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Ingestive behaviour, performance, and methane emissions of pregnant alpacas grazing cultivated pastures in the high Peruvian Andes

Laura B. Gualdrón-Duarte [a,b], Cecilia Loza [a,c], José I. Gere [a,d], Nancy F. Huanca-Marca [a,e], Francisco E. Franco [a], Yemi Sanca Uscamayta [a], Rubén García-Ticllacuri [a,f], Guadalupe Orellana Ligas [a], Diannett Benito López [a,g], Feliciano Rivera Pachiño [a], Juan E. Moscoso-Muñoz [h], Medardo A. Díaz-Céspedes [i], Lizbeth L. Collazos Paucar [a,j], César S. Pinares-Patiño [a], Víctor M. Vélez-Marroquín [a,*], Edward H. Cabezas-Garcia [a,k,*]

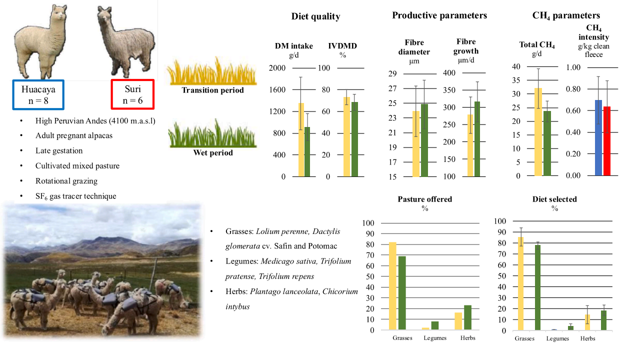
[a] *Estación IVITA—Maranganí, Facultad de Medicina Veterinaria, Universidad Nacional Mayor de San Marcos (UNMSM), Cusco 08258, Peru*
[b] *Departamento de Producción Animal, Instituto Nacional de Tecnología Agropecuaria INTA - Balcarce. Ruta 226km 73.5, Balcarce, Argentina*
[c] *Departamento de Producción Animal y Pasturas, Centro Regional Sur, Facultad de Agronomía, Universidad de la República, Canelones 9000, Uruguay*
[d] *Unidad de Investigación y Desarrollo de las Ingenierías, Universidad Tecnológica Nacional (UTN), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires C1179, Argentina*
[e] *Departamento de Producción Animal y Ciencia de los Alimentos, Universidad de Zaragoza, Miguel Servet 177, Zaragoza 50013, Spain*
[f] *Facultad de Ciencias Agrarias – E P Ingeniería Agroindustrial, Universidad Nacional de Huancavelica, Jr. Victoria Garma N 330, Huancavelica, Peru*
[g] *Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional del Altiplano (UNAP), Puno, Peru*
[h] *Facultad de Ciencias Agrarias, Universidad Nacional San Antonio Abad del Cusco (UNSAAC), Cusco 08006, Peru*
[i] *Facultad de Zootecnia, Universidad Nacional Agraria de La Selva (UNAS), Tingo María 10131, Peru*
[j] *Facultad de Ingeniería, Universidad Nacional Autónoma de Alto Amazonas (UNAAA), Yurimaguas 16501, Peru*
[k] *International Livestock Research Institute (ILRI), PO Box 30709, Nairobi 00100, Kenya*

HIGHLIGHTS

- Season and pregnancy stage were main drivers of alpacas' performance.
- Alpaca variety did not influence neither on forage selectivity or intake.
- Feed intake of alpacas prior to parturition decreased up 1.6 % of live weight.
- Huacaya alpacas tended to emit more methane per unit of metabolic live weight.
- Methane intensity increased up to 0.805 g/kg of clean fleece prior to parturition.

GRAPHICAL ABSTRACT

Overview of experimental conditions and some key findings.



Abbreviations: DMI, Dry matter intake; P1, Transition period; P2, wet period; SAC, South American camelids.

* Corresponding authors.

E-mail addresses: vvelezm@unmsm.edu.pe (V.M. Vélez-Marroquín), E.H.Garcia@cgiar.org (E.H. Cabezas-Garcia).

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ABSTRACT

This study compares grazing patterns, animal performance, and enteric methane emissions (CH_4) of female alpacas (Huacaya and Suri) at two periods of their late pregnancy. Animals were rotationally grazed on a mixed sward at high Peruvian Andes conditions. This study involved two experimental periods (P1 and P2), each lasting 26 days. P1 took place in November 2021, corresponding to the end of dry season ('Transition period'), whereas P2 was conducted in January 2022, with this coinciding with the beginning of rainy season ('Wet period'). Forage selectivity was measured using hand plucking of forage harvested, whereas grazing behaviour was recorded by visual observation. Dry matter intake (DMI) was estimated from total faecal collection and *in vitro* forage digestibility. Fleece characteristics were measured following standard procedures adopted by the local industry. The sulphur hexafluoride (SF_6) gas-tracer technique was used to estimate CH_4 emissions. Data were analysed by a repeated measures model including both alpaca variety and period as fixed effects, whereas alpaca within variety was considered as random. No differences were detected among alpaca varieties either in terms of forage selectivity, grazing behaviour (except for biting rate) or feed intake. Regardless of period, grasses were the main dietary components ($\geq$ 78 %). The proportion of leaves consumed lowered from 84 to 70% and presence of both legumes and herbs increased during P2. Increased nutritional requirements prior to parturition in conjunction with reduced forage quality and DMI ($P = 0.004$) during P2 led to negative energy and protein balances. This was aligned with increases in fibre growth and diameter, prior to parturition ($P \leq 0.035$). Suri displayed faster fibre growth than Huacaya ($P = 0.005$). Although Huacaya females tended to emit more enteric CH_4 per unit of metabolic live weight than Suri ($P = 0.056$), this was not reflected either on variety differences in emissions per unit of intake or fleece produced. On average, CH_4 intensity decreased from 0.805 (P1) to 0.530 g/kg clean fleece at P2 ($P = 0.032$). Results are valuable towards fine-tuning the effect of pregnancy for sustainable alpaca farming.

Introduction

Llamas and alpacas, the domestic species of South American camelids (SAC), form an integral part of livelihoods and traditional farming practices of indigenous communities in the high-altitude Andean region, playing a multifaceted role as sources of fibre, and meat, but also as highly resilient and fully adapted to fragile Andean ecosystems, hence contributing to their conservation (Miranda-de la Lama and Villarroel, 2023). In 2019, global alpaca population was estimated in 4.5 million heads (Wurzinger and Gutiérrez, 2022), with Peru having the largest herd, and also being the main exporter of alpaca fibre (MINCETUR, 2022; MINAGRI, 2019). There are two alpaca varieties coexisting in traditional production systems in Peru, namely, Huacaya and Suri, but the second has remained in small proportions (around 10%) since mid of the 20th century in part due to low appreciation of their fibre in the international textile market (Bustinza Choque, 2023).

Alpaca farming in the Peruvian highlands is primarily conducted through extensive production systems, frequently in mixed flocks with llamas and/or sheep, and employing traditional grazing techniques (herding) on native pastures at high altitude conditions ($\geq$ 3500 m above the sea level; m.a.s.l.). These systems rely heavily on climate conditions for forage production. Climate in this region is characterised by a short rainy season from December to March, with precipitation ranging from 441 to 837 mm, and a long dry season from April to November. Annually, mean temperature can vary from $\leq -5\,^{\circ}$C up to $\geq 15\,^{\circ}$C, reaching up to 20 °C difference within a day (Imfeld et al., 2021). Dry season native grassland vegetation biomass grazed by free-ranging alpacas can experience a 42% dry matter basis decline (Reiner et al., 1987), with this being often encompassed with significant crude protein reduction (often below 8%; Chino Velasquez et al., 2022; Reiner et al., 1987).

Perennial grasses, particularly from *Festuca, Calamagrostis, Stipa, Muhlenbergia, and Poa* genera, constitute the primary vegetation of Andean native grasslands (Farfán Loaiza and Farfán Tenicela, 2012; Flórez Martínez, 2005). However, the decline in both forage availability and nutritional quality, driven by poor management practices (e.g., overgrazing) as well as climate change, poses a significant threat to these ecosystems (Pérez et al., 2010). Although SAC have shown adaptability to harsh conditions of the arid Altiplano (e.g., high temperature variability, UV radiation, low O_2 pressure, etc.), because of accelerated impacts of climate change on these ecosystems, now require a reassessment of livestock management to enhance survival, productivity, and ecological balance (Vélez Marroquín et al., 2021; Pérez et al., 2010).

Over the past decade, government initiatives have been directed towards enhancing alpaca' industry (MINAGRI, 2020). Despite official extension endeavours promoting establishment of cultivated forages for SAC (Bazán Blas et al., 2014; Farfán Loaiza and Farfán Tenicela, 2012), their adoption by local farmers remains notably low and has limited impact, with this being attributed to inadequate support for proper pasture management (Pinares-Patiño et al., 2021). Early studies on forage selection focused in comparing SAC and sheep, both on native grasslands and cultivated pastures (San Martin and Bryant, 1989), but to date no previous research attempted to compare the two alpaca varieties on this regard. The effects of implementation of cultivated mixed pastures (grass and legumes) with these also including herbs (e.g., chicory and plantain) in the diet on alpacas' performance has not been reported in Peruvian highlands conditions. A recent agronomic evaluation conducted in the northern Andes (Cajamarca region) shown that two cultivars of chicory (*Cichorium intybus* L.) and one of plantain (*Plantago lanceolata L.*) are promising to be used as a nutritional supplement in ruminants when associated with grasses (Vallejos-Fernández et al., 2024).

In Peru, it is estimated that the livestock sector contributes up to 15% of the national greenhouse gas (GHG) emissions (MINAM, 2016). Recent research has focused on quantifying *in vivo* enteric methane (CH_4) emissions from domestic ruminants (Díaz-Céspedes et al., 2021; Gómez et al., 2021; Moscoso et al., 2017) and SAC (Febres et al., 2021; Gómez et al., 2021; Roque et al., 2020). However, data remains limited, hindering efforts to improve estimates for more accurate national livestock greenhouse gases (GHG) inventories. Both DM intake (DMI) and diet digestibility are main factors driving enteric CH_4 emissions in ruminants (Ribeiro et al., 2020; van Lingen et al., 2019; Niu et al., 2018; Ramin and Huhtanen, 2013), and in SAC, as the available evidence indicates (Febres et al., 2021; Dittmann et al., 2014; Nielsen et al., 2014; Pinares-Patiño et al., 2003).

Pregnancy is a physiological stage carrying severe changes in feed intake, nutrient requirements and metabolism (Cruz et al., 2017; Burton et al., 2003). Therefore, an improved plane of nutrition, especially at the final stages of pregnancy, positively impacts on an adequate finalization of foetal development and increased milk production post parturition (Folkesson, 2007; Burton et al., 2003). As far as we are aware of, in the high Andes of Southern Peru, there are no studies yet focusing on improving plane of nutrition of late pregnant alpacas by using cultivated mixed pastures. Moreover, the possible effects of alpaca variety (Huacaya and Suri) modulating animal responses and CH_4 emissions at this physiological stage remain unknown, particularly when taking their phenotypic differences into account (Pezo et al., 2014). The objective of this work was to quantify the effects of both alpaca variety and period (season), which in this study is linked to two late pregnancy stages, on ingestive behaviour, animal performance, fleece characteristics, and enteric CH_4 emissions in alpacas when offered a high quality cultivated mixed pasture which includes grasses, legumes, and herbs on a rotational grazing system.

Materials and methods

This study was carried out on an approved license (number: CEBA 2021–35) from the Veterinary Faculty (Universidad Nacional Mayor de San Marcos, Peru), following guidelines provided by their Institutional Animal Care and Use Committee in order to minimize unnecessary animal stress and suffering.

Experimental site

This *in vivo* trial was conducted at the Peruvian high Andes (Puna ecoregion) in cultivated pastures under grazing, in two periods. Pastures were established 11 months prior to commencement of the study (December 2020). First experimental period (P1) was conducted during the annual transition from the dry to rainy season (November 2021; hereafter referred as 'transition period'), whereas the second period (P2) was conducted 60 days apart, at the beginning of rainy season (January 2022; hereafter referred as 'wet period'). Each experimental period comprised of 26 days, with 14 days of acclimatization to the pasture, and 12 days of samples collection. A detailed description of experimental activities (timeline) conducted within each of these periods is presented in Table 1.

The study was located at the 'Punapampa' sector which is part of 'Sangarará' indigenous community (district) in Cusco, Peru (13° 55′ 1.9″ S, 71° 37′ 14.7″ W) at 4141 m above the sea level (m.a.s.l.). Environmental temperature (°C) and relative humidity (%) were recorded every 10 min using an EXTECH RHT10® thermo-hydro USB datalogger (Industrial Electronics, Inc., Knoxville, TN, USA) during the 5-day period when both intake and CH_4 emissions were recorded (Day 15 to 19 in Table 1). Trends for daily variation (hourly mean values) of these weather variables are shown in Fig. S1 of the Supplementary material. During the transition period, average daily temperature was 10.0 °C ± 6.62, and relative humidity 72 % ± 24.8, whereas for the wet period, values were 8.1 °C ± 5.05 and 80 % ± 18.4, respectively. Rainfall was also recorded during the same time frame on a daily basis using a manual rain gauge (0.5 mm scale). Recorded daily rainfall amount was 1.6 ± 0.89 and 2.6 ± 0.98 mm/d on average for the transition and wet periods, respectively.

Pasture establishment and specifications

Pastures used in this study were purposely established on a site that was previously used to cultivate potatoes. This area was not in cultivation over the last two years and hence was covered by a secondary plant community dominated by species belonging to *Festuca, Calamagrostis,* and *Alchemilla* genera, respectively. A total of 2.5 ha of pasture was established. Soil texture was silt loam and the site had a flat relief (slope of $\leq$ 5 %). Ploughing of the land took place at the beginning of the rainy period (November 2020) and was performed to a depth of 40 to 50 cm by using a three-disc plow attached to a CASE IH FARMALL 60A - 40 HP tractor. Manual sowing of the pasture seeds was done during the first week of December 2020, at seeding rate of 37 kg/ha. Seed mix was composed of perennial grasses (68%), legumes (16%) and herbs (16%). Five varieties of perennial ryegrass (*Lolium perenne*) and two cultivars of orchard grass (*Dactylis glomerata*, cv. 'Safin' and 'Potomac'), represented 80 and 20% of grass share in the seed mix, respectively. Lucerne (*Medicago sativa*), red clover (*Trifolium pratense*) and white clover (*Trifolium repens*) respectively contributed 59, 26, and 15% of legume share in the seed mix. In turn, plantain (*Plantago lanceolata*) and chicory (*Chicorium intybus*), each contributed in equal parts to the share of herbs in the mix. The sowing depth was maintained at approximately 2–3 cm using planting rakes. Inorganic fertilizers were applied at sowing (urea, 21 kg N/ha; diammonium phosphate (DAP), 8.2 kg N, and 21 kg of P/ha (P as P_2O_5)).

Following sowing, pasture was monitored for seedlings survival, soil cover, and overall pasture condition. A rain gun sprinkler system was used for pasture irrigation purposes (Fig. S2A in the Supplementary material). Both frequency and volume of irrigation were contingent upon weather conditions. Despite this, pasture establishment was affected by severe drought and frosts during the dry season. Of the new pasture (2.5 ha), a subarea of 1.0 ha was chosen for conducting this study (P1 and P2) based on the higher and uniform forage biomass production. Prior to the beginning of each experimental period, the experimental sward (1.0 ha) was sequentially trimmed in strips previously defined to an average height of 2 inches using a string trimmer, and DAP subsequently top-dressed at a rate of 9 kg N and 23 kg of P_2O_5 per hectare. The objective was for pasture breaks to typically have a growing period of 60 days on average before grazing (rest period). Given that SAC consistently defecate and urinate in the same spots, excreta piles were uniformly spread across the pasture, and this labour was combined with irrigation and chemical fertilisation.

Table 1
Timetable of activities conducted during each of the experimental periods.

Days of the experiment	Activities
	Preparation of grazing paddocks
−1	Measure forage biomass for adjustment of grazing strip areas for alpacas on a rotational grazing system.
−1	Setting up of the grazing strips with portable solar-powered electric fence and watering trough.
	Adaptation phase
1 – 11	Alpacas enter daily into the grazing strips from 7:00 to 17:00 without additional experimental routines on them[a].
12	Harnesses and bags for both faecal output and CH_4 samples collection were placed on the alpacas to become accustomed to these[a].
12 – 14	Alpacas get used to harnesses and bags prior samples collection[b].
	Samples collection
15 – 19	Estimation of enteric CH_4 emissions by the SF_6 gas-tracer technique[c].
15 – 19	Measures of total daily faecal output using sampling collection bags.
16, 18, 20	Hand plucking of forage samples imitating grazing behaviour of each individual alpaca[d].
21	Measure of fibre growth and fleece characteristics on each individual animal.
22, 24, 26	Visual assessment of morning ingestive behaviour (8:00 to 12:00).
26	End of experimental period.

[a] The experimental animals were withdrawn daily from grazing paddocks at 17:00 and moved to a corral shelter nearby (located no more than 200 m distance) until to their return the following day in the morning (7:00).

[b] The faecal samples collected during this period were discarded.

[c] Five days of collection period counted from the assembly of the SF_6 collection lines and vessels until the removal of the gas collection system.

[d] Up to five individual alpacas were evaluated per day for their selective grazing. Samples of plant species and their parts were hand plucked from 25 grazing stations per animal within the grazing strip.

Experimental design

Adult animals, eight Huacaya and six Suri females (5.1 ± 1.89 years old and 8.4 ± 0.35 months of pregnancy at the beginning of P1), were obtained from a mixed (alpacas and llamas) extensive grazing production system in May 2021 and moved to the experimental pasture in September 2021. Previous to the commencement of this study, animals were grazing on native grasslands dominated by *Calamagrostis eminens, Calamagrostis. vicunarum, Festuca dolichophylla*, and *Muhlenbergia fastigiata* (without concentrate supplementation) at 'La Raya' Research Station (4200 m.a.s.l.; 14°28′55''S; 70°59′22''W). Pregnancy status was confirmed before moving alpacas to the experimental area by transrectal ultrasound examinations. Upon arrival to the new location, each individual animal underwent a comprehensive veterinary examination, which included weighing and deworming, to ensure their optimal health status.

The day before of the commencement of each period (Day = - 1), paddocks were set up on rotational grazing (3-day strip occupation) with portable solar-powered electric fence and watering trough (Table 1). Area of grazing strips was controlled by back and front fences, with this being adjusted when required depending on the forage biomass, to guarantee an herbage allowance of at least 2.5 % live weight on a DM basis) per alpaca per day. This is assumed to be unconstrained forage allocation when high-quality pastures are offered (Van Saun, 2014). Alpacas were kept in the grazing area from 7:00 to 17:00. Thereafter, they were moved to a night enclosure (a corral shelter) for protection from predators (e.g., pumas, foxes, etc.) and extreme weather conditions (e.g., frosts, extreme winds). No concentrate supplementation was offered to the experimental animals.

Herbage measurements

Herbage on offer was measured using quadrats of 0.25 m^2. Forage within these quadrats were cut above of 5.0 cm from ground using hand-operated grass clippers. Plant biomass collected within each quadrat was immediately weighed and then pooled across the quadrats ($n = 10$ samples). A subsample of approximately 1 kg (fresh basis) of the pooled forage was used to determine botanical composition of the pasture, whereas two other subsamples of harvested forage (approximately 200 g, each), for each individual quadrat, were used to determine DM contents by oven-drying at 60 °C for 48 h, and then ground through a 1-mm sieve. A single composite pooled sample of the 10 individual quadrats was obtained and stored for chemical composition analyses. Botanical composition was determined visually by uniformly spreading the fresh herbage after field collection on a 100 square grid (surface: one square meter). Within each grid, three forage attributes were measured to obtain a total of 100 measurements per attribute (% contribution): 1. functional groups (grasses and graminoids, legumes, and herbs), 2. plant parts (leaf, stem, or inflorescence), and 3. green and senescent biomass, according to Hodgson et al. (1994).

Grazing behaviour

Grazing behaviour was recorded for each individual alpaca by visual observation every other day in three different days (Days 22, 24, and 26 in Table 1) during morning grazing period (8:00 to 12:00). This assessment was not conducted on the afternoon to avoid risk to observers and disturbance in animal behaviour arising from high likelihood of lightning strikes. Observations were recorded every 10 min according to Silva et al. (2006). Recorded animal activities included: grazing, ruminating, drinking, resting, and idling. The number of bites per minute (biting rate) was recorded once at the end of each hour of measurements interval.

Forage selectivity

During days 16, 18, and 20, of each experimental period (Table 1), 25 grazing stations visited by each alpaca were sampled using the hand-plucking method (Wallis De Vries, 1995). Each day were sampled up to 5 animals (randomly chosen) until completing the total of 14 females in the three evaluation days. Grazing stations were defined as the semi-circle area in front of each alpaca (30 cm diameter) within which the animal grazed before it stops to eat and move. These spots were identified by 'flags' that were nailed to the ground and a distinct colour was used for each individual animal. Forage samples (≈ 30 to 50 g) were collected manually at each point (Fig. S2B in the Supplementary material), mimicking the grazing depth of the animal (≈ 1-inch height). Fresh forage samples (pooled per animal) were taken to the laboratory for evaluation of botanical composition as described above. The Steinhaus or Sorensen/Czekanowsky coefficient (S_s) was used to evaluate the percentage of similarity in forage selectivity performed by alpaca variety as described by (Kent, 2011), following the equation:

$$Ss = 2a/2a + b + c$$

where a = number of species common to both quadrats/samples from each alpaca variety, b = number of species in quadrat/sample for the first variety only, and c = number of species in quadrat/sample from the second variety only. The obtained coefficient is then multiplied by 100 to give a percentage for the similarity index value. After botanical composition and forage selectivity evaluations, two subsamples (≈ 200 g each) were oven dried, ground, and stored for later wet chemistry analyses as previously mentioned.

Faecal output and forage intake

Measurements of daily faecal output (g/d) were performed throughout the first five days of sampling collection period (day 15 to 19, Table 1) by using faecal collection bags with a harness system attached to the perineal area of alpacas. Routinely, faeces collected in the bags were weighed daily at 7:30 for assessing total faecal production, and representative samples of approximately 200 g were taken and immediately frozen at −20 °C for later dry matter and chemical analyses. Since collection of faecal samples from female alpacas often come together with urine, nylon braided multifilament thread-faecal bags with a pore size of 5 mm × 5 mm were used and thus most of urine was separated from faeces by gravity. Dry matter intake (DMI; g/d) was calculated as (Lippke, 2002):

$$DMI = \frac{FO}{(1 - IVDMD)}$$

where, FO is total daily faecal output (g of DM), and IVDMD is *in vitro* DM digestibility of forages (%) as later described.

Chemical composition, diet digestibility analyses, and estimated balances of nutrients

Forage (offered and selected) and faecal samples were dried in a forced-air oven at 60 °C for 48 h or until constant weight. Dry samples were ground in a knife mill using a 1-mm screen. Afterward, wet chemistry analyses were performed as follows according to official AOAC methods (2000): ash (method 942.05), crude protein (CP; method 968.06), ether extract (EE; method 920.39). Ash content was determined after 6 at 500 °C in a muffle furnace. Ether extract was extracted with petroleum ether after acid hydrolysis to recover saponified fat (Soxhlet System HT Tecator, Hillerød, Denmark). Both neutral detergent fibre (NDF) and acid detergent fibre (ADF) fractions were measured in an ANKOM Fibre Analyser (A220, ANKOM Technologies, Fairport, NY, USA) according to (Mertens et al. (2002) and AOAC (2000), respectively. The NDF fraction was determined with the inclusion of sodium sulphite and alpha amylase (aNDF). All nutrients are expressed as percentages of DM. Gross energy concentrations in megajoules (MJ) per kg of DM (GE; MJ/kg DM) of forages were estimated from analysed chemical composition according to Jentsch et al. (2003):

$$GE = \frac{[(23.6 \times CP) + (39.8 \times EE) + (17.3 \times NFC) + (18.9 \times aNDF)]}{100}$$

where, the dietary concentrations of nutrients are expressed in percentage (% of DM) and coefficients in MJ per kg of DM. Non-fiber carbohydrates fraction (NFC; % DM) was calculated by difference (NRC, 2001) as: NFC = 100 – (aNDF + CP + EE + Ash). Forage digestibility was determined *in vitro* by using the Daisy^{II} incubator following recommended procedures provided by the manufacturer (ANKOM Technology Corp., Fairport, NY). Each of the *in vitro* incubation of forages was conducted for 48 h using a mixture of bovine ruminal fluids collected from three slaughtered cattle. Rumen fluid was gathered immediately during the evisceration process while still warm and stored in pre-warmed thermal flasks which were filled to the top. The inoculum was transported to the laboratory within 15 to 20 mins, ensuring optimal conditions before starting the anaerobic incubations. Metabolisable energy contents (ME; MJ/kg DM) of forages were assessed by the equation proposed by Givens et al. (1990) which was obtained from sheep data:

$$ME = 0.37 + (0.142 \times DOMD) + (0.077 \times CP)$$

where, DOMD = digestible organic-matter content of the dry matter (% of DM), and CP = crude protein contents (% of DM). DOMD was estimated from IVDMD according to CSIRO (2007) as: (0.84 × IVDMD) + 7.32. Estimated nutritional balances (ME and CP) were computed as the difference between the nutrient consumed (input) and the sum of nutritional requirements for maintenance, activity, pregnancy stage, and fibre production (outputs) following a factorial approach (Van Saun, 2014). Further details on calculations and assumptions made are presented with examples (Tables S1 and S2 in the Supplementary material for the ME and CP balances, respectively).

Enteric CH_4 emissions

Total CH_4 emissions from alpacas were measured during five days concurrently with total faecal collection (day 15 to 19; Table 1) by using the sulphur hexafluoride (SF_6) gas-tracer technique (Johnson and Johnson, 1995), which was modified by Gere and Gratton (2010) and after validated by Pinares-Patiño et al. (2012). Pre-calibrated permeation capsules containing the SF_6 gas at a known constant release rate (5.5 ± 1.0 mg/d, on average) were inserted orally with a bolus applicator into compartment one (C-1 forestomach) of each alpaca at least 20 days before commencement of the first experimental period (P1). These capsules were manufactured in the Animal Nutrition Lab at Universidad Nacional San Antonio Abad del Cusco (UNSAAC; Cusco, Peru) following recommendations provided by Loza et al. (2024). Breath sampling from each individual animal was carried out continuously (over the 5-day gas collection period) and in duplicate. Two PVC collection canisters (1L volume each) were mounted on each animal using an adjustable vest (Deighton et al., 2020); Fig. 2C in the Supplementary material).

Background SF_6 and CH_4 concentrations were determined using two sampling sets, identical to those used with experimental animals, placed in the field near to the grazing flock. Breath and background gas samples were analysed for CH_4 and SF_6 concentrations by gas chromatography (Perkin Elmer, Clarus 680), equipped with an electron capture detector (ECD) and a flame ionization detector (FID) following methodology by Gere et al. (2019). Daily CH_4 emissions were calculated from SF_6 release rate and the ratio between CH_4 and SF_6 concentrations in breath samples, after correction for background gas concentrations (Loza et al., 2024):

$$CH_4 = PRSF_6 \times \frac{[CH_4 - BGCH_4]}{[SF_6 - BG_{SF6}]}$$

where, CH_4 is total enteric methane emission (in g/d), $PRSF_6$ is release rate of the SF_6 of each capsule in the C-1 forestomach (g/d), both $[CH_4]$ and $[SF_6]$ refers to concentrations of these gases in the breath sample. Finally, $[BG_{CH4}]$ and $[BG_{SF6}]$ are background atmospheric concentrations of CH_4 and SF_6 respectively. Atmospheric pressure measured from empty collection vessels at the beginning of both experimental periods averaged 608 mbar.

Live weight and fleece production

Alpacas were weighed at the beginning and at the end of each experimental period after an overnight fast on a digital scale (Tru-TestTM - Econo Plus; Auckland, NZ) connected to a platform ramp and monitored for fleece growth at day 21 of each experimental period (Table 1). Fibre samples were collected from two different areas. On the right side of the animal, centred between the 5th and the 6th rear rib (central saddle) was taken as a fleece sampling site (ca. 10 cm × 10 cm), and fibre clipped as described by Wuliji et al. (2000), using small animal clippers with a comb Size 30. A previous cut was performed 28 days previous to monitor fleece growth at each experimental period. Fibre collected for each animal was weighted to get it expressed as the daily rate of greasy fleece production (g/d). Clean fleece weight was obtained after carefully washing, drying, and picking the initial greasy samples according to the procedures described in Pezo et al. (2014). On the left side of the animal, in the central saddle area, growth fibre in longitude (μm/d) was measured using a Vernier calliper following the dye banding technique (McCloghry, 1997). A fibre sample was cut at skin level and then minicored at 2 mm snippets to be analysed using an Optical Fibre Diameter Analyser (OFDA 2000), as described by Brims et al. (1999), to determine fibre diameter (μm).

Statistical analyses

Grazing behaviour, feed intake, live weight, fibre growth, and CH_4 emissions data were analysed by the MIXED procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC) according to the following model (2 × 2 factorial arrangement):

$$Y_{ijkl} = \mu + V_i + P_j + A_k(V_i) + V_iP_j + e_{ijkl}$$

where Y_{ijkl} = response variable; μ = overall mean; V_i = fixed effect of alpaca variety i; P_j = fixed effect of period j; A_k (V_i) = random effect of alpaca k within variety i; V_iP_j = interaction of variety and period; and e_{ijkl} = residual, normally distributed. Individual alpaca within variety was included in the REPEATED statement. Denominator degrees of freedom were estimated by using the Kenward-Roger option in the MODEL statement. The main effects of alpaca variety (V) and experimental period (P), along with their interaction ($V \times P$), were significant at $P \leq 0.05$ and showed trends at $P \leq 0.10$. All data are expressed as least squares means (LSM) and the pooled standard error of the means (SEM).

Results

Botanical composition of grazing paddocks offered to alpacas are outlined in Table 2. In both experimental periods, grasses were predominant in the pasture, followed by herbs and legumes. The share of grasses in total forage on offer decreased by 13% from transition to the wet period, whereas the share of legumes, plantain, and chicory increased by 6 %, 2 %, and 5 %, respectively. These trends were encompassed by a decreased proportion of leaves (- 4 %) and an increased green biomass (+ 5 %). Chemical composition of offered swards to the experimental animals is shown in Table 3. Wet period pastures displayed lower CP (- 1.5 % DM), estimated ME contents

Table 2

Composition of plant functional groups, plant parts, phenological stage, and forage biomass of the pasture forage offered to pregnant alpacas during the transition and the wet experimental periods at the high Peruvian Andes.

Item[a]	Period[b]	
	Transition	Wet
Functional group (%)		
Grasses	82.0	69.0
Legumes	2.0	8.0
Plantain	9.0	11.0
Chicory	7.0	12.0
Parts of the plant (%)		
Leaves	74.0	70.0
Stems	25.0	26.0
Inflorescence	1.0	4.0
Phenological stage (%)		
Green	74.0	79.0
Senescent	26.0	21.0
Forage biomass (kg DM/ha)[c]	2,000 ± 300	1,888 ± 471

[a] Botanical composition of the herbage on offer (% on a fresh basis) was obtained from a single composite sample harvested from the fresh biomass collected in ten quadrats of 0.25 m^2 (forage cut above of 5.0 cm from ground) which were randomly chosen for the total experimental area (1.0 ha).

[b] The experimental periods in this study were aligned with two stages of late pregnancy: 8 to 9 months (*'Transition'*) and 10 to 11 months (*'Wet'*), respectively.

[c] Mean ± SD values calculated from individual values obtained from each of the ten quadrats of 0.25 m^2 of the herbage on offer after determination of their DM contents.

Table 3

Dry matter contents, chemical composition, and digestibility of the pasture forage offered to pregnant alpacas during the transition and the wet experimental periods at the high Peruvian Andes.

Item[a]	Period[b]	
	Transition	Wet
Nutrient (in % DM unless otherwise stated)		
Dry matter (%)	34.8	34.4
Ash	6.0	4.6
Crude protein	9.0	7.5
Neutral detergent fibre (aNDF)[c]	39.0	38.3
Acid detergent fibre	19.6	20.0
Ether extract	1.5	1.3
Non-fibrous carbohydrates[d]	44.5	48.3
Gross energy (MJ/kg DM)[e]	17.8	17.9
Metabolisable energy (MJ/kg DM)[f]	10.5	9.8
ME/GE[g]	0.59	0.55
Forage digestibility (%)[h]		
IVDMD	70.6	65.7
IVOMD	75.1	68.9

[a] Nutritional composition and *in vitro* digestibility of the herbage on offer was obtained from a single composite sample harvested from the fresh biomass collected in ten quadrats (0.25 m^2 each) taken from the experimental area (1.0 ha) to calculate its forage biomass production.

[b] The experimental periods in this study were aligned with two stages of late pregnancy: 8 to 9 months (*'Transition'*) and 10 to 11 months (*'Wet'*), respectively.

[c] Neutral detergent fibre assayed with a heat-stable amylase and expressed inclusive of residual ash (Mertens, 2002).

[d] Estimated by difference according to NRC (2001).

[e] Estimated from chemical composition using the equation proposed by Jentsch et al. (2003).

[f] Estimated from the digestible organic-matter content of the dry matter (DOMD, %) and crude protein contents according to Givens et al. (1990).

[g] Diet metabolisability (AFRC, 1993).

[h] *In vitro* digestibilities of the dry (IVDMD) and organic matter (IVOMD) obtained from forage samples after incubation in bovine rumen fluid for 48 h.

(−0.70 MJ/kg DM), and IVDMD (- 4.9%) compared with transition period pastures with this reflecting on decreased diet metabolisability (9.8 vs 10.5 ME/GE, for the referred periods respectively).

Botanical characteristics of the forage selected by pregnant alpacas is presented in Table 4. For all of these variables, the effect of alpaca variety × period interaction was not significant ($P \geq 0.306$). Overall, diets consumed by the experimental animals had a greater share of grasses in their diet, followed by herbs, and, to a much lesser extent, legumes. Wet period led to a decreased proportion of grasses (- 7.7 %), and increased participation of legumes (+ 3.7 %) and plantain (+ 4.8 %), whereas the share of chicory slightly decreased (- 0.8 %). Except for inflorescence, there were period effects in the share of plant parts in the diet ($P < 0.001$). The proportion of leaves was higher during the transition period than in the wet period, whereas the opposite was observed for stems. In turn, the share of green forage material was higher in transition than in the wet period, whereas the opposite was observed for the share of senescent material. Alpaca varieties did not differ in the share of botanical functional groups or plant parts selected ($P = 0.546$). However, diets of Huacaya alpacas had higher presence of green material than of their Suri counterparts ($P \geq 0.030$). Values for S_s indexes (Steinhaus) were rather close to 1.0 for all parameters including functional group, parts of the plant, and phenological stage and no significant differences were observed among alpaca varieties.

Grazing and ingestive behaviour variables are shown in Table 5. On average, grazing, rumination and resting times accounted for 77 %, 7.8 %, and 15.7 % within the evaluated 4-h morning grazing time frame (8:00 to 12:00). There were no alpaca variety × period interaction effects on grazing and ingestive behaviour ($P \geq 0.149$). However, rumination time during transition period by Huacaya alpacas was longer than that of Suri ($P = 0.050$). Neither there were effects of alpaca variety or period on the behaviour parameters ($P \geq 0.266$), exception was that Huacaya alpacas had higher rate of biting than Suri counterparts ($P = 0.046$) and that rate of biting during transition period was 13.6 bites per minute faster than that observed during wet period ($P < 0.001$).

Live weight (LW), LW change, feed intake (DM and its constituents), diet metabolisability, *in vitro* digestibility, and balances of ME and CP are presented in Table 6. There were no variety or variety × period effects on the above-mentioned variables ($P \geq 0.235$). Period did not affect either mean live weight of alpacas or metabolisability of gross energy intake (ME/GE). On the contrary, there were period effects on LW change, feed intakes (and chemical constituents), and balances of ME and CP. Alpacas lost live weight during the transition period (P1; Suri having numerically much higher live weight loss), whereas they had a live weight gain during the wet period (P2; $P = 0.013$). Feed intakes were significantly higher during transition period ($P \leq 0.053$). In line with the latter, estimated balances of ME and CP were positive, whereas the balances were negative during the wet period ($P \leq 0.001$). The *in vitro* digestibility of the diet was not affected by variety, period, or variety × period effects ($P \geq 0.408$). Although there was a numerical reduction in IVDMD for the wet period diets compared to those during transition period (73.1 vs 68.5 % on average, respectively), no statistical difference was detected with this displaying considerable variation among animals (CV = 9.5 and 8.9 % on average for P1 and P2, respectively).

Fleece characteristics (fibre growth, greasy and clean fleece weight, and fibre diameter) are shown in Table 7. There was no alpaca variety × period interaction effect on these variables ($P \geq 0.109$). Period did not affect either grease or clean fibre growth ($P \geq 0.179$), whereas alpaca varieties differed ($P = 0.020$) in grease fibre growth (Huacaya having higher growth than Suri). Fibre growth expressed as length (μm/d) was faster in Suri than Huacaya ($P = 0.005$). Increased fibre length was observed during the wet period (+ 38.5 μm/d on average) when compared to transition ($P = 0.028$). Fiber diameter (μm) increased during the wet period ($P = 0.035$), but there was no effect of alpaca variety on this production trait ($P = 0.161$).

Metrics for enteric CH_4 emissions are presented in Table 8. There

Table 4
Composition of plant functional groups, plant parts and phenological stage of the forage selected by pregnant alpaca varieties (Huacaya and Suri) during the transition and the wet experimental periods at the high Peruvian Andes.

Item	Period[a]	Variety		SEM	*P*-value		
		Huacaya	Suri		Variety	Period	*V* × *P*
Functional group (%)							
Grasses	Transition	85.3	85.5	2.25	0.614	<0.001	0.499
	Wet	78.8	76.7	0.88			
Legumes	Transition	0.25	0.17	0.166	0.723	<0.001	0.850
	Wet	4.00	3.74	0.635			
Plantain	Transition	11.4	11.2	2.27	0.827	0.016	0.701
	Wet	15.5	16.6	1.28			
Chicory	Transition	3.13	3.17	1.02	0.546	0.478	0.510
	Wet	1.69	3.11	1.13			
Parts of the plant (%)							
Leaves	Transition	85.0	82.5	2.77	0.683	<0.001	0.406
	Wet	70.4	70.4	1.85			
Stems	Transition	12.0	14.8	3.07	0.668	<0.001	0.306
	Wet	26.3	26.2	1.38			
Inflorescence	Transition	0.79	0.91	0.838	0.608	0.364	0.932
	Wet	0.84	0.83	0.832			
Phenological stage (%)							
Green	Transition	90.0	86.5	2.29	0.030	0.002	0.949
	Wet	80.7	77.5	1.06			
Senescent	Transition	10.0	13.5	2.29	0.030	0.002	0.949
	Wet	19.3	22.5	1.06			
Steinhaus Index (S_s)[b]	Transition	Wet					
Functional group	0.97	0.98					
Parts of the plant	0.97	0.98					
Phenological stage	0.97	1.00					

[a] The experimental periods in this study were aligned with two stages of late pregnancy: 8 to 9 months (‘*Transition*’) and 10 to 11 months (‘*Wet*’), respectively.

[b] S_s = Steinhaus coefficient evaluates the percentage of similarity on forage selectivity performed by two alpacas’ varieties (comparison) at two different periods.

Table 5
Grazing and ingestive behaviour during the morning grazing period (8:00 to 12:00) by pregnant alpaca varieties (Huacaya and Suri) during the transition and the wet experimental periods at the high Peruvian Andes.

Item[a]	Period[b]	Variety		SEM	*P*-value		
		Huacaya	Suri		Variety	Period	*V* × *P*
Grazing (min)	Transition	198	200	8.13	0.529	0.384	0.149
	Wet	201	185	9.06			
Rumination (min)	Transition	23.9	10.6	4.66	0.417	0.266	0.050
	Wet	19.6	25.4	4.09			
Resting and idling (min)	Transition	38.5	37.2	5.51	0.691	0.397	0.498
	Wet	39.5	46.2	6.93			
Biting rate (bites/min)	Transition	44.0	41.3	0.99	0.046	<0.001	0.878
	Wet	30.3	27.8	1.25			

[a] Only one drinking event for an individual animal was recorded during the six morning evaluations performed (three days for each experimental period).

[b] The experimental periods in this study were aligned with two stages of late pregnancy: 8 to 9 months (‘*Transition*’) and 10 to 11 months (‘*Wet*’), respectively.

were no alpaca variety × period interaction effects ($P \geq 0.478$) on CH_4 emissions expressed either on absolute basis (g/d) or emissions expressed per unit of metabolic live weight (g/kg $LW^{0.75}$), or feed intake (g/kg DM or % of GEI), as well on clean fleece growth basis (g/kg). Alpaca variety did not influence any of CH_4 emission metrics ($P \geq 0.111$), except that Huacaya alpacas tended to have larger emissions per unit of metabolic live weight than their Suri counterparts ($P = 0.051$). Total CH_4 emissions during transition period were significantly higher ($P = 0.041$) than that during wet period (32.0 vs 23.6 g/d on average, respectively). Nevertheless, CH_4 yield (either per unit of feed DM or GE intakes) did not differ with period ($P \geq 0.968$). There were differences between periods in CH_4 emissions expressed per unit of metabolic live weight size and per unit of clean fleece weight, with emissions being considerably higher during transition period ($P \leq 0.032$).

Discussion

Grazing management, period, and animal effects on diet composition and ingestive behaviour

Although forage DM contents were rather similar for both grazing periods (34.6 % on average), pasture forage biomass (kg DM/ha) decreased up to 16 % during wet period and this was despite of the greater precipitation recorded. It was expected that with increased precipitation, cultivated pasture maintained on moderate levels of grazing pressure and at the same fertilization rate, would produce greater total forage mass than the measured during transition period (Patton et al., 2007). This is explained by decreased proportion of grasses during wet period (69 vs 82 % for wet and transition period, respectively). In mixed swards, grasses usually produce larger biomass per hectare meter than legumes and herbs (Moloney et al., 2020).

Decreased proportion of grasses from transition to the wet period may indicate a slow post-grazing recovery and that warmer and wetter conditions favoured the growth of non-grass species. Also is plausible

Table 6
Mean live weight and LW change, feed intake (of DM and chemical constituents), *in vitro* digestibility of the diet consumed, and estimated metabolisable energy and crude protein balances for pregnant alpaca varieties (Huacaya and Suri) during the transition and the wet experimental periods at the high Peruvian Andes.

Item	Period[a]	Variety		SEM	*P*-value		
		Huacaya	Suri		Variety	Period	*V* × *P*
Live weight (kg)	Transition	56.3	58.9	2.05	0.443	0.463	0.932
	Wet	57.4	59.8	2.72			
LW change (g/d)[b]	Transition	−42.5	−120	37.6	0.235	0.013	0.305
	Wet	20.8	16.7	27.6			
Feed intake and diet metabolisability							
Dry matter (g/d)	Transition	1344	1361	190.5	0.540	0.004	0.461
	Wet	809	1013	89.1			
DM (as a % of LW)	Transition	2.37	2.25	0.28	0.641	0.001	0.324
	Wet	1.40	1.77	0.20			
DM (g/kg LW $^{0.75}$)[c]	Transition	65.1	62.7	7.99	0.612	0.007	0.351
	Wet	38.6	48.4	5.04			
Organic matter (g/d)	Transition	1255	1274	176.2	0.531	0.004	0.462
	Wet	763	953	82.1			
Crude protein (g/d)	Transition	133	130	21.4	0.669	0.005	0.452
	Wet	68.5	88.6	10.7			
Neutral detergent fibre (aNDF, g/d)	Transition	524	509	84.1	0.640	0.014	0.422
	Wet	296	383	46.2			
Acid detergent fibre (g/d)	Transition	276	268	44.3	0.604	0.053	0.386
	Wet	178	227	27.4			
Ether extract (g/d)	Transition	17.7	17.1	2.83	0.658	0.004	0.364
	Wet	9.6	12.5	1.15			
Gross energy (MJ/d)	Transition	23.7	24.1	3.38	0.542	0.004	0.457
	Wet	14.3	17.9	1.58			
Metabolisable energy (MJ/d)	Transition	15.2	14.8	2.40	0.640	0.007	0.439
	Wet	8.3	10.7	1.24			
ME/GE[d]	Transition	0.63	0.60	0.019	0.695	0.106	0.422
	Wet	0.57	0.58	0.021			
Forage digestibility (%)[e]							
IVDMD	Transition	74.9	71.3	2.65	0.698	0.137	0.415
	Wet	67.8	69.1	2.98			
IVOMD	Transition	80.1	76.1	3.01	0.699	0.125	0.408
	Wet	71.9	73.5	3.37			
Balances of nutrients[f]							
ME balance (MJ/d)	Transition	4.79	4.11	2.26	0.736	<0.001	0.456
	Wet	−7.09	−5.05	1.32			
CP balance (g/d)	Transition	34.2	26.9	19.8	0.807	<0.001	0.513
	Wet	−79.6	−64.0	12.7			

[a] The experimental periods in this study were aligned with two stages of late pregnancy: 8 to 9 months (‘*Transition*’) and 10 to 11 months (‘*Wet*’), respectively.
[b] LW change = (LW end – LW start) / 26 days of experimental period.
[c] $LW^{0.75}$ = metabolic live weight.
[d] Diet metabolisability (AFRC, 1993).
[e] *In vitro* digestibilities of the dry (IVDMD) and organic matter (IVOMD) obtained from forage samples after anaerobic incubation in bovine rumen fluid for 48 h.
[f] Estimates of metabolisable energy (ME) and crude protein (CP) balances were performed using equations in Van Saun (2014). Further details can be found in the supplementary material.

Table 7
Fibre growth, weight, and diameter during the 26-day measurement period for pregnant alpaca varieties (Huacaya and Suri) during the transition and the wet experimental periods at the high Peruvian Andes.

Item	Period[a]	Variety		SEM	*P*-value		
		Huacaya	Suri		Variety	Period	*V* × *P*
Fibre growth (μm/d)	Transition	237	319	12.5	0.005	0.028	0.288
	Wet	293	340	20.7			
Greasy fleece weight (g/100cm^2)	Transition	2.07	1.62	0.133	0.020	0.179	0.720
	Wet	2.22	1.71	0.144			
Clean fleece weight (g/100cm^2)[b]	Transition	1.61	1.36	0.115	0.138	0.492	0.741
	Wet	1.64	1.46	0.113			
Fibre diameter (μm)	Transition	22.3	25.5	1.18	0.161	0.035	0.109
	Wet	23.9	25.8	1.24			

[a] The experimental periods in this study were aligned with two stages of late pregnancy: 8 to 9 months (‘*Transition*’) and 10 to 11 months (‘*Wet*’), respectively.
[b] Fibre samples were cleaned following standard procedures as described in Pezo et al. (2014).

that at high altitude conditions, perennial grasses are of slower establishment than dicotyledons (Bartl et al., 2009; Dong et al., 2007). In this study, mean pasture forage mass was found in the range 1900 – 2000 kg DM/ha, which is slightly higher than that (1600 kg DM/ha) presented by San Martín (1987) for the Altiplano region (4000 m.a.s.l.), whereas it is lower than that (3040 kg DM/ha) reported by Salas-Riega et al. (2022) at a lower altitude (3260 m.a.s.l.) in the Central Peruvian Andes, for similar post-grazing resting periods.

Reductions in CP contents and *in vitro* digestibility of the forage on offer during wet period could be attributed to the slightly increased

Table 8

Enteric CH_4 emissions by pregnant alpaca varieties (Huacaya and Suri) during the transition and the wet experimental periods at the high Peruvian Andes.

Item	Period[a]	Variety		SEM	*P*-value		
		Huacaya	Suri		Variety	Period	*V* × *P*
Total CH_4 (g/d)	Transition	34.2	29.8	3.37	0.111	0.041	0.870
	Wet	25.2	22.0	1.92			
CH_4 (g/ kg $LW^{0.75}$)[b]	Transition	1.69	1.30	0.167	0.051	0.031	0.478
	Wet	1.21	1.03	0.058			
CH_4 yield (g/kg DMI)	Transition	31.3	26.5	8.65	0.310	0.968	0.668
	Wet	34.7	23.7	4.93			
Y_m (% of GEI)[c]	Transition	9.85	8.36	2.68	0.314	0.978	0.669
	Wet	10.9	7.45	1.56			
CH_4 intensity (g/kg clean fleece)[d]	Transition	0.852	0.757	0.130	0.566	0.032	0.754
	Wet	0.544	0.515	0.055			

[a] The experimental periods in this study were aligned with two stages of late pregnancy: 8 to 9 months ('*Transition*') and 10 to 11 months ('*Wet*'), respectively.

[b] Methane emissions on a metabolic live weight basis.

[c] Y_m = Methane conversion rate indicates the fraction of the estimated gross energy intake partitioned into CH_4 energy. The estimated GE intake was calculated as: GEI = DMI × GE (Jentsch et al., 2003). The calorific value of 55.65 MJ/kg CH_4 was used to estimate the energy emitted as enteric methane (Niu et al., 2018).

[d] Calculations were performed on a clean fleece weight basis by extrapolating the amount the daily amount of CH_4 emitted per alpaca (g/d) to one kilogram of clean fleece produced. Measurements of fleece weight taken from the central saddle of the animal (10 cm × 10 cm square; g/d) were used for obtaining this metric.

stem/leaf ratio and the shift of nutrients to inflorescences. However, no substantial differences were observed in the proportions of structural carbohydrates (aNDF and ADF) despite biomass of wet period contained higher green material and lower senescent material than that observed at transition. There are few studies comparing dietary preferences of alpacas under cultivated pastures. Similar to the study conducted by Pinares-Patiño et al. (2013), pregnant alpacas in this study preferred leafier material and tended to avoid legumes consumption. Regardless of period, percentages of similarity on forage selectivity observed for both alpaca varieties, as assessed by the Steinhaus coefficient (Ss) on hand plucked samples, were rather high ($\geq$ 0.97). To compensate for reduced proportion of leaves in the pasture on offer in P2, alpacas selected increased proportion of legumes (3.9 vs 0.2 %, for the wet and the transition periods, respectively). The larger share of plantain in the diet of alpacas compared to that of chicory observed is a first report of its kind. Although there is evidence supporting that chicory could be a highly palatable forage for sheep (Niderkorn et al., 2019) and increased IVDMD has been observed in chicory cultivars when compared to plantain in high Andes conditions (Vallejos-Fernández et al., 2024), the reasons for alpacas' low selection observed in this study remain to be understood.

Ingestive behaviour was not affected by as dictated by seasonal forage availability (period) as it could be expected in native Andean forages with more contrasting differences in terms of their nutritional quality (Bryant et al., 1989; Pfister et al., 1989; Bryant and Farfan, 1984). Both varieties of alpaca spent much more time grazing than rumination and resting and idling (77, 7.8, and 15.7% of time spent on average in each activity from 8:00 to 12:00, respectively). In a study conducted in New Zealand with both alpaca females with young at foot and non-lactating sheep offered mixed pastures, the authors found that during the 7:00 to 13:00 timeframe, 63% of time spent by alpacas was on grazing and few rumination events were recorded (Sharp et al., 1995). Although increased proportion of greener material consumed by Huacaya alpacas during both periods are in line with their increased biting rate, this forage preference was not reflected in differences either on DMI or digestibility. Biting rates displayed by both varieties of alpacas during the transition period (44 and 41 bites/min for Huacaya and Suri, respectively) are similar with the 41 bites/min reported by Sharp et al. (1995).

Live weight change, feed intake, estimated balances of nutrients, and in vitro digestibility of the diet

Live weight losses of pregnant alpacas during transition period ($\approx$ 8 months of gestation at the beginning of P1) are not consistent with observed feed intake. Although LW loss and a decline in body condition score are common clinical signs of pregnant females experiencing protein-energy malnutrition (Van Saun, 2006; Carmalt, 2000; Oetzel, 1988), positive estimated ME and CP balances do not support the magnitude of LW losses observed in this study at P1. Even though pregnant alpacas should not lose LW, especially when offered high-quality forages, it is possible that factors that were not under our control (i.e., stress) led to mobilisation of body tissues to counteract their effects. For example, cold stress is a prevailing factor at high altitude ecosystems, and in line with this, we often observed alpacas (especially Suri) shivering at early morning when temperatures were < 5 °C (Fig. S1 in the Supplementary material). Regardless of period, Suri females in this study consistently displayed decreased body temperature (−1.5 °C on average during both experimental periods) as it was recently reported (Benito-López et al., 2024). The last may contribute to observed LW losses during transition period.

Substantial reductions in DMI (as a % of LW), especially in Huacaya alpacas, were observed at the wet period (last month of pregnancy), with these representing up to 42 and 21% of observed consumption at the transition period, respectively. Neither variety of alpaca experienced a loss in LW at P2, on the contrary, both Huacaya and Suri alpacas gained LW (20.8 and 16.7 g/d, respectively). These findings are in line with expected physiological responses of pregnant females as a result of the rapid foetal growth prior to parturition as documented in farm animals (Jainudeen and Hafez, 2000). mobilisation of body tissues likely continued because of reduced nutrients' supply from swards to cope with the increased nutrient requirements of late pregnancy and mammary gland development (McNeill et al., 1997; Bauman and Currie, 1980). Estimated negative balances for ME (−7.09 vs −5.05 MJ/d, for Huacaya and Suri respectively) and CP (−79.6 and −64.0 g/d for Huacaya and Suri, respectively) were observed during wet period. These estimates may suggest that late pregnant alpacas just prior to parturition time would require either improved forage quality or additional concentrate supplementation to tackle reduced voluntary intake as a result of physical constraint effect on forestomach size. This observation is consistent with reduced CP contents and *in vitro* digestibility values of the diet. The DMI reduction observed in the experimental animals prior to parturition was perhaps more related to decreased pasture quality (reduced CP levels) as camelids retain lower quality forage longer to facilitate greater microbial fermentation (Van Saun, 2006) than the reduced forestomach volume capacity caused by the accelerated foetal growth, as described in early studies in sheep with increased likelihood of multiple foetuses (Forbes, 1970).

In order to better understand the dynamics of body tissue mobilisation and energy status, future studies should implement monitoring body condition score (BCS). Late pregnant animals should have slightly high body condition (3.5 of 5, or 6 of 9) to have reserves in support of impeding lactation (Van Saun and Herdt, 2014). Undertaken approach for estimating ME and CP balances in pregnant animals should better account for differences in LW (not only associated to pregnancy) and thermic regulation as late pregnant alpacas, at least in this study, appeared to be very sensitive to the harsh conditions of the experimental area. Therefore, there is a necessity of more *in vivo* local validation studies of currently available factorial approaches.

Decreased quality of forage fibre (assessed by difference as hemicellulose = aNDF – ADF) in addition to reduced CP contents, numerical reductions in IVDMD, and the two-fold increase in senescent material of the forage consumed, may help to explain the inconsistency in observations relative to estimated ME and CP balances, as well as lowered diet metabolisability (ME/GE; AFRC, 1993) observed during wet period. Apparently, increased legumes consumption during this period did not compensate the reduced CP supply as a result of the decreased proportion of leaves in swards. The lowering effect of legumes on IVDMD in regrowth herbage has been previously reported as a result of dominance of generative shoots (highly lignified) with lower leaf/stem ratio (Steinshamn et al., 2016; Buxton and Brasche, 1991). In future studies, lignin fraction should be analysed and the lignin/aNDF ratio be calculated for better assessing the effect of fibre digestibility on forage quality of cultivated mixed swards.

In this study, feed intake calculations relied on both *in vitro* digestibility and faecal output measurements. The last was reliable obtained via total collection using proper bags. Conversely, *in vitro* digestibility procedures and values can be questioned. Firstly, source of rumen fluid (cattle) and diet of the donor animals substantially differed from experimental animal species and diet. Secondly, incubation time (48 h) seemed short. In fact, alpacas are characterised by longer retention times of the digesta in their forestomach (Pinares-Patiño et al., 2003). Unfortunately, fine-tuning of ANKOM technique aiming for more realistic predictions of *in vitro* digestibilities of forages using stomach fluid taken from C-1 forestomach of fistulated alpacas and fed the same diet and samples being incubated for a longer period (i.e., 72 h), was found impractical to be successfully implemented when considering conditions of this study. The IVDMD values of the diet consumed by the alpacas in this study, which were obtained with the standard ANKOM procedure, could be considered slightly high, especially at P1.

Fleece characteristics

Data reporting the effect of pregnancy linked to nutrition on fleece characteristics in alpacas are scarce. Growth in length (μm/d) for Huacaya females is in accordance with the values reported for females in the Mediterranean region of Chile (Castellaro et al., 1998) and in the Peruvian Andes (Franco et al., 2009). However, growth in mass of clean fibre (mg/cm^2) observed in this study was lower when compared to findings reported by Wuliji et al. (2000) and Franco et al. (2009). The latter could be partly attributed to differences in nutritional quality of the offered diets as well as other environmental factors. In addition to differences associated with alpaca variety, age of alpacas has a remarkable impact in driving fleece quality variables as mentioned by Olarte-Daza (2022) and Bustinza (2001) with higher fibre production between the age of 2 and 4 years. In this study, mean age of the females at the beginning of the study was 5.1 ± 1.89 years-old, and fibre diameter exhibited a closer value to those reported by Olarte-Daza (2022) for adult pregnant alpacas (27.3 ± 0.59 μm).

A long-term study conducted in an experimental farm in the Peruvian region of Puno demonstrated that lactation appeared as an important effect decreasing fibre diameter of Huacaya and Suri pregnant females, while pregnancy appeared to be a much less relevant factor influencing diameter (Cruz et al., 2017). Fibre diameter values reported for Huacaya (22.3 and 23.9 μm for transition and wet periods, respectively) and Suri alpacas (25.5 and 25.8 μm for transition and wet periods, respectively) were higher when compared to those reported by Wuliji et al. (2000) for Huacaya female alpacas under grazing conditions in New Zealand, but within range of studies conducted in the Peruvian Highlands (Pinares et al., 2023; Olarte-Daza, 2022; Franco et al., 2009).

McGregor (2002), demonstrated that live weight loss in nutrition-stressed adult alpacas was associated with both reduced fibre growth and a decrease in fibre diameter. Conversely, in a study conducted by Russel and Redden (1997) in Scotland, involving male alpacas fed below and above maintenance requirements, the authors found that improved nutrition had a positive effect on fibre weight, but the effect was more noticeable on fibre length than diameter. Contrary to the expectations, in this study, improved plane of nutrition during transition period (P1), which reflected in increased DMI, did not result in a greater fibre diameter, which may be attributed to the observed live weight losses.

Methane emissions

Understanding the relationship between diet and enteric CH_4 production is essential towards reducing uncertainty in GHG emission inventories and to identifying effective mitigation strategies. Similar to ruminants, DMI has been recognized as the main driver of enteric CH_4 production in SAC (Riek et al., 2019; Dittmann et al., 2014; Pinares-Patiño et al., 2013). When CH_4 emissions were expressed per unit of $LW^{0.75}$, Suri alpacas tended to exhibit lower emissions compared to Huacaya, particularly during the transition period. A similar emission pattern was reported by Gómez-Oquendo (2023) in male alpacas grazing native pastures at the end of dry season in the Andean altiplano, with values around 1.4 g/kg $LW^{0.75}$. These results are considerably higher than those observed by Dittmann et al. (2014) under confinement conditions (0.74 g/kg $LW^{0.75}$), and those by Pinares-Patiño et al. (2003), which reported values between 0.66 to 0.99 g/kg $LW^{0.75}$ from both indoor and grazing studies on a mixed grass-legume or legume pastures, respectively. Also, in llamas, previously reported values (ranging from 0.59 to 0.82 g/kg $LW^{0.75}$; Febres et al., 2021; Dittmann et al., 2014) are lower than those observed in this study.

It is possible that the numerical increase in diet digestibility contributed to increased total CH_4 at transition period, but this was not reflected in greater CH_4 yield emissions as previously reported by Pinares-Patiño et al. (2013). Obtained values for this metric (33 and 25 g/kg DMI on average for Huacaya and Suri alpacas, respectively) are in accordance with those calculated from tabulated values by Pinares-Patiño et al. (2003) in alpacas grazing a ryegrass + white clover pasture. Probably, the increased alpacas' consumption of plantain observed at P2 may have influenced the numerical reductions in digestibility and thus contributing with decreased total CH_4 emissions as shown in a recent study conducted with dairy cows (Della Rosa et al., 2022).

Mean estimated Y_m emissions (CH_4 as % of GEI) for this study ranged from 7.5 to 10.9% and are in line with the value reported by Pinares-Patiño et al. (2003) in alpacas on perennial ryegrass and white clover pastures (Y_m = 9.4 %). It is worth mentioning that specific Y_m values for each alpaca variety have not been reported before. Further studies are required to be conducted in SAC for allowing adjustments in terms of sex, physiological stages, production level, and diet quality, as it is currently implemented in The Intergovernmental Panel on Climate Change (IPCC) guidelines for GHG inventory purposes in ruminant animals (Gavrilova et al., 2019).

Methane emission intensity quantifies the production of enteric CH_4 relative per unit of output product. In domestic ruminant species, this trait is expressed as g of CH_4 per kg of live weight gain (beef), milk (dairy), and fleece (sheep), respectively. We believe that CH_4 intensity in alpacas (g/kg of clean fleece) should be more often reported in future *in vivo* studies as a means for improving modelling efforts towards carbon footprint assessments. From the daily mass growth of fibre produced in a specific area (g/d of clean fleece in 100 cm^2), we extrapolated the daily amount of total CH_4 emissions to be equivalent to produce 1.0 kg of clean fleece. Overall, CH_4 intensity values decreased from 0.805 (transition) to up to 0.530 (wet) g/kg clean fleece, respectively. We preferred to use clean fleece instead of greasy fibre as especially Huacaya alpacas tended to dirtier fleeces than Suri due to differences in behaviour.

Conclusions

This study demonstrated that period (transition vs wet), which was aligned with two different late stages of pregnancy, had a stronger influence than animal variety (Huacaya vs. Suri) on feed intake, animal performance, and enteric CH_4 emissions of alpacas grazing a cultivated mixed sward. Poorer forage quality as observed during wet period (just prior to expected parturition time) in addition with the expected higher nutritional requirements, led to estimated negative ME and CP balances. However, these figures have to be taken with caution due to limitations of factorial approach undertaken for a more reliable representation of Peruvian Andes conditions. Reasons for reduced forage quality in P2 (pasture regrowth) are not fully clear and deserve further research on adaptation mechanisms of introduced plant species to these ecosystems as such kind of studies are particularly lacking. Overall, except for some fleece characteristics (e.g., fibre growth in length and weight), forage selectivity, and production responses were rather similar among alpaca' varieties. Although Suri alpacas displayed an increased biting rate when compared to Huacaya, this was not reflected on differences in DMI or CH_4 emissions either per unit of feed intake (yield) or final product (intensity). Results found in this study for late pregnant alpacas under cultivated mixed swards may confirm no need for specific enteric CH_4 emission factors addressing alpaca variety.

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Data availability

Data will be made available upon reasonable request.

CRediT authorship contribution statement

Laura B. Gualdrón-Duarte: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Cecilia Loza:** Writing – review & editing, Validation, Methodology, Investigation. **José I. Gere:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization. **Nancy F. Huanca-Marca:** Writing – review & editing, Investigation, Data curation. **Francisco E. Franco:** Writing – review & editing, Validation, Methodology. **Yemi Sanca Uscamayta:** Writing – review & editing, Investigation, Data curation. **Rubén García-Ticllacuri:** Writing – review & editing, Supervision, Investigation, Data curation. **Guadalupe Orellana Ligas:** Writing – review & editing, Investigation, Data curation. **Diannett Benito López:** Writing – review & editing, Investigation, Data curation. **Feliciano Rivera Pachiño:** Writing – review & editing, Investigation, Data curation. **Juan E. Moscoso-Muñoz:** Writing – review & editing, Resources. **Medardo A. Díaz-Céspedes:** Writing – review & editing, Resources. **Lizbeth L. Collazos Paucar:** Writing – review & editing, Project administration. **César S. Pinares-Patiño:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Víctor M. Vélez-Marroquín:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Edward H. Cabezas-Garcia:** Writing – original draft, Visualization, Supervision, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

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