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Design, operation, and validation of metabolism crates for nutrition studies in alpacas (*Vicugna pacos*)

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ABSTRACT

Metabolism studies in farm animals require reliable measurements of feed intake and water consumption, as well as the accurate collection of feces and urine. When designing metabolism crates, aspects such as: animal behavior, minimization of injury risks, and animal cleanliness should be considered. In the present study, morphometric measures taken from 30 Huacaya male alpacas (five years old) were the basis for designing an initial prototype of metabolism crates, which was built largely of metal for strength and durability, and has replaceable parts. Two of these metabolism crates were built and tested with experimental animals sequentially allocated in pairs during five days each. Based on the observed performance, both length and width of metabolism crates were adjusted (2.00×0.55 m respectively) for an up-graded design, which was tested during the validation phase. These features provided greater comfort and ease of handling of the experimental animals. Metabolism crates are illustrated by photos and drawings, which give dimensions and details of construction. At the end, five units were built. Validation of metabolism crates comprised the evaluation of animal performance of five alpacas fed at maintenance level of intake in terms of: daily feed intake and water consumption, fecal and urine excretion, and diet digestibility over a two-week experimental period. Data were analyzed by descriptive statistics. Overall, both dry matter intake per kg of metabolic body weight (DMI: 42.7 ± 1.29 g/kg BW^{0.75}), and water consumption (100 \pm 7.1 ml/kg of BW^{0.75}), which was equivalent to 2.3 times the observed DMI (kg/d), were in line with a number of previous studies conducted with alpacas for this feeding regime. Due to differences mainly related to diet composition, direct comparisons with studies in the literature in terms of fecal production and diet digestibility are difficult to draw. However, these results appear to be in line with expected performance for the given diet (70:30 oat hay and alfalfa pellets on a fed basis). Same applies to urine volume (428 ± 34.2 ml/ d), which in the present study represented 19% of the daily water consumption. It is concluded that proposed metabolism crates are suitable for conducting nutritional studies with alpacas.

1. Introduction

In Peru, alpacas' production is entirely under grazing, mostly on native grasslands. Andes ecosystems are characterized by such environmental conditions (e.g. high altitude, variability and scarcity of forage quality and supply, high UV radiation, etc.), that can be challenging for raising any ruminant livestock (i.e. cattle, sheep). Although alpacas (camelids) have successfully evolved to deal with these stressful conditions, the accelerated deleterious effects of climate change-related factors have increased the pressure on these ecosystems over the last decades and it may compromise sustainability of this livestock production system in the near future (Pinares-Patiño, 2017).

Knowledge on animal energetics, protein and fiber utilization from roughages, and digestive physiology of domestic South American camelids (SAC; alpacas and llamas) is still rather limited and in occasions parameters being extrapolated from ruminant species (National Research Council NRC., 2007, 2001; Van Saun, 2014). This may lead either to under or overestimate their nutritional requirements,

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especially when referring to differences in dry matter intake (DMI) capacity between camelids and domestic ruminants (Van Saun, 2009). For studies aiming to calculate balance of nutrients, a factorial approach has been traditionally adopted by the conventional feeding systems worldwide (i.e. Agricultural and Food Research Council AFRC., 1993; National Research Council NRC., 2007, 2001; Institut National de la Recherche Agronomique INRA., 2018), based on the assumption that the total requirements of energy or other nutrients are the amounts to be fed to the animal to fulfill specific requirements for maintenance and productive functions (e.g. growth, pregnancy, lactation).

To conduct such studies, a controlled supply of feed and water is required to determine their daily intakes accurately. For that purpose, an easy collection of feed refusals, feces, and urine must be guaranteed by use of metabolism cages (Nelson et al., 1954; Aschbacher, 1970; Michell, 1979) to properly carry out animal nutrition experiments such as nutrient digestibility and balance studies (Hristov et al., 2019; Danesh Mesgaran et al., 2020). Moreover, as the animal comfort should be a major component in the design of metabolism crates, these structures must be in concordance with animal morphology to minimize as much as it is possible further constraints on animal behavior, especially when metabolism crates are incorporated within respiration chambers (Pinares-Patiño and Waghorn, 2014).

Historically, much of the scientific basis on the study of nutrient requirements that support contemporary ruminant livestock farming has been traditionally developed under highly controlled conditions in developed countries. Studies using indoor facilities with metabolism crates to conduct basic research on this field are particularly lacking in the Andean countries and much less when referring to SAC. At least in ruminant animals, there are acceptable procedures to extrapolate research findings obtained from suitable indoors research facilities (controlled environments) to simulate a number of scenarios representing free-range conditions (Osuji, 1974; Milne et al., 1978; Gross et al., 1996; Pinares-Patiño et al., 2007). Therefore, similar principles could be also replicated in studies conducted with SAC.

The objectives of this study were to evaluate the operability of metabolism crates and the performance of alpacas housed inside and fed at maintenance level of intake. This kind of research is of major importance for establishing a suitable laboratory to conduct research on animal energetics, balance trials and metabolism studies with alpacas aimed to be conducted in the Andes region.

2. Materials and methods

Three experimental phases (initial design, prototype adjustment, and validation phase) were carried out to establish the feasibility of proposed metabolism crates for conducting animal nutrition studies with alpacas located at 3800 m above the sea level. All trials were conducted between August to October 2019 (dry season) at the Maranganí Research Station of the Veterinary Institute for Tropical and High Altitude Research (San Marcos University in Cusco, Peru). The environmental temperature during the time frame that the study was conducted varied between - 8.4–25.8 °C, and the relative humidity was 64.5 \pm 7.82% (SENAMHI National Service of Meteorology and Hydrology of Peru, 2021).

The present study was carried out on an approved license (number: CEBA 2021-35) from the Veterinary Faculty, under the supervision of a local animal welfare committee at the Maranganí Research station. All efforts were made to minimize unnecessary suffering. At the beginning of the study, the alpacas were gradually trained to the indoors' conditions (confinement in metabolism crates) following recommendations by Lund et al., (2012).

2.1. Initial design

A number of morphometric measures were taken using a measuring tape (2 m' length) from thirty adult male alpacas (Huacaya breed) born

between 2013 and 2015 (five years old on average). For that purpose, alpacas were stand up on a flat surface with a companion person beside. Once the animal was properly stopped on the flat surface, the following measures were taken: 1. height at withers (inter-scapular space), 2. rump height (middle point of the hip joint), 3. total length (distance between the shoulder joint to the hip joint), 4. Height to the forehead (measure taken from the ground to the upper frontal edge), 5. hips width (distance between the middle point of hip joint to the same joint opposite). Once tape-measurements were completed, the animals were weighted in an electronic scale with a sensitivity of \pm 0.5 kg (Tru-Test^{\textsc{tm}} - Econo Plus; Auckland, NZ). Descriptive statistics of biometric measures are presented in Table 1. These were the basis for developing the initial design of the metabolism crates (two stainless steel units) following the standard model for herbivores proposed by Milne et al., (1978). This model comprises a cubicle for head and neck, containers for feed and water (feeders and drinkers both located in the front side of the metabolism crate), and a receptacle for collecting urine located underneath (Fig. 1). Once the initial prototype was designed, two metabolism crates were built and placed in a proper ventilated room before the beginning of the study under indoor conditions.

Alpacas were sequentially allocated in pairs (one animal per crate; n = 15 pairs of alpacas) and stay for a period of five days inside the crates. Metabolism crates were equipped with a ramp for the easy access and withdrawal of the animals. Alpacas were fed twice daily (0700 h and 1900 h) for ad libitum intake with a mixture of oat hay and alfalfa pellets (70:30 proportion on an as fed basis). Oat hay was chopped using a forage chopper to obtain a mean particle size of 2 cm approximately. Feed refusals were collected and weighted just before morning feeding at 0700 h. An extra drinker was used to correct daily intakes for water evaporation (similar as the one already included in the metabolism crate) and this was placed out of reach of the animal.

Experimental alpacas were fitted with a harness system attached to the perineal area of the animal and feces collection bags were placed (Fig. 2). Routinely, feces collection bags were placed on the animals at 0700 h prior morning feeding and replaced by empty ones at 1800 h, thus allowing two changes during the day. Fecal production was weighed at those times and recorded for each animal on a daily basis. Trajectories of urine ejection for each animal were also recorded using a measuring tape to test the effectiveness of the urine collection system. A solution of 10% sulfuric acid was added to the urine collected in a receptacle underneath crate to prevent its deterioration as a result of the microbial activity (Raimundo-Giménez, 2010).

Animal vocalizations were considered as a sign of animal discomfort (Fowler, 2008). In the present study, the absence of vocalizations while the animals were handled by the operators (e.g. feeding times, changes of feces collection bags, etc.), in ten-minutes intervals, was assumed to be an indicator of animal comfort. Also, a visual assessment of ears and tail positions was considered as an indicator either of animal comfort or stress according to the scale proposed by Fowler (2008) for SAC.

Table 1

Descriptive statistics of morphometric measures taken from male alpacas during the initial phase of the study (n = 30).

Item ¹	Mean	SD	Minimum	Maximum
Total body length (cm)	99.6	4.72	109.0	91.0
Height at withers (cm)	94.7	3.58	90.0	100.1
Rump height (cm)	94.0	3.91	85.0	98.2
Total length (cm)	99.6	4.72	109.0	91.0
Height to the forehead (cm)	143.9	6.32	151.2	131.1
Hips width (cm)	37.0	2.73	40.0	31.0
Body weight (kg)	71.2	5.04	63.1	74.0

¹ Except body weight, all variables were measured with a measuring tape (2 m['] length).



Fig. 1. Initial design of metabolism crates prototypes for alpacas. A: side view; B: top view.



Fig. 2. Aspects of feces collection bags used in the present study. A. Bag measurements; B. Feces collection bag placed in an alpaca.

2.2. Prototype adjustment

The initial prototype of the metabolism crate was adjusted primarily

according to the observed animal behavior during the initial phase of the study. Measurements of both side and back panels of the metabolism crates, as well as the heights of both feeders and drinkers were further adjusted to guarantee the maximum comfort for the alpacas kept inside them. At the end, five modified stainless steel metabolism crates were built to proceed with the validation phase of the study.

2.3. Validation phase

Five alpacas were chosen based on their good behavior during the initial phase and similarity to the mean morphometric measures obtained from 30 animals during the initial design phase (Table 1). The experimental diet fed to the animals was the same as the one used in the initial phase (70:30 oat hay and alfalfa pellets on an as fed basis).

However, for validation purposes these alpacas were fed at maintenance level of intake. Diet was formulated to meet the energy requirements for maintenance (ME_m) of 0.346 mega joules (MJ) per kilogram of metabolic weight ($BW^{0.75}$) according to the recent comparative slaughter study by Roque et al., (2020). Protein requirements for maintenance (MP_m) were assumed to be 3.5 g of crude protein per kg of $BW^{0.75}$ as reported by Huasasquiche (1974).

Chemical composition of single feeds and experimental diet is presented in Table 2. Feed analyses were carried out in duplicate. Feed and fecal samples were analyzed following the official AOAC methods (AOAC International, 2000). Composite samples of both feeds and feces were oven dried (60 °C) and dry matter contents (DM) quantified (method 934.01). Dry samples were processed in a knife mill using a 1-mm screen sieve. Subsequently, wet chemistry analyses were performed as follows: ash (method 942.05), crude protein (CP; method 968.06), ether extract (EE; method 920.39), and neutral detergent fiber assayed with a heat stable amylase and expressed inclusive of residual ash (aNDF) according to Mertens (2002). Alpacas were fed and drinkers filled twice a day (0700 h and 1900 h). Both feed intake (g/d) and water consumption (l/d) were recorded for each individual alpaca on a daily basis throughout the trial (during 15 days instead of five days as set in the initial phase). Feed intake was calculated as the difference between offered feed and orts. Total feces collection and urine were also measured as described earlier. Apparent digestibility of the dry matter (DMD) was then calculated from feed intake and total fecal collection (Cochran and Galyean, 1994) as:

$$DMD \quad (g/kg) = \frac{DM \quad intake \quad - \quad DM \quad feces}{DM \quad intake} \times 1000$$

Thirty minutes before feeding times (0630 h and 1730 h) animals were released from metabolism crates for allowing them physical exercise (Robinson et al., 2005).

2.4. Statistical analysis

The experimental data (intakes, fecal and urine production, and DMD) were analyzed by descriptive statistics (means and standard deviations) using the MEANS procedure of SAS (version 9.4; SAS Institute

Table 2

Chemical composition of the experimental dietary ingredients (g/kg DM unless otherwise stated).

Nutrient	Oat hay	Alfalfa pellets	Diet ¹
Dry matter (g/kg)	948	921	939
Ash	44.6	90.4	58.3
Crude protein	56.7	146.9	83.8
Ether extract	6.60	23.1	11.6
Neutral detergent fiber (aNDF) ²	432	325	400
Non-fibrous carbohydrates (NFC) ³	460	415	447

¹ Diet is a 70:30 blend of oat hay and alfalfa pellets (fed basis). Diet composition is calculated from dietary ingredients.

² NDF assayed with a heat stable amylase and expressed inclusive of residual ash (Mertens, 2002).

 3 Calculated as NFC (g/kg DM) = 1000 - (NDF + (CP - NDIP) + Ether extract + Ash (NRC, 2001); NDIP: neutral detergent insoluble protein.

Inc., Cary, NC).

3. Results and discussion

3.1. Evaluation of the initial design: animal comfort

Overall, the initial design of the metabolism crates performed well in terms of practicality for measuring feed intake and water consumption, as well as fecal and urine production. It is worth to emphasize that animal performance (feed intake and water consumption) was not the main focus during this experimental phase. Instead, an animal behavior assessment with animals housed inside metabolism crates was performed in order to evaluate their reactions to the confinement in crates and further adaptation to these conditions. In general, alpacas did not show significant signs of discomfort, which were confirmed by the absence of animal vocalizations indicating stress. In addition, visual signs of aggressiveness were rather seldom observed. The last was assessed by both ears and tail positions (Fowler, 2008).

Two minor modifications to the original frame of the metabolism crates were implemented. Firstly, the length of the metabolism crate was extended from 1.30 to 2.00 m (segment A-B in Fig. 1) because it was observed that when the animals were laving down (prone position), they were not able to fully extent their bodies. Secondly, a 10 cm reduction in the crate width was implemented (segment C - D in Fig. 1), and this resulted in a final measure of 55 cm. This adjustment was justified as the alpacas were able to turn around on their own body axis, and thus they had the possibility to contaminate feed and water with feces and urine. Photos of adjusted metabolism crates in operation are shown in Fig. 3. Modifications to the original design of metabolism crates are presented in Figs. 4 and 5 (in Fig. 4, the modified segments are denoted as A'- B', and C-D, respectively). These measurements were rather similar to those reported for the metabolism crates (2.00 m length \times 0.60 m width) used in the N metabolism study conducted with female Huacaya alpacas (no lactating; three years-old; 55.4 ± 5.40 kg BW) by Condori-Mamani (2017) in the region of Puno, Peru.

Alpacas are a herd animal and have a gregarious social instinct (Matthews et al., 2020). When an individual is isolated from its herd, the animal became anxious and emit vocalizations as a clear sign of stress. In the present study, each individual alpaca within a pair was able to see its partner housed in the companion metabolism crate and this strongly contributed to enhance animal welfare. This preference has been considered in the design of respiration chambers studies conducted with sheep and cattle (Pinares-Patiño and Waghorn, 2014). Another important consideration is that the alpacas in our study were already familiar with a number of routine procedures in the farm performed by their caretakers and this may have contributed to reduce animal stress when experimental routines were carried out (Windschnurer et al., 2021).

3.2. Validation of adjusted metabolism crate design: animal performance at maintenance level of intake

Validation of metabolism crates comprises the evaluation of animal performance of the chosen five alpacas fed at maintenance level of intake in terms of: feed intake and water consumption, fecal and urine excretion, and diet digestibility over a 15 days' experimental period on a daily basis. This time-period should provide reliable estimates of these variables. Feed intake and body weight data are presented in Table 3. Experimental diet mean (\pm SD) DMI was 964 \pm 28.9 g/d, which is equivalent to 42.7 g/kg BW^{0.75}. This value is in line with the value set by Roque et al., (2020) for maintenance level of intake (40.0 g/kg BW^{0.75}). However, alpacas in the latter study lost weight (-14.6 g/d), whereas in the present study alpacas gained weight (73.3 g/d). Discrepancies on this may arise from differences in the age of the animals, energy content of the diet, and the length of the experimental period.

Similar DMI values at maintenance level as those found in the present study were also reported in the study by Paredes et al., (2014) in



Fig. 3. Adjusted metabolism crates in operation. A. Front view; B. Rear view.



Fig. 4. Schematic views of the adjusted metabolism crates for alpacas (A: side view; B: top view), and details of inside (C). Each crate has a slanted receptacle tray for urine collection by gravity flow into a container.

alpacas fed with oat hay (DMI ranged from 36.3 to 45.7 g/kg BW^{0.75}), and by López et al., (1996) in alpacas fed with quinhuilla hay (Chenopodium album; 37.6 g/kg BW^{0.75}). Variation observed in the former study was related to the proportion of leaves in the oat hay. As expected, the increased proportion of leaves (aiming to increase CP, and decrease fiber proportion of the diet) was directly associated with increased digestibility and that was also reflected in increased DMI. Alfalfa hays offered to the animals in the study by López et al., (1996) suggested increased nutritive quality of these roughages as feed intakes (54.6, 48.6, 53.3 g /kg $BW^{0.75}$ for the first, second and third cut respectively) were higher when compared to the value reported by Paredes et al., (2014). Apparently these numbers may suggest a strong influence of chemical composition of the offered roughages to the animals on DMI responses per kg of BW^{0.75} of alpacas fed at maintenance level of intake. However, results of a one-way t-test mean comparison indicated that there is not statistical difference between value obtained in the present study (42.7 \pm 1.29 g/kg BW^{0.75}) with the collective mean of treatments

 $(44.0 \pm 7.04 \text{ g/kg BW}^{0.75})$ published for the referred studies above (P = 0.60). Although all of these studies used the same breed and gender (Huacaya males) as in the present study, there were differences in terms of the age and body weight between animals. No study was found in the peer-review literature using a similar diet as the one fed to the animals in the present study.

A number of studies have indicated that SAC are more prone to suffer a drastic reduction of feed intake as a result of abrupt changes in the diet (e.g. type, frequency of meals, etc.) when compared to domestic ruminants (Provenza and Balph, 1987). For that reason, it is often recommended that alpacas should be trained for indoors feeding since a young age (Castro et al., 2017). In the present study, alpacas fed at maintenance level of intake showed a preference towards alfalfa pellets vs oat hay (selectivity of 98.8% and 91.3% respectively).

Water consumption is presented in Table 4. Mean value was 2259 ml/alpaca/d with this being equivalent to 2.3 times the DMI or per unit of metabolic weight 100 ml/kg BW^{0.75}. Despite of data on water



Fig. 5. Schematic views of the adjusted metabolism crates for alpacas. A: rear and front view; B: in the middle it is shown both removable feeder and drinker; C. alpaca inside of the metabolism crate (front view).

Table 3

Feed intake and body weight of male alpacas (n = 5) housed in metabolism crates and fed at maintenance level of intake.

Item	Mean	SD	Minimum	Maximum
Offered feed (g DM)				
Oats hay	756	21.9	736	780
Alfalfa pellets	309	1.12	308	311
Refusals (g DM)				
Oat hay	65.4	27.2	21.7	134.9
Alfalfa pellets	3.76	2.58	1.12	9.27
Selectivity ¹ (%)				
Oat hay	91.3			
Alfalfa pellets	98.8			
Daily diet intake				
DMI, g/d	964	28.9	893	1011
DMI as a % of BW	1.52	0.049	1.43	1.61
DMI per kg of BW ^{0.75}	42.7	1.29	40.1	45.3
Body weight, kg				
Initial	62.8	7.01	53.0	72.0
Final	63.9	6.68	54.0	72.0
Average daily gain (g/d) ²	73.3	-	-	-

¹ Expressed as = 100 - ((consumed feed / offered feed) * 100).

² Calculated as = (finish BW – start BW) / d.

Table 4

Daily water consumption of male alpacas (n = 5) housed in metabolism crates and fed at maintenance level of intake.

Item	Mean	SD	Minimum	Maximum
Water consumption (ml)				
Offered	3140	250.1	3000	3900
Refusal	864	231.5	616	1469
Evaporation ¹	16	8.3	0	25
Daily intake				
ml/d	2259	152.3	2050	2638
ml/kg of BW	36	3.0	33	43
as a % of BW	3.6	0.31	3.3	4.3
ml/kg of BW ^{0.75}	100	7.1	92	121

¹ Water evaporation was assessed by using an extra drinker located far away of the one available for the experimental animal.

consumption in alpacas are particularly lacking in the literature, values found in the present study are within the recommendations summarized by Van Saun (2006) for SAC to fulfill their nutritional requirements for maintenance. However, this value is slightly lower when compared to a more recent value reported by the same author for animals fed at maintenance level of intake assuming isothermal conditions (122 ml/BW $^{0.75}$; Van Saun, 2014). In a study conducted by Ríos (1991) in Central Chile (574 m above the sea level) with alpacas fed alfalfa hay as unique feed, the water consumption was considerably higher (2900 ml) than the one found in the present study at a higher altitude (3800 m above the sea level). Water consumption in alpacas is greater with dry roughage when compared to pellets (Van Saun, 2014), and this may help to explain the increased water consumption in the Chilean study. Moreover, differences in protein content might also influence water consumption and needs. To the best of our knowledge, there is no study aiming to quantify the effect of altitude as such on water requirements in SAC.

Earlier studies have indicated that the water consumption of alpacas and llamas is considerably lower than sheep, with this being positively related to decreased DMI (San Martín, 1987; San Martín, 1996). Nevertheless, in those studies the ratio between water consumption and DMI was rather similar for alpacas and sheep (2.2) whereas, llamas displayed a significant lower value (1.6).

Fecal production, urine excretion and DMD are presented in Table 5.

Table 5

Daily excretion of feces and urine, and apparent digestibility of the dry matter of male alpacas (n = 5) housed in metabolism crates and fed at maintenance level of intake.

Item	Mean	SD	Minimum	Maximum
Daily excretion				
Feces (g of DM)	320	35.6	234	385
Urine (ml)	428	34.2	380	480
Excretion as % of intake				
Feces (on DM basis) ¹	33.2	4.22	23.2	40.1
Urine (on ml basis) ²	19.0	2.11	14.6	22.6
Apparent digestibility				
DMD ³ (g/kg)	668	42.2	599	768

 1 (Fecal output / DMI) \times 100.

 2 (Urine output / water consumption) \times 100.

³ Calculated as DMD = (DM intake – DM feces) / (DM intake) \times 1000.

Total fecal production in the present study (320 g of DM/d) is similar to the one found by Huareccallo (2017) for alpacas fed with a mixture of oat hay and alfalfa hay (50:50 on a DM basis; 302 g of DM/d). Fecal production as percentage of the DMI was almost two-fold in the former study (62.9 vs 33.2%, respectively), with this mainly explained by differences in diet digestibility among studies. Although diet offered to alpacas in the study by Huareccallo (2017) was virtually the same as the one used by Roque et al., (2020), differences in diet quality (CP: 92 vs 124; aNDF: 545 vs 516 g/kg DM for the former and latter study respectively) reflected into increased DM digestibility at maintenance level found in the latter study (590 vs 619 g/kg respectively). Fecal production for alpacas fed at maintenance level in the study by Roque et al., (2020) was 39.5% of the total diet intake on a DM basis, which is in closer agreement with the value found in the present study. Finally, it is worth noting that in both studies by Roque et al., (2020) and Huareccallo et al., (2017), DMD did not change as a result of the gradual increase of DMI from maintenance up to ad libitum level.

It has been well documented that SAC are more efficient than domestic ruminants in digestibility of low and medium quality diets (San Martín, 1987; López et al., 1998; Sponheimer et al., 2003; Nielsen et al., 2014). Although Pinares-Patiño et al., (2003) did not find differences between alpacas and sheep when animals were fed alfalfa hay ad libitum in DMD (636 vs 639 g/kg respectively), alpacas were slightly more efficient than sheep in digesting the neutral detergent fiber (NDF) fraction of the offered roughage (478 vs 461 g/kg DM respectively). These results are also in agreement with those reported by Liu et al., (2009) in alpacas as the digestibilities of DM, OM and EE were not affected by forages sources. Whereas, digestibilities of NDF were greater for sorghum-sudan diets than for alfalfa hay diets and fresh alfalfa diets (542, 453, and 433 g/kg DM respectively).

The increased efficiency in fiber digestibility shown by the SAC compared to ruminant animals has been related to a number of physiological mechanisms such as: increased retention time of the solid phase in the gastrointestinal tract (GIT), increased contractions of fermentative compartments, high saliva flow, and the presence of glandular sacs in the first two compartments of the GIT (San Martín, 1987; San Martín and Van Saun, 2014).

South American camelids when fed poor quality diets are able to maintain high concentrations of ammonia N and this pool represents an effective N source for cellulolytic microbes and thus fiber degradation is optimized (Hinderer and Engelhardt, 1974; Engelhardt and Schneider, 1977). This evolutionary advantage was confirmed by San Martín et al., (1985) who reviewed several in vivo digestibility trials in alpacas and sheep. When alpacas were fed low levels of dietary CP (75 g/kg DM), they displayed increased digestibility compared to sheep. However, in the same study when CP contents increased up to 105 g/kg DM, no differences were found between both animal species. Condori-Mamani (2017) reported that alpacas fed at a restricted level of intake (1.5% of BW) and low levels of CP in the diet (40, 60, and 80 g/kg DM), DMI did not differ among treatments. However, in the referred study, the lowest CP supplementation level led to decreased N digestibility of the diet (166, 414, and 500 g/kg for the 40, 60, and 80 g/kg of CP respectively), and this was encompassed with slightly BW losses (-0.076 g/d). Alpacas showed a lower N requirement to meet metabolic needs than llamas, which is likely related to the smaller body size of the alpaca (Davies et al., 2007). In the same study, although neither DMD nor N digestibility were unaffected by either diet or SAC species (llama vs alpaca), llamas displayed a much higher increase in N retention compared to alpacas (3.8 vs 1.1 g/d respectively) when consuming a high protein diet (120 g/kg DM; 80:20 barley-alfalfa blend).

Urine volume in the present study represented 19% of the daily water consumption. The study by Huareccallo (2017) reported two-fold urine excretion that the observed in the present study (847 vs 428 ml/d respectively), unfortunately water consumption was not reported. Assuming isothermal conditions (Van Saun, 2014), the water requirement for the alpacas (BW = 60 kg) in the study by Huareccallo (2017)

gives an estimated water consumption of 2630 ml/d. Thus, urine volume should be equivalent to 32% of the daily water intake, which is much higher than the found in the present study.

4. Conclusions

Initial design of metabolism crates was slightly modified to facilitate cleaning, allow easy collection of feed and water refusals, and ensure comfort of alpacas housed in. Length of the metabolism crate prototype was enlarged to 2.00 m, and width reduced to 55 cm. Adjusted design allowed for a rapid adaptation of the animals to the experimental conditions. This was verified by the absence of animal vocalizations and further signs of discomfort. Moreover, the metabolism crates were designed to allow easy allocation and withdrawal of alpacas inside, with this facilitating to take the animals out for short periods of time allowing them physical exercise and thus improving animal welfare. Observed data of feed intake and water consumption, excretion (feces and urine), and DMD are within the ranges reported in the literature for alpacas fed at maintenance level of intake. It is concluded that metabolism crates described in the present study are suitable for conducting nutritional studies in mature alpacas fed at maintenance level of intake.

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Conflict of interest statement

- All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.
- This manuscript is not under review at, another journal or other publishing venue.
- The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript.

Declaration of Competing Interest

The authors have not stated any conflicts of interest.

References

- Agricultural and Food Research Council (AFRC). 1993. Energy and Protein Requirements of Ruminants; CAB International, Wallingford, UK.
- AOAC International, 2000. Official Methods of Analysis, 17th Ed. AOAC International, Gaithersburg, MD.
- Aschbacher, P.W., 1970. An adjustable metabolism stall for cattle. J. Anim. Sci. 31, 741–744. https://doi.org/10.2527/jas1970.314741x.
- Castro, J., Chirinos, D., Rojas, R., 2017. Aprendizaje temprano a la ingesta de concentrado en alpacas Huacaya. Rev. de Investig. Vet. del Peru. 28, 71–77. https:// doi.org/10.15381/rivep.v28i1.11841.
- Cochran, R., Galyean, M., 1994. Measurement of in vivo Forage Digestion by Ruminants. Forage Quality, Evaluation and Utilization. American Society of Agronomy, Madison, Wisconsin, USA.
- Condori-Mamani, K.E., 2017. Determinación de nitrógeno endógeno total: metabólico fecal, urinario y dérmico en alpacas (Vicugna pacos) hembras de tres años de edad. BSc. thesis. Faculty of Veterinary Medicine and Animal Science. Universidad Nacional del Altiplano de Puno. Puno, Peru. (In Spanish with abstract in English). (http://repositorio.unap.edu.pe/handle/UNAP/5146).
- Danesh Mesgaran, S., Kuhla, B., Baumont, R., Cantalapiedra-Hijar, G., Noziére, P., Lund, P., Humphries, D., Dijkstra, J., 2020. Nutrient digestibility and balance studies. In: Mesgaran, S.D., Baumont, R., Munksgaard, L., Humphries, D., Kennedy, E., Dijkstra, J., Dewhurst, R., Ferguson, H., Terré, M., Kuhla, B. (Eds.), Methods In Cattle Physiology and Behaviour: Recommendations from the SmartCow

V.M. Vélez-Marroquín et al.

Consortium. (https://books.publisso.de/de/publisso_gold/publishing/books/overvi ew/53/overview%20chapters).

- Davies, H.L., Robinson, T.F., Roeder, B.L., Sharp, M.E., Johnston, N.P., Christensen, A.C., Schaalje, G.B., 2007. Digestibility, nitrogen balance, and blood metabolites in llama (*Lama glama*) and alpaca. (*lama pacos*) fed barley or barley alfalfa diets. Small Rumin. Res. 73, 1–7. https://doi.org/10.1016/j.smallrumres.2006.10.006.
- Engelhardt, W.V., Schneider, W., 1977. Energy and nitrogen metabolism in the llama. Anim. Res. Dev. 5, 68–72.
- Fowler, M., 2008. Behavioral clues for detection of illness in wild animals: Models in camelids and elephant's. In: Zoo and Wild Animal Medicine Current Therapy, Sixth Ed. Elsevier Saunders, St. Louis. Missouri.
- Gross, J.E., Alkon, P.U., Demment, M.W., 1996. Nutritional ecology of dimorphic herbivores: digestion and passage rates in Nubian Ibex. Oecologia 107, 170–178. https://doi.org/10.1007/BF00327900.
- Hinderer, S., Engelhardt, W.V., 1974. Urea metabolism in the llama. Comp. Biochem. Physiol. 52A, 619–622. https://doi.org/10.1016/S0300-9629(75)80012-0.
- Hristov, A.N., Bannink, A., Crompton, L.A., Huhtanen, P., Kreuzer, M., McGee, M., Nozière, P., Reynolds, C.K., Bayat, A.R., Yáñez-Ruiz, D.R., Dijkstra, J., Kebreab, E., Schwarm, A., Shingfield, K.J., Yu, Z., 2019. Nitrogen in ruminant nutrition: a review of measurement techniques. J. Dairy Sci. 7, 5811–5852. https://doi.org/10.3168/ ids.2018-15829.
- Huasasquiche, A., 1974. Balance del nitrógeno y digestibilidad en alpacas y ovinos. BSc. Thesis. Faculty of Veterinary Medicine, Universidad Nacional Mayor de San Marcos. Lima, Perú. (In Spanish).
- Huareccallo, J.C., 2017. Efecto del nivel de consumo sobre la digestibilidad y valor energético de concentrado fibroso en llamas y alpacas. BSc. thesis. Faculty of Veterinary Medicine and Animal Science, Universidad Nacional del Altiplano de Puno. Puno, Perú. (In Spanish with abstract in English). (http://repositorio.unap.edu .pe/handle/UNAP/5574).

Institut National de la Recherche Agronomique (INRA), 2018. INRA Feeding System for Ruminants. Wageningen Academic Publishers, Wageningen, The Netherlands.

- Liu, Q., Dong, C.S., Li, H.Q., Yang, W.Z., Jiang, J.B., Gao, W.J., Pei, C.X., Qiao, J.J., 2009. Effects of feeding sorghum-sudan, alfalfa hay and fresh alfalfa with concentrate on intake, first compartment stomach characteristics, digestibility, nitrogen balance and energy metabolism in alpacas (Lama pacos) at low altitude. Livest. Sci. 126 (1–3), 21–27. https://doi.org/10.1016/j.livsci.2009.05.013.
- López, A., Maiztegui, J., Cabrera, R., 1998. Voluntary intake and digestibility of forages with different nutritional quality in alpacas (*Lama pacos*). Small Rumin. Res. 29, 295–301. https://doi.org/10.1016/S0921-4488(97)00135-1.
- López, A., Cabrera, R., Rojas, E., 1996. Digestibilidad aparente de forrajes secos por la alpaca (Lama pacos). I. Henos de alfalfa (Medicago sativa) de tres calidades y heno de quinhuilla (Chenopodium albun). Av. Cienc. Vet. (Impr.) 11, 5–9. (In Spanish). (https://revistadesociologia.uchile.cl/index.php/ACV/article/view/4759).
- Lund, K.E., Maloney, S.K., Milton, J.T., Blache, D., 2012. Gradual training of alpacas to the confinement metabolism pens reduces stress when normal excretion behavior is accommodated. ILAR J. 53, E22–E30. https://doi.org/10.1093/ilar.53.1.22.
- Matthews, P.T., Barwick, J., Doughty, A.K., Doyle, E.K., Morton, C.L., Brown, W.Y., 2020. Alpaca field behaviour when cohabitating with lambing ewes. Animals 10, 1605. https://doi.org/10.3390/ani10091605.
- Mertens, D.R., 2002. Gravimetric determination of amylase-treated neutral detergent fibre in feeds with refluxing beakers or crucibles: collaborative study. J. AOAC Int. 85, 1217–1240. https://doi.org/10.1093/jaoac/85.6.1217.
- Michell, A.R., 1979. Robust and inexpensive metabolism cages for sheep. Br. Vet. J. 135, 294–296. https://doi.org/10.1016/s0007-1935(17)32891-9.
- Milne, J.A., Macrae, J.C., Spence, A.M., Wilson, S., 1978. A comparison of the voluntary intake and digestion of a range of forages at different times of the year by the sheep and the red deer (*Cervus elaphus*). Br. J. Nutr. 40, 347–357. https://doi.org/10.1079/ BJN19780131.
- Nelson, A.B., Tillman, A.D., Gallup, W.D., MacVicar, R., 1954. A modified metabolism stall for steers. J. Anim. Sci. 13, 504–510. https://doi.org/10.2527/ iac1054 132504x
- Nielsen, M.O., Kiani, A., Tejada, E., Chwalibog, A., Alstrup, L., 2014. Energy metabolism and methane production in llamas, sheep and goats fed high- and low-quality grassbased diets. Arch. Anim. Nutr. 69, 171–185. https://doi.org/10.1080/ 1745039X.2014.912039.

National Research Council (NRC), 2007. Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids. National Academies Press, Washington, DC. https://doi.org/10.17226/11654.

- National Research Council (NRC), 2001. Nutrient Requirements of Dairy Cattle, Seventh Ed. National Academy Press, Washington, DC. https://doi.org/10.17226/9825.
- Osuji, P.O., 1974. The physiology of eating and the energy expenditure of the ruminant at pasture. J. Range Manag. 27, 437–443. https://doi.org/10.2307/3896717.

- Paredes, G., San Martín, F., Olazábal, J., Ara, M., 2014. Efecto del nivel de fibra detergente neutra sobre el consumo en la alpaca (*Vicugna pacos*). Rev. de Investig. Vet. del Peru 25, 205–212. https://doi.org/10.15381/rivep.v25i2.8492.
- Pinares-Patiño, C.S., 2017. Cambio Climático y Ganadería en los Andes Peruanos: retos para su rentabilidad y sostenibilidad, in: Tercera Conferencia de Gases de Efecto Invernadero en Sistemas Agropecuarios de Latino-América. 4 al 6 de octubre del 2017. INIA – La Estanzuela – Colonia. Uruguay. (In Spanish).
- Pinares-Patiño, C.S., Waghorn, G., 2014. Technical Manual on Respiration Chambers. Ministry of Agriculture and Forestry (MPI). Wellington, New Zealand. (http://dx. doi.org/10.13140/RG.2.1.4511.6244).
- Pinares-Patiño, C.S., Waghorn, G.C., Machmüller, A., Vlaming, B., Molano, G., Cavanagh, A., Clark, H., 2007. Methane emissions and digestive physiology of nonlactating dairy cows fed pasture forage. Can. J. Anim. Sci. 87, 601–613. https://doi. org/10.4141/CJAS06023.
- Pinares-Patiño, C.S., Ulyatt, M.J., Waghorn, G.C., Lassey, K.R., Barry, T.N., Holmes, C.W., Johnson, D.E., 2003. Methane emission by alpaca and sheep fed on lucerne hay or grazed on pastures of perennial ryegrass/white clover or birdsfoot trefoil. J. Agric. Sci. 140, 215–226. https://doi.org/10.1017/S002185960300306X.
- Provenza, F., Balph, D., 1987. Diet learning by domestic ruminants: theory, evidence and practical implications. Appl. Anim. Behav. Sci. 18, 211–232. https://doi.org/ 10.1016/0168-1591(87)90218-8.
- Raimundo- Giménez, A., 2010. Determinación de las necesidades de mantenimiento en dos razas de ovejas autóctonas españolas: Manchega y Guirra. MSc. Thesis. Universitat Politécnica de Valencia, Valencia, Spain (In Spanish with abstract in English). (http://hdl.handle.net/10251/11475).
- Ríos, J., 1991. Requerimiento de manutención, digestibilidad y consumo de agua en Alpacas. BSc. Thesis. Facultad de Agronomía, Pontificia Universidad Católica de Chile, Santiago, Chile. (In Spanish).
- Robinson, T.F., Roeder, B.L., Schaalje, G.B., Hammer, J.D., Burton, S., Christensen, M., 2005. Nitrogen balance and blood metabolites of alpaca (*Lama pacos*) fed three forages of different protein content. Small Rumin. Res. 58, 123–133. https://doi.org/ 10.1016/j.smallrumres.2004.09.005.
- Roque, H.B., Bautista, P.J., Beltrán, B.P., Calsín, C.B., Medina, S.J., Aro, A.J., Araníbar, A. M., Sumari, M.R., Benito, L.D., Marca, C.U., Huareccallo, M.J., Pari, H.J., Ramírez, A.J.E., Condori, A.E., Roque, H.E., Chui, B.H., Pinares-Patiño, C.S., 2020. Requerimientos de energía metabolizable para mantenimiento y ganancia de peso de llamas y alpacas determinados mediante la técnica de sacrificio comparativo. Rev. de Investig. Vet. del Peru 31 (4), e16738. https://doi.org/10.15381/rivep.v31i4.16738.
- San Martin, F., Van Saun, R., 2014. Applied digestive anatomy and feeding behavior. In: Cebra, C., Anderson, D.E., Tibary, A., Van Saun, R.J., Johnson, L.W. (Eds.), Llama and Alpaca Care: Medicine, Surgery, Reproduction, Nutrition, and Herd Health. Elsevier Inc, St. Louis, MO, USA, pp. 51–58.
- San Martín F., 1996. Nutrición en alpacas y llamas. Fondo Contravalor Perú-Suiza, CISA/ IVITA, Faculty of Veterinary Medicine, Universidad Nacional Mayor de San Marcos. Pub. Cient. IVITA N° 27, 3–21. (In Spanish).
- San Martín F., 1987. Comparative forage selectivity and nutrition of South American camelids and sheep. PhD. Thesis. Lubbock, TX, Texas Tech University.
- San Martín, F., Farfán, F., Valdivia, R., 1985. Digestibilidad comparativa entre alpacas y ovinos, in: V Convención Internacional sobre Camélidos Sudamericanos. Cuzco, Perú. Resumen. (In Spanish).
- SENAMHI (National Service of Meteorology and Hydrology of Peru) 2021. (htt ps://www.senamhi.gob.pe/) (Accessed on 13 June 2021).
- Sponheimer, M., Robinson, T., Roeder, B., Hammer, J., Ayliffe, L., Passey, B., Cerling, T., Dearing, D., Ehleringer, J., 2003. Digestion and passage rates of grass hays by llamas, alpacas, goats, rabbits, and horses. Small Rum. Res. 48, 149–154. https://doi.org/ 10.1016/S0921-4488(03)00002-6.
- Van Saun, R.J., 2014. Nutritional Requirements. In: Cebra, C., Anderson, D.E., Tibary, A., Van Saun, R.J., Johnson, L.W. (Eds.), Llama and Alpaca Care: Medicine, Surgery, Reproduction, Nutrition, and Herd Health. Elsevier Inc., St. Louis, MO, USA, np. 59–80
- Van Saun, R.J., 2009. Nutritional requirements and assessing nutritional status in camelids. Vet. Clin. Anim. Food 25, 265–279. https://doi.org/10.1016/j. cvfa.2009.03.003.
- Van Saun, R.J., 2006. Nutrient requirements of South American camelids: a factorial approach. Small Rumin. Res. 61, 165–186. https://doi.org/10.1016/j. smallrumres.2005.07.006.
- Windschnurer, I., Fischer, L., Yanagida, T., Eibl, C., Franz, S., Waiblinger, S., 2021. Caretaker attitudes and animal training are associated with alpaca behaviour towards humans—an online survey. Appl. Anim. Behav. Sci. 236, 105224 https:// doi.org/10.1016/j.applanim.2021.105224.