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Mineral and nutrient content of corn stover silage with livestock excreta and by-products rich in carbohydrates

Contenido mineral y de nutrientes de ensilados de rastrojo de maíz con excretas pecuarias y subproductos ricos en carbohidratos



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ABSTRACT

This research was carried out to determine the effect of corn stover silage with three non-protein nitrogen sources plus two carbohydrate sources on its mineral content. Nitrogen sources (NS) were agricultural urea (AU), dehydrated poultry manure (PM) and swine manure fresh (SM) and sources of carbohydrates (CS) sugar cane molasses (SCM) and bakery by-products (BBP). After 30 d of fermentation, the microsilages were opened and the concentration of Ca, P, Na, K, Mg, Co, Cu, Mn, Fe, and Zn were measured. The results were subjected to analysis with a completely randomized design with a 3x2 factorial arrangement of treatments and under a mixed effects model. There were ($P<0.01$) NSxCS interactions on the mineral content of all silages. The PM+SCM silage was higher ($P<0.01$) in Ca, P, Na, Mg and K. The SM silage had higher ($P<0.01$) Cu content, and the PO-based treatments had higher ($P<0.01$) Mn content. In general, the highest mineral content was observed in silages based on PL and SM. It is concluded that corn stover silage enriched with PM with SCM can provide macro and micro minerals in ruminant feed.

Keywords: minerals, silage, poultry litter, swine manure, sugar cane molasses.

RESUMEN

Esta investigación se realizó para determinar el contenido mineral del ensilado de rastrojo maíz con tres fuentes de nitrógeno no proteico y dos fuentes de carbohidratos. Las fuentes de nitrógeno (NS) fueron urea agrícola (UA), pollinaza deshidratada (PO) y cerdaza fresca (CF); y las fuentes de carbohidratos (CS) fueron melaza de caña (MC) y subproductos de panadería (SP). Después de 30 d de fermentación, los microensilados se abrieron y se midió la concentración de Ca, P, Na, K, Mg, Co, Cu, Mn, Fe, y Zn. Los resultados fueron sujetos a análisis con un diseño completamente al azar con arreglo factorial 3x2 y modelo de efectos mixtos. Hubo interacciones ($P<0.01$) NSxCS sobre el contenido mineral de todos los ensilados. El ensilado PO+MC fue mayor ($P<0.01$) en Ca, P, Na, Mg y K. Los ensilados con CF tuvieron mayor ($P<0.01$) contenido de Cu, y los tratamientos a base de PO tuvieron mayor ($P<0.01$) contenido de Mn. En general, los mayores contenidos de minerales se observaron en ensilados a base de PO y CF. Se concluye que el ensilado de rastrojo de maíz enriquecidos con PO con MC puede proveer macro y microminerales en la alimentación de rumiantes.

Palabras clave: minerales, ensilado, pollinaza deshidratada, cerdaza fresca, melaza de caña.



INTRODUCTION

In tropical areas of Mexico and Central America, the household economy of poor farmers is based on the cultivation of corn and the raising of ruminants. Maize grain is used as human food, and animals are fed mainly on maize stubble. Nutrients in the stubble are marginal for ruminants in maintenance (NRC, 2007; NASEM, 2016), in addition, crude protein and minerals such as P, Mg, Co, Cu and Zn are low (Zinn *et al.*, 1996). It is documented that fresh sow and poultry manure can be included as sources of nitrogen for ruminants (Jayathilakan *et al.*, 2012; Bórquez *et al.*, 2018), but at the same time, they can be sources of minerals due to their high ash content. Livestock excreta contaminate the environment with pathogens and bad odors (Pell, 1997; Schiffman, 1998); but the process of anaerobic fermentation (ensiling) eliminates bad odors and considerably decreases the pathogen load (López-Garrido *et al.*, 2014), and therefore can be used for ruminant feeding (Denton *et al.*, 2005; Seok *et al.*, 2016) in the form of silage (Bórquez *et al.* 2010). The ensiling process requires soluble carbohydrates to initiate and maintain fermentation (González-Muñoz *et al.*, 2022). Sugar cane molasses has high concentration of carbohydrates (So *et al.*, 2020) and minerals (ppm): Mn (18), Zn (34), Cu (4.9), Bo (3), Co (0.6) and Fe (115) (NASEM, 2016; Senthilkumar *et al.*, 2016; Tendonkeng *et al.*, 2018). Sugar cane molasses (Trujillo *et al.*, 2014) and bakery by-products can be used as a source of rapidly fermentable carbohydrates in silages (França *et al.*, 2012; Mahmoud *et al.*, 2017; Salama *et al.*, 2019).

In Mexico, regulations on livestock waste are limited to the management of discharges into water bodies and on national assets (Pinos-Rodríguez *et al.*, 2012), as well as the health and agri-food safety of agricultural products (Acevedo *et al.*, 2017). However, there are no regulations on the use of excreta from livestock species in ruminant feed.

Therefore, it was postulated that corn stover silages with livestock excreta combined with by-products high in soluble carbohydrates can be a source of minerals for ruminants. Therefore, the objective of this research was to determine the content of Ca, P, Na, Mg, K, Co, Cu, Mn Fe and Zn in silages based on corn stover with dehydrated poultry manure, fresh sow manure and agricultural urea mixed with sugar cane molasses or bakery by-products.



MATERIAL AND METHODS

Location of the experiment

This research was carried out at the Animal Production Research and Teaching Unit and the Bromatology Laboratory of the Faculty of Veterinary Medicine and Animal Husbandry of the Autonomous University of the State of Mexico (FMVZ-UAEMex), located in Toluca, Mexico at 19°16`W and 2745 m altitude.

Ingredients and preparation of microensilates

Fresh swine manure (feces and urine; SM) was obtained from fattening pigs from the experimental farm of the FMVZ-UAEMex. Inputs such as dehydrated poultry manure (PM), agricultural urea (AU), sugar cane molasses (SCM), bakery by-product (BBP) and corn stover were obtained from commercial vendors. Samples of the inputs were obtained, were stored in glass jars and were kept in dark conditions until proximate chemical analysis. Nitrogen sources (AU, PM and SCM) and carbohydrate sources (SCM and BBP) were mixed to prepare six micro-silages (treatments; Table 1) based on corn stover and water: AU+SCM (control) AU + BBP (control) PM + SCM, PM + BBP, SM + SCM and SM + BBP. The proportions of silage ingredients were based on the treatments tested by Trujillo et al (2014). Microsilos with ten replicates per treatment were made with PVC tubes (10 cm diameter and 20 cm long). In each tube was placed 2.5 kg of material mixed with tap water ($\pm 60\%$) (Cobos *et al.*, 1997), and this was compacted to expel oxygen. Microsilos were sealed then with a plastic film to prevent deterioration by oxygen. The fermentation period lasted 30 d at room temperature under shade.

Bórquez *et al.* (2010) and Mejía *et al.* (2013) suggested the levels of ingredients used in silages.

Table 1. Composition and ingredient inclusion proportions (% DM) of the pre-silage treatments

Ingredients	Treatments*					
	AU		PM		SM	
	SCM	BBP	SCM	BBP	SCM	BBP
Corn stubble	63	63	38	38	30	30
Agricultural urea	3	3	0	0	0	0
Dehydrated poultry manure	0	0	39	39	0	0
Fresh pig manure	0	0	0	0	53	53
Sugar cane molasses	34	0	23	0	17	0
Bakery by-product	0	34	0	23	0	17

*All treatments were inoculated with Sil-All 4x4 (10 mg kg DM⁻¹) containing a mixture of *Lactobacillus plantarum*, *Pediococcus acidilactii*, *Enterococcus faecium* and *Lactobacillus salivarius*. AU, agricultural urea; PM, dehydrated poultry manure; SM, fresh swine manure. SCM, sugar cane molasses; BBP, bakery by-product.



Chemical composition and mineral determination

Once the fermentation period was completed, microsilos were opened and 100 g were taken for each replicate to obtain a composite sample of 1000 g per treatment. The samples per treatment were divided into two 500 g subsamples. The first was placed in a 1000 mL Erlenmeyer flask with 500 mL of distilled water and 4 drops of thymol were added, sealed with plastic and stored for 24 h. The next day, the pH of the sample was measured in a 1000 mL Erlenmeyer flask with 500 mL of distilled water. The next day, pH was measured with a portable potentiometer (Hanna H198130; Hanna Instruments Italy). The second was dried in a forced-air oven at 65°C for 72 h (AOAC, 1990) to determine dry matter (DM) and ground in a Wiley Mill (1 mm mesh; Model 4 Thomas Scientific Swedes, NJ). Crude protein (Kjeldjhal, N×6.25) and organic matter were determined according to AOAC (1990). Neutral detergent fiber, acid detergent fiber and acid detergent lignin were determined according to Van Soest *et al.* (1991) and ANKOM method. For mineral analysis, 2 g DM of each treatment was used with six replicates, which were digested in 8 mL of 10 % trichloroacetic acid, centrifuged and the supernatant was used to determine P in a spectrophotometer (Genesis 20) with visible ultraviolet light (Harris & Popat, 1954). The minerals Ca, Mg, Cu, Fe, Zn, Mn, and Co were determined by atomic absorption spectrophotometer (Perkin Elmer 3110) (Fick *et al.*, 1976). Na and K were analyzed by flammety (Corning 410). Table 2 shows the pre-silage mineral content of the ingredients used in the micro-silages.

Table 2. Mineral content (% DM) of ingredients used to prepare the microsilos

Mineral	Ingredientes ¹				
	Corn stubble	Bakery by-product	Sugar cane molasses	Dehydrated poultry manure	Fresh pig manure
Calcium, %	0.30	0.15	0.65	2.15	2.61
Phosphorus, %	0.07	0.19	0.07	0.28	0.18
Magnesium, %	0.10	0.10	0.36	2.15	2.61
Potassium, %	1.10	0.35	3.70	1.95	3.16
Sodium, %	0.06	0.72	0.18	0.21	1.38
Cobalt, mg/kg	Nd	Nd	Nd	3.32	2.90
Copper, mg/kg	4.00	5.0	15.0	86.2	93.8
Manganese, mg/kg	37.0	10.0	20.0	299.7	342.7
Iron, mg/kg	180.0	20.0	180.0	2417.6	1449.9
Zinc, mg/kg	16.0	16.0	19.0	585.9	305.6

¹Values were determined in the laboratory using six replicates per ingredient. Nd, not detected



Experimental design and statistical analysis

A Completely Randomized Design was used with a 3x2 factorial arrangement of treatments with six replicates per treatment. Statistical analysis was performed with PROC MIXED (SAS Institute Inc., 2004) and comparison of means with Tukey's test ($P \leq 0.05$). The composite sample of each treatment was considered as random subject and the treatments as fixed effects with TYPE3 estimation method. Figures were performed with R-project version 4.1.0 (R Core Team, 2022). The mathematical model was: $y_{ijk} = \mu + a_i + b_j + a*b_{(ij)} + e_{ijk}$, where: y_{ijk} = response variable, μ = overall mean, a_i = effect of nitrogen source (AU, PM, SM), b_j = effect of carbohydrate source (SCM, BBP), $a*b_{(ij)}$ = effect of nitrogen x carbohydrate interaction, e_{ijk} = experimental error, $e_{ij} \sim N(0, \sigma^2)$. To determine the effect of the main factors nitrogen source, carbohydrate source and interaction, the SOLUTION option of MODEL (SAS Institute Inc., 2004) was used.

RESULTS

Macrominerals

The chemical composition of silages (Table 3) and the effect of NS and CS on mineral concentration are presented in Table 4. Figure 1 shows the NSxCS interaction of treatments for macrominerals. The solutions of the model parameter estimates show that the sum of the main effects and interactions result in the total effect of the independent variables on the dependent variable. Thus, the interaction is explained as, a part of the effects of the nitrogen source depend on the effect of the carbohydrate source and vice versa.

Table 3. Chemical composition (% DM) of corn stover-based silages after 30 d of fermentation

Ingredients	Treatments					
	AU		PM		SM	
	SCM	BBP	SCM	BBP	SCM	BBP
Dry matter, %	42.20	33.80	43.10	41.60	36.9	34.20
Crude protein, %	12.62	14.30	12.42	13.57	13.71	14.56
ME, Mcal/kg	2.05	2.29	2.16	2.30	2.16	2.27
Neutral detergent fiber, %	38.30	44.40	42.50	38.50	35.10	37.00
Acid detergent fiber, %	23.70	25.00	29.00	21.40	20.10	19.80
Acid detergent lignin, %	4.80	5.20	6.10	6.00	4.40	4.40
Ash	7.30	9.10	11.00	11.10	9.60	7.70
pH	4.20	4.20	4.00	4.10	4.10	4.20

AU, agricultural urea; PM, dehydrated poultry manure; SM, fresh swine manure. SCM, sugar cane molasses; BBP, bakery by-product. ME, metabolizable energy, calculated from published values of ingredients (NRC, 2007; NASEM, 2016)



The highest ($P < 0.01$) Ca content was observed in the PM-based treatments. A positive effect of PM \times SCM and SM \times SCM was observed, which generated an additional effect of 0.23 and 0.32 % in Ca ($P < 0.01$), respectively (Figure 1).

Table 4. Results of the effect of nitrogen and carbohydrate sources on the mineral concentration of corn stover microsilos

Mineral	Treatments						SEM	Main effects		
	AU		AU		SM			NS	CS	NS x CS
	SCM	BBP	SCM	BBP	SCM	BBP				
Macrominerals, % DM										
Ca	0.154 ^e	0.378 ^d	1.151 ^a	1.143 ^a	0.779 ^b	0.682 ^c	0.009	< 0.01	< 0.01	< 0.01
P	0.004 ^d	0.027 ^c	0.105 ^a	0.075 ^b	0.112 ^a	0.116 ^a	0.004	< 0.01	0.71	< 0.01
Na	0.561 ^b	0.452 ^c	0.928 ^a	0.574 ^b	0.362 ^d	0.062 ^e	0.018	< 0.01	< 0.01	< 0.01
Mg	0.168 ^d	0.330 ^c	0.499 ^a	0.411 ^b	0.477 ^a	0.405 ^b	0.005	< 0.01	0.91	< 0.01
K	0.859 ^d	2.746 ^a	2.839 ^a	1.770 ^c	2.267 ^b	0.980 ^d	0.079	< 0.01	0.02	< 0.01
Microminerals, mg/kg DM										
Co	1.426 ^c	0.391 ^d	3.171 ^a	2.242 ^b	2.216 ^b	1.916 ^b	0.083	< 0.01	< 0.01	< 0.01
Cu	0.77 ^e	6.15 ^d	18.23 ^b	14.07 ^c	25.60 ^a	25.69 ^a	0.805	< 0.01	0.69	< 0.01
Mn	128.00 ^d	103.30 ^e	214.95 ^a	219.48 ^a	178.37 ^b	160.71 ^c	2.65	< 0.01	< 0.01	< 0.01
Fe	517.60 ^d	660.33 ^c	740.65 ^c	1102.97 ^a	898.21 ^b	994.19 ^{ab}	27.33	< 0.01	< 0.01	< 0.01
Zn	20.24 ^e	79.91 ^d	116.11 ^c	120.30 ^c	169.86 ^b	198.07 ^a	5.61	< 0.01	< 0.01	< 0.01

UA, agricultural urea; PM, dehydrated poultry manure; SM, fresh swine manure. SCM, sugar cane molasses; BBP, bakery by-product. SEM, standard error of the mean. NS, effect of nitrogen source; CS, effect of carbohydrate source. ^{a-e}Means with different literals in the same row are different ($P < 0.01$)

In relation to phosphorus (P), its content was affected ($P < 0.01$) by the nitrogen source. An increase of 0.04 % P was observed when PM was used instead of AU; similarly, when SM was used instead of UA, 0.08 % Ca was produced. The SM \times SCM interaction produced higher P content (> 0.01 %), and this effect was greater in PM \times SCM (≥ 0.05 % P) vs PM \times SCM. Figure 1 shows that the AU treatments were lower in P, and this effect was more evident in the SCM-based silage. In addition, there was a linear increase in P with the use of excreta in the silages. Regarding sodium (Na) concentration, this was higher ($P < 0.01$) in PM \times SCM than the rest (Table 4). In addition, an interaction effect ($P < 0.01$) of NS \times CS was observed. The interaction effect on Na concentration of PM \times SCM and SM \times SCM additionally yielded 0.24 and 0.19 %, respectively ($P < 0.01$). The interaction plot (Figure 1) shows that the combination of SCM or BBP with dehydrated poultry manure increased the Na content of the silages, but when were combined with fresh swine manure there was a negative effect.

The highest ($P < 0.01$) magnesium (Mg) accumulation was in silages based on SCM with excreta. The effect of N source on this mineral was observed. This effect was evident when comparing AU vs PM, which showed higher Mg content (0.08 %) in the latter.



However, when SM was used instead of AU, there was an increase in Mg of 0.07 %. The effect of NS×CS showed an additive effect ($P < 0.01$) in PM ×SCM (+0.25% Mg) vs PM ×BBP and SM×SCM (+0.23% Mg) vs CF×SPP, respectively; this caused higher Mg accumulation (Figure 1).

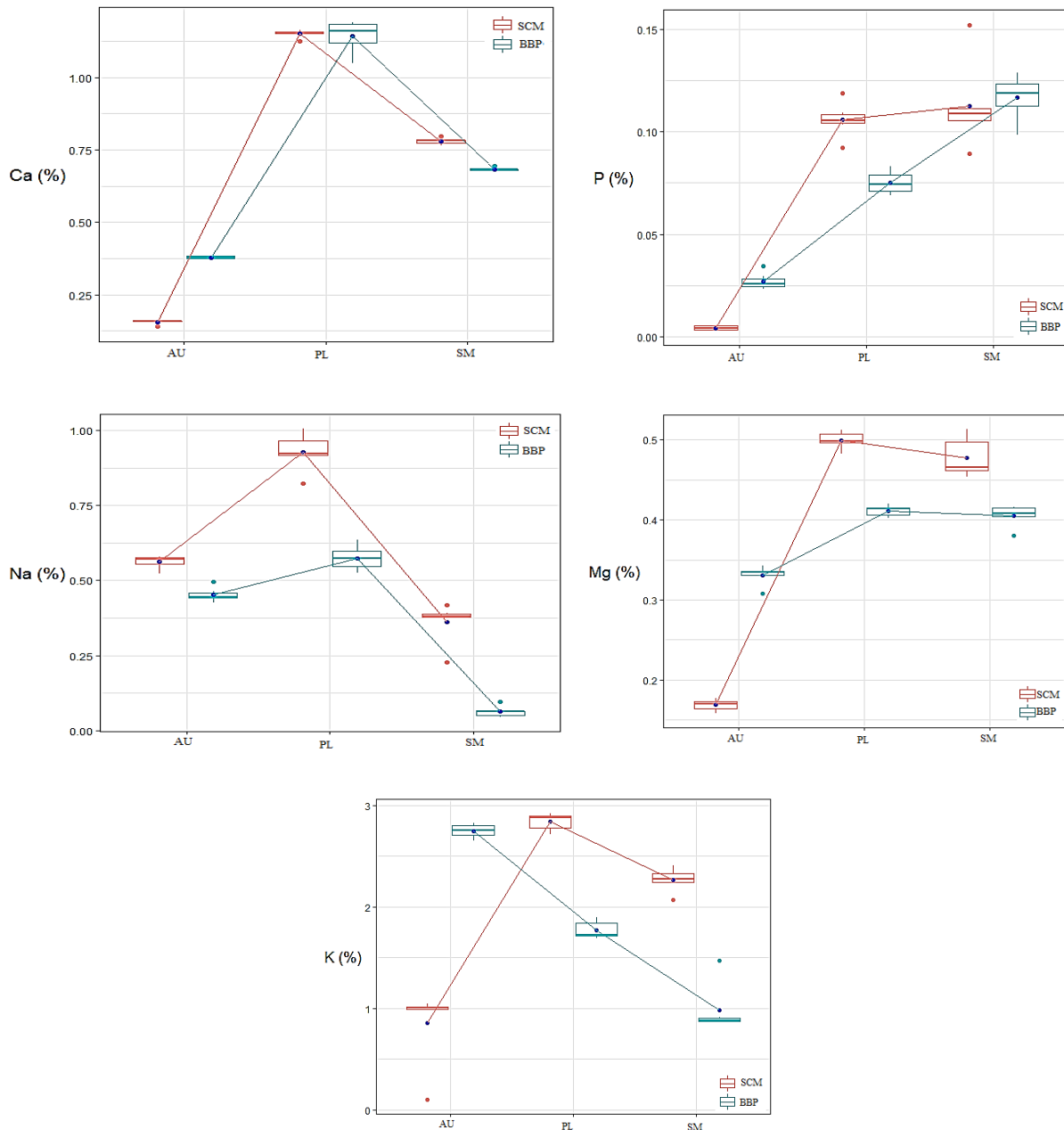


Figure 1. Interaction effect of nitrogen and carbohydrate sources on macrominerals content. Nitrogen sources: AU, agricultural urea; PL, dehydrated poultry manure; SM, fresh swine manure. Carbohydrate sources: SCM, sugar cane molasses; BBP, bakery by-product



Microminerals

The concentration (mg/kg DM) of all microminerals resulted from the effect of the NS×CS interaction (Table 4 and Figure 2). Cobalt (Co) concentration was higher ($P<0.01$) in the PL×SCM silage. The effect of nitrogen source showed that using PM instead of agricultural urea produced more than 1.85 mg/kg Co in silages. Similarly, using SM instead of AU increased Co content by 1.52 mg/kg. In addition, using SCM instead of BBP increased Co concentration in silages by 1.03 mg/kg. Copper (Cu) content was higher ($P<0.01$) in silages with SM. It was observed that in the control treatments (AU), the addition of SCM to the silage had a negative effect (-5.38 mg/kg Cu) compared to BBP; but in the rest of the treatments the carbohydrate source had no effect ($P=0.69$) alone, on Cu concentration. The effects of PM ×SCM and SM×SCM produced 9.54 mg/kg and 5.29 mg/kg Cu, respectively; than the contribution of nitrogen source and carbohydrate source alone. This allowed higher Cu concentration for SCM compared to BBP when mixed with PM; however, SM-based silages combined with both carbohydrate sources were similar (Figure 2).

Manganese (Mn) concentration was higher ($P<0.01$) in PM-based silages due to the NS×CS effect (Table 4, Figure 2). As for the effect of nitrogen source, PM-based silages were higher than control silages (AU) by 116.18 mg/kg Mn; likewise, SM was higher than AU by 57.41 mg/kg Mn. In addition, there was a positive effect of the addition of SCM to the silage, which resulted in a higher accumulation (24.70 mg/kg Mn) compared to the effect of BBP inclusion. A similar pattern was observed in the interaction graph (Figure 2) for silages with both soluble carbohydrate sources. Iron (Fe) content was higher ($P<0.01$) in the PM×BBP silage. The use of PM instead of AU had a beneficial effect by increasing the Fe concentration in silages by 442.64 mg/kg. Similarly, when SM was used instead of AU, there was an increase of 333.85 mg/kg Fe. The effect of PM×SCM interaction decreased ($P<0.01$) the concentration (-219.59 mg/kg) of Fe compared to PM×BBP but when SM×SCM interacted, Fe increased 46.75 mg/kg. However, this effect was not sufficient to match the Fe content of the SM×BBP interaction (Figure 2).

Finally, zinc (Zn) concentration was higher ($P<0.01$) in the silage with BBP. It was observed that with SCM the Zn content decreased when combined with AU (-59.67 mg/kg), but when mixed with PM or SM there was an additive effect (Figure 2). In this regard, the use of PM instead of AU increased Zn concentration by 40.38 mg/kg, and the PM×SCM interaction additionally yielded 55.48 mg/kg. Likewise, when AU vs SM was compared the latter produced more than 118.16 mg/kg Zn, and when SM was mixed with SCM an additional ≥ 31.45 mg/kg Zn was produced. Therefore, the positive effect of these components in the silages was sufficient to equal the Zn concentration (198.07 mg/kg) of the SM×BBP combination.

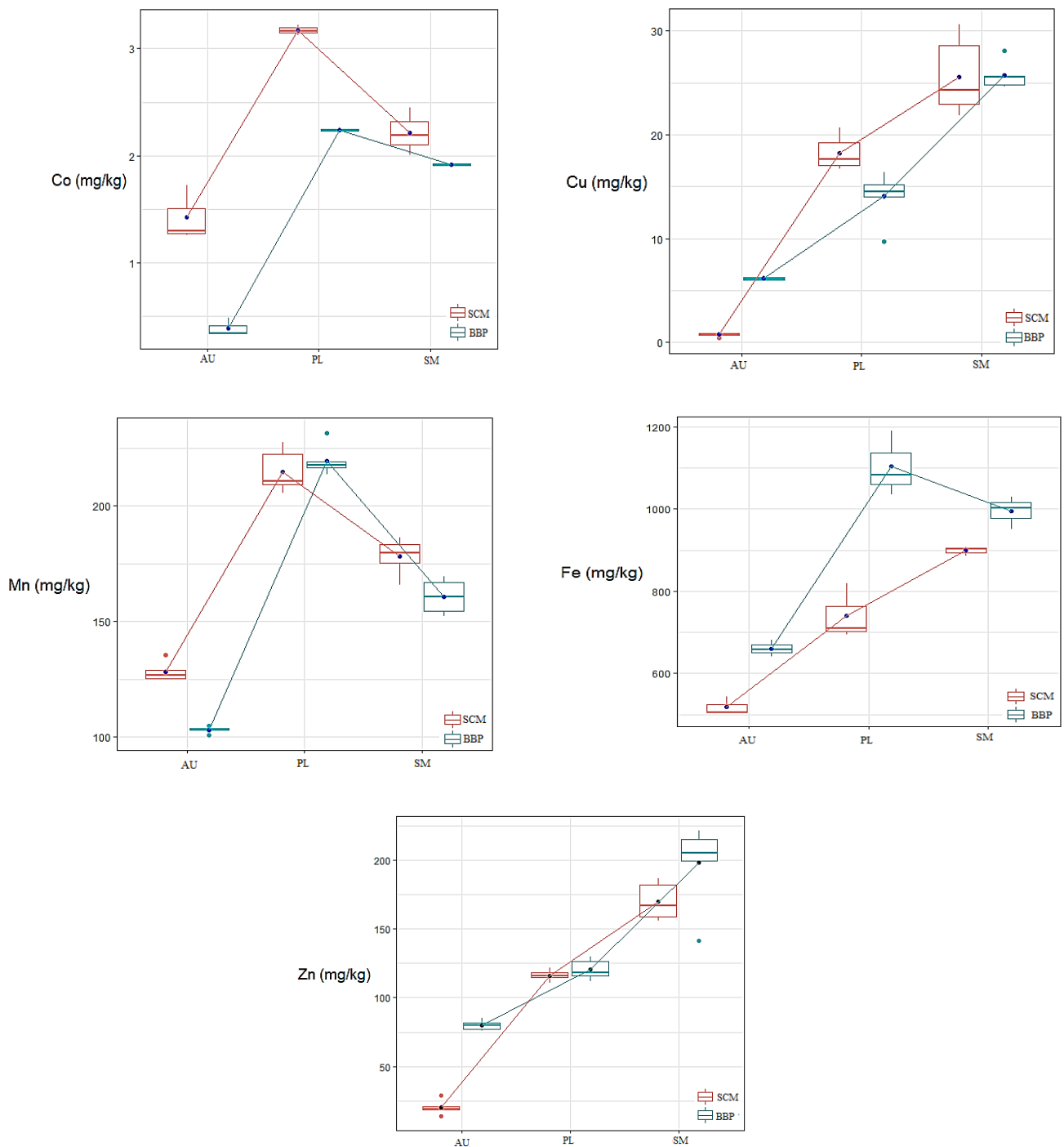


Figure 2. Interaction effect of nitrogen and carbohydrate sources on micro mineral content. Nitrogen sources: AU, agricultural urea; PM, dehydrated poultry manure; SM, fresh swine manure. Carbohydrate sources: SCM, sugar cane molasses; BBP, bakery by-product.



DISCUSSION

According to the results, the lactic-acid fermentation process affected the mineral content of the silages, due to the interaction effects of excreta combined with sugarcane molasses or bakery by-product. In addition, silages based on excreta with cane molasses showed higher macrominerals content. The effect of carbohydrate source was observed in most treatments, except for Mg and Cu. It was not surprising that PM increased the mineral content (Ca, P, Na, Mg, K, Co, Mn and Fe) in the silages, due to its high ash content (Kwak, 2006). The contribution of SM to high P, Mg, Cu and Zn contents of silages was also expected, because CuSO_4 is frequently used as an antimicrobial agent in swine diets (Shannon & Hill, 2019), and ZnO is used to decrease diarrhea symptoms (Zhou *et al.*, 2017); whereas, ZnSO_4 has antimicrobial effect. In addition, a high concentration of Ca, K, Cu, Mn, and Fe was expected because of the use of SCM in silages due to their high content of these minerals in the extracted sugar cane juice residues and their contamination with soil residues (Senthilkumar *et al.*, 2016; Tendokeng *et al.*, 2018) during handling.

In this research all treatments were inoculated with *Lactobacillus plantarum*, *Pediococcus acidilactii*, *Enterococcus faecium* and *Lactobacillus salivarius*; which produced good quality silages (Trujillo *et al.*, 2014) with pH between 4.1 - 4.2. The pH value is an important factor in determining the quality of the fermentation process in silages (Cañeque & Sancha, 1998); its values were around 4.1 and are considered appropriate for corn silages with sufficient grain (Basso *et al.*, 2014) but high pH values may indicate insufficient amount of soluble carbohydrates. The NS×CS interaction was observed in all treatments; this is relevant since in culture media with sufficient concentration of sugars (glucose >1 %) the expression of the enzyme phytase is induced (Palacios *et al.*, 2005). In the culture and growth of lactic acid bacteria (LAB), the essential mineral elements are Mg^{2+} , Mn^{2+} , Ca^{2+} , Fe^{2+} , Na^+ , and K^+ (Saeed & Salam, 2013); other elements such as Hg^{2+} , Cu^{2+} , Ni^{2+} , Zn^{2+} and Co^{2+} optimize and control their enzymatic activity.

Effect of silage on macro- and microminerals content

In the early stages of the fermentation process, rapid colonization of ingredients depends on the availability of fermentable soluble carbohydrates (Wood, 1998). Thus, oxygen consumption and oxygen depletion in silage depends on bacterial mechanisms that reduce aerobic conditions. In establishing anaerobic fermentation conditions, LAB in silage at $\text{pH} < 4$ have developed alternative mechanisms to the catalase enzyme to scavenge highly reactive oxygen radicals. Among these are a) enzymatic defense by superoxide dismutase and b) ability to accumulate Mn (Mn II) which prevents the endogenous toxic oxygen effect ($\text{O}_2^- + 2\text{H}^+ + \text{Mn}^{2+} \rightarrow \text{H}_2\text{O}_2 + \text{Mn}^{2+}$) (Pahlow *et al.*, 2003).



In LAB metabolism, Mn^{2+} is an effector (Vos *et al.*, 2009) in the structure and activation of numerous enzymes. In this investigation, the initial Mn content in the ingredient mixture before the ensiling process was low for treatments based on AU (26-30 mg/kg) and PM (133-135 mg/kg), but high with SM before and after ensiling (194-196 mg/kg). However, an increase in Mn concentration was observed in AU- and PM-based silages, but stable levels with SM before and after ensiling. This could be due to an increased protective response of LAB in the presence of oxygen. In this regard, the addition of nitrogen sources (AU or PM) could improve the anaerobic stability of silages due to the buffering effect of enterobacteria in the initial phase of ensiling through. a) Reduction of nitrogen sources to ammonia (buffering capacity), a temporary beneficial effect for LAB species (Pahlow *et al.*, 2003), and b) inhibition of clostridia concentration, due to the reduction of ammonia (NH_3) to NO and NO_2 in non-toxic amounts; this effect may have extended to inhibition at $pH < 4$. Enterobacteria are epiphytic species present up to 100 times more than LAB, clostridia, yeasts and fungi (Behrendt *et al.*, 1997) in dry and senescent forages. In cereal grains, Mg and other divalent cations can be chelated by phytic acid (Serna-Saldivar, 2010). LAB have been shown to have acid phosphatase activity (APs). López *et al.* (2000) showed that *Ln. Mesenteroides* S38 has APs activity, which decreases phytic acid activity as lactic acid production increases. Similarly, increased solubility of Ca and Mg in whole-wheat flour as a culture medium was observed by the expression of PAs.

Magnesium (Mg^{2+}) is an essential element in the growth and metabolism of LAB (Vos *et al.*, 2009). In the glycolysis pathway, the first reaction in the pathway is the formation of a complex between the Mg^{2+} cation, ATP and glucose, which binds hexokinase (Miesfeld & McEvoy, 2017). The importance of this metabolism is to obtain pyruvate and lactate under anaerobic conditions (Wood, 1998) from lactic fermentation (Madigan *et al.*, 2022) and through the enzyme lactate dehydrogenase (Mozzi *et al.*, 2010). In this investigation, the initial Mg content was higher in SCM than in BBP. However, the carbohydrate source had no significant effect by itself, but there was NS \times CS interaction effect that produced higher Mg concentrations in SCM-based silages with PM or SM, so these combinations of ingredients promoted higher LAB growth during fermentation; this was reflected in the pH values of both treatments (Table 3). It is interesting to mention that the SM-based treatments contained higher amounts of Mg before ensiling, but this trend changed during the fermentation process due to the effect of N available to LAB in the excreta.

Phosphorus (P) is a necessary element in the metabolism of lactic acid bacteria. The Embden-Meyerhof-Parnas pathway (glycolysis) requires ATP hydrolysis to generate the free energy needed to activate hexokinase, phosphofructokinase-1 and phosphoglycerate kinase (Miesfeld & McEvoy, 2017); also in the phosphorylation step of phosphoenolpyruvate to pyruvate. In cattle excreta, 80 % of P is contained in the form of phytic acid (myoinositol hexakisphosphate), mainly in monogastrics on high-grain diets



(Leytem & Maguire, 2007). Before ensiling, the AU-based treatments (SCM and BBP) contained higher amounts of P (0.068 and 0.109 % DM), but after 30 d of lactic fermentation the concentration changed (0.004 and 0.027 % DM, respectively), However, the manure-based silages maintained a constant amount of P before and after the ensiling process. This could be due to the use and depletion of available P by LAB in the AU-based silages, whereas, in the excreta-based treatments there was P release through the activity of phytases. Moreover, the expression of acid phosphatase enzyme in LAB (*Lactobacillus plantarum*) can be stimulated by the presence of Ca^{2+} , Mg^{2+} , Mn^{2+} , and Cu^{2+} (Saeed & Salam, 2013) and thus increase P availability from 29 to 34 % (López et al., 2000); in the same way *Pediococcus acidilactici* has expressed extracellular and intracellularly expressed phytases activity (Cizeikiene et al., 2015). Therefore, the increase in solubility and availability of P in excreta silages and mineral content in the treatments confirmed this behavior. However, acid phosphatase can decrease its effect by Co^{2+} and be inhibited by Fe^{2+} (Palacios et al., 2005); in AU-based silages, there was an increase in the availability of these elements after the ensiling process.

Lactic fermentation (pH 3.6 to 3.8) with LAB (*Streptococcus lactis* and *Lactobacillus* strains) plus the addition of 10 to 50 mg of phytase increased the availability (>200 %) of Fe from corn and sorghum meal (Svanberg et al., 1993). In *Lactobacillus pentosus* CECT 4023, a stimulatory effect of Co^{2+} and an inhibitory effect of Ca^{2+} on the enzymatic activity of PAs (65 % homologation with *Lactobacillus plantarum* PAs) have been observed; however, this has not been observed in other LAB (Palacios et al., 2005). The presence and abundance of lactic acid bacteria such as *Lactobacillus plantarum* showed significant correlation ($r_{xy}=0.25$) with Na concentration in drinking water (Minervini et al., 2019); in all treatments an increase of Na was observed after ensiling. In drinking water, the presence and abundance *Lactobacillus plantarum* has a correlation of $r_{xy}=0.86$ with K concentration (Minervini et al., 2019). Zn^{2+} (ZnCl_2) and Hg^{2+} (HgCl_2) are strong inhibitors of PAs in *Lactobacillus curvatus* (Abdallah et al., 1999). In *Lactobacillus sanfranciscensis* CB1, Hg^{2+} and Fe^{2+} are inhibitors of the phytase enzyme; furthermore, increasing NaCl in the medium had a negative effect on the enzyme activity (De Angelis et al., 2003). This supports that the availability of mineral elements in the silage based on livestock excreta of this research may be influenced by the inoculation of lactic acid bacteria and by the interaction of nitrogen and soluble carbohydrate sources. Finally, the above implies that the ensiling process and the reuse of nutrients from monogastric excreta in diets for fattening sheep can supply mineral requirements in order to reduce feed costs. From the bioethical point of view, the recycling of excreta nutrients through the ensilage process allows providing a harmless input to ruminant feed, reducing the sanitary problems that can originate without previous treatment but despite its routine and necessary use in animal feed, no regulations have been established in this regard.



CONCLUSION

The results suggest that corn stover silages with dehydrated poultry manure or fresh swine manure in combination with cane molasses or bakery by-product change the concentration of minerals in the mixture. The PM-based silage with SCM had the highest content of macrominerals and some microminerals, therefore, it can be a source of supply for ruminants.

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