the village of Caquena ($18^{\circ}03'S$; $69^{\circ}11'W$; 4405 m.), Arica and Parinacota Region of Chile, were studied. The herd of about 400 alpacas maintains year-round males and females grazing together in the Caquena bofedal. The reproductive state of alpacas was determined using rectal palpation and trans-rectal ultrasonography with a linear

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probe (7.5 MHz, Samsung Madison, Korea) to identify the presence of an echogenic embryo or fetus, and it took place in October 2015, previous to the breeding period. Lactation was excluded by palpation of the mammary gland and age was determined using dental chronometry. The alpacas body live weight (BLW) were obtained using a mechanical scale (± 0.1 kg), and the body condition score (BCS) was determined according to Van Saun (2009). A blood sample was taken (4ml; heparinised tubes) from the jugular vein to determine the plasma concentration of cholesterol (CHODPAP method; HUMAN[®]), non-esterified fatty acids (enzymatic colorimetric method; RANDOX[®]) and triglycerides (GPO-PAP method; HUMAN[®]). The samples were centrifuged immediately at 1500 x g for 15 minutes; plasma was transported by plane to Universidad Católica de Temuco, where it was stored at -80°C. Protein and metabolites were analysed using a Metrolab 2300 Plus, Random Access Clinical Analyzer (Wiener Lab, Argentina), at the Veterinary Sciences Laboratory, Universidad Católica de Temuco, Chile. Following the breeding period (May 2016), pregnancy was determined by rectal palpation and trans-rectal ultrasonography and animals were assigned to the Pregnant or Non-pregnant groups. The Shapiro-Wilks test was used to determine the normal distribution of the data and the Levene's test to determine the homoscedasticity of the variances. Data from both groups were compared using the Mann-Whitney U test, using the SPSS V. 20.0 statistic programme (SPSS Inc, Chicago IL, USA) with a $P < 0.05$ significance level.

RESULTS AND DISCUSSION

Twenty alpacas out of 35 (57%) were pregnant at the end of the breeding period. However, it is possible that

some pregnant females had not been identified because pregnancy can be positively diagnosed by trans-rectal ultrasound from day 20 in camelids (Herrera *et al* 2002). The Pregnant and Non-pregnant groups were similar in age, BLW, BCS and plasma concentration of cholesterol, triglycerides, and NEFA, before the breeding period (table 1).

The BLW of animals agrees with that obtained in Chilean herds (Raggi *et al* 1997, Muñoz 2007). Age did not differentiate groups; animals ranged from 1 year 10 months to 3 years 10 months before the breeding period. Alpaca females are usually first bred at 12 months of age, but many breeders delay their first breeding until 15-18 months old (Vaughan and Tibary 2006), which was the age of the youngest alpacas during the breeding period of this study. The BCS of the groups indicates an ideal fat cover (Van Saun 2009) which was not expected, considering that the critical levels of forage quality and productivity extended from June to October in the Chilean Puna (Raggi and Parraguez 2005). The mean BCS of both groups was according the mating recommendation for small ruminant species (3-3.5 BCS), (Molina *et al* 1994, Vatankhah *et al* 2012).

Both groups had similar plasma concentrations of cholesterol, triglycerides and NEFA prior to the breeding period, thus indicating that energy metabolic status does not explain the differences obtained in their reproductive success. The plasma concentrations of cholesterol and triglycerides were within the reference intervals for adult alpacas (table 1). However, Siguas *et al* (2007) obtained lower values than our study in plasma concentrations of triglycerides during the dry season (0.1 ± 0.06 mmol/l) and the wet season (0.08 ± 0.04 mmol/l) in alpacas from the Peruvian Puna; probably due to a lower lipid intake from their forage (Aboelmaaty *et al* 2008). The NEFA levels were in the upper limit of the reference interval obtained

Table 1. Age and nutritional variables values (mean \pm SD, range) from non-pregnant alpacas (n=35) before the breeding period. Pregnancy diagnosis was carried out at the end of the breeding period.

Variable	Pregnant group (n = 20)	Non-pregnant group (n = 15)	P	References intervals
Age (m)	30 \pm 7 (22-46)	31 \pm 7 (22-46)	0.6	
BLW (kg)	47 \pm 9 (35-67)	53 \pm 11 (35-70)	0.1	
BCS (1-5)	3.0 \pm 0.4 (2.5-4)	3.1 \pm 0.4 (2.5-4)	0.5	
Cholesterol (mmol/l)	1.0 \pm 0.4 (0.5-1.8)	0.9 \pm 0.3 (0.5-1.6)	0.4	0-1.5 mmol/l (Fowler 1998) 0.2-2.2 mmol/l (Foster <i>et al</i> 2009)
Triglycerides (mmol/l)	0.3 \pm 0.1 (0.2-0.5)	0.4 \pm 0.1 (0.2-0.5)	0.8	0.1-0.5 mmol/l (Foster <i>et al</i> 2009) 0-0.5 mmol/l (Husakova <i>et al</i> 2014)
NEFA (mmol/l)	0.6 \pm 0.4 (0.2-1.4)	0.8 \pm 0.6 (0.2-1.5)	0.1	0.1-0.7 mmol/l (Husakova <i>et al</i> 2014)

BLW = body live weight; BCS = body condition score; NEFA = non-esterified fatty acids; P values of Mann-Whitney U test.

in alpacas from to Central Europe farms (Husakova *et al* 2014). Indeed, the non-pregnant group slightly exceeded this range. The fat mobilization increased NEFA in camelids (Tornquist *et al* 2001, Norambuena *et al* 2013) with values about 2 mmol/l in llamas submitted to five days of fasting (Wenswoort *et al* 2001). In addition, the NEFA values obtained in this study were very similar to those obtained in pregnant vicuñas (*Vicugna vicugna*) in their last third of gestation, in the same geographical area (Mussa 2017). Nevertheless, it is necessary to have a reference range of alpacas NEFA from hypoxic environments, since it has been determined that the magnitude of their feed fermentation was greater at high altitude than at sea level (San Martín and Bryant 1989).

In conclusion, the nutritional status obtained before the breeding period did not explain the differences in the reproductive success obtained in alpacas from the Chilean Puna. Further investigations are needed to determine other possible causes behind low fertility indices, such as malnutrition events or drinking water shortages during the breeding period, low male/female ratio, particularities of sexual behaviour and infectious or genetic reproductive pathologies.

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