Veterinary Integrative Sciences 2024; 22(3): 1073 - 1087 DOI; 10.12982/VIS.2024.072

Faculty of Veterinary Medicine
Chiang Mai University

Vet Integr Sci
Veterinary Integrative Sciences

ISSN; 2629-9968 (online)

Website; www.vet.cmu.ac.th/cmvj

Research article

Rumen kinetics of nutrient degradability of forage barley *(Hordeum vulgare L.)* with different levels of quinoa *(Chenopodium quinoa)* residues supplementation

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Abstract

The study aimed to determine the kinetic parameters of in situ degradation of dry matter (DM), crude protein (CP), and neutral detergent fiber (NDF) in barley as a winter forage, supplemented with four levels (0, 10, 20, and 30%, based on natural matter) of quinoa residue (QR). The experimental design used was a randomized complete block with a 4 x 6 factorial scheme (4 levels, 6 incubation times) and three replications. Three adult rumen-fistulated cattle with an average live weight of 434.33 + 4.04 kg were employed. Five grams of samples were incubated at 0, 6, 12, 24, 48, and 72 hours. Time 0 was used for calculating the soluble fraction. There was no interaction between level and time in the degradability of DM, CP, and NDF. CP and NDF degradability were influenced by the levels. The maximum disappearances of CP (60.41 and 62.36%) were obtained at 24 and 48 hours of ruminal incubation with the addition of 8.77 and 11.57% QR to barley, respectively. The potential degradability of DM, CP, and NDF, although showing variations, averaged around 70%. Higher potential and effective degradability of NDF in barley were observed with 10% QR compared to barley with 0, 20, and 30% QR. Barley supplemented with 10 and 30% QR promotes greater effective degradability of CP, suggesting increased utilization of this nutrient. The highest degradation rates (DR) of CP and NDF were observed at the 10% QR level, a parameter that classifies barley with QR as a high-quality forage resource.

Keywords: Cattle, Disappearance, Forage barley, In situ degradability, Kinetic parameters, Quinoa.

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Article history; *received manuscript: 9 January 2024,*
revised manuscript: 13 February 2024,
accepted manuscript: 16 February 2024,
published online: 4 March 2024
Academic editor: *Korakot Nganvongpanit*

INTRODUCTION

In the highland region of Peru, pasture production is characterized by seasonality, primarily due to the irregular distribution of rainfall. This results in providing animals with low-quality forage, leading to inadequate nutrient intake and compromising animal production. One way to address the current situation is to develop alternatives for utilizing forages and agricultural by-products during the colder and drier months of the year (Contreras et al., 2019b).

Forage barley, as a winter crop, is sown between January and April (rainy season), constituting one of the options used to alleviate the extreme shortage of forage during the dry season. Under tropical conditions, winter cereals (grain and forage), such as the BRS Marciana barley variety, contain 22.04% crude protein (CP) and 61.60% neutral detergent fiber (NDF) in their composition (Meinerz et al., 2011). Furthermore, this species is characterized by showing potential and effective degradability for a passage rate of 5% per hour of 78.51% and 58.79% for dry matter (DM), and 71.35% and 62.94% for crude protein (CP), respectively (Cordero et al., 2018). However, during the dry period, its use in animal feeding is either as exclusive forage or in a mixture with barley straw and/or oats, with these being considered as agricultural residues of moderate quality (Contreras et al., 2019b).

The Huancavelica region possesses a great diversity of crops. Therefore, the processing of various products generates large quantities of residues, which can be utilized in animal feeding. This not only reduces environmental pollution but also lowers animal production costs since feed constitutes between 60 and 70% of these expenses (Dutra et al., 1997). When harvesting quinoa grain in agricultural areas, by-products such as grain remnants, leaves, and stems are generated, constituting approximately 50 to 70% of the production of this crop. According to Contreras et al. (2019b), quinoa residues contain 8.25% crude protein (CP), 12.41% ash (mineral matter), 52.87% neutral detergent fiber (NDF), and 34.41% acid detergent fiber (ADF). Additionally, they exhibit crude protein degradation rates of 7.66% per hour and effective degradability at a passage rate of 2% per hour of 71.15%, classifying them as high-quality nutritional feed resources (Contreras et al., 2019b). Therefore, the inclusion of quinoa residues in winter forage barley in the diet of ruminants could be a viable alternative to improve ruminal fermentation patterns, fostering synchronization between nitrogen and energy availability when forages constitute the sole source of energy in the rumen (Russell et al., 1992).

Among the techniques employed to assess ruminal degradation, the *in-situ* method is the most commonly used for estimating the ruminal degradation of feeds (Martins et al., 2007; Costa et al., 2017). This technique involves determining the disappearance of components from the feed samples enclosed in nylon bags or other synthetic materials, which are then incubated in the rumen for specific incubation periods.

In modern systems for formulating diets for ruminants, the kinetics of degradation of different feed fractions are taken into account. This considers the maximum efficiency of microbial protein synthesis and the reduction of energy and nitrogen losses resulting from ruminal fermentation. This approach allows for the observation of synchronization in the degradation of nitrogen and carbohydrates in the rumen among different feed sources (Fox et al., 1990; Carvalho et al., 2008). In the Peruvian highlands, especially in the Huancavelica region, information regarding the nutritive value of forages and agro-industrial residues is limited, particularly in terms of studies focused on their *in-situ* degradation characterization and their use in the formulation of balanced diets. This is essential to address the microbial and host animal requirements adequately. Given all the aforementioned considerations, the objective of the study was to assess the in situ ruminal fermentation kinetics of dry matter (DM), crude protein (CP), and neutral detergent fiber (NDF) of forage barley (*Hordeum vulgare L.*) containing four levels of quinoa residue (*Chenopodium quinoa*).

MATERIALS AND METHODS

The methodologies and ethical considerations governing this research adhered strictly to the principles delineated in the "Code of Ethics for Scientific Research." Explicit approval for the research procedures was granted under the official sanction of letter Nº 004-GRJ-DRA-AAC-PERÚ-2023, dated January 20, 2023. Furthermore, the execution of all research protocols was conducted with due diligence and in full compliance with the explicit permission and authorization obtained from the pertinent overseeing authorities. The research was conducted in strict accordance with both international and national guidelines governing the ethical care and utilization of research animals.

Study Area

The study was conducted at the laboratory for animal nutrition and feed evaluation belonging to the Professional School of Animal Science at the National University of Huancavelica (UNH). The university is located in the district, province, and region of Huancavelica, Peru, at 11º 59' 10" south latitude and 75º 48' 30" west longitude. The climate is characterized as subtropical highland climate and desert climate. The region experiences two well-defined seasons: a rainy season (October to March) and a dry season (April to September). The average annual temperature ranges from 6 to 14°C. The majority of the soils in the region are classified as superficial (Litosols) (Flores, 1993).

Animals and distribution

Three male Brown Swiss cattle, equipped with permanent ruminal cannulas (Figure 1C) and an average weight of 534.33 + 4.04 kg, aged between 4 and 4.5 years, were used in the study. The experiment was conducted following animal welfare standards. A 12-day adaptation period was allowed, during which the animals were fed a diet consisting of equal parts of alfalfa (*Medicago sativa L.*) and winter forage barley (*Hordeum vulgare L.*) (Figure 1B) provided at 08:00 and 17:00 hours, with access to water and mineral salt ad libitum. The mixture's proportion remained constant during the 7-day experimental period. The studied treatments (4 nevels) were as follows: T0 (control): Forage barley; T1: Forage barley + 10% quinoa residue (QR); T2: Forage barley + 20% QR; T3: Forage barley + 30% QR; the addition of quinoa residue (QR) was based on natural matter. The other independent variable is the incubation times (6 levels) were 0, 6, 12, 12, 24, 48, and 72 hours (Figure 1D). To determine the degradation, the chemical composition of the treatments was determined (Table 1). For this purpose, proximate analysis (Rêgo et al., 2010) and its calculation were performed for DM, which was added to 100 g of sample and placed in a 100°C oven, then a difference between the initial weight of the sample (100%) and for the case of CP, EE, and NDF, the known proximate test was performed, all of which were carried out in the animal nutrition and feeding laboratory following the recommendations of Nocek (1997).

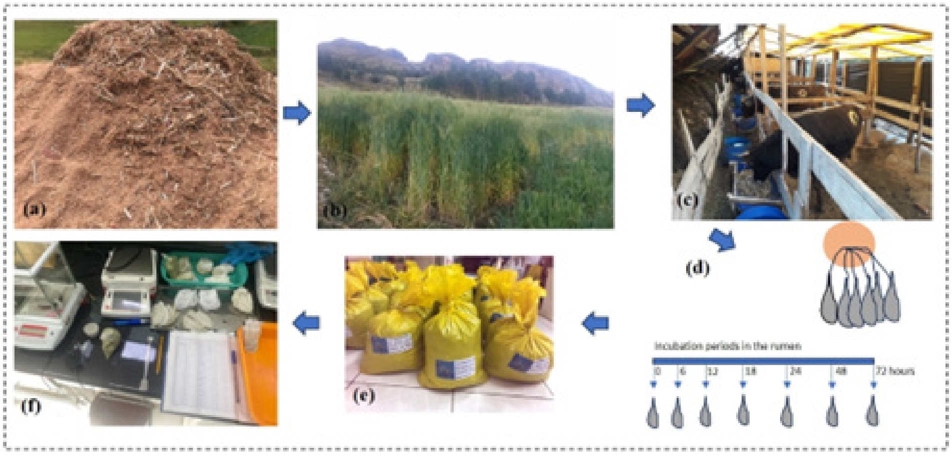

Figure 1 Methodology for data collection. (a) quinoa (Chenopodium quinoa) residues; (b) feed barley (Hordeum vulgare L.); (c) Brown Swiss male bovines, with permanent ruminal cannula; (d) Incubation periods in the rumen; (e) samples collected; (f) laboratory analysis and data collection.

Samples

Barley was sown using broadcast seeding and received no fertilization or irrigation. At 150 days of growth, when the barley reached an average height of 70 cm, it was manually harvested 10 cm above the ground. Subsequently, using a stationary chopping machine, the barley was fragmented into particles of approximately 2 cm. Quinoa residue (QR) was obtained from local agricultural producers. Six replications per treatment were prepared in jute sacks and stored in a covered shed for 30 days. After this period, the sacks were opened, and samples from each treatment were dried in a forced ventilation oven at 65°C for 72 hours. Subsequently, the samples were ground in a Willy-type mill using a 3 mm sieve.

Rumen degradation

Following the recommendations of Nocek (1997) and Orskov et al. (1980), samples of approximately 5g [on a DM (dry matter) basis] were weighed and placed in 7 x 12 cm nylon bags (Ankom) with a porosity of 30 μm, pre-weighed. All samples were incubated in duplicate and simultaneously in the rumen. For incubation, the bags were attached to a stainless-steel chain suspended by a nylon thread in the cannula. The incubation times were 0, 6, 12, 24, 48, and 72 hours (Figure 1D). Bags corresponding to the zero hours (t0) were not incubated but were immersed in water at 39 °C for 15 minutes and then dried in a forced ventilation oven at 60 ºC for 24 hours.

After removal from the rumen, the bags were continuously washed in cold water for 5 minutes to interrupt microbial activity until the water appeared clean. Subsequently, the bags were dried in a forced ventilation oven at 60 ºC for 24 hours and then re-weighed. The in situ disappearance of DM was determined using the following formula: [(Incubated sample, g - Residue, g)/ Incubated sample, g]*100. A fraction of each residue sample was used for the determination of crude protein (CP: Kjeldahl) (Silva and Queiroz, 2002), and NDF (neutral detergent fiber) using an Ankom 200 fiber analyzer (Ankom Technology Corp., Macedon, NY).

The kinetics characteristics of ruminal degradation of DM (dry matter), CP (crude protein), and NDF (neutral detergent fiber) were studied using the model PD = a + b (1 – e-CT) described by Orskov and McDonald (1979), where PD = potential degradability of DM, CP, and NDF calculated as the sum of a + b; a = soluble fraction of nutrients (SF); b = potentially degradable fraction of nutrients (PDF) at a specific degradation rate; C = degradation rate (DR) of PDF; T = incubation time (hours). The undegraded fraction of nutrients was calculated as follows: 100 - (SF + PDF).

The effective degradability (ED) of DM, CP, and NDF in the rumen was calculated based on the constants a, b, and c using the formula described by Orskov and McDonald (1979), ED = a + [(b x c)/(c + k)], where k (%/h) is the estimated rate of particle passage in the rumen (other parameters were described in the previous equation). The parameters a, b, and c were estimated using the Solver add-in in Microsoft Excel (Fernández, 2002). The ED of DM, CP, and NDF were estimated for each level of quinoa residue, considering estimated rates of particle passage in the rumen of 2, 5, and 8%/h, attributed to low, medium, and high levels of feed intake, respectively, according to AFRC (1995).

Statistical Analysis

The experiment was conducted using a completely randomized block design (3 animals) with a 4 x 6 factorial arrangement (levels, times), with the linear model: $Yij=\mu+\tau i+\beta j+(\tau\beta)ij+bk+eijk$, where Yij is the response variable (parameters of in situ degradation), μ is the overall mean, τi is the effect of quinoa residue levels (i = 1, 2, 3, 4), βj is the effect of ruminal incubation times (j = 1, 2, 3, 4, 5, 6), $(\tau\beta)ij$ is the interaction effect between the levels and times, bk is the effect of blocks, and $eijk$ is the experimental error.

Analysis of variance and regression were performed on data related to the degradability of DM, CP, and NDF, using the GLM (generalized linear model) procedure in SAS (2009). Mean differences were compared using the Tukey test at a 5% significance level. The selection of regression models was based on the significance of linear, quadratic, and cubic coefficients, determined by the *Student's t-test* at levels of 0.1%, 1%, and 5%.

RESULTS

Disappearance of Nutrients

Degradability as opposed to chemical composition was shown in Table 1. The average disappearance at time zero and during ruminal incubation times (hours) of DM (dry matter), CP (crude protein), and NDF (neutral detergent fiber) in barley supplemented with quinoa residue (QR) are presented in Table 2. Significant differences were reported for treatments, incubation time, and interaction of treatments ($p<0.05$). For DM disappearance with different levels of QR, it showed an increasing linear relationship ($p<0.05$) at an incubation time of 6 hours, with an attributed increase of 0.18% in DM disappearance for each 1% inclusion of QR. A cubic response was observed in the disappearance of CP for each incubation time, except for the 72-hour incubation, with no significant difference ($p>0.05$). With 8.05, 8.22, 8.77, and 11.57% inclusion of QR in barley for 6, 12, 24, and 48 hours of incubation, the maximum CP disappearances were 43.29, 54.03, 60.41, and 62.36%, respectively, according to the regression equations in Table 2. Regarding the disappearance of NDF in barley with quinoa residue (QR), no significant differences were observed at incubation times of 0, 6, 24, 48, and 72 hours. However, at the 12-hour incubation, the maximum estimated disappearance of NDF was 51.35%, occurring with a 9.07% QR level, resulting in a 24.36% increase in NDF disappearance. With a 24.52% QR level, representing the minimum point, a 44.36% disappearance of NDF was obtained.

Table 1 Chemical composition of the treatments.

Composition	Treatments			
	0%	10%	20%	30%
DM (%)	17.42^{b}	26.09^{a}	26.58^{a}	23.26^{a}
CP[1]	13.52^{c}	14.21^{b}	14.34^{b}	15.62^{a}
EE[1]	6.58^{b}	7.26^{ab}	8.58^{a}	8.12^{ab}
MM[1]	9.52^{a}	10.5^{a}	10.63^{a}	10.58^{a}
NDF[1]	49.77^{a}	41.64^{c}	44.51^{b}	43.55^{b}

1: % within the DM.

a, b, c Different superscripts within rows indicate statistical difference ($p<0.05$).

Table 2 **Table 2** Average disappearance of dry matter (DM), crude protein (CP), and neutral detergent fiber (NDF) of barley added with quinoa residue levels at time zero, and ruminal incubation times (hours).

Time (hours)	Quinoa residue levels (% QR)				Mean	SD*	Regression equation
	0	10	20	30			
Dry matter							
0	6.56	7.21	10.53	10.45	8.68	3.13	ns
6	15.33	15.63	17.65	20.54	17.29	2.91	1
12	22.02	28.55	26.19	28.04	26.20	3.88	ns
24	42.66	38.32	40.72	39.05	40.19	4.17	ns
48	53.46	48.82	48.81	45.70	49.20	5.73	ns
72	64.92	63.31	62.81	58.94	62.49	3.68	ns
Crude protein							
0	23.45	31.93	30.53	34.40	30.08	2.31	2
6	34.11	42.96	36.72	43.86	39.41	4.07	3
12	39.77	53.59	43.77	51.23	47.09	3.93	4
24	49.67	60.25	54.19	58.69	55.70	3.54	5
48	55.78	62.98	57.98	63.46	60.05	2.79	6
72	65.46	71.44	70.57	71.15	69.66	3.31	ns
Neutral detergent fiber							
0	23.73	26.94	26.26	26.15	25.77	3.22	ns
6	35.47	39.47	35.79	39.82	37.64	3.45	ns
12	41.29	51.22	45.57	47.10	46.29	3.48	7
24	55.28	57.84	56.94	56.59	56.66	3.78	ns
48	62.29	64.88	62.28	59.95	62.35	4.68	ns
72	66.57	70.44	70.46	68.39	68.97	5.22	ns

* Standard deviation of the mean

[1] $Y = 14.636 + 0.177^{*}N$ ($R^2 = 0.3559$), [2] $Y = 23.447 + 1.849^{**}N – 0.125^{*}N^2 + 0.00253^{*}N^3$ ($R^2 = 0.8240$), [3] $Y = 34.107 + 2.589^{**}N - 0.218^{**}N^2 + 0.00474^{**}N^3$ ($R^2 = 0.6056$), [4] $Y = 39.767 + 3.929^{**}N - 0.323^{**}N^2 + 0.00682^{**}N^3$ ($R^2 = 0.7509$), [5] $Y = 49.673 + 2.796^{**}N – 0.219^{*}N^2 + 0.00453^{*}N^3$ ($R^2 = 0.6711$), [6] $Y = 55.777 + 2.088^{**}N – 0.175^{*}N^2 + 0.00378^{*}N^3$ ($R^2 = 0.6732$), [7] $Y = 41.287 + 2.530^{*}N – 0.191^{*}N^2 + 0.00379^{*}N^3$ ($R^2 = 0.6094$).

*** P<0.001, **P<0.01, * P<0.05.

Average values of nutrients

The estimations of the mean values for the soluble fraction (SF), potentially degradable fraction (PDF), indegradability (I), and the degradation rate of PDF (DR) of DM, CP, and NDF were influenced (p<0.05) by the levels of QR (Table 3).

Dry Matter (DM): The soluble fraction (SF) of barley DM with QR levels showed an increase, ranging from 5.46 to 11.67%, for levels from 0 to 30%, respectively. These increases were linearly related to QR levels, with an increase of 0.21% in DM solubility for each unit of added QR. The potentially degradable fraction (PDF) of DM varied between 68.48% for the lowest level and 52.33% for the highest level of QR, being influenced cubically by QR levels (p<0.001). The minimum and maximum values of DM PDF were estimated at 61.79% and 62.17% for QR levels of 10.86% and 17.33%, respectively, according to the regression equation Y = 68.483 – 1.558N + 0.1167N2 – 0.00276**N3 (Table 3). Barley free of QR had a degradation rate (DR) of 2.82%/hour in DM. From this QR level onwards, there was a quadratic reduction in this parameter, with 14.16% QR allowing a minimum of 2.55%/hour in DM degradation in barley evaluated at 130, 150, and 170 days. It was observed that as the QR level increased, there was a non-linear increase in the indigestible fraction (I) of DM, corresponding to the third-degree model. According to this behavior, the minimum value of 27.65% for the indigestible fraction of DM was estimated, with a minimum QR level of 20.09%.

Crude protein (CP): The QR levels had a cubic effect ($p<0.001$) on the CP soluble fraction (SF) of barley, ranging from 24.68 (0% QR) to 34.97% (30% QR), with estimated maximum and minimum contents of 32.12% and 30.85% SF, respectively, with the addition of 11.09% and 21.45% QR. The PDF of CP in the treatments was influenced ($p<0.001$) by QR levels, showing a cubic response (Table 3), varying from 45.10 (0% QR) to 35.31% (30% QR). The minimum and maximum values were 36.03% and 45.04% with additions of 7.34% and 22.33% QR, respectively. QR levels influenced the CP degradation rate (DR) cubically, ranging from 3.33%/h (0% QR) to 6.41%/h (10% QR), with estimated maximum and minimum values of 6.78% and 2.01%/h, with the addition of 7.16% and 23.71% QR, respectively. It was observed that there were no significant differences for QR levels of 0, 10, and 30% for the indigestible fraction (I). However, differences were reported for the mentioned levels compared to the 20% QR with 24.14%.

Neutral detergent fiber (NDF): For the soluble fraction (SF), significant differences were reported between 0% (23.63%) and the levels 10% (27.23%), 20% (26.12%), and 30% (26.43%) of QR. The PDF of the NDF in the treatments was influenced ($p<0.001$) by QR levels, showing a cubic response (Table 3), ranging from 44.11% (0% QR) to 39.44% (30% QR). Similarity was reported between 44.11% and 44.11% for 0% and 20% QR. QR levels influenced the NDF degradation rate (DR) cubically, finding similarity between 0% and 20% QR and similarly between 10% and 30% QR. It was observed that there were significant differences for QR levels of 0%, 10%, 20%, and 30% for the indigestible fraction (I), with 32.26%, 30.70%, 29.77%, and 34.03%, respectively.

Table 3 Mean values of soluble fraction (SF), potentially degradable fraction (PDF), undegradability (I), and degradation rate (DR) of dry matter (DM), crude protein (CP), and neutral detergent fiber (NDF) of barley feed with quinoa residue levels added.

Parameters	Quinoa residue levels (% QR)				Mean	SD*	Regression equation
	0	10	20	30			
Dry matter							
SF (%)	5.46[c]	7.86[bc]	10.45[ab]	11.67[a]	8.86	2.81	1
FPD (%)	68.48[a]	61.82[b]	61.95[b]	52.33[c]	61.15	4.25	2
I (%)	26.05[b]	30.32[b]	27.60[b]	36.00[a]	29.99	5.08	3
TD (%/hora)	2.82[ab]	2.73[ab]	2.43[b]	2.96[a]	2.74	0.57	4
Crude protein							
SF (%)	24.68[c]	32.11[b]	31.00[b]	34.97[a]	30.69	2.46	5
FPD (%)	45.10[a]	36.82[b]	44.85[a]	35.31[b]	40.52	3.66	6
I (%)	30.22[a]	31.06[a]	24.14[b]	29.58[a]	28.75	3.76	7
TD (%/hora)	3.33[c]	6.41[a]	2.62[c]	4.60[b]	4.24	1.10	8
Neutral detergent fiber							
SF (%)	23.63[b]	27.23[a]	26.12[a]	26.53[a]	25.88	2.57	9
FPD (%)	44.11[a]	42.07[ab]	44.11[a]	39.44[b]	42.43	5.64	10
I (%)	32.26[ab]	30.70[bc]	29.77[c]	34.03[a]	31.69	5.04	11
TD (%/hora)	4.63[b]	6.23[a]	4.63[b]	6.46[a]	5.56	1.17	12

* Standard deviation of the mean.

[a, b, c, d:] Different superscripts within rows indicate statistical difference (p<0.05).

[1] $Y= 5.681 + 0.212^{***}N$ ($R^2 = 0.4232$), [2] $Y= 68.483 – 1.558^{***}N + 0.1167^{**}N^2 – 0.00276^{***}N^3$ ($R^2 = 0.6602$), [3] $Y = 26.050 + 1.378^{**}N – 0.1253^{**}N^2 + 0.00302^{**}N^3$ ($R^2 = 0.3711$), [4] $Y= 0.0288 – 0.000453^{*}N + 0.0000155^{*}N^2$ ($R^2 = 0.0719$), [5] $Y = 24.68 + 1.624^{***}N – 0.1108^{***}N^2 + 0.00227^{***}N^3$ ($R^2 = 0.7123$), [6] $Y = 45.103 – 2.773^{***}N + 0.251^{***}N^2 – 0.00564^{***}N^3$ ($R^2 = 0.6145$), [7] $Y = 30.220 + 1.143^{**}N – 0.139^{***}N^2 + 0.00335^{***}N^3$ ($R^2 = 0.3546$), [8] $Y = 0.03324 + 0.0107^{***}N – 0.00097^{***}N^2 + 0.000021^{***}N^3$ ($R^2 = 0.6426$), [9] $Y = 23.633 + 0.803^{***}N – 0.0547^{**}N^2 + 0.00104^{*}N^3$ ($R^2 = 0.2282$), [10] $Y = 44.232 – 0.120^{*}N$ ($R^2 = 0.0550$), [11] $Y = 32.483 – 0.393^{*}N + 0.0146^{*}N^2$ ($R^2 = 0.0887$), [12] $Y = 4.942 + 0.48429^{***}N – 0.0461^{***}N^2 + 0.001054^{***}N^3$ ($R^2 = 0.3280$).

*** P<0.001, ** P<0.01, * P<0.05, ns not significant.

Potential degradability (PD) and effective degradability (ED)

Table 4 presents the results of potential degradability (PD) of dry matter (PDDM), crude protein (PDCP), and neutral detergent fiber (PDNDF), as well as effective degradability (ED): EDDM, EDCP, and EDNDF for passage rates of 2, 5, and 8%/h. A third-degree behavior (p<0.001) was observed in PDDM, where initially, values showed an increasing trend with the addition of QR up to the 20% level, followed by a decrease with the subsequent level. The minimum PDDM obtained (69.40%) was with the addition of 7.57% QR.

Table 4 Potential (PD) and effective (ED) degradabilities at three passage rates of dry matter (DM), crude protein (CP), and neutral detergent fiber (NDF) of barley supplemented with quinoa residue levels.

Parameters	Quinoa residue levels (% QR)				Mean	SD*	Regression equation
	0	10	20	30			
	Dry matter						
DP (%)	64.10[b]	69.69[a]	72.40[a]	64.10[b]	70.03	5.09	1
DE (2%/h)	45.11[a]	43.54[ab]	44.15[ab]	42.12[b]	43.73	3.05	2
DE (5%/h)	29.86[a]	29.71[a]	30.56[a]	30.59[a]	30.18	2.77	ns
DE (8%/h)	23.10[a]	23.61[a]	24.79[a]	25.43[a]	24.23	2.65	3
	Crude protein						
DP (%)	69.78[b]	68.94[b]	75.86[a]	70.42[b]	71.25	3.76	4
DE (2%/h)	51.85[c]	59.90[a]	56.03[c]	59.65[a]	56.86	2.06	5
DE (5%/h)	42.18[a]	52.53[a]	46.16[b]	51.95[a]	48.21	2.30	6
DE (8%/h)	37.66[c]	48.28[a]	41.89[b]	47.93[a]	43.94	2.25	7
	Neutral detergent fiber						
DP (%)	67.74[bc]	69.30[ab]	70.23[a]	65.97[c]	68.31	5.21	ns
DE (2%/h)	54.73[c]	58.72[a]	56.86[b]	56.13[bc]	56.61	3.08	8
DE (5%/h)	45.26[c]	50.17[a]	47.26[b]	48.23[b]	47.73	2.40	9
DE (8%/h)	40.23[c]	45.30[a]	42.23[b]	43.72[b]	42.87	2.25	10

* Standard deviation of the mean.

a, b, c Different superscripts within rows indicate statistical difference ($p<0.05$)..

1 Y = 73.950 – 1.374**N+ 0.1248**N 2 – 0.00300**N 3 (R2 = 0.3652), 2 Y = 44.981 – 0.0834*N (R2 = 0.0874), 3 Y = 23.011 + 0.0816**N (R2 = 0.1086), 4 Y = 69.780 – 1.143**N + 0.1394***N 2 – 0.00335***N 3(R2 = 0.3546), 5 Y = 51.847 + 2.048***N – 0.156***N 2 + 0.00323***N 3 (R2 = 0.7280), 6 Y = 42.183 + 2.833***N – 0.228***N 2 + 0.0048***N 3 (R2 = 0.7858), 7 Y = 37.663 + 2.893***N – 0.232***N 2 + 0.00491***N 3 (R2 = 0.8040), 8 Y = 54.727 + 0.924**N – 0.0641*N 2 + 0.00116*N 3 (R2 = 0.1876), 9 Y = 45.260 + 1.273***N – 0.0977***N 2 + 0.00195***N 3 (R2 = 0.3656), 10 Y = 40.233 + 1.337***N – 0.1042***N 2 + 0.00212**N 3 (R2 = 0.4232).

*** P<0.001, **P<0.01, * P<0.05, ns not significant.

DISCUSSION

Degradable nutrient

Regarding the SF of dry matter (DM), the increase in SF in the treatments can be attributed to the higher solubility of QR. Compared to QR-free barley, the other treatments show higher solubilities in barley. Carvalho et al. (2008) reported similar behavior for the SF of DM when adding cocoa bran and urucum grain byproduct (*Bixa Orellana L.*) to elephant grass (*Pennisetum purpureum Schum*) silage, respectively. Cordero et al. (2018), evaluating the degradability of barley as winter forage in alpacas, found a value of 19.15% for SF, which is higher than the one found in this study. The mean of 61.15% for the PDF observed in this study is higher than the PDF value of 59.36% obtained for barley as a winter crop in alpacas (Cordero et al., 2018). These differences in this parameter of DM forages, which is potentially degradable by rumen microorganisms, may be related to the levels of NDF of the species under study, which allows a decrease in forage degradability (Razz et al., 1999).

Regarding DR (degradation rate), Contreras et al. (2018) reported degradation rates of 2, 1.67, and 2.33%/hour for the DM of barley, values that are in line with those obtained in this study. In degradability studies in alpacas, Cordero et al. (2018) observed a DR of 10.06%/h in the DM of barley as a winter crop. It is concluded that the quality of barley added with RQ can be considered high when evaluated by the degradation rate, whose values ranged from 2.82 (0% QR) to 2.96%/h (30% QR). Sampaio (1988) suggests that good-quality plant foods have degradation rates of 2 to 6%/h.

Concerning the SF of crude protein (CP), the results obtained are supported by Cordero et al. (2018), who, when evaluating the degradability of barley, found 31.53% in SF, a value that falls within the range observed in this study. However, Contreras et al. (2018) reported values of 53.56, 51.97, and 59.64% for barley evaluated at 130, 150, and 170 days of growth. It can be inferred that the high figures for CP SF in this species are related to particle size (Villela et al., 1999) or the high content of non-nitrogenous compounds (urea, free amino acids, and small peptides), which are released in the rumen and quickly converted into ammonia nitrogen (Guerrero et al., 2010; Contreras et al., 2019A). According to Klopfenstein et al. (2001), the contribution of this nitrogen to microbial protein production is relevant; however, there is a limit above which the SF fraction is not physiologically acceptable, as it should not exceed 40% of the effective degradability of CP (EDCP) (AFRC, 1995). Considering EDCP (Table 4) at a rumen particle passage rate of 5%/h, barley containing 20 and 30% QR showed equal and the highest SF: EDCP compared to barley with 0 and 10% QR (58.51 and 61.13%, respectively). These results indicate nitrogen losses by ruminants. Higher losses of this nutrient were observed in barley added with 0, 10, 20, and 30% QR (61.35, 70.88, 73.41, and 80%, respectively) when considering EDCP at an 8%/h flow rate. Contreras et al. (2018) reported SF: EDCP ratios of 81.15, 78.49, and 85.76% for barley at 130, 150, and 170 days of growth, respectively. These values, higher than the recommended < 40% (AFRC, 1995), can be attributed to the growth stage of the species, fertilization, and management.

Regarding the values of CP PDF, Cordero et al. (2018) observed 39.82% for this parameter in barley, a figure slightly higher than the minimum value obtained in this study. Values of 39.92, 25.73, and 22.61% for CP PDF in barley at 130, 150, and 170 days of growth were found by Contreras et al. (2018). Rêgo et al. (2010) observed an increase in CP PDF in elephant grass silage by adding urucum grain byproduct (UGB) (*Bixa Orellana L*) from 19.86% (0% UGB) to 31.38% (12% UGB). The magnitude of CP PDF is a function of the time the food remains subjected to the enzymatic activity of rumen microorganisms (NRC, 2007).

Like SF and PDF, the indigestible fraction (I) of CP was affected by QR levels. In the regression analysis, a cubic effect was observed ($p<0.001$), with estimated maximum and minimum values of 32.88 and 23.73% of CP I in barley, with the addition of 5.02 and 22.64% QR, respectively. The DR values for CP confer good nutritional potential to ruminants in barley added with QR (Salado et al., 2007). According to NRC (1985), the DR of CP PDF varies from 2 to 8%. Despite variations in SF and FPD, CP degradation rates remained constant, ranging from 2.62 to 4.60%/h, except for barley added with 10% QR, which was 6.41%/h. Similarly, DM degradation rates remained stable, with DR ranging from 2.43%/h (20% QR) to 2.96%/h (30% QR). A cubic effect ($p<0.001$) of QR levels on the SF of NDF in barley was observed, with variations from 23.63 (0% QR) to 27.23% (10% QR). This behavior presented maximum and minimum estimated values of 27.24 and 25.77%, with 10.46 and 24.60% QR addition, respectively. Normally, the SF of NDF should be close to zero (0) (Huhtanen and Krizsan, 2023), as this parameter is represented by particles lost through bag pores since cell wall constituents are insoluble in water.

The addition of increasing amounts of QR to barley resulted in a linear decreasing effect on NDF PDF (Y = 44.232 – 0.120*N). That is, for each 1% addition of QR, NDF PDF decreased by 0.12% between the levels of 0 and 30% QR. DR varied from 4.63 (0% QR) to 6.46%/h (30% QR) with maximum and minimum estimated values of 6.44 and 4.51%/h for QR levels of 6.88 and 22.27%, respectively. As a comparative reference, Contreras et al. (2018) reported DR values between 2.77 and 3.39%/h in barley at three different ages, which are lower than those obtained in barley added with QR. According to Mertens (1993), for forages to be considered of quality, their NDF degradation rates should range from 2 to 6%/h. According to this classification, it is inferred that barley, with or without QR addition, is suitable forage for ruminants in terms of quality.

Potential degradability (PD) and effective degradability (ED)

Effective degradability (ED), measures the magnitude of the reduction in potential degradability (PD) due to the effect of the particle passage rate through the rumen. Barley with 0% and 20% QR showed reductions in PD of 14.50% (54.73 – 40.23) and 14.63% (56.86 – 42.23), respectively, compared to levels of 10% and 30% QR, whose reductions were 13.42% (58.72 - 45.30) and 12.41% (56.13 – 43.72) as particle passage rates increased from 2 to 8%/h (Table 3). Considering the potential degradability of NDF (PDNDF) and the value of k = 8%/h decreases in degradability were similar: 40.61% and 39.87% for 0% and 20% QR treatments, respectively. Associated with the potentially degradable fraction (PDF = 44.11%) and degradation rate (DR = 4.63%/h) of each of these treatments, more time was needed to degrade the cell walls of both treatments (Yang et al. 2023) compared to barley with 10% and 30% QR, whose depressive effects on the passage rate of 8%/h on NDF degradability were 34.64 and 33.73%, respectively. These treatments presented lower proportions of PDF (42.07 and 39.44%) and higher DR (6.23 and 6.46%/h), respectively. Consequently, NDF in barley added with 10% and 30% QR disappeared from the rumen in shorter times.

Table 4 presents the results of potential degradability (PD) of DM (PDDM), CP (PDCP), and NDF (PDNDF), and effective degradability (ED): EDDM, EDCP, and EDNDF for passage rates of 2, 5, and 8%/h. A third-degree behavior ($p<0.001$) was observed in PDDM, where initial values showed an increasing trend due to the addition of QR up to the level of 20%, then decreased with the next level. The minimum PDDM obtained (69.40%) was with the addition of 7.57% QR. The observed PDDM (72.40%) for the 20% QR treatment is close to 78.71% of PDDM for alfalfa, a high-quality forage (Evangelista et al., 2002; Viégas and Marques, 2011). Under tropical conditions, Meinerz et al. (2011) obtained 66.20% of the estimated dry matter digestibility (DMD) for dual-purpose BRS Marciana winter barley, a value that is close to 69.69% of the observed PDDM in barley added with 10% QR. When comparing the results observed in this study, which were lower, with 86.71 and 82.835 (Contreras et al., 2018) for PDDM of barley at 130 and 170 days of growth, respectively, this can be attributed to the varieties used and the state of maturation.

At a passage rate of 2%/h, all treatments showed EDDM below 50%, decreasing linearly, and the addition of 30% QR favored a decrease of 2.5 percentage units in EDDM for the treatments. The opposite behavior was observed for the passage rate of 8%/h, with a linear increasing response ($p<0.01$), allowing the highest QR level (30%) to increase by 2.5 percentage points in EDDM. At each of the QR levels studied, EDDM decreased as the passage rate (k) increased.

PDCP and EDCP at the passage rate of 2%/h were above 50%. For levels of 0, 10, and 30% QR, PDCP was similar ($p>0.05$), differing from the 20% QR level (75.86% of PDCP). This degradability is close to the 77.69% obtained by Contreras et al. (2018) in barley at 150 days of growth. The PDCP (70.42%) of barley added with 30% QR is consistent with what was observed (71.35%) by Cordero et al. (2018) in studies with alpacas. Through regression, it was possible to observe a third-degree behavior ($p<0.001$), where PDCP values reached maximum values (76.48%) with the addition of 22.74% QR. For passage rates of 2 and 8%/h, EDCP was higher ($p<0.05$) in barley added with 10% QR compared to treatment with barley alone. Statistical analysis at all three passage rates revealed cubic responses ($p<0.001$). For passage rates of 2, 5, and 8%/h, the maximum EDCP reached was 60, 52.74, and 48.51% with the addition of 9.18, 8.49, and 8.56% QR, respectively. For these same passage rates, the estimated minimum EDCP was 55.76, 45.13, and 41.21% with the addition of 23.76, 23.18, and 22.94% QR, respectively.

The regression analysis of QR levels did not show an effect ($p>0.05$) on the PDNDF of barley. However, a trend was observed in the increase in PD, reaching the highest values (69.30 and 70.23%) with the addition of 10 and 20% RQ, and then decreasing with the higher level used. Showing that for NDF, these treatments have the highest disappearances (70.44 and 70.46%, respectively). There was an increase of 2.49 percentage points in PDNDF between 0% and 20% QR, equivalent to a 4% increase in PD. Contreras et al. (2018), for the PDNDF of barley at 150 days of growth, report 72.81%, a value that exceeds by three percentage units what was obtained (70.23%) in this study. The observed EDNDF at the passage rate of 2%/h exceeds 50%, compared to the other rates. At rates of 2, 5, and 8%/h, barley with 0% QR had the lowest coefficients (54.73, 45.26, and 40.23%, respectively). Corresponding to each of them, third-degree responses ($p<0.01$, $p<0.001$, and $p<0.001$, respectively), where EDNDF reached maximum values of 59, 49.68, and 45.37% with the addition of 10, 12.62, and 8.75% QR, respectively. EDNDF decreased as the passage rate increased for all levels of QR addition. This behavior coincides with literature reports (Rêgo et al., 2010; Contreras et al., 2018), and is due to the shorter residence time of the food in the digestive tract for the action of the ruminal microbiota.

CONCLUSIONS

The highest disappearances of crude protein occurred at 6, 12, 24, and 48 hours of ruminal incubation of barley with levels of quinoa residue ranging from 8.05% to 11.57%

Barley with 10 or 30% quinoa residue stood out for their higher values of the soluble fraction (SF) and effective degradability of crude protein, contrary to the other levels of quinoa residue.

Due to the degradation rates (DR) ranging from 2.43 to 2.96%/h for dry matter and between 2.62 and 6.41%/h for crude protein, barley with or without quinoa residue can be considered as good-quality feed.

Barley with 20% quinoa residue constitutes forage with the highest potential degradability of dry matter and crude protein, and lower coefficients of the indigestible fraction.

Barley with 10 or 20% quinoa residue stood out for the highest potential and effective degradability of neutral detergent fiber, and lower indigestible fractions.

ACKNOWLEDGEMENTS

Acknowledgments to the Socioeconomic Development Funds of the Camisea-FOCAM Project (Graduate Students) for funding the project execution titled 'Nutritional Value and Estimation of Barley (Hordeum vulgare L.) Consumption as Winter Forage with Different Levels of Quinoa Residue (Chenopodium quinoa)'. We also extend our gratitude to the Animal Nutrition and Feed Evaluation Laboratory for providing fistulated cattle for the in-situ degradability trials.

CONFLICT OF INTEREST

There is no conflict of interest among the authors regarding the publication of this article.

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How to cite this article;
Espinoza Abregu Diego, Huaman Jurado Rodrigo, Castrejon Valdez Manuel, Mayhua Mendoza Paul, Contreras Paco José Luis, Cordero Fernández Alfonso and Jordan Ninahuanca Carhuas. Rumen kinetics of nutrient degradability of forage barley (*Hordeum vulgare L.*) with different levels of quinoa (*Chenopodium quinoa*) residues supplementation.Veterinary Integrative Sciences. 2024; 22(3): 1073 - 1087