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Kinetics of rumen degradation of oat silage-potato foliage content *Saccharomyces cerevisiae* in Peru

Cinética de degradação no rúmen do teor de silagem de aveia e folhagem de batata com Saccharomyces cerevisiae no Peru

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ABSTRACT

The aim of this study was to assess the *in situ* degradation of dry matter (DM), crude protein (CP), and neutral detergent fiber (NDF) in oat silage-potato foliage (OSPF) (70:30) containing four levels (0, 25, 50, 75 g of *Saccharomyces cerevisiae* yeast/kg of fresh forage) as an additive. A randomized complete block design was employed in a 4 x 8 factorial scheme (levels, times) with three replications to determine the impact of yeast levels and incubation times on the degradation of variables. Three Brown Swiss cattle with ruminal fistula were used. Samples of 5 g were incubated in nylon bags for 4, 8, 12, 24, 48, 72, and 96 hours. Time zero (t0) allowed for estimating the soluble fraction. The degradation parameters of DM, CP, NDF, and acid detergent fiber (ADF) were analyzed using a randomized complete block design with three replications. The potential degradation (PD) of DM, NDF, and ADF in silages was not influenced by yeast levels, with means of 89.55, 85.22, and 83.45%, respectively. This trend was also observed for effective degradations at 2, 5, and 8%/hour of DM, NDF and ADF. Silages containing 25, 50, and 75 g of yeast/kg of OSPF, which did not differ from each other, showed significant superiority ($p<0.05$) in CP degradation compared to yeast-free silage. In the latter silage, the indigestible rate (i) of CP was higher than in silages containing yeast, with degradation rates (c) of 2%/hour. Among the silages, the one containing 25 g/kg of OSPF exhibited high potential CP degradation (93.18%), effective at 2%/hour (87.48%), and a low indigestible rate (6.82%), classifying it as a forage resource with good nutritional quality.

KEYWORDS: degradation rate; indigestible fraction; potential; hours of incubation.

RESUMO

O objetivo foi avaliar a degradação *in situ* da matéria seca (MS), proteína bruta (PB) e fibra detergente neutro (FDN) na silagem de aveia-folhagem de batata (AFB) (70:30) contendo quatro níveis (0, 25, 50, 75 g de levedura *Saccharomyces cerevisiae*/kg de forragem fresca) como aditivo. Foi utilizado um delineamento experimental de blocos completos ao acaso, em um esquema fatorial 4 x 8 (níveis, tempos) com três repetições para determinar o impacto dos níveis de levedura e dos tempos de incubação na degradação das variáveis. Três bovinos da raça Brown Swiss com fístula ruminal foram utilizados. Amostras de 5 g foram incubadas em sacos de nylon por 4, 8, 12, 24, 48, 72 e 96 horas. O tempo zero (t0) permitiu estimar a fração solúvel. Os parâmetros de degradação da MS, PB, FDN e FDA foram analisados utilizando um delineamento de blocos completos ao acaso, com três repetições. A degradação potencial (DP) da MS, FDN e FDA nas silagens não foi influenciada pelos níveis de levedura, com médias de 89,55, 85,22 e 83,45%, respectivamente. Isso também foi observado para as degradações efetivas a 2, 5 e 8%/hora da MS, FDN e FDA. As silagens contendo 25, 50 e 75 g de levedura/kg de AFB, que não diferiram entre si, mostraram superioridade significativa ($p<0,05$) na degradação da PB em comparação com a silagem sem levedura. Na última silagem, a taxa indigestível (i) da PB foi maior do que nas silagens contendo levedura, com taxas de degradação (c) de 2%/hora. Entre as silagens, aquela contendo 25 g/kg de AFB apresentou alta degradação potencial de PB (93,18%), efetiva a 2%/hora (87,48%) e baixa taxa indigestível (6,82%), classificando-a como um recurso forrageiro de boa qualidade nutricional.

PALAVRAS-CHAVE: taxa de degradação; fração indigestível; potencial; horas de incubação.

INTRODUCTION

Certain areas of the Huancavelica region in Peru offer excellent conditions for dairy farming (CONTRERAS et al. 2024). However, in most areas where dual-purpose South American cattle, sheep, and camelids are primarily raised, there is a limited supply of forage, especially during the dry season, and the forage mass is of low nutritional value (ESTREMADOYRO et al. 2024). On the other hand, during the rainy season, pastures are composed of alfalfa (*Medicago sativa* L.), clover (Trifolium pratense L.), dactylis (Dactylis glomerata L.), and ryegrass (Lolium perenne L.). Among the annual crops, oat varieties (*Avena sativa* L.), such as Mantaro 15, Strigosa, and Silvestre, are grown together with barley (Hordeum vulgare L.) and triticale (Triticosecale spp.) (LUIS et al. 2020).

In the agricultural context, potato (*Solanum tuberosum* L.) is an important food resource for humans. At the end of the tuber growth phase, the foliage, consisting of stems, leaves, and flowers, becomes available and is used in the feeding of ruminants (providing 26% of the daily requirement of copper; 17 to 18% potassium, phosphorus, iron; and between 5 and 13% zinc, magnesium, and manganese; and up to 50% vitamin C), especially cattle, directly without any treatment (LÓPEZ et al. 2020). In general, forage is often discontinued or underutilized in animal feed because of a lack of knowledge of its nutritional value (BAUTISTA et al. 2024). Therefore, potato foliage can be an alternative for ensiled with oats to supply forage in ruminants during the dry season. This shortage becomes critical and compromises the productivity rates of the systems.

Among the forage conservation techniques, ensiling stands out because it allows for prolonged preservation of the forage and reduces nutrient losses during the fermentation process (ZHOU et al. 2019).

Saccharomyces cerevisiae yeast are characterized by high protein content, ranging from 40% to 50% of its dry weight, and excellent quality based on its essential amino acid profile (JACH et al. 2022, AGBOOLA et al. 2021). Furthermore, it improves ruminal functions, leading to an increase in the passage of nitrogen to the duodenum and a reduction in methane emissions, especially in animals fed starch-rich diets (CATTANEO et al. 2023). It is also recognized that yeasts are undesirable microorganisms in silage because they are the main initiators of aerobic deterioration during the fermentation process and silo opening (SOUNDHARRAJAN et al. 2021). However, its ability to consume oxygen has had a positive effect on silage quality, ensuring the emergence of anaerobic microorganisms during the fermentation of the ensiled material (MUCK et al. 2018).

The use of additives or inoculants in silage improves nutritional quality and regulates intestinal microflora (ZHOU et al. 2019). Incorporation into forage during the ensilage phase exerts positive effects on nutritional quality. For example, FERNÁNDEZ et al. (2021) observed protein concentrations of 17.06% and 16.81% in oat silage at 21 and 42 days of fermentation, and in the case of barley silage, protein concentrations were 17.98% and 17.11% at the same fermentation times with the addition of 45 g of yeast/kg of fresh forage, respectively. This was compared with silage without this additive (0%), which had oat protein concentrations of 8.86% and 9.17% and barley protein concentrations of 9.13% and 9.56%, respectively, at the mentioned storage times.

It is advantageous to have information about degradation rates and food flow. Under the conditions of the Huancavelica region in Peru, there are few or no studies aimed at characterizing the nutrient degradation profile of silage containing additives. Taking these aspects into consideration, the aim of the study was to evaluate the *in situ* degradation kinetics of dry matter (DM), crude protein (CP), and neutral detergent fiber (NDF) in oat and potato leaf silage containing four levels of yeast (S. cerevisiae).

MATERIALS AND METHODS

The study was conducted in accordance with national guidelines for the care and use of research animals. According to the directive "This is authorized by letter No. 009-GRJ-DRA-AAC-PERÚ-2023, issued on December 22, 2023".

Study Area

Ruminal incubation and stomatological analysis were performed at the facilities and in the Animal Nutrition and Food Evaluation Laboratory of the Professional Academic School of Animal Science of the Faculty of Engineering Sciences of the National University of Huancavelica (UNH) (Figure 1). The university is located in the district, province, and region of Huancavelica at a longitude of 74°58'21.58"W and latitude of 12°46'57.4"S. The area is at an altitude of 3,704 m above sea level, with an average annual temperature ranging from 6°C to 14 °C.

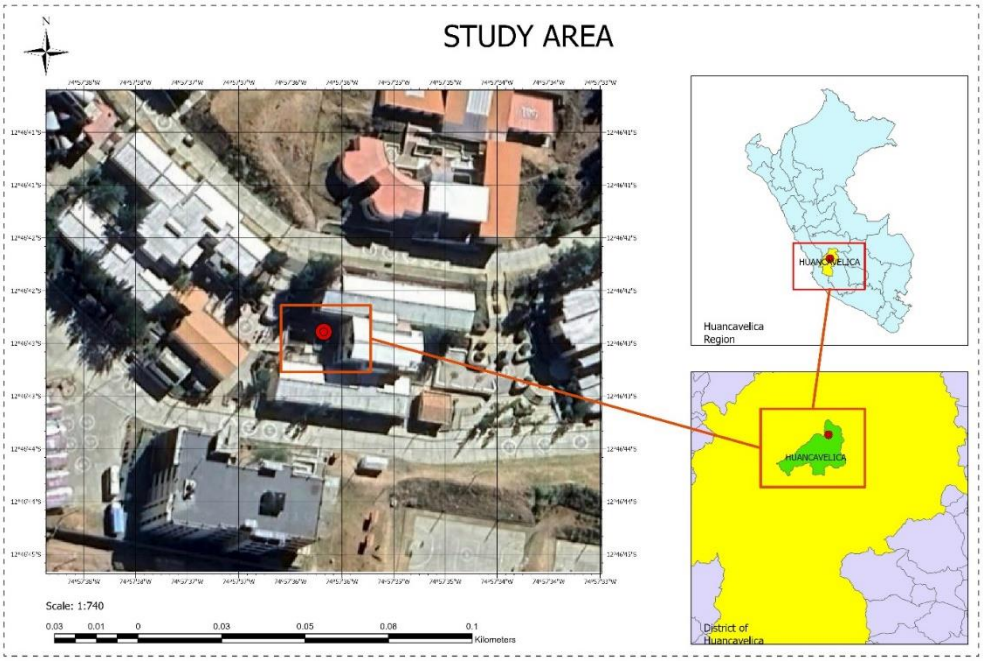

Figure 1. Study area location.

Treatments

Plastic buckets with a capacity of 20 L and pressure lids were used as experimental silos. These were distributed into four levels of *S. cerevisiae* yeast inclusion (LV) (0, 25, 50 and 75 g/kg oat-potato foliage, OPF), in a ratio of 70:30. Each treatment consisted of three replicates.

The silages were prepared using the Mantaro 15 oat variety harvested at 180 days of growth and foliage of the Unique potato variety and collected at four months of age from January to April 2023. Both forages were disintegrated into particles approximately 2 cm in diameter using a stationary Retsch grinder. The additive was included based on the natural material of the ensiled mass, with each silo containing 20 kg of fresh material. After 60 days of fermentation, the silos were opened, and 250 g samples were collected. These samples were pre-dried in a forced ventilation oven at 65 °C for 72 h and then ground in a Willy-type mill through a 3-mm sieve. The volume of each experimental silo was 0.05 m^3, and the storage density was calculated as 400 kg/m3

***In situ* experiments**

To determine the degradation (degradability) of AFP silage supplemented with different levels of yeast, the *in situ* nylon bag technique was used. To evaluate the degradation kinetics of dry matter (DM), crude protein (CP), and neutral detergent fiber (NDF), three male Brown Swiss cattle with ruminal fistulas were used. These animals were aged between 3 and 3.5 years and weighed an average of 545 kg live weight and were kept in confinement throughout the experimental period. They were fed twice daily *ad libitum*, in the morning (08:00 h) and in the evening (17:00 h), with equal portions of alfalfa (Medicago Sativa) and fresh oats. The animals were housed individually in pens equipped with feeders, drinkers and mineral salt boxes. Nylon bags (ANKOM) measuring 7 x 12 cm with a porosity of 50 µm, were used. Five grams of sample (on a dry matter basis) were placed in each nylon bag and incubated in the rumen for the following morning intervals: 4, 8, 12, 24, 48, 72, and 96 h after feeding. All samples were incubated in duplicate at each time point. The samples at time zero (t0) corresponded to the washing of bags in cold running water to determine the soluble fraction. Subsequently, the bags were dried in a forced ventilation oven at 60 °C for 72 h (SULTANA et al. 2021).

After the incubation period, the bags were removed from the rumen, washed manually in running water to remove external impurities, and then frozen for subsequent dehydration, weighing, and analysis. At the end of the incubation period, the bags were dehydrated in a forced ventilation oven at 60 °C for 72 h and weighed to determine the degradation (disappearance) of dry matter (DM). In both samples and

residues, the concentrations of crude protein (CP) and neutral detergent fiber (NDF) were analyzed following the methodology described by HALL et al. (1997).

The *in situ* degradation data of DM (DISM), CP (DISPC), and NDF (DISFDN) were obtained by calculating the weight difference of each component between the measurements performed before and after ruminal incubation, expressed in percentages .

The model described by ORSKOV & MCDONALD (1979) was used to determine the degradation potential of dry matter (DM), crude protein (CP), and neutral detergent fiber (NDF) according to the following formula: $DP = a + b\ (1\text{-}exp^{-Ct})$, (Equation I), where: DP = actual percentage of nutrient degraded after t hours of ruminal incubation, a = immediately soluble fraction of material in the nylon bag, b = potentially degradable fraction of material remaining in the nylon bag after time zero, c = degradation rate of the fraction remaining in the nylon bag after time zero, t = ruminal incubation time in hours, exp = the base of the natural logarithms.

The nondegradable fractions of DM, CP, and NDF were determined by the difference. [i = 100 – (a + b)], which is the percentage sum of the immediately soluble fractions (a) of the material in the nylon bag, b (potentially degradable fraction of the material remaining in the nylon bag after time zero), and i (non-degradable) is equal to 100.

Parameters (a), (b), and (c) were estimated using the Solver tool in Microsoft Excel and used to calculate the effective degradation of DM, CP, and NDF in the rumen using the following equation by ORSKOV & MCDONALD (1979): DE = a + [(b x c)/(c + k)], (Equation II), where: k = estimated solid flow rate in the rumen. The other parameters were described previously. The k values used to calculate the DE were 2, 5 and 8%/h. A flow rate of 5%/h corresponds to growing animals with milk production below 15 kg/day, whereas a flow rate of 8%/h corresponds to cows with higher production above 15 kg/cow/day. This can be attributed to low, medium, and high feed intake levels, according to the AFRC (1993).

Statistical analysis

To determine the animal effects, yeast levels, incubation times, and interaction between the last two factors, a randomized block design (three animals) with a 4 x 8 factorial arrangement (levels, times) was used. Regression analysis was performed according to the following model: $Y_{ijk} = \mu + n_i + t_j + n \times t_{ij} + a_k + e_{ijk}$where Y_{ijk} = response variable (degradation or disappearance of DM, PB and NDF), μ= global average, n_i = effect of yeast levels (i = 1, 2, 3, 4), t_j = effect of ruminal incubation times (j = 0, 4, 8, 12, 24, 48, 72, 96 h), $n \times t_{ij}$ = effect of interaction between level and time factors, a_k= effect of animals (k = 1, 2, 3), e_{ijk} = random error associated with each observation.

In situ analysis of DM, CP, and NDF degradation parameters was performed using a randomized block design with three replicates, following the following model: $Y_{ij} = \mu + n_i + a_j + e_{ij}$where: Y_{ij}= curve parameter for yeast level i in relation to animal j, μ = global average, n_i = effect of yeast levels (i = 1, 2, 3, 4), a_j= effect of animals (j = 1, 2, 3), e_{ij}= random error associated with each observation. The passage rate (k) is the estimated rate of solid flow into the rumen.

RESULTS AND DISCUSSION

Nutrient degradation (disappearance)

For incubation times of zero (t0), the soluble fractions of DM in silage supplemented with 50 and zero (0) g of LV/kg of OSPF varied between 20.94% and 28.03%, respectively (Table 1). The first value agrees with the soluble fraction of 19.19% observed in genotype cv. BR 701 by MOLINA et al. (2003). Regression analysis of variance revealed a quadratic effect (p = 0.0083) of LV levels on DM degradation. According to the equation, the minimum point corresponds to 22.04% of DM degradation with 65./33% LV. No significant differences (p>0.05) were observed in the degradation of DM in the silage after 8 h of incubation. However, a quadratic response was observed, with a minimum DM degradation of 28.58% with 64.29 g of LV. For the other incubation times based on LV levels, no significant differences (p>0.05) were recorded in the degradation of DM in the silage.

In yeast-free silage (0%), the soluble DM fractions varied between 28.03% at time zero (t0) and 71.67% at 96 hours of incubation (Table 1). High values of DM degradation at t0 can be attributed to the fact that, at very fine grindings, the material can easily and quickly come out of the incubation bags. MICHALET-DOREAU & OULD-BAH (1992) indicated that particles that escape from the incubation bags and are not degraded would not affect the final degradation of the incubated material. The incubation times of yeast-free silage (0%) showed stabilization of the average DM degradation after 24 h, not differing

(p>0.05) from those recorded at 48 h and without significant differences (p>0.05) in degradation between 72 and 96 h of incubation. This behavior indicates that incubations for 96 h are appropriate to achieve maximum DM degradation (i.e., the asymptote is reached. This silage, based on incubation times, showed a quadratic response, with an increase and decrease in DM degradation of 0.73 and 0.003%/h, respectively.

Throughout the incubation times in silage containing 25, 50, and 75 g of yeast/kg AFP, the greatest DM degradation was achieved after 24 h of incubation (Table 2). Likewise, no significant differences (p>0.05) were observed in DM degradation between 48 and 72 h of incubation in silages added with 50 and 75 g of yeast/kg of AFP, differing from degradation at 96 h of incubation.

For t0, the degradation (disappearance) of CP varied between 61.26% for yeast-free silage and 78.48% for silage with the inclusion of 75 g of yeast/kg of AFP. This result was quadratic , estimating a maximum degradation of 80.82% with 51.25-g yeast/kg of AFP. According to MOLINA et al. (2003), degradation values represent soluble nitrogen or material finely ground into small particles to exit the incubation bags. Furthermore, there was a quadratic effect at 4, 8, 24, and 96 h of incubation in CP degradation, estimating maximum degradations of 97.98%, 82.46%, 89.28%, and 92.46%, with the inclusion of 50.95, 51.00, 52.00, and 50.89 g of yeast/kg of AFP, respectively (Table 1). At 12 h of incubation, a positive linear response (p = 0.0386) in CP degradation was observed, with a 0.15% degradation of CP for each gram increase in yeast/kg AFP. There was no significant effect of yeast concentration on degradation at 48 and 72 h of incubation, with averages of 84.70% and 87.33%, respectively.

Table 1. Mean degradation (disappearance) of dry matter (DM), crude protein (CP), and neutral detergent fiber (NDF) of oat-potato foliage (AFP) silage at different ruminal incubation times as a function of yeast levels. Regression equations and coefficients of determination.

Time	Yeast level (g/kg)				Regression equation	
(hours)	0	25	50	75	Value of $\hat{Y}$	R^2
DM degradation (%)						
T_0	28.03	24.45	20.94	25.62	$\hat{Y} = 28.442 - 0.196^{**}L + 0.0015^{**}L^2$	0.6555
4	30.40	27.25	24.80	26.91	Ns	-
8	32.59	28.56	29.42	30.43	$\hat{Y} = 32.344 - 0.117^{*}L + 0.00091L^2$	0.6423
12	37.72	34.79	33.37	37.05	Ns	-
24	44.55	41.85	44.93	46.05	Ns	-
48	55.69	55.31	51.51	54.03	Ns	-
72	65.41	65.23	58.98	63.18	Ns	-
96	71.67	71.56	69.69	73.57	Ns	-
CP degradation (%)						
T_0	61.26^{B}	81.23^{A}	75.62^{A}	78.48^{A}	$\hat{Y} = 62.96 + 0.697^{*}L - 0.0068^{*}L^2$	0.6126
4	63.67^{B}	82.17^{A}	76.77^{A}	79.46^{A}	$\hat{Y} = 65.27 + 0.642^{*}L - 0.0063^{*}L^2$	0.6973
8	65.43^{B}	82.68^{A}	78.20^{A}	80.46^{A}	$\hat{Y} = 66.85 + 0.612^{*}L - 0.0060^{*}L^2$	0.6981
12	67.98^{B}	84.18^{A}	79.43^{A}	82.39^{A}	$\hat{Y} = 72.73 + 0.154^{*}L$	0.4618
24	71.69^{B}	85.87^{A}	83.11^{A}	84.84^{A}	$\hat{Y} = 72.76 + 0.520^{**}L\ \ 0.0050^{*}L^2$	0.6854
48	77.55^{B}	89.13^{A}	85.06^{A}	87.04^{A}	Ns	-
72	81.49^{B}	91.07^{A}	87.36^{A}	89.38^{A}	Ns	-
96	84.60^{B}	92.45^{A}	90.74^{A}	91.69^{A}	$\hat{Y} = 85.21 + 0.285^{***}L - 0.0028^{*}L^2$	0.7019
NDF degradation (%)						
T_0	49.55^{AB}	47.57^{B}	49.32^{AB}	51.40^{A}	Ns	
4	52.65	50.10	51.79	53.67^{A}	Ns	
8	55.07^{AB}	51.66^{B}	54.76^{AB}	55.90^{A}	Ns	
12	58.50^{AB}	55.63^{B}	57.29^{AB}	60.10^{A}	Ns	
24	63.05^{AB}	60.43^{B}	64.70^{AB}	65.80^{A}	Ns	
48	70.47	69.59	68.92	70.86	Ns	
72	75.56	75.06	73.70	76.13	Ns	
96	79.76	78.81	80.57	81.28	Ns	

A, B: Values followed by different letters in the same line differ from each other (p<0.05) by “t” test. *5%; **1%; ***0.1%, NS (not significant at 5% probability by t-test), implies the differences in probability, respectively.

There was a positive linear effect of incubation time on CP degradation in silage when yeast doses of 0, 25 and 50 g were used, with increases of 0.24, 0.12 and 0.15% of CP degradation per hour of ruminal incubation, respectively (Table 1). This behavior demonstrates that the CP degradation values stabilized after 48 h of incubation, with no significant differences observed between degradations at 48, 72, and 96 h of ruminal incubation. This result reveals that incubations between 72 and 96 h are sufficient to achieve maximum CP degradations; in other words, the asymptote is reached.

The results of this study are in agreement with the observations of MOLINA et al. (2003) in silages of six sorghum genotypes; CONTRERAS et al. (2019), in forages isolated or associated with alfalfa or dactyls; and CARRASCO et al. (2022) in oat-potato foliage silages (70:30), including wheat bran at different levels (0, 10, 20 and 30%).

The combination of incubation times with the inclusion of 75 g yeast/kg AFP at ensiling time led to a quadratic response: $\hat{Y} = 78.95 + 0.229^{**}T - 0.0011T^2$ ($R^2 = 0.9099$) in CP degradation, with the maximum CP degradation of 90.87% occurring at 96 h of incubation. SARTI et al. (2005) mentioned that a high rate of silage degradation (disappearance) favors a high concentration of ammonia in the rumen.

Table 2. Mean percentages of dry matter (DM), crude protein (CP), and neutral detergent fiber (NDF) degradation of oat-potato-potato foliage (OAP) silage (PFA) with the addition of yeast as a function of different ruminal incubation times. Regression equations and coefficient of determination.

Yeast level (g/kg AFP)	Incubation time (hours)							
	t_0	4	8	12	24	48	72	96
PC degradation								
0	61.26^{D}	63.67^{D}	65.43^{CD}	67.98^{CD}	71.69^{BC}	77.55^{AB}	81.49^{A}	84.60$^{A, 5}$
25	81.23^{E}	82.17^{DE}	82.68^{DE}	84.18^{CD}	85.87^{C}	89.13^{B}	91.07^{AB}	92.45$^{A, 6}$
50	75.62^{E}	76.77^{E}	78.20^{E}	79.43^{DE}	83.11^{CD}	85.06^{BC}	87.36^{AB}	90.74$^{A, 7}$
75	78.48^{G}	79.46^{F}	80.46^{F}	82.39^{E}	84.84^{D}	87.04^{C}	89.38^{B}	91.69$^{A, 8}$
NDF degradation								
0	49.55^{F}	52.65^{EF}	55.07^{DF}	58.50^{CD}	63.05^{C}	70.47^{B}	75.56^{A}	79.76$^{A, 9}$
25	47.57^{G}	50.10^{FG}	51.66^{F}	55.63^{E}	60.43^{D}	69.59^{C}	75.06^{B}	78.81$^{A, 10}$
50	49.32^{F}	51.79^{EF}	54.76^{DE}	57.29^{D}	64.70^{C}	68.92^{BC}	73.70^{B}	80.57$^{A, 11}$
75	51.40^{G}	53.67^{FG}	55.90^{EF}	60.10^{E}	65.80^{D}	70.86^{C}	76.13^{B}	81.28$^{A, 12}$

Values followed by different letters on the same line differ from each other ($p<0.05$). *5%; **1%; ***0.1% probability, by the "t" test, respectively.

Analysis of variance showed no significant effect ($p>0.05$) of yeast levels on NDF degradation at each ruminal incubation time (Table 2). The soluble fractions of NDF at t0 were similar for yeast levels of 0, 25, and 50 g/kg AFP. The value of 47.57% degradation of this variable at t0 and without yeast coincides with the value of 47.10% recorded for oat-potato silage (70:30), including 10% wheat bran (CARRASCO et al. 2022). In this type of silage, at other levels, NDF degradation was greater (56.55% and 57.28%) than in this study. This difference can be attributed to the lack of homogeneity in the incubated samples. For treatments with 0, 25, 50, and 75 g of yeast/kg of AFP combined with incubation times of 4, 48, 72, and 96 h, the average degradation did not show statistically significant differences ($p>0.05$).

NDF degradation in silage with different yeast levels was quadratically correlated with incubation time (Table 2). The estimated maximum NDF degradation was 79.76%, 78.81%, 80.57%, and 81.28% with a ruminal incubation time of 96 h, respectively.

With the exception of silages containing 25, 50, and 75 g of yeast/kg of AFP, the results demonstrate that yeast-free silages generate degradations of 75.56% and 79.76% for NDF, with no statistically significant differences ($p>0.05$), with incubations for 72 and 96 h, respectively. Therefore, for this type of silage, incubations between these hours are adequate to achieve maximum NDF degradation.

Ruminal degradation parameters of DM, CP, and NDF

DM degradation

The passage rate (k) is the estimated rate of solid flow in the rumen, and its values for calculations were chosen as 2, 5 and 8%/h. Analysis of variance showed no significant effects ($p > 0.05$) of equidistant LV levels on effective degradations of MS (DEMS) at passage rates of 2, 5, and 8%/h (Table 3). DEMS values, ranging from 28.43 to 37.91%, did not differ significantly between the LV levels for any of the passage rates. The degradation rates (c) of MS, ranging from 1.10 to 1.37%/h for the lowest and highest concentrations of LV, respectively, indicated that the LV levels did not influence the degradation speed of the silage under study. It is noteworthy that the DEMS values for the silages were below 50% at each passage rate and, with the exception of the silage with 75% LV at 5%/h of passage, there were decreases in degradation at each passage rate. Similarly, there were decreases in DEMS as approval ratings increased.

At a passage rate of 2%/h, the DEMS values were higher than those reported by CARRASCO et al. (2022) for LV-free AFP silage at passage rates of 2, 5, and 8%/h, which were 36.76, 24.94, and 20.73%, respectively. For winter oats, CORDERO et al. (2018) observed DEMS values of 70.48, 60.64, and 54.23% for the same passage rates. In the passage rates considered in this study, SARTI et al. (2005) reported 65.2, 55.8 and 51.4% for corn silage. Differences in DEMS values can be attributed to the variety, maturity status, fertilization, and time of use of forage species (CARRASCO et al. 2022).

CP degradation

The results obtained from the degradation kinetics: (a), (b), (i) and DP, and the effective degradation of CP (DEPC) for the passage rate of 2%/h, showed significant differences ($p<0.05$) due to the effect of LV addition to AFP at the ensiling moment (Table 3). Regression analysis ($p = 0.0050$) of LV levels in the immediately soluble fraction (a) of PB silage, ranging from 61.71 (0 g of LV) to 81.86% (25 g of LV), respectively. The silage with the highest soluble fractions (a) had the highest CP contents (9.57 to 13.60%) compared with the LV-free controls (6.60%).

SARTI et al. (2005) observed values of 51.5% and 43.9% for the fraction (a) of CP in corn and *Pennisetum purpureum* Schum. variety Cameroom silages, respectively, including enzymatic-bacterial inoculant. CARRASCO et al. (2022) reported soluble proteins originating from fraction (a) between 31.35 and 47.07% in AFP silage, including wheat bran levels ranging from 0% to 30%. On the other hand, the variability in the CP fractions (a), (b), and (c) can be attributed to the characteristics of the ensiled material (chemical composition) and the quality of forage fermentation in the silo (SARTI et al. 2005, DIEGO et al. 2024). Furthermore, GUERRERO et al. (2010) indicated that high soluble fractions (a) are related to particle size or high levels of non-nitrogen compounds (urea, free amino acids and small peptides), which are released when the feed reaches the rumen and rapidly converted into ammoniacal nitrogen. SHAIKH et al. (2023) suggested that nitrogen from non-nitrogenous compounds contributes to microbial protein production. There is a limit above which fraction (a) is not physiologically acceptable, as it should not exceed 40% of DEPC (AFRC 1993).

Therefore, considering DEPC (Table 3) at a particle flow rate of 5%/h, silages containing 25 g/kg AFP presented the highest ratio of the soluble fraction (a) of CP to DEPC; (a)/DECP = 102.89% (81.86*100/79.56) compared to LV-free silages, with 50 and 75 g of LV/kg AFP, whose (a)/DEPC ratios were 89.32, 92.39 and 94.68%, respectively. Consequently, silage with and without LV levels exceeded the threshold of <40% DEPC, leading to nitrogen loss by ruminants. High (a)/DEPC ratios were also observed at a particle flux rate of 8%/h (92.31, 104.68, 93.84, and 96.19% for 0, 25, 50, and 75 g LV/kg AFP, respectively). CARRASCO et al. (2022) reported (a)/DEPC ratios of 69.85, 75.44, 76.96, and 77.47% in AFP potato silages including 0, 10, 20, and 30% wheat bran, respectively. Similarly, nitrogen losses were observed in alfalfa, alfalfa red clover, and oats (60.52, 64.52 and 62.00%, respectively) compared to alfalfa dactylis (24.18%) when relating (a)/DEPC to a particle flux rate of 2%/h (CONTRERAS et al. 2019).

Regarding the potentially degradable insoluble fraction (b) of CP, the regression analysis of LV levels showed no significant effect ($p = 0.1060$). However, comparison of the means for coefficient (b) indicated that silage with 25 g of LV/kg of AFP was significantly lower (11.32%) than silage free of LV (27.33%), and

did not differ from silage with 20 and 75 g of LV/kg of AFP. The values of the CP fraction (b) in the silages of this study are lower than those of the AFP silages (36.68, 32.14, 30.59 and 31.71% for 0, 10, 20 and 30% wheat bran, respectively) (CARRASCO et al. 2022). CP fraction (b) coefficients of 39.92, 25.73 and 22.61% for barley at 130, 150 and 170 days of growth were found by CONTRERAS et al. (2019). Under tropical conditions, SILVA et al. (2020) observed an increase in coefficient (b) of 47.27, 47.13, 50.86, and 57.60% in *P. purpureum* Schum. silage added with 0, 4, 8, 12, and 16% of annatto grain by-product. According to the NRC (2007), the amount of potentially degradable insoluble fraction of crude protein (b) depends on the time the feed remains subjected to the enzymatic activity of ruminal microorganisms.

The indigestible fraction (i) of the silages was influenced by the LV levels ($p<0.05$), where LV-free silage showed superiority (i = 10.96%) compared with other silages that did not differ from each other (Table 3). The data resulted in a quadratic effect ($p = 0.0069$), with the inclusion of 54 g of LV/kg of AFP leading to a minimum estimate of 6.48% of indigestibility (i) of CP in the silages. CARRASCO et al. (2022) obtained similar behavior in AFP silage with wheat bran levels, with the inclusion of 27.47% wheat bran corresponding to the minimum (i) of 20.68%.

The degradation rate (c) of CP did not differ ($p>0.05$) between the silages under study. This is in agreement with the results of corn silage (*P. purpureum* Schum. cv. Cameroom silage was included with enzymatic-bacterial inoculant, and this species had a coefficient (c) of 3%/h for each silage (SARTI et al. 2005). Regarding the CP degradation potential (DPPC), the LV-free AFP silage was lower (89.04%) ($p<0.05$) compared to LV-including silage (Table 3). This is consistent with the lower CP content (6.60%) in LV-free silage compared with the other silages, which ranged in CP content from 9.57 to 13.60%. Based on the equation: $\hat{Y} = 89.31 + 0.159^{**}L \ \ 0.0015^{*}L^{2}$, the optimal estimate of DPPC was 93.52%, with 53 g of LV/kg of AFP. CHEN et al. (2021) reported that increased CP degradation is generally related to higher ammonia levels, which may contribute to the growth of microbial population and activity in the rumen, leading to an increase in microbial nitrogen supply to the small intestine and maximizing the consumption of high-fiber diets.

The DPPC values of AFP silage, including wheat bran, obtained and recorded by CARRASCO et al. (2022) were on average 74.96%, for corn silage 80.90% (SARTI et al. 2005), and for alfalfa, red clover-alfalfa, alfalfa-dactylis, and oats 97.62, 95.06, 86.91, and 80.20%, respectively. Forages degrade rapidly in the rumen, especially tender ones, degrading by up to 73% (SHAIKH et al. 2023).

The effective degradation of CP (DEPC) at 2%/h for silages including 25, 50 and 75% LV showed higher values ($p<0.05$) compared to LV-free silage. Regression analysis led to an estimate of the optimal DEPC at 87.66% with the inclusion of 46.72 g of LV/kg of AFP. Likewise, in Table 3, it can be observed that the LV-free silage was most affected by the passage rates, as it presented the lowest fraction (a) (61.71%) and the highest fraction (b) (27.33%) in relation to the other silages, whose fractions (a) varied from 81.86 to 78.59%, and the fractions (b) varied from 11.32 to 14.68%.

Silages with different yeast grades at passage rates of 5 and 8%/h showed linear increasing effects on DEPC, with increases of 0.18 and 0.19% for each g of LV, respectively. Based on the DEPC data, it can be stated that almost all the protein available in the silage, including the LV levels, was degraded in the rumen, remaining available for ruminal microbial growth. For DEPC in AFP silage including wheat bran, CARRASCO et al. (2022) found average values of 63.26, 56.05, and 52.54% at passage rates of 2, 5, and 8%/h, respectively, which were lower than those presented in this study.

NDF degradation

The source of variation, LV levels included in the statistical model, with the exception of the immediately soluble fraction (a) of the NDF, did not significantly influence ($p>0.05$) the parameters (b), (i), and (c), as well as the potential (DP) and effective (DE) degradation of the NDF for flow rates of 2, 5, and 8%/h (Table 3).

The soluble fraction (a) of NDF in LV-free silage did not differ significantly ($p>0.05$) from silages with additives, showing statistical differences only between silages with 25 and 75 g of VM/kg of AFP (a = 47.42 vs. a = 51.66%) (CARRASCO et al. 2022). Similar values for (a) in AFP silage with and without wheat bran were reported: 30.90, 48.29, 56.97 and 58.21%. In oat, alfalfa, and *alfalfa-dactyl* forages, (a) the coefficients were 17.48, 15.50, and 23.42%, respectively (CONTRERAS et al. 2019). The silages in this study presented a degradation rate (c) of 2%/h, with incomplete digestions (i) ranging from 15.49% to 14.06%,

indicating low and slow degradation.

Table 3. Immediately soluble fraction (a), insoluble potentially degradable fraction (b), indigestible fraction (i), degradation rate (c), potential degradation (DP)1, and effective or real degradation (DE)2 of dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) of oat-potato foliage (AFP) silage supplemented with yeast. Regression equations and coefficient of determination.

Degradation parameters	Yeast levels (g/kg AFP)				Regression equation Value of Ŷ	R^2
	0	25	50	75		
			Dry matter			
a (%)	27.68	23.74	21.96	25.74	$\hat{Y} = 27.825 - 0.0254^{**}L + 0.0029^{**}L^2$	0.6269
b (%)	62.83	66.51	54.64	64.23	Ns	-
i (%)	9.49	9.75	23.40	10.03	Ns	0.5173
c (%/hour)	1.10	1.53	1.93	1.37	$\hat{Y} = 1.053 + 0.035^{*}L - 0.0004^{*}L^2$	0.4616
DP (%)	90.51	90.25	76.60	89.97	Ns	-
DE (%); 2%/h	50.64	49.25	47.89	40.48	Ns	-
DE (%); 5%/h	39.67	38.78	36.62	38.21	Ns	-
DE (%); 8%/h	35.30	34.35	32.22	33.46	Ns	-
			Crude protein			
a (%)	61.71^{B}	81.86^{A}	75.59^{A}	78.59^{A}	$\hat{Y} = 61.71 + 1.79^{**}L - 0.049^{*}L^2 + 0.00037^{*}L^3$	0.7821
b (%)	27.33^{A}	11.32^{B}	16.88^{AB}	14.68^{AB}	Ns	-
i (%)	10.96^{A}	6.82^{B}	7.53^{B}	6.73^{B}	$\hat{Y} = 10.69 - 0.159^{**}L + 0.0015^{*}L^2$	0.6692
c (%/hour)	2	2	2	2	Ns	
DP (%)	89.04^{B}	93.18^{A}	92.47^{A}	93.27^{A}	$\hat{Y} = 89.31 + 0.159^{**}L - 0.0015^{*}L^2$	0.6692
DE (%); 2%/h	74.83^{B}	87.48^{A}	85.04^{A}	83.85^{A}	$\hat{Y} = 75.65 + 0.514^{**}L - 0.0055^{**}L^2$	0.6843
DE (%); 5%/h	69.09	79.56	81.82	83.01	$\hat{Y} = 71.77 + 0.176^{*}L$	0.6841
DE (%); 8%/h	66.85	78.20	80.55	81.70	$\hat{Y} = 69.79 + 0.188^{*}L$	0.6829
			Neutral detergent fiber			
a (%)	49.86^{A} ^{B}	47.42^{B}	49.86^{AB}	51.66^{A}	Ns	-
b (%)	34.39	38.39	34.74	34.27	Ns	-
i (%)	15.49	14.19	15.40	14.06	Ns	-
c (%/hour)	2	2	2	2	Ns	-
DP (%)	84.51	85.81	84.60	85.94	Ns	-
DE (%); 2%/h	67.10	65.47	65.88	60.96	Ns	-
DE (%); 5%/h	59.77	58.61	58.5	59.46	Ns	-
DE (%); 8%/h	56.86	55.62	55.66	56.46	Ns	-
			Acid detergent fiber			
a (%)	47.27^{A}	42.06^{B}	40.21^{B}	47.94^{A}	$\hat{Y} = 47.58 - 0.387^{***}L + 0.0052^{***}L^2$	0.8666
b (%)	35.87	42.30	41.79	36.35	$\hat{Y} = 35.97 + 0.340^{**}L - 0.0047^{**}L^2$	0.6141
i (%)	16.86	15.63	18.01	15.71	Ns	-
c (%/hour)	2	2	2	2	Ns	-
DP (%)	83.14	84.37	81.99	84.29	Ns	-
DE (%); 2%/h	65.17	61.95	60.40	58.22	Ns	-
DE (%); 5%/h	57.49	54.97	51.84	56.41	$\hat{Y} = 57.91 - 0.238^{*}L + 0.0028^{*}L^2$	0.5875
DE (%); 8%/h	54.42	51.72	48.50	53.06	$\hat{Y} = 54.84 - 0.248^{*}L + 0.0029^{*}L^2$	0.5855

[1] Obtained by the DP model = a + b (1-exp -Ct); 2 Obtained by the DE model = a + [(b x c)/(c + k)]. $^{A, B:}$ Values followed by different letters on the same line differ from each other ($p<0.05$). Ns, no significance.

SARTI et al. (2005) reported (c) coefficients of 2%/h for corn silage and P. purpureum Schum. with additives. In contrast, CARRASCO et al. (2022) reported higher values of (c) (3.00 to 10.00%/h) and (i) (15.64 to 32.21%) for AFP silage with varying levels of wheat bran. The insoluble fraction (b) did not differ significantly ($p>0.05$), with an average of 35.45%. SARTI et al. (2005) reported higher values (b): 71.8, 64.3 and 78.0% for corn silage and *P. purpureum Schum*. with bacterial and enzymatic-bacterial inoculants.

The potential for NDF degradation (DPNDF) in oat silages with or without LV did not differ significantly ($p>0.05$), with an average of 85.22% and a presenting coefficient (i) of 14.78% (100 - 85.22%), improving the ruminal environment for the activity of microorganisms (Table 3). Similar DPNDF values were reported for corn silage (80.40%) and AFP silage with 20-30% wheat bran (81.92-84.36%) (SARTI et al. 2005, CARRASCO et al. 2022). No significant differences ($p>0.05$) were observed in the effective degradation of NDF (DENDF) at flow rates of 2, 5, or 8%/h. Silages with 0, 25, and 50 g of LV/kg of AFP showed slight reductions in DENDF when passing from flow rates of 2 to 8%/h (67.10 to 56.86%, 65.47 to 55.62% and 65.88 to 55.66%, respectively), indicating a decrease in degradation and energy value of 10.29, 9.85, and 10.22%, respectively. Silage supplemented with 75 g of LV/kg of AFP showed a reduction of 4.50% (60.96 to 56.46%). These reductions are relevant for high-production animals with rapid gastric content transit, such as early lactating cows and heavily fattened calves (DEVANT & MARTI 2020)

In silage containing 25 g of LV, at DPNDF and K = 8%/h, NDF degradation decreased by 35.18%, linked to the potentially degradable insoluble fraction (b) = 38.39%, degradation rate of 2%/h, and fibrosity (i) = 14.19%, indicating slow degradation of the cell wall. For silages with 50 and 75 g of yeast, the depressive effect at 8%/h was similar (34.21% vs. 34.30%), with degradation rates of 2%/h, fractions (b) of 34.74% and 34.27%, and fibrosities of 15.40% and 14.06%, respectively. VE-free AFP silage showed a reduction of 32.72% at 8%/h with (a) = 2%/h, (b) = 34.39%, and i = 15.49%, leading to faster ruminal disappearance. The DENDF values in AFP silages with LV were lower than those reported by CARRASCO et al. (2022) for AFP silage with wheat bran, with an average of 69.08%, 63.37%, and 60.38% at flow rates of 2, 5, and 8%/h, respectively. For corn silage, the DENDF values were 43.3%, 29.1%, and 23.8% at respective passage rates (SARTI et al. 2005).

CONCLUSION

It is evident that yeast-free oat-potato leaf silage samples incubated in rumen for 72–96 h are suitable for obtaining maximum values for the degradation of dry matter, crude protein, and neutral detergent fiber. Likewise, it has been demonstrated that oat-potato-greens silages, including 25 or 50 grams of yeast/kg of oat-potato-greens, suspended in the rumen of cattle for 96 h provide sufficient time to achieve the maximum degradation of crude protein.

Yeast levels had no effect on the indigestible fraction, degradation rate, potential degradation, or effective degradation of silage at flow rates of 2, 5 and 8% per hour.

For the set of parameters under study, the inclusion of 25, 50, and 75 g of yeast/kg of oat-potato foliage showed an improvement in the potential and effective degradation of crude protein.

Yeast levels were not efficient in the potential and effective degradation of neutral detergent fiber in silage.

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