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REGULAR ARTICLES

Intake and digestibility of four rations with different fiber levels in alpacas (*Vicugna pacos*)

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Abstract

The aim of this study was to evaluated the impact of different fibre levels in alpaca diet on voluntary feed intake and apparent digestibility, and to estimate the digestibility of organic matter (OMD) from the content of crude protein (CP) in feces. The study was carried out with twelve alpacas (36.7 ± 6.4 kg body weight- BW), which were offered 4 treatments with different neutral detergent fiber content (NDF. T1: 40.3%; T2: 62%; T3: 68%; T4: 72%) under a switch back design. Absolute daily dry matter intake (DMI) was higher for T1 (678 g/d) than T4 (312 g/d) ($p \geq 005$). NDF intake was similar between treatments when related to BW or MW (on average 1% BW and 22 g/kg MW. $p \geq 0.05$). Water intake (L/kg DMI) was higher in T1 compared to the other treatments, with values ranging from 2.9 L/kg DMI(T1) to 2.8 L/kg DMI(T4), respectively ($p \leq 0.05$). Digestibility of dry matter, organic matter and CP was higher in T1 than in the other treatments, with average values ranging from 72% for T1 to 32% for T4 ($p \leq 0.05$). NDF digestibility was similar among treatments ($p \geq 0.05$). The regression equation generated to predict OMD (y) was as follows: $y = 0.360 + 0.08294$*fecal CP (g/kg OM). Further studies will indicate whether faecal nitrogen can be used to estimate digestibility and hence diet quality in South American camelids.

Keywords Digestibility · Faecal nitrogen · Nutrient intake · South American camelids

Introduction

Peru has approximately 56% of the global population of South American Camelids (SAC) with an estimated 4,500,000 animals (INEI 2012). Animals are raised in an extensive system, where feeding is based on grazing of natural pastures (Chino et al. 2022). The extreme Andes conditions make grasslands scarce and with high fiber content, this causes rich in fiber content, this negatively affects animal productivity (Cordero et al. 2018:). In this regard, Van Saun (2006) concluded that a combination of a higher degree of degradability, along with a higher microbial performance, provides llamas and alpacas with a greater advantage when dealing with coarse and low quality feeds, in contrast to other feeds, ruminants and herbivores. Likewise, Sponheimer et al. (2003) determined that SAC were more efficient than goats for the digestion of C4 grass (*Cynodon dactylon*) hays, possibly due to their relatively longer mean particle retention times (71 and 54 h, respectively). The SAC feed consists mostly of a low-quality forage, which means neutral detergent fiber (NDF) concentration is the factor that predominantly regulates intake and hence passage time, because it affects stomach filling and dry matter digestibility (DMD. Arelovich et al. 2008). Across domestic and nondomestic mammalian herbivores, voluntary forage intake typically decreases with increasing fibre level (Meyer et al. 2010). In contrast, the relationship between a wider range of feed fiber levels and feed intake has not been well studied in alpacas.

According to Van Saun (2006), dry matter intake (DMI) varies between 1.25 and 1.5% of body weight (BW) for alpacas on maintenance diet and pregnant females, while another study has reported a range of 1.08 to 2.3% with an average of 1.8% of the BW for maintenance (San Martin and Bryant 1989). When directly compared to domestic sheep or goats, food intake of SAC is typically lower, which has been linked to a lower metabolic rate (Dittmann et al. 2014). The available information about feed digestibility for SAC

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is relatively scarce. However, there is a concordance among authors which suggests these animals are more efficient than other species, in terms of the use of high fibre rations with low crude protein (CP) content (López et al. 2000).

Several equations have been developed to estimate digestibility of organic matter (OMD) from different variables, such as the content of crude protein in feces (CPF), which allows to estimating the OMD of the diets ingested by animals during grazing without the need of collecting representative ingested forage samples (Lukas et al. 2005; Wang et al. 2009).

These regression equations for the prediction of OMD have been performed taking into account the digestive capacity of the different animal species (cattle: Leite and Stuth 1990; Lukas et al. 2005; Peripolli et al. 2011; sheep/goats: Boval et al. 2003; Oliveira et al. 2007; Wang et al. 2009; Horses: Mésochina et al. 1998). However, until now, there is no equation that allows the estimation of OM digestibility in alpacas.

The aim of this study was to evaluate the impact of different fibre levels in alpaca diet on voluntary feed intake and apparent digestibility. For this aim, a regression equation was generated to estimate the OMD from the CP content in faeces.

Material and methods

Location and time of the study

The experiment was performed at the Center of Investigation and Production (CIP) "Quimsachata" of the National Institute of Agrarian Innovation (INIA), located on the district of Santa Lucía, in the department of Puno, at 4 200 m.a.s.l. It took place during January and February 2019. The experiment was approved by the animal use committee of the National Agrarian University La Molina, within the framework of the evaluation of projects of the Master's Degree in Nutrition carried out by CIENCIAACTIVA-CONCYTEC-FONDECYT and UNALM.

Experimental animals

Twelve 2-year-old male "Huacaya" alpacas (*Vicugna pacos*) were used for the experiment, with an average weight of 36.7 ± 6.4 kg, clinically healthy and well adapted to the region. These animals were kept in individual roof-covered pens (6 m^2), designed to protect the alpacas from rain, and equipped with plastic drinkers and feeders that allowed for control of water and feed intake. Each animal had a harness attached to a collecting bag for daily total feces collection.

Experimental rations

The experiment with alpacas was carried out at the Agrarian Experimental Station "Illpa" of the National Institute of Agrarian Innovation (INIA- *spanish acronyms*), located in the district of Paucarcolla, province and department of Puno, at 3822 m.a.s.l. To achieve the objective of this research, four diets were formulated from the following raw materials:: oat (*Avena sativa*) hay variety INIA 902 and variety INIA 904, common vetch (*Vicia sativa*) and *Stipa ichu (*Ruiz & Pav.*)* Kunth. These forages from the rations were cut using a mincer, obtaining cut sizes of 2 cm, and only the proportion that passed through a 5 mm sieve was offered to the animals. Each ration had a different fiber level (Table 1). The animals did not receive any other type of supplementation.

Experimental design

The study was conducted with a *Switch Back* design for four treatments, which uses the same animals in three different experimental periods and where a treatment is tested on the animal in both the initial and final periods, and then compared with the second (Jones and Kenward 2003. Diagram 1). In each period, the animals were distributed in three blocks and in turn, in each block the four treatments were tested, that is, one animal per treatment was distributed in each block. Each of the periods had a total duration of 18 days where the first 8 days was a phase of adaptation of the animals to the diets, the following 10 days was an experimental phase for the evaluation of voluntary feed and

Table 1 Chemical composition of diets offered to alpacas (% of dry matter, except dry matter expressed as % as fed)

Treatment	Composition	DM (%)	OM (%)	CP (%)	NDF (%)
T1	100% INIA 902-African Oat + common vetch	92.3	92.4	7.3	40.3
T2	100% INIA 904-Vilcanota Oat + common vetch	93.7	92.7	9.2	62.1
T3	50% *Stipa ichu* + 50% INIA 904-Vilcanota Oat + common vetch	94.1	93.6	6.1	67.7
T4	80% *Stipa ichu* + 20% INIA 904-Vilcanota Oat + common vetch	94.7	94.3	5.4	71.5

Abbreviations: DM = dry matter, *OM* = organic matter, *CP* = crude protein, *NDF* = neutral detergent fiber

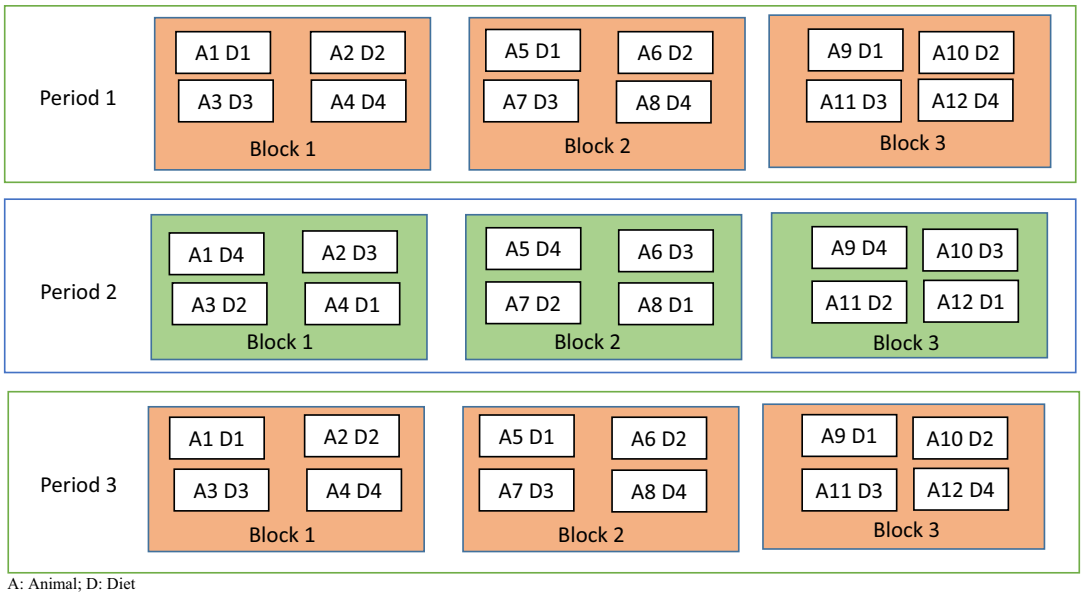

Diagram 1 Experimental design of the research. A: Animal; D: Diet

water intake (from days 8 to 13) and the evaluation of apparent digestibility (from days 13 to 18).

During the first five days of the experimental phase, the evaluation of voluntary feed and water intake was carried out. For this purpose, alpacas were fed ad libitum with each of the corresponding experimental rations. The amount of feed supplied at 07:00 h was calculated to obtain a 10% of refused feed at 06:00 h the following day. Both supply and refusal were weighed to obtain by difference the voluntary intake. The feed was weighed on a digital scale with a precision of ± 10 g. Simultaneously, the same methodology was used for the evaluation of water intake, during the experiment was offered in an amount of 5 L/day at 08:00 h. During the last five days, an apparent digestibility test was performed, using the controlled feed supply and total fecal collection methodology (Tapia 1993). For this purpose, alpacas were fed daily at 07:00 h with 90% of the registered voluntary intake, while the feces were collected and weighed daily at 05:00 h on the next day. Finally, 20% of the feces were stored at -20 °C for further analysis.

Sample management and laboratory analysis of samples

At the beginning of each experimental period, a sample of each ration was collected, which totaled 12 samples (3 per ration) at the end of the experiment. Likewise, at the end of each period, five daily fecal samples obtained from each animal were unfrozen and mixed, which resulted in a total amount of 36 fecal samples at the end of the experiment (Tapia 1993).

The conditioning and analysis of samples were performed at the Laboratory of Feed Nutritional Evaluation (LENA) of the Faculty of Zootecnics, at the Universidad Nacional Agraria La Molina, during March and April- 2019. Furthermore, experimental rations and collected feces were tested for DM (AOAC 2005; 950.46), OM (AOAC 2005; 950.05), CP (AOAC 2005; 920.115) and NDF (Van Soest et al.1991 in the Ankom Fiber Analyzer AN 200 (Ankom® Technology Corp. USA)).

Statistical analysis

The evaluation of the effects of each ration on the intake and apparent digestibility coefficients was estimated by the analysis of variance (ANOVA) for a *Switch Back* design for four treatments. The statistical software SAS 9.4 was used (SAS Institute 2012). The comparison between means was performed with the Tukey Test ($p < 0.05$). The model is as follows:

$$Y_{ijk} = \mu + +\pi_i + \tau_j + \varsigma_k + (i-2)S_k + \delta_{ijk}$$

where: Y_{ijk} = Response variable; μ = Effect of the general mean; π_i = Effect of the i-nth period; τ_j = Effect of the j-nth treatment; ς_k = Effect of the k-nth block; S_k = Effect of the variation between periods; δ_{ijk} = Random effect of the experimental error.

To describe the relationship between the ration OMD and the fecal OM nitrogen or CP concentration, a equation regression was generated. The linear regression model of the statistical software SAS 9.4 was used (SAS Institute 2012). The model is as follows:

Table 2 Dry matter intake (DMI), Neutral detergent fiber intake (NDFI) and Water intake (WI) in alpacas

Treatment	BW (Kg)	DMI			NDFI		WI[1] (L/kg DMI)
		g/day	BW[1] (%)	MW[2] (g/kg $BW^{0.75}$)	BW[1] (%)	MW[2] (g/kg $BW^{0.75}$)	
T 1	35.2 ± 7.0[a]	677.5 ± 103.2[a]	1.9 ± 0.1[a]	47.0 ± 1.2[a]	1.0 ± 0.1[a]	23.8 ± 1.7[a]	2.9 ± 0.1[a]
T 2	36.9 ± 5.1[a]	611.3 ± 62.9[a]	1.7 ± 0.07[a]	41.0 ± 1.8[a]	1.0 ± 0.1[a]	23.1 ± 3.7[a]	2.8 ± 0.1[a]
T 3	36.7 ± 4.2[a]	514.3 ± 127.3[a]	1.3 ± 0.3[b]	32.8 ± 7.0[b]	0.9 ± 0.1[a]	22.2 ± 3.8[a]	2.8 ± 0.1[a]
T 4	34.0 ± 8.2[a]	312.4 ± 84.7[b]	0.8 ± 0.1[c]	20.5 ± 2.7[c]	0.9 ± 0.1[a]	20.9 ± 2.7[a]	2.8 ± 0.1[a]
p-value	0.75	< 0.00	< 0.00	< 0.00	0.05	0.48	0.21
SEM	40.16	9497	0.02	15.35	0.0047	9.32	0.005

[a, b, c]Superscripts with different letters within columns are statistically different from each other ($p < 0.05$). [1] Intake based on body weight (expressed as percentage). [2] Intake based on metabolic weight (g/kg $BW^{0.75}$). *Abbreviations*: *DMI* dry matter intake, *NDFI* neutral detergent fiber intake, *WI* Water intake, T1: 100% INIA 902-African Oat + common vetch; T2: 100% INIA 904-Vilcanota Oat + common vetch; 50% *Stipa ichu* + T3: 50% INIA 904-Vilcanota Oat + common vetch; T4: 80% *Stipa ichu* + 20% INIA 904-Vilcanota Oat + common vetch.

$$Y = a + bx + e_{ij}$$

where: Y_{ij} = OM digestibility on the *i*th ration (%);
a and b= Fixed effect parameters;
X_{ijk} = Concentration of CP in fecal OM (g/kg OM);
e_{ijk} = Residual error.

Results

Assessment of voluntary intake

When analyzing the obtained DMI values in terms of BW and MW, ranges of 0.8 to 1.9% BW, and 20.5 to 47.0 g/kg $BW^{0.75}$ were found (Table 2). There were significant differences ($p < 0.05$) between the absolute intake values obtained for the experimental rations T1 and T4. A comparison of the neutral detergent fiber intake (NDFI) expressed on the basis of BW (%) and MW (g/kg $BW^{0.75}$) was made. Relative intake, either on a BW or MW basis, did not differ between the treatments.

Additionally, water intake values were recorded that ranged between 2.8 to 2.9 L/kg DMI, the minimum and maximum values correspond to treatments T4 and T1, respectively ($p < 0.05$. Table 2)

Assessment of apparent digestibility

In the experiment, the protein content in the feces was quantified, which ranged from 5.8 to 10.7% for the four treatments (Table 3). They also found the digestibility coefficients for DM, OM, CP and ADF. For the first three parameters (DMD, OMD, CPD) it was observed that T1 was characterized by the highest digestibility values (74.3, 76.0, 67.2%, respectively), while treatment 4 had the lowest coefficients (36.7, 41.2 and 20.4%). As for the NDFD parameter, no differences were observed between treatments (43% on average).

Prediction equation

The following equation estimates the digestibility of the ration OM based on the CP content in the fecal OM in alpacas (Fig. 1):

$$y = 0.360 + 0.08294^{*} fecalCP(g/kgOM)$$

where: y = ration OM digestibility (%).

Probability values of the estimated parameters were higher than 0.05. The R^2 was 0.96 and the 95% Confidence interval was -0.41 (lower) and 1.13 (Upper).

Discussion

Assessment of voluntary intake

The results of the present study show that as the NDF content of the alpaca diet increased, there was a progressive decrease in absolute DMI based on body weight (expressed as a percentage) and intake based on metabolic weight (g/kg $BW^{0.75}$). Our results are similar to those reported by Paredes et al. (2014), who observed a variation of the DMI from 1.4 to 1.7% of the BW (36.3 to 45.7 g/kg $BW^{0.75}$) when they offered alpacas four rations made from leaves, stems and oat hay, with an NDF range between 70.2 to 58.2%. Likewise, López et al. (2001) found a similar trend when comparing four diets based on red clover hay Italian ryegrass, bean and oat straw, where alpacas DMI decreased from 38.8 to 20.9 g/kg $BW^{0.75}$ by increasing the amount of NDF from 53.5 to

Table 3 Apparent digestibility coefficients for the parameters of DM, OM, CP and NDF in alpacas

Treatment	FCP (%)	DMD (%)	OMD (%)	CPD (%)	NDFD (%)
T 1	10.7 ± 0.8[a]	74.3 ± 6.0[a]	76.0 ± 4.8[a]	67.2 ± 4.5[a]	57.9 ± 10.1[a]
T 2	8.7 ± 0.8[a]	60.6 ± 4.0[b]	62.8 ± 5.0[b]	56.8 ± 4.2[a]	57.1 ± 5.8[a]
T 3	6.5 ± 0.8[a]	46.8 ± 6.2[c]	49.9 ± 5.2[c]	38.6 ± 3.6[b]	49.8 ± 5.6[a]
T 4	5.8 ± 0.8[a]	36.7 ± 7.4[c]	41.2 ± 6.6[c]	20. ± 15.6[c]	46.2 ± 4.2[a]
p-value	< 0.00	< 0.00	< 0.00	< 0.00	0.039
SEM	0.44	36.47	29.57	73.71	46.36

[a, b, c]Superscripts with different letters within columns are statistically different from each other ($p < 0.05$). *Abbreviations*: T1: 100% INIA 902-African Oat + common vetch; T2: 100% INIA 904-Vilcanota Oat + common vetch; 50% *Stipa ichu* + T3: 50% INIA 904-Vilcanota Oat + common vetch; T4: 80% *Stipa ichu* + 20% INIA 904-Vilcanota Oat + common vetch. *DM* = dry matter, *OM* = organic matter, *CP* = crude protein, *NDF* = neutral detergent fiber, *FCP* = fecal crude protein, *SEM* standard error of mean, *nd* not determined

78.5% in the diet. Similarly, López et al. (2000) studied three rations of alfalfa hay in combination with wheat straw with increasing percentage of NDF from 46.3 to 58.4 and found that alpacas reduced DMI from 45.8 to 33.2 g/kg $BW^{0.75}$.

San Martín (1987), reported two experiments. In the first, llamas were fed with mixed rations containing 7, 11 and 15% of CP, and 69, 55 and 27% of NDF; which resulted in DMIs of 54, 58 and 55 g/kg $BW^{0.75}$, respectively. These values were higher than the ones we obtained in our alpacas using rations of similar levels of CP and NDF. In the second experiment, the authors fed llamas with isonitrogenous rations (11–13% CP) with low, medium and high NDF levels (42, 58 and 68% dry matter basis), obtaining OMIs of 53, 50 and 47 g/kg $BW^{0.75}$, which are also higher than those of our study. For their part, Stölzl et al. (2014) fed llamas with hay of different qualities: hay 1 (15.1% CP and 52.6% NDF) and hay 2 (6.6% CP and 64.3% NDF per kg DM); which resulted in DMIs of 1.26 and 0.89% of the BW, respectively. These values were lower than the ones we obtained using rations of similar levels of CP and NDF.

The decreasing tendency of the ration DMI while its NDF content increases can be attributed to the presence of high cell wall content in the forage (Mertens 1994), which provide physical fill and stimulate the mechanoreceptors of the muscular layer (Forbes 1996), thus limiting feed intake. However, many other factors affect fill, including particle size, chewing frequency and effectiveness, particle fragility, indigestible NDF fraction, rate of fermentation of the potentially digestible NDF, and characteristics of reticular contractions (Allen 1996).

Regarding the NDFI, our results (Table 3) coincide with those found by Paredes et al. (2014), who reported an average NDFI of 0.9 ± 0.1% BW (24.2 ± 1.6 g/kg $BW^{0.75}$) for a range of NDF from 58.2 to 70.2%, and pointed out that regardless of the NDF level of the rations, alpacas have a similar intake of this component when it is between 60 to 70% in the ration. Our results coincide with the NDFI

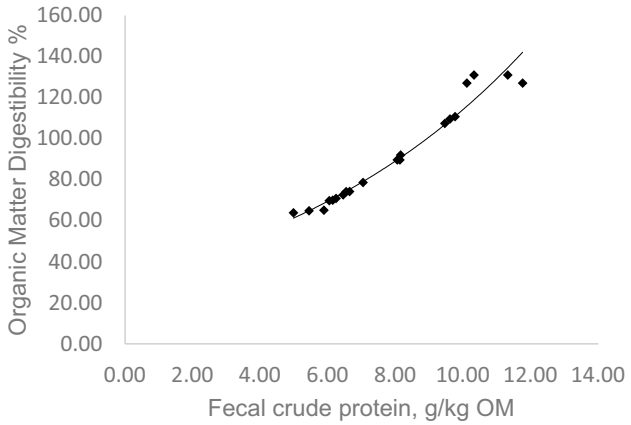

Fig. 1 Equation proposal for organic matter digestibility (OMD, %) estimation using fecal crude protein (g/kg OM) in alpacas

(0.9% ± 0.3% BW) in alpacas and llamas reported by San Martín and Van Saun (2014) from a collection of data in which individual maintenance DMI and forage nutrient content were determined. These data suggest dietary NDF content could be used to predict the potential capacity for intake. The expected maintenance DMI would be based on a NDFI in the range of 0.6 to 1.2% BW, with the lowest and highest values representing low and high fibrous forages, respectively (San Martín and Van Saun 2014).

Our results support what was mentioned by Van Saun (2006), who suggested that the intake potential in llamas and alpacas was related to the crude protein content of the diet. It is mentioned that the reduced intake in South American camelids is the result of low protein diets. In ruminant animals, microbial fermentation of fiber is the rate-limiting step in intake. Furthermore, the NDF content in the diet is highly associated with the regulation of feed intake.

The results of the present study show a decrease in WI as DMI decreases and NDF content increases in the experimental rations. This trend is similar to that reported by Llanos

et al. (2018), who observed a reduction in WI of 4.3 to 0.5 L/day in llamas fed barley hay and straw hay, at the same time that the DMI decreased from 3.3 to 1.3 kg of DM, and the crude fiber (CF) content increased from 1.8 to 28.3% in the experimental rations. Likewise, our results coincide with those reported by San Martin (1996), who mentions the existence of a directly proportional relationship between the DMI and WI. On the other hand, Velez-Contacayo et al. (2011) reported a reduction of the WI from 1.9 to 0.2 L/day in llamas fed with oats and Brazilian grass, at the same time that the DMI increased from 2.1 to 2.2 kg of DMI, and the CF content decreased from 52.5 to 47.7%, respectively. This could be due to Brazilian grass presenting a higher water content than oat.

Assessment of apparent digestibility

Several comparative studies addressing in vivo digestibility have been conducted in SACs and ruminants. San Martin et al. (1997) review several in vivo digestibility trials in alpacas and sheep, in which animals were separated into groups on the basis of the dietary CP content being less than 7.5% or greater than 10.5%. For diets containing less than 7.5% CP, digestibility was found to be higher and more favorable to alpacas, whereas no difference between species was observed in diets with level higher than 10.5% CP. Additional studies on comparative digestibility have confirmed these findings (San Martin (1987); Sponheimer et al., (2003); López et al. (1998)). Thus, SACs show higher digestibility compared with ruminants consuming low-to medium-quality diets, whereas comparable digestibility was observed between the two species for diets of high quality or low fibre content.

The results of this study show a decrease in the apparent digestibility coefficients obtained for DM, OM and CP parameters as the percentage of NDF in the experimental diets increases. Several studies conducted in llamas and alpacas fed rations made with forages show a similar trend in which the apparent digestibility for DM and CP decreases as the energy-protein levels decrease and the percentage of NDF increases. For example, San Martín (1987) and López et al. (2001), supplied forage-based diets to llamas with different levels of NDF (42 to 78%) and obtained values of MOD between 67 to 53.4%, PCD ranging from 61 to 52%, respectively. In some cases, these values were lower than in our study, perhaps because there are other differences not measured in the present study, such as the acid detergent lignin content, which has an inverse relationship with the MOD and a correlation coefficient of 0.96 (Saho et al., 2010). Adding to the above, the indigestible component of NDF plays an important role in regulating digestibility and feed intake in ruminants (Harper and McNeil 2015).

Although the decreasing trend is evident, no statistically significant differences were found between the NDF digestibility coefficients of our 4 experimental rations, this is in agreement with the results reported by Paredes et al. (2014), who suggest that NDFD may be maintained as ration fibre content increases. The above findings may be due to a greater capacity of SAC to utilize structural carbohydrates (López et al. 2001) or a longer residence time in rumen compartment one with low quality forages (Yaranga, 2009).

Prediction equation

The R^2 of our prediction equation was high, suggesting that model accuracy and fit of the equation is good. Also, this parameter washigher than that obtained by Wang et al. (2009), who obtained an $R^2 = 0.82$, working with 721 observations obtained from sheep subjected to 159 types of rations with ranges of crude fiber between 178 and 373 g/kg DM; or Peripolli et al. (2011) who estimated intake and digestibility in grazing ruminants from fecal nitrogen and obtained $R^2 = 0.36$ and MPE = 0.13 or Oliveira et al. (2007) in sheep ($R^2 = 0.24$, MPE = 0.17).

The difference between the RMSE and the MPE value of our equation and that obtained for the equation of Wang et al. (2009) could be associated to the number of observations used for the elaboration of the equation. Boval et al. (2003) state that the predictive reliability of OMD from fecal CP content depends on the range and number of observations of the in vivo digestibility tests and the regression model applied.

Conclusion

Under the conditions in which the present study was carried out, it was concluded that DMI (g/d) in alpacas was lower as the level of fibre content of the rations increased, while the NDFI did not vary according to fibre content. The digestibility of DM, OM and CP in alpacas was significantly lower as the level of fibre content of the rations increased, while the NDFD did not vary as the level of fibre content increased. The regression equation generated to predict OMD (y) was as follows: $y = 0.360 + 0.08294$*fecal CP (g/kg OM) with high R^2 value, suggesting that the model accuracy and equation fit are good. Further studies will indicate whether fecal nitrogen can be used to estimate digestibility and hence diet quality in South American camelids.

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Author contributions The conceptualization was performed by Ana Obregón Cruz, Carlos Alfredo Gómez Bravo and Cesar Mauro Osorio

Zavala. The methodology was designed by Ana Obregon and Carlos Gómez. The formal analysis and investigation were performed by Ana Obregon. The writing-original draft preparation was prepared by Ana Obregon. The writing-review and editing was performed by Carlos Gomez and Robert John Van Saun. The funding acquisition was conducted by Carlos Gomez and Cesar Osorio. The general supervision was performed by Carlos Gomez. All authors commented on previous versions of the manuscript, and all authors read and approved the final manuscript.

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Data availability The datasets generated and analyzed during the current study are available in the UNALM repository on the following link: https://repositorio.lamolina.edu.pe/handle/20.500.12996/5295

Declarations

Statement of animal rights The animals were cared in accordance with Peru's Law on Animal Protection and Welfare, No. 30407.

Conflict of interest This research was financing and supported by "CIENCIAACTIVA- CONCYTEC- FONDECYT".

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