NUTRITIVE VALUE OF BARLEY SILAGE (*Hordeum vulgare L.*) WITH DIFFERENT LEVELS OF *SACCHAROMYCES CEREVISIAE* AT DIFFERENT ENSILING LENGTHS

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ABSRTACT

The objective of this study was to evaluate the effects of yeast (Saccharomyces *cereviceae*) as additive for barley silage preparation at different ensiling times. Small scale silages were prepared from barley forage and four yeast levels were evaluated (0 (control), 5, 10 and 15 g/kg FM). Silos were opened at 6, 12 and 24 days. Three silage-replicates were prepared for each yeast level × ensiling time combination. Silage quality in terms of CP and NDF and ADF concentration appeared to improve when yeast was added at 5 and 10 g/kg, compared with the control, but the quality decreased again when 15 g yeast/kg FM were aded to the silage. However, gas production, metabolisable energy and organic matter digestibility linearly decreased by yeast addition. This indicates a likely negative effect of yeast over the fermentation process during fermentation of barley silage. No time effects were observed for most of the parameters with the exception of ether extract concentration. Based on the results of this study, yeast addition is detrimental to the nutritional quality of barley silage. Mechanisms for this still remain unknown, but an undesirable fermentation provoked by yeast addition might be an explanation for our findings.

Keywords: Digestibility, yeast, forage, neutral detergent fibre

القيمة الغذائية لسايلج الشعير(*Hordeum valgare* L) المعامل بمستويات مختلفة من الخميرة (SACCHAROMYCES CEREVISIAE) في سايلوات السايلج المختلفة

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المستخلص

الهدف من الدراسة تقييم تأثير الخميرة (SACCHAROMYCES CEREVISIAE) كمضاف الى مواعيد السايلج المختلفة . ثم استعمال سايلوات سايلج صغيرة مع استعمال اربعة مستويات من الخميرة (صفر للمقارنة , 5 , 10 , 15 غم/ كغم FM . فتحت السيايلوات بعد 6 و 12 و 24 يوم . استعمل ثلاثة مكررات لكل معاملة . كانت نوعية السايلج بتراكيز مختلفة ADF , NDF,, CF . اظهرت النتائج بأن الخميرة بكميات 5 و 10 غم / كغم تفوقا مقارنة مع معاملة المقارنة الا ان النوعية انخفضت باضافة الخميرة 15 غم / كغم خميرة , كما ان نتائج الغاز والطاقة والمواد العضوية المهضومة انخفضت باضافة الخميرة . هذا دليل على التأثير السلبي للخميرة عند اضافته في تحضير سايلج الشعير , حيث ظهرت ان الخميرة حددت نوعية سايلج الشعير .

كلمات مفتاحية : قابلية الهضم, خميرة , علف , الالياف

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INTRODUCTION

Barley (*Hordeum vulgare* L.) silage production has been proposed as an option for conserved forage in areas where maize cultivation is limited by e.g. climate, soil conditions, irrigation or machinery (13). Barley ensiling, however, may be challenging due to the high moisture content at the ideal time of harvest and a low concentration of water soluble carbohydrates, compared with other grasses (9), making it imperative to investigate the potential of additives to enhance the ensilability of this material. Even though yeasts are generally recognized as undesirable microorganisms in the silage, because they are the primary initiators of aerobic spoilage during ensiling and opening (12), their ability to consume oxygen has made researchers to believe that they could have a positive effect on silage quality (10), particularly to ensure the onset of anaerobic fermentation. Furthermore, when directly fed to ruminants certain yeasts have shown to increase the population of cellulolytic bacteria and to enhance growth rate and milk production, while also excluding zoonotic pathogens from the intestinal tract (8), which would be an additional advantage of using yeasts as silage inoculant if those microorganisms are able to withstand the ensiling. Therefore, the objective of this study was to evaluate the changes that yeast inoculant have over early stages of conservation of barley silage on the nutritional characteristics of the forage.

MATERIALS AND METHODS

The experiment was conducted at the Faculty of Engineering Sciences of the University of Huancavelica (UNH). Barley forage was harvested from a local farms from the Antaccocha municipality in the Huancavelica district, in the Western chain of the Peruvian Andes. Barley forage was manually harvested at an age of 5 months and chopped at a particle length of 2 to 2.5 cm using a mechanical chopper for the ensiling. Small scale silos were prepared by storing 5 kg of fresh forage in polyethylene bags. *Saccharomyces cerevisiae* yeast (Active Dry Yeast, Fleischmann's, ACH Food Companies, Inc., Oakbrook Terrace, IL) was added to the fresh forage before ensiling at four levels: 0 (Control), 5, 10 and 15 g/kg fresh matter (FM). The yeast was uniformly spread and thoroughly mixed by hand with the forage material. The silages were stored for 6, 12 and 24 days to study the effects of the yeast during the ensiling time. In total 36 small scale silos were prepared, corresponding to three replicates for each yeast level and time evaluation. At opening, 500 g of sample were collected from each silage, dried at 65 °C for 72 h and ground with a hammer meal. All samples were analysed for crude protein (CP), organic matter (OM) and ether extract (EE) following the procedures of (15). Neutral (NDF) and acid (ADF) detergent fiber were analysed following the procedure of (17). These analyses were done at the facilities of the Laboratory of Animal Nutrition and Feedstuffs Evaluation (LUNEA) of UNH, Peru. Additionally, the Hohenheim *in vitro* gas test was utilized to determine gas production (GP) in silage samples following the procedure of (7) at the facilities of the Institute of Agricultural Sciences in the Tropics, Section Animal Nutrition and Rangeland Management in the Tropics and Subtropics, of the University of Hohenheim. Metabolisable energy (ME) and organic matter digestibility (OMD) was estimated with the following equations based on GP (2):

$$ME = 2.2 + 0.136 \times GP + 0.057 \times CP + 0.00285 \times CL^2 \quad \text{(Eq. 1)}$$

Where: ME = Metabolisable energy in MJ/kg DM; GP = Gas production in ml/200 mg of substrate dry matter (DM) after 24 h; CP = Crude protein concentration of grass hay in g/kg DM; CL= crude lipid concentration of grass hay in g/kg DM.

$$OMD = 14.88 + 0.889 \times GP + 0.045 \times CP + 0.065 \times CA \quad \text{(Eq. 2)}$$

Where: OMD = Organic matter digestibility in g/100 g; GP = Gas production in ml/200 mg of substrate DM after 24 h; CP = Crude protein concentration of grass hay in g/kg DM; CA= crude ash in g/kg DM.

Statistical analysis

Statistical analyses were conducted using the software SAS 9.4 (SAS Institute Inc., Cary, NC, USA). The main effect of yeast level and time and their interaction was tested using the GLM procedure according to:

$$Y_{ij} = \mu + Y_i + T_j + (Y \times T)_{ij} + e_{ij}$$

Where: Y_{ij} = dependent variable; μ = overall mean; Y_i = effect of the ith Yeast level; T_j = effect of jth ensiling Time; $(Y\times T)_{ij}$ = the interaction effect of Yeast level and ensiling Time; and eij = residual error of experiment. Significant effects of Yeast level were characterized using orthogonal polynomial contrasts testing the probability of linear or quadratic responses. Differences within means of Yeast level or ensiling Time were estimated by the Tukey test. The values reported are means and standard errors. Differences between treatments were declared significant at $p < 0.05$, whereas tendencies were declared at $p < 0.10$.

RESULTS AND DISCUSSION

There were no differences caused by the ensiling length on the nutrients concentration on barley silage, with the exception of ether extract which increased with longer ensiling time (Table 1). Conversely, the supplementation of yeast had an effect (quadratic) on all nutrients other than OM (Table 1). Crude protein was highest and ADF and EE lowest for yeast levels of 5 and 10 g/kg FM; whereas NDF was highest with the yeast level of 15 g/kg FM while all other levels remained unaffected. There was an ensiling Time × Yeast level interaction effect for CP, EE, NDF and ADF. Where CP increased at yeast levels of 5 and 10 g/kg FM for the length of ensiling of 6 and 12 days, but decreased for the length of ensiling of 24 days; NDF and ADF had the opposite trend; and EE changed the proportional differences between Yeast levels depending on ensiling Time. Gas production, metabolisable energy and organic matter digestibility linearly decreased with increasing yeast level and were always higher in silages with no yeast addition. No time effects were observed for these parameters. In recent years some yeast species have been studied as inoculants because of their ability to inhibit detrimental silage microorganisms (10), and because of the benefits that yeasts may have on microbial ecology of the digestive tract of livestock (10). However, yeast are also lactate-assimilating microorganisms, and are therefore undesired in the silage because they are the primary initiators of aerobic spoilage in silage (12). A number of studies have evaluated the effects of yeast on silage quality. It is difficult, however, to compare among those studies because of the differences in the type of yeast utilized, including specific strains of *Saccharomyces cerevisiae* and baker's yeast of different manufacturers. Additionally, the amounts of yeast added to the silage is not always presented in comparable units or it cannot be calculated back to the concentrations per unit of fresh matter. These are important considerations to keep in mind for the remaining of the discussion. Similar to the current study, increases in CP concentration by yeast addition to silages has been reported by Ok et al (11), who found that a strain of *S. cereviseae* treatment (20 g/kg FM) increased CP concentration of a rice straw silage compared with no yeast addition. Similarly, Saeed (14) found an increase in CP concentration upon addition of a commercial baker's yeast (100 g/kg FM) to reed silage. The main reason for an increase in CP concentration in a silage is related to the disappearance of other substrates (regularly sugars) coupled with a minimal degradation of protein to ammonia-N with consequent reduced losses of N in the effluents. It appeared that adding yeast at 5 and 10 g/kg FM to barley forages prevents the degradation of protein, however, once the concentration of yeasts increases a negative effect on CP appeared, likely reflecting an undesired fermentation where excessive production –and loss- of ammonia occurred. The reasons for this phenomenon cannot be explained with the data available from the current study, but it could be hypothesized that an excessive amount of yeast caused a shift in the microbial population, with the possibility that yeast started consuming lactate and/or caused aerobic spoilage of the silage. The NDF and ADF concentrations followed a pattern that can be similarly explained by the changes in CP concentration, where CP increased upon addition of yeast at 5 and 10 g/kg FM, but decreased when added at 15 g/kg FM, which would indicate an effect of dilution or concentration of the fiber components, when other substrates' concentration change. A study of (16) inoculated king grass with 3 inoculant treatments (untreated, *Lactobacillus plantarum*, and *Lactobacillus plantarum* plus *S. cerevisiae*) along with 3 different levels of

rice bran (0, 5, and 10 g/100 g FM). After 21 d of ensiling *in vitro* ruminal degradation over 48 h measured by gas production was greatest for the *L. plantarum* treatment and this was higher than that of the *L. plantarum* plus *S. cerevisiae* treatment. This might indicate an effect of yeast on lactobacilli, where the presence of yeast hindered some of the positive effects of the lactic acid-producing bacteria. If this theory was truth it would explain the detrimental effects of yeast observed in this study, particularly in terms of *in vitro* OMD and ME, which strongly decreased upon addition of yeast, partially agreeing with the observations of Sofyan et al. (16). Similarly, GP decreased linearly with increasing yeast level, in agreement with Dunniere et al. (3) who found a numerical decrease in gas production for two strains of *S. cerevisiae* of up to 7 %. It appears that yeast addition during ensiling is detrimental to the quality of the forage, particularly in terms of rumen degradability. The reasons for the decreased GP, ME and OMD are not clear, but it is obvious that the quality of a forage will decrease with decreasing CP and increasing NDF and ADF. Yeast supplements have demonstrated *in vitro* and *in vivo* to increase the populations of cellulolytic bacteria (4,6), which would enhance the OM degradation. This effect, however, was not observed and it must be an evidence that not a significant amount of yeast remained available for rumen fermentation after the ensiling. No effects of time were observed on the quality of the silages, with the exception of EE, indicating that the characteristics of the silage may remain similar once the silage environment has stabilized (roughly after 3-4 days). Finally, there are two further considerations to make about these results. First, the quality of the original forage was much higher than that of the silages, including the control silage. For example GP for the original sample was 54.3 ml/200 mg DM, indicating a greater substrate degradability of the original forage material compared with the silages. A higher overall quality of fresh forages compared with their silages is commonly reported, but such an extreme difference as seen here can only indicate the difficulties to produce high quality barley silage, as argued by McDonald eta l.(9). A second consideration is that 21 days of ensiling is close to the minimum time a silage would be stored before opening. Therefore, the silage quality that better reflects the forage that an animal would be consuming is that of the 21 days length of ensiling, which does not always correspond to the averages across all ensiling times. It still remains unclear what would the effects be for longer ensiling times, as several studies have reported increases in e.g. OM and starch degradability with advancing ensiling length (1,5), and how those longer conservation lengths would interact with the addition of yeast.

Table 1. Nutrients concentration of barley silage at different ensiling times with increasing levels of yeast addition.

Item	Ensiling time (days)[1]	Yeast level (g/kg FM) 0	5	10	15	Ensiling time average[2]	SEM	p-values Yeast	Time	Yeast*Time
Organic matter	6	87.9	87.6	87.7	86.5	87.4	0.224	0.51	0.79	0.56
	12	87.7	86.8	86.1	88.0	87.2				
	24	87.9	87.0	87.0	88.3	87.6				
	Yeast level average[1]	87.8	87.2	86.9	87.6					
Crude protein	6	15.4	18.7	16.9	15.0	16.5	0.245	<0.01, Q	0.11	<0.01
	12	14.4	15.9	18.2	14.6	15.8				
	24	16.7	15.2	15.8	16.2	16.0				
	Yeast level average[1]	15.5[b]	16.6[a]	17.0[a]	15.2[b]					
Ether extract	6	3.37	2.64	2.10	2.30	2.6[b]	0.110	<0.01, Q	<0.01	<0.01
	12	3.37	2.78	3.39	3.94	3.4[a]				
	24	4.08	3.30	3.30	3.74	3.6[a]				
	Yeast level average[1]	3.61[a]	2.91[b]	2.93[b]	3.33[a]					
Neutral detergent fiber	6	52.7	56.9	61.6	60.0	57.8	0.634	<0.01, L, Q	0.95	<0.01
	12	60.8	52.7	55.8	62.3	57.9				
	24	58.7	60.4	52.9	60.2	58.0				
	Yeast level average[1]	57.4[b]	56.7[b]	56.8[b]	60.8[a]					
Acid detergent fiber	6	34.6	33.4	37.7	37.7	35.8	0.428	<0.01, Q	0.65	<0.01
	12	39.1	33.0	35.3	38.5	36.5				
	24	37.7	37.3	33.1	37.0	36.2				
	Yeast level average[1]	37.1[a]	34.6[b]	35.4[b]	37.7[a]					

[1] Means with different superscripts within a row indicate statistical differences ($p < 0.05$)
[2] Means with different superscripts within a column indicate statistical differences ($p < 0.05$)

Table 2. Gas production, metabolisable energy and organic matter digestibility of barley silage at different ensiling times with increasing levels of yeast addition

Item	Ensiling time (days)	Yeast level (g/kg FM) 0	5	10	15	Ensiling time average[2]	SEM	p-values Yeast	Time	Yeast*Time
Gas production (ml/200 g DM)	6	46.1	40.1	37.6	38.7	40.6	0.52	<0.01, L, Q	0.48	0.75
	12	44.1	40.3	38.6	38.4	40.4				
	24	45.0	39.4	39.0	38.4	40.5				
	Yeast level average[1]	45.1[a]	39.9[b]	38.4[b]	38.5[b]					
Metabolisable energy (MJ/kg DM)	6	8.97	8.74	8.29	8.33	8.58	0.071	<0.01, L, Q	0.64	0.51
	12	9.32	8.60	8.53	8.30	8.69				
	24	9.32	8.46	8.43	8.39	8.65				
	Yeast level average[1]	9.20[a]	8.60[b]	8.42[bc]	8.34[c]					
Organic matter digestibility (g/100 g)	6	68.0	67.0	64.0	64.8	65.9	0.472	<0.01, L	0.68	0.45
	12	70.3	66.4	66.5	63.3	66.6				
	24	70.2	65.2	65.1	63.9	66.1				
	Yeast level average[1]	69.5[a]	66.2[b]	65.2[bc]	64.0[bc]					

[1] Means with different superscripts within a row indicate statistical differences ($p < 0.05$)
[2] Means with different superscripts within a column indicate statistical differences ($p < 0.05$)

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CONCLUSIONS

Yeast supplementation to barley silage had overall detrimental effects on silage quality, particularly at yeast level of 15 g/kg FM. Even though some advantage could be seen with lower yeast levels, as CP concentrations increased and NDF concentrations decreased, *in vitro* OMD and ME linearly decreased upon yeast addition. This may indicate negative effects of yeast on the fermentation process during ensiling, likely promoting the excessive degradation of soluble sugars and starch. Further research is needed to elucidate the modes of action of yeast when used as an additives for Barley silage.

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