



Chemical changes in sunflower silage associated with different additives

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ABSTRACT. Thirty-six experimental silos arranged in a completely randomized 4 x 3 factorial design were provided to evaluate the chemical changes of sunflower silage treated with soybean hulls, sunflower crushed and urea at 14, 21 and 28 days of ensilage. The additives were based on 5% natural matter, whereas control consisted of silage with 100% sunflower plant. OM, NDIP, and MM had average rates 911.2; 86.6 and 92.9 g kg⁻¹ of dry matter respectively. The addition of soybean hulls and sunflower crushed increased DM rates after 28 and 21 days. Urea increased nitrogen fractions and the addition of soybean hulls increased total carbohydrate content of silage by 5.1%, whereas the addition of sunflower crushed decreased the same by 2.18%. NDF, ADF and hemicellulose average rates were 625.4, 460.3 and 165.2 g kg⁻¹ of DM. The addition of soybean hulls and sunflower crushed reduced the *in vitro* dry matter digestibility by 8.3 and 5.97%. The addition of 5% sunflower crushed and soybean hulls improved the nutritional value of sunflower silage and the addition of urea improved the protein rates.

Keywords: sunflower crushed, lignin, NDF, pH, soybean hulls, urea.

Alterações bromatológicas da silagem de girassol associada com aditivos

RESUMO. Para se avaliar as alterações bromatológicas da silagem de girassol com casca de soja, torta de girassol e uréia com 14, 21 e 28 dias de ensilagem, foram confeccionados 36 silos experimentais, em delineamento inteiramente casualizado num esquema fatorial 4 x 3. Os aditivos foram adicionados, na base de matéria natural de 5%; e o controle foi a silagem de 100% da planta de girassol. Os teores de MO, PIDN e MM, apresentaram médias de 911,2; 86,6 e 92,9 g kg⁻¹ de MS. A adição de casca de soja e torta de girassol elevou o teor de MS após 28 e 21 dias. A uréia incrementou as frações nitrogenadas e, a adição de casca de soja proporcionou aumento no teor de carboidratos totais em 5,1% e a adição de torta de girassol, redução de 2,18%. Os teores médios de FDN, FDA e hemicelulose, foram de 625,4; 460,3 e 165,2 g kg⁻¹ de MS. A adição de casca de soja e torta de girassol reduziu a digestibilidade *in vitro* da MS em, 8,3 e 5,97%. A adição de 5% de torta de girassol e de casca de soja melhora o valor nutricional da silagem de girassol e a adição de uréia o perfil protéico.

Palavras-chave: torta de girassol, lignina, FDN, pH, casca de soja, ureia.

Introduction

The sunflower (*Helianthus annuus* L.) is resistant to drought and frost and has a great adaptability to different climatic conditions. Since its yield is not influenced by latitude, altitude and photoperiod, it is an option for crop rotation in all producing regions in Brazil (ELTZ et al., 2010) and an alternative for silaging.

Sunflower silage has a higher energy and protein rates than those of corn silage traditionally used in ruminant feed. It may represent economic advantages in balanced diets when compared to other forages.

A certain amount of nutrient losses in silage are avoidable and therefore the efficiency of their

preservation is related with the fast filling of the silo and proper sealing conditions which are essential for the rapid establishment of anaerobic conditions with pH reduction.

Different additives have been used to provide favorable conditions to maximize recovery of energy, better quality and conservation of silage through the modulation or addition of more nutritional value. The additives most commonly used are stimulating microbial fermentation or moisture-absorbing additives.

The moisture-reducing additives are usually carbohydrate sources (cereals, bran etc.) used to raise dry matter content by reducing the production of effluents and increasing the nutritional value of

the silage. The use of byproducts, such as soybean hulls, becomes an interesting option, since its addition at ensiling increases the nutritional value and reduces the production of silage effluent (RIBEIRO et al., 2009). Urea is indicated to reduce nutrient losses, increase protein fraction and decrease yeasts and molds. In fact, the ammonia released by urea hydrolysis changes fermentation rates and makes soluble the cell wall components, especially hemicellulose (FERNANDES et al., 2009), with positive results on the cellular digestibility.

Due to the presence of several agribusinesses in Brazil, many by-products are available and evaluated as alternative additives. In fact, oil seeds crushed during the oil production of biofuel may be used in silage production (OLIVEIRA et al., 2011).

Current study evaluates the bromatological changes in sunflower silage to which soybean hulls, sunflower crushed or urea were added at different times of silo opening.

Material and methods

Sunflower Rumbosol 91 was planted, harvested and ensiled on a reserved area of the Regional Campus at Umuarama, Paraná State, Brazil, of the *State University of Maringá*, between September and December 2007. Sunflower plants were harvested after 123 days of growth (200 g kg⁻¹ of DM and 100 g kg⁻¹ of CP) and cut at a height of 20 cm, with an average chopping size of 1.5 cm.

According to Koeppen's classification, climate is Cfa, characterized as mesothermal humid subtropical, with hot summer months and rare frosts in winter, with an average temperature above 22°C during the hottest months and below 18°C during the coldest months. Average annual rainfall is 1.500 mm. The soil is classified as Oxisol A with a typical horizon of sandy texture and subsurface diagnostic horizons of Oxisols B sandy-loam (EMBRAPA, 1999).

The experimental area was prepared in a conventional way, with a double plow and harrow. Ten days before planting, the area was dried by glyphosate (glyphosate 1.080 g e. a. ha⁻¹). The sowing of sunflower was performed mechanically with 0.90 m spacing between rows and density of five seeds per meter.

Further, 24 kg ha⁻¹ N, 60 kg ha⁻¹ P₂O₅, 60 kg ha⁻¹ K₂O were applied through 300 kg ha⁻¹ of 8-20-20 and 1 kg ha⁻¹ of B with boric acid as source on the sowing of the sunflower. Nitrogen fertilization was undertaken with 26 kg ha⁻¹, with urea as a source, 28 days after emergence.

Immediately after cutting, the fresh forage was homogenized and enriched with 5% of soybean hulls (SH), 5% of sunflower crushed (TG) and 5% urea (U), as recommended by Fernandes et al. (2009) (Table 1). The additives were based on natural matter. Control consisted of silage exclusively made from sunflower plants (SG). Thirty-six experimental silos made from plastic bottles, 150 mm diameter and 252 mm height each, with a 500 kg m⁻³ compression. All the silos were sealed with plastic stops, using adhesive tape, and stored in a covered shed.

Table 1. Chemical composition of additives used, in g kg⁻¹ of dry matter.

Feeds	DM	OM	CP	NDF	ADF	MM	EE
Soybean hulls	892.7	956.6	85.6	684.0	505.2	43.4	16.0
Sunflower crushed	900.3	953.5	214.0	322.6	208.3	46.5	100.0

After ensiling, silos were transported to the Animal Nutrition Laboratory of the Agricultural Sciences College at Federal University of Grande Dourados - UFGD, Dourados, Mato Grosso do Sul State, Brazil, where the silos were opened at 14, 21 and 28 days of silage. Approximately 60 grams of samples were collected, placed in a container of 250 ml containing 50 mL of distilled water for approximately 30 minutes to determine silage pH by digital pH meter, as described by Silva and Queiroz (2002).

The material removed from the silos were pre-dried in forced air oven at 60-65°C for 72 hours, processed in a mill-type "Willey" with 1 mm sieves and stored in plastic bottles. Part of the samples was transported to the Laboratory of Animal Nutrition of the Federal University of Goiás (UFG) in Goiania, Goiás State, Brazil, where dry matter (DM), organic matter (OM), crude protein (CP), extract ether (EE) and mineral matter (MM) were determined following methodology by Silva and Queiroz (2002); acid detergent insoluble protein (ADIP) and neutral detergent insoluble protein (NDIP) were estimated according Krishnamoorthy et al. (1982). Rates of neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose (HCEL), cellulose (CEL) and lignin (LIG) were determined by the sequential method (SILVA; QUEIROZ, 2002) using 25 mL of 1% thermostable amylase added at the start of boiling.

NDF and ADF analyses were performed with Tecnal® (TE-149), using TNT bags with 100 g m⁻², size 5.0 x 5.0 cm (CASALI et al., 2008). Sequential extraction method with potassium permanganate (KMnO₄) was employed to determine lignin and cellulose concentrations that were later burned in a muffle furnace at 600°C (SILVA; QUEIROZ, 2002).

Total carbohydrates (TC) were determined according to Sniffen et al. (1992) by equation $TC = 100 - (\% CP + \% EE + \% MM)$. The *in vitro* digestibility of dry matter (IVDM) was determined at the Animal Nutrition Laboratory in the Federal University of Grande Dourados following method by Tilley and Terry (1963), using the *in vitro* Tecnal® (TE-150) incubator, with 5.0 x 5.0 cm TNT bags (100 g m⁻²).

The experimental design was completely randomized and treatments had a 4 x 3 factoring (four silages, three days of opening), with three replicates. Data were interpreted by statistical package SAEG 9.1 (UFV, 2007) and means compared to 5% probability by Tukey's test, according to the model: $\hat{Y}_{ijk} = \mu + \tau_i + d_j + e_{ijk}$; in which: \hat{Y}_{ij} = value observed in experimental unit with additive i, at repetition j, in opening k; μ = overall average; τ_i = effect of addition of additive i, where i = 1, 2, 3, 4; d_k = effect caused by the day of opening k, where k = 1, 2, 3; and e_{ijk} = random error associated with each observation.

Results and discussion

According to Jobim et al. (2007), the variables used to evaluate the efficiency of the silage fermentation are dry matter, pH, ammonia and organic acids. Only pH and dry matter content were evaluated in current study. The sharp decrease in silage's pH reduced proteolytic activity and arrested the growth of undesirable aerobic microorganisms, such as clostridia sensitive to acid conditions (TAVARES et al., 2009).

Moreover, pH values between 3.8 and 4.2 were used to preserve silage in appropriate conditions, since range restricted the action of proteolytic enzymes in the plant and that of other undesirable microorganisms (TOMICHI et al., 2004). With the exception of urea addition, the sunflower silage had a decrease in pH (Table 2), influenced by the addition of different agro-industrial residues and by the time of opening of the silo (p < 0.05).

Table 2. Mean values of pH of sunflower silage ensiled with additives on different days of opening.

Treatments	Days of opening		
	14	21	28
Sunflower silage (SG)	3.14Ac	3.08Ab	3.12Ab
SG + 5% soybean hulls	3.41Ab	3.28Ab	3.26Ab
SG + 5% sunflower crushed	3.41Ab	3.19Bb	3.17Bb
SG + 5% urea	7.88Ba	8.10Aa	8.15Aa
CV (%)	2.39		

Means followed by the same uppercase letter on the line and by the lowercase letter in column do not differ by Tukey's test (p < 0.05).

Since adequate pH for the efficient conservation of ensiled forage depended on moisture content, pH should not be taken in isolation but together with the dry matter content of forage (GONÇALVES

et al., 2005). Tomich et al. (2004) evaluated sunflower varieties and found that pH value was positively related to dry matter content, indicating that silages have more humidity at lower pH rates.

Jobim et al. (2007) registered that, in the case of silages with low DM contents, pH continues to be a good indicator of the quality of fermentation. The sunflower silage (SG) and silage with soybean hulls and sunflower crushed showed mean rates of 220.9 g DM kg⁻¹ and pH 3.22. With the exception of urea-treated silage, all silages achieved pH rates below 4.2, and thus favorable to good forage conservation. Results also provide good quality fermentation (McDONALD et al., 1991).

Gonçalves et al. (2005) emphasized that sunflower silages registered high pH rates. Only the sunflower silage associated with urea showed a high pH in current assay. The highest CP rate (Table 3), due to the addition of urea, may have affected the drop in pH by the alkaline effect generated by the addition of the same (BUMBIERIS JR. et al., 2009). Urea produced nitrogen compounds which neutralized the lactic acid due to the dissociation of H⁺, and the formation of NH₄⁺ promoting the buffer effect (McDONALD, et al., 1991). The pH rates were similar to those found by Souza et al. (2005) for silages of sunflower genotypes, and lower than 5.14 found by Possenti et al. (2005).

Table 3. Regression equations for dry matter (DM) and ether extract (EE) of sunflower silage associated with additives depending on the opening day.

Treatments	Regression equation
Dry matter	
Sunflower silage (SG)	$\hat{y} = 0.5671*x + 10.18$ (r ² = 0.97)
SG + 5% soybean hulls	$\hat{y} = 0.1347*x^2 - 5.4286*x + 81.33$ (r ² = 0.99)
SG + 5% sunflower crushed	$\hat{y} = -0.1079*x^2 + 4.6564*x - 18.48$ (r ² = 0.98)
SG + 5% urea	$\hat{y} = 23.08$
Ether extract	
Sunflower silage (SG)	$\hat{y} = -0.0864*x + 7.417$ (r ² = 0.86)
SG + 5% soybean hulls	$\hat{y} = 0.0207*x + 3.3217$ (r ² = 0.60)
SG + 5% sunflower crushed	$\hat{y} = 0.0268*x^2 - 0.9121*x - 11.46$ (r ² = 0.98)
SG + 5% urea	$\hat{y} = -0.0914*x + 7.33$ (r ² = 0.92)

*(p < 0.05).

Possenti et al. (2005) highlighted that high protein levels in silage might neutralize lactic acid. This was due to the production of nitrogen compounds resulting from the decomposition of silage protein, or rather, a stable pH was not obtained because of carbohydrate deficiency or excess moisture.

In the case of the variables DM and EE, an interaction occurred between time of opening of silos and additives evaluated. The addition of 5% soybean hulls and sunflower crushed respectively increased DM content of silage at 21 and 28 days of silo opening.

DM content is an important variable in the ensiling process and is related to the presence of

harmful microorganisms, the production of effluents that carried highly digestible nutrients to the solution and essential compounds for the occurrence of a good fermentation of forage and the reduction of DM intake (McDONALD, et al., 1991). The low dry matter content of sunflower silage is the main limitation for its use. The inclusion of 5% soybean hulls improved dry matter content in 32.06% after 28 days of ensiling, whereas sunflower crushed brought an addition of 48.94% after 21 days of ensiling (Figure 1a). Rodrigues et al. (2001) obtained DM rates of 19.05% for sunflower silage, similar to current assay after 14 days of ensiling. Urea, with an average 23.08%, did not affect DM in silage.

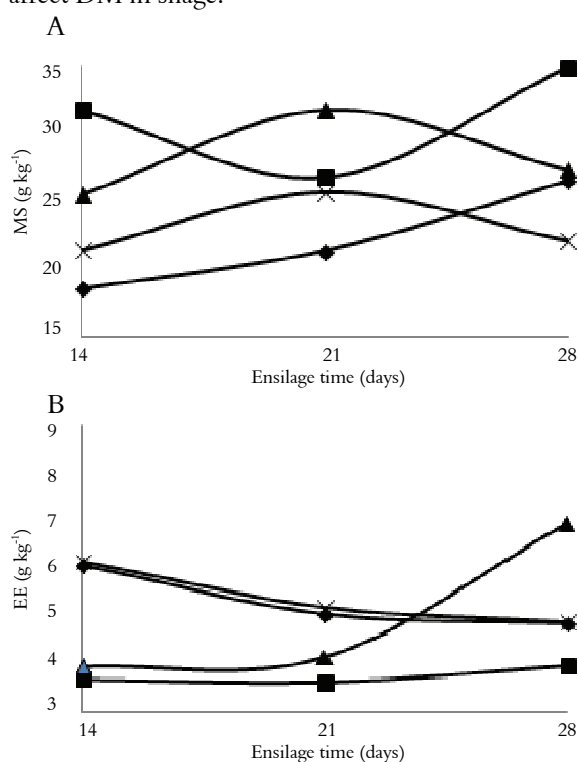


Figure 1. Contents of dry matter (DM) and ether extract (EE) of sunflower silage with additives, according to opening days (◆ Sunflower silage - SG, ■ SG + 5% soybean hulls, ▲ SG + 5% of sunflower crushed, x SG + 5% urea).

DM content of sunflower silage without additives amounted to 22.09% due to the age of plant cutting for silage (123 days after planting). Possenti et al. (2005) reported rates of 22% DM for the cultivar Rumbosol 91, similar to those found in this study.

In current analysis, the sunflower silage had the lowest EE rate, or 20%, at the time of opening of the silo (Figure 1b). The sunflower stores its energy in the form of grains in oil and thus its silage has a high EE concentration of about 100 g kg⁻¹ DM (POSSENTI et al., 2005). The addition of

sunflower crushed increased silage EE when an oil extraction byproduct with high EE rates was included (GOES et al., 2008, 2010).

The high levels of sunflower EE may be a limiting factor for its use as exclusive roughage in ruminant diet. This fact indicates need for combination with other forages, since diets containing more than 7 g kg⁻¹ DM of lipids may be related with reductions in ruminal fermentation, fiber digestibility and passage rate (GONÇALVES et al., 2005). In current assay, only sunflower silage associated with sunflower crushed had any similarity (6.96 g kg⁻¹ DM) to this limit, at 28 days of fermentation. This fact would limit its use as the sole roughage source.

Averages of MO, NDIP and MM were 911.2, 86.6 and 92.9 g kg⁻¹ DM respectively. There was a significant effect when additives were used in the case of CP, ADIP and TC, albeit not for the opening days (Table 4).

Table 4. Concentrations of organic matter (OM), crude protein (CP), neutral detergent insoluble protein (NDIP), acid detergent insoluble protein (ADIP), total carbohydrates (TC) and mineral matter (MM) in g kg⁻¹ DM of sunflower silage ensiled with different additives.

Treatments	OM	CP	NDIP	ADIP	TC	MM
Sunflower silage (SG)	922.1a	121.5b	85.1a	09.0b	731.1a	94.1a
SG + 5% soybean hulls	915.4a	108.9b	93.6a	07.5b	768.9a	84.6a
SG + 5% sunflower crushed	887.5a	122.3b	55.3a	07.6b	715.1ab	112.5a
SG + 5% urea	919.8a	235.0a	112.5a	12.7a	630.6b	80.2a
CV (%)	7.23	22.95	80.21	24.80	10.57	68.19

Means followed by same letters in column do not differ by Tukey's test ($p < 0.05$).

CP rates of 121.5, 108.9 and 122.3 g kg⁻¹ DM respectively obtained for sunflower silage (SG) and SG associated with soybean hulls and sunflower crushed agree with the rate 116 g kg⁻¹ provided by Possenti et al. (2005). When used in balanced diets, high CP levels may represent an economic advantage for sunflower silage when compared to other forages, which may reduce the need for an additional supply of nutrients.

Urea provided an increase in nitrogen fraction and therefore higher CP and ADIP levels, consistent with the purpose of applying urea in the silage, or rather, to increase nitrogen levels in the silage, since a significant part of the NNP is retained in material (BUMBIERIS JR. et al., 2009). The addition of 5% soybean hulls and sunflower crushed provided an increase in the levels of TC silage, possibly due to their high carbohydrate rates. The lowest level of carbohydrates for the addition of urea to the silage may be due to increased consumption of soluble carbohydrates during fermentation (SIQUEIRA et al., 2009), or to an increase in CP (SNIFFEN et al., 1992).

There was no interaction between opening day and additives with regard to NDF, ADF and hemicellulose, respectively with mean rates 625.4, 460.3 and 165.2 g kg⁻¹ DM (Table 5). In their assays on different genotypes of sunflower silage, Porto et al. (2006) reported no effect on NDF when 0.5% urea was added. Rates ranged between 416 and 563 g kg⁻¹ DM, or rather, they were lower than those reported in current study. Fernandes et al. (2009) registered a linear effect as doses of urea in silages increased. This fact was probably due to the ammonia released by urea hydrolysis on the constituents of the cell wall. As a rule, NDF and hemicellulose decreased linearly when there was an increase in ammonia addition (PIRES et al., 1999).

Table 5. Concentrations of neutral detergent fiber (NDF), acid detergent fiber (ADF) and hemicellulose (HCEL) in g kg⁻¹ DM of sunflower silage ensiled with different additives.

Treatments	NDF	ADF	HCEL
Sunflower silage (SG)	609.7 ^a	438.0 ^a	171.6 ^a
SG + 5% soybean hulls	646.4 ^a	487.3 ^a	159.1 ^a
SG + 5% sunflower crushed	613.1 ^a	463.3 ^a	149.8 ^a
SG + 5% urea	632.3 ^a	452.1 ^a	180.1 ^a
CV (%)	7.75	9.34	38.25

Means followed by same letters in column do not differ by Tukey's test (p < 0.05).

Compared to data from the literature, NDF variation was probably related to different cutting times, amount of grain and silage cultivar. Moreover, late cuttings entail an increase in fiber fraction (VAN SOEST, 1994).

Mean FDA amounted to 460.2 g kg⁻¹ DM and might be related to a greater loss of cellular contents due to the additives' action on the cell wall (BUMBIERIS JR. et al., 2009). Tomich et al. (2003) studied different cultivars of sunflower silage and registered a variation between 289 and 406 g kg⁻¹ DM, whereas Souza et al. (2005), who worked with different cutting ages, enhanced on ADF increase for 111-day old plants for ensilage. In current assay, the cutting of sunflower plants was performed after 123 days and this fact must have contributed to the high ADF rates. Pereira et al. (2007) pointed out that sunflower silage had a high concentration of ADF and lignin that might interfere in the digestibility of the fiber fraction.

Rates for silage with urea in ADF are in agreement with Porto et al. (2006) who failed to register any difference when urea was added to different sunflower genotypes. NDF and ADF rates provided by sunflower silage at 609.7 and 438.0 g kg⁻¹ DM were compatible with plants with low hemicellulose levels, such as the sunflower (RODRIGUES et al., 2001). The addition of additives did not alter HCEL concentration with a mean rate of 165.2 g kg⁻¹ DM.

Lignin (LIG) and cellulose (CEL) contents were effect by additives (p < 0.01). The addition of 5% of soybean hulls and sunflower crushed provided conditions for lignin content decrease and, consequently, CEL increased contents (Table 6). Van Soest (1994) stated that LIG and CEL levels were stable with the progress of the fermentation process, but the addition of 5% of sunflower crushed or soybean hulls produced lower LIG levels, or possibly because of the effect of diluting the concentration of the materials added.

Table 6. Concentrations of lignin (LIG) and cellulose (CEL) of sunflower silage ensiled with additives, expressed in g kg⁻¹ DM.

Treatments	LIG	CEL
Sunflower silage (SG)	208.3 ^a	154.9 ^b
SG + 5% soybean hulls	97.9 ^b	304.9 ^a
SG + 5% sunflower crushed	98.7 ^b	274.4 ^a
SG + 5% urea	217.5 ^a	207.5 ^{ab}
CV (%)	43.48	34.14

Means followed by same letters in column do not differ by Tukey's test (p < 0.01).

High LIG contents for sunflower silage might be associated with the plant's harvest, or the Maillard reaction, which might have occurred during the fermentation process, since they did not reflect the rates found for *in vitro* digestibility (IVDMD).

The addition of soybean hulls and sunflower crushed did not improve the *in vitro* digestibility of DM (p < 0.05) of silage (Table 7), which might be due to higher levels of cell wall. During the plant's maturation, the DM digestibility was reduced (SOUZA et al., 2005). The addition of urea improved *in vitro* digestibility by 9.1%. The ammonia released from urea hydrolysis might have caused the breakage of ester bonds of LIG, CEL and HCEL and increased IVDMD (PORTO et al., 2006).

Table 7. Mean *in vitro* digestibility of dry matter (DM) of sunflower silage ensiled with additives.

Treatments	IVDMD (g kg ⁻¹ de MS)
Sunflower silage (SG)	534.6 ^{ab}
SG + 5% soybean hulls	490.1 ^b
SG + 5% sunflower crushed	502.7 ^b
SG + 5% urea	583.3 ^a
CV (%)	8.70

Means followed by same letters in column do not differ by Tukey's test (p < 0.05).

A negative correlation was registered between IVDMD and cell wall constituents (Table 8) which indicated that silage digestibility increased the concentration of fibrous forage, complying with results by Tomich et al. (2004) and Porto et al. (2006). High ADF and Lignin rates in sunflower silage might restrict the quality of the fiber and, consequently, its use to highly demanding animal categories (TOMICH et al., 2004).

Table 8. Correlation matrix for variables involving cell wall components and *in vitro* digestibility of dry matter of sunflower silage ensiled with additives.

	IVDMD	NDF	ADF	HCEL	CEL	LIG
NDF	-0.1438	-	-	-	-	-
ADF	-0.0719	-0.0090	-	-	-	-
HCEL	-0.1465	0.4851*	-0.6974**	-	-	-
CEL	-0.3313*	0.3738*	0.1279	-0.0489	-	-
LIG	-0.3661*	-0.1840	-0.0067	0.0493	-0.8351**	-

*(p < 0.05), ***(p < 0.01), ns = not significant.

Conclusion

A 5% addition of sunflower crushed and soybean hulls changed the chemical composition of sunflower silage and provided suitable conservation standard, whereas the addition of urea improved protein profile of sunflower silage.

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