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Investigation of the strength of copper and zinc bonds with other constituents of ruminant feedstuffs

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ABSTRACT - The objective of the present study was to evaluate the effect of feed type, pH, and Cu and Zn concentration on Cu and Zn binding with different feedstuffs after *in vitro* incubation in water. For this purpose, six feedstuffs (wheat straw, grass hay, corn silage, dried distillers grains with solubles, ground corn, and soybean meal) were incubated in ultrapure water (pH≈6.4) with supplemental Cu concentrations equivalent to 0, 5, 10, 15, 20, 30, and 40 mg Cu/kg DM or supplemental Zn concentrations equivalent to 0, 15, 30, 45, 60, 90, and 120 mg Zn/kg DM for 48 h. This experiment was repeated; however, following the 48-h incubation in ultrapure water (pH≈6.4), samples were incubated for one additional hour at a fitted pH of 2.3. Following incubation, the indigestible residue was analyzed for the presence of Cu and Zn. Copper and zinc bind to other dietary constituents at a pH similar to that of the rumen, regardless of the type of feedstuffs (concentrate or roughage). However, in a pH condition similar to that of the abomasum, part of these bonds is broken. In these pH conditions, there is greater breakdown of bonds in concentrate feedstuffs than in roughages, regardless of the mineral analyzed. Comparing these minerals, zinc forms weaker bonds than copper.

Keywords: copper, roughages, rumen, zinc

1. Introduction

Mineral deficiencies in livestock can be classified as primary or secondary according to the clinical and chemical signs of development. A primary mineral deficiency is caused by the consumption of a diet devoid of a specific mineral. This deficiency type develops over time. A secondary deficiency is a result of consumption of a diet that contains one or more antagonistic minerals and/or compounds that inhibit the absorption of an essential mineral. A simple analysis of the essential mineral in the diet may suggest that the essential element concentration is adequate; however, the presence of antagonists may reduce the availability of the element, which may result in deficiency (Spears, 2003; Arthington and Swenson, 2004; Suttle, 2010; NRC, 2016).

Numerous experiments have been conducted focusing on the antagonistic interactions between nutrients and the competition between minerals for absorption sites in the gastrointestinal tract of ruminants, mainly between metallic cations (Phillippo et al., 1987; Puls, 1994; Pérès et al., 2001; Suttle, 2010). However, a possible formation of lignocellulosic-metal clusters in the rumen may occur, ultimately reducing the availability of minerals to the animal (Moreira et al., 2013), which needs more investigation.

Lignin, cellulose, and hemicellulose of lignocellulosic-metal clusters are structurally flexible and contain several “coordinate sites” that contain oxygen atoms. The oxygen atoms can function as electron pair donor sites to form coordinate covalent bonds with Lewis acids (electron pair receptors), such as metallic cations (Ralph et al., 1998). The coordination of a given metallic cation through several donor atoms of the same macromolecule can generate a type of metallic complex, i.e., a type of coordination compound with the respective cations acting as coordination centers (Lindoy, 1989). Considering the inherent size and number of donor sites of these biomolecules, the effective interaction between these macromolecules and the metallic cations would characterize the so-called “macrocyclic effect” (an accentuated chelating effect), which provides high structural and thermodynamic stabilities and results in the formation of metallic complexes. Therefore, a significant number of cations could be coordinated by, at least, three electron donor atoms. Once formed, these bonds are difficult to break (Moreira et al., 2013).

Copper and zinc are metallic cations essential to animal metabolism, and their availability could be affected by this type of interaction. Therefore, we hypothesized that Cu and Zn can form insoluble complexes or with low solubility, which impair the absorption of the two minerals. Thus, the present research is focused on determining the influence of feed type, pH, and Cu and Zn concentration on the availability of these elements *in vitro*.

2. Material and Methods

2.1. Treatments and *in vitro* analysis

For the investigation of the possible formation of lignocellulosic-metal cluster, an *in vitro* assay procedure was performed. Six independent feedstuffs [wheat straw, grass hay, corn silage, dried distillers grains with solubles (DDGS), ground corn, and soybean meal] (Table 1) were dried at 60 °C in a forced-air drying oven for 72 h and then ground in a Wiley Mill to pass through an 1.0-mm screen.

Copper ($CuSO_4{\cdot}5H_20$) and Zn ($ZnSO_4{\cdot}7H_20$) stock solutions were prepared by mixing the appropriate amount of Cu or Zn with ultrapure water. Appropriate dilutions were then made to create the following Cu and Zn treatment concentrations in relation to the mass of experimental feedstuff for *in vitro* incubation: Cu = 0, 5, 10, 15, 20, 30, and 40 mg Cu/kg, and Zn = 0, 15, 30, 45, 60, 90, and 120 mg Zn/kg. These concentrations were chosen to equal 0.5, 1, 1.5, 2, 3, and 4 times the NRC (2016) recommendation, and the control treatment.

In vitro incubations were conducted as follows: 2 g of each feedstuff were placed in 50 mL Falcon tubes in quintuplets (water pH: 6.4; SEM: 0.025) to mimic ruminal pH. Tubes were then capped, vortexed for 30 s, and then incubated at room temperature with constant agitation for 48 h. After incubation, samples were filtered through pre-weighted ashless filter paper, dried, and then dry-ashed and analyzed for Cu and Zn.

Table 1 - Chemical composition of experimental feedstuffs

Feedstuff	DM (g/kg)	CP (g/kg)	ADFom (g/kg)	aNDFom (g/kg)	Copper (mg/kg)	Zinc (mg/kg)	ME (Mcal/kg)
Wheat straw	834.94	45.00	487.20	753.70	3.39	12.51	1.33
Grass hay	834.46	111.30	392.90	638.20	7.05	16.48	1.79
Corn silage	334.59	78.50	300.90	476.60	3.70	15.85	2.46
DDGS	920.50	276.70	138.90	333.80	4.85	51.46	2.70
Ground corn	887.50	79.90	34.80	100.30	1.74	18.07	2.90
Soybean meal	906.50	473.50	44.00	73.10	14.79	37.46	2.92

DM - dry matter; CP - crude protein; ADFom - acid detergent fiber; aNDFom - neutral detergent fiber; ME - metabolizable energy; DDGS - dried distillers grains with solubles.

To determine the influence of pH on the ability of each feedstuff to bind to Cu and/or Zn, the sample preparation, as described above, was repeated. However, after the 48-h incubation at a pH of 6.4 (SEM: 0.025), the pH was reduced to 2.3 (SEM: 0.030) with the addition of 12 N HCl (to simulate abomasum pH conditions). The sample was then agitated for 1 h, filtered, and analyzed for Cu and Zn.

The *in vitro* incubations described above were repeated five times (five runs). Each run was considered a block and represented the experimental unit.

2.2. Analytical procedures

All samples were analyzed for dry matter (DM; method 934.01; AOAC, 2012), ash (method 930.05; AOAC, 2012), and total nitrogen (N, method 981.10; AOAC, 2012). Crude protein (CP) content was obtained by multiplying the percentage N by 6.25. Neutral detergent fiber (aNDFom) content was determined according to Mertens (2002) without the addition of sodium sulfide and with the addition of thermostable alpha-amylase. The NDF content was corrected for protein and ash for all samples (Licitra et al., 1996).

Copper and Zn concentrations for all samples were determined as follows. Briefly, after filtration through pre-weighted Whatman 541 ashless filter paper, all samples were dried at 60 °C for 48 h, weighed, and then dry-ashed in a muffle furnace at 600 °C for 12 h. Samples were then removed and placed in a desiccator for an additional 30 min to cool. Finally, samples were weighed and re-suspended with 5 mL of HCl (1.2 N HCl). Copper and Zn concentrations of the samples were determined through simultaneous/sequential ICP-AES analysis with cross flow nebulization.

2.3. Statistical analysis

Data was analyzed as a randomized block design. The statistical model was as follows:

$$y_{ijkl} = \mu + \alpha_i + \gamma_j + \delta_k + b_l + \alpha\gamma_{ij} + \alpha\delta_{ik} + \gamma\delta_{jk} + \alpha\gamma\delta_{ijk} + e_{ijkl}$$

in which μ is the general mean, α_i corresponds to the effect of the i-th feed (i = 1 to 6), γ_j is the effect of the j-th pH (j = 1,2), δ_k represents the effect of the k-th level of supplemented mineral (copper or zinc; k = 1 to 7), b_l represents the effect of block (each run was considered one block; Montgomery, 2005), and the other terms were the respective interactions. The effects of feed, pH, and level of supplemented mineral were considered fixed and block b_l and error e_{ijkl}, assuming iid N $(0, \sigma^2)$, were considered random.

After analysis of the main effect and interactions, data were subjected to regression analyses (testing the linear, quadratic, and cubic regression equations) using the proc mixed of SAS (Statistical Analysis System, version 9.4). The Box-Cox transformation (Box and Cox, 1964) was used to correct lack of normality. The transformed y (yt) was calculated:

$$\begin{cases} y_t = \ln y \quad ; \quad \lambda = 0 \\ y_t = \dfrac{y^\lambda - 1}{\lambda} \; ; \quad \lambda \neq 0 \end{cases}$$

in which λ is the transformation parameter and ln the natural logarithm.

The parameters retransformation for the original scale was estimated as (Sakia, 1990):

$$\begin{cases} E[y] = \exp\left(\hat{\beta} + \dfrac{\hat{\sigma}^2}{2}\right); & \hat{\lambda} = 0 \\ E[y] = (1 + \hat{\lambda}\hat{\beta})^{1/\hat{\lambda}}\{1 + (1 - \hat{\lambda})\dfrac{\hat{\sigma}^2}{2}(1 + \hat{\lambda}\hat{\beta})^2\}; & \hat{\lambda} \neq 0 \end{cases}$$

in which $E[y]$ is the estimate of the conditional mean, $\hat{\lambda}$ is the estimated parameter of transformation, $\hat{\beta}$ is the estimated mean transformed, and $\hat{\sigma}^2$ is the estimated variance.

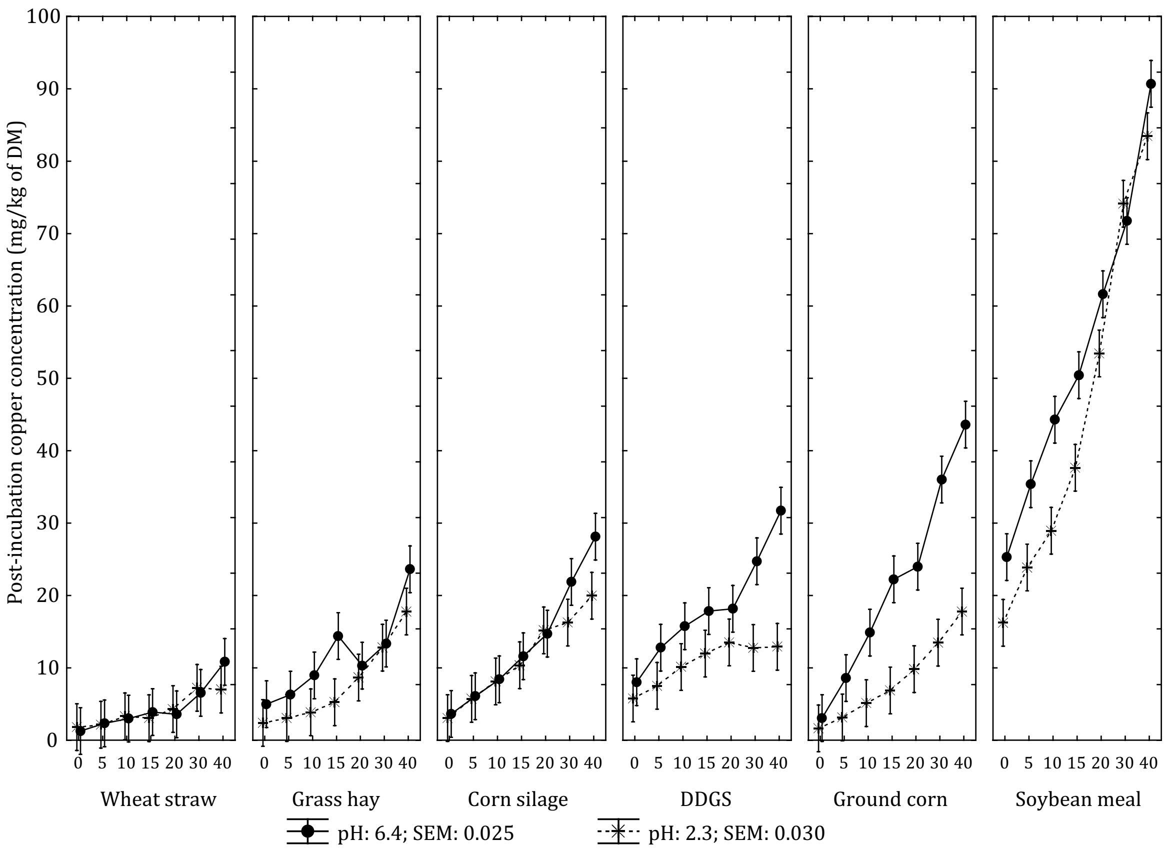
The decision about the similarity of the estimated parameters of the regression equations was done by comparing the 95% confidence limits. If there was intersection between the 95% confidence limits, the parameters of treatments were not considered significantly different.

3. Results

The amount of Cu and Zn remaining in the indigestible residue after 48 h of incubation in water was affected by feedstuff, pH, and mineral concentration (P<0.0001; Figures 1 and 2). Also, an interaction (P<0.0001) among feedstuff, pH, and mineral concentration was observed. Therefore, the regression analysis of the post-incubation concentration of Cu and Zn as function of the Cu and Zn dose was performed for each feedstuff within each pH (P<0.0001).

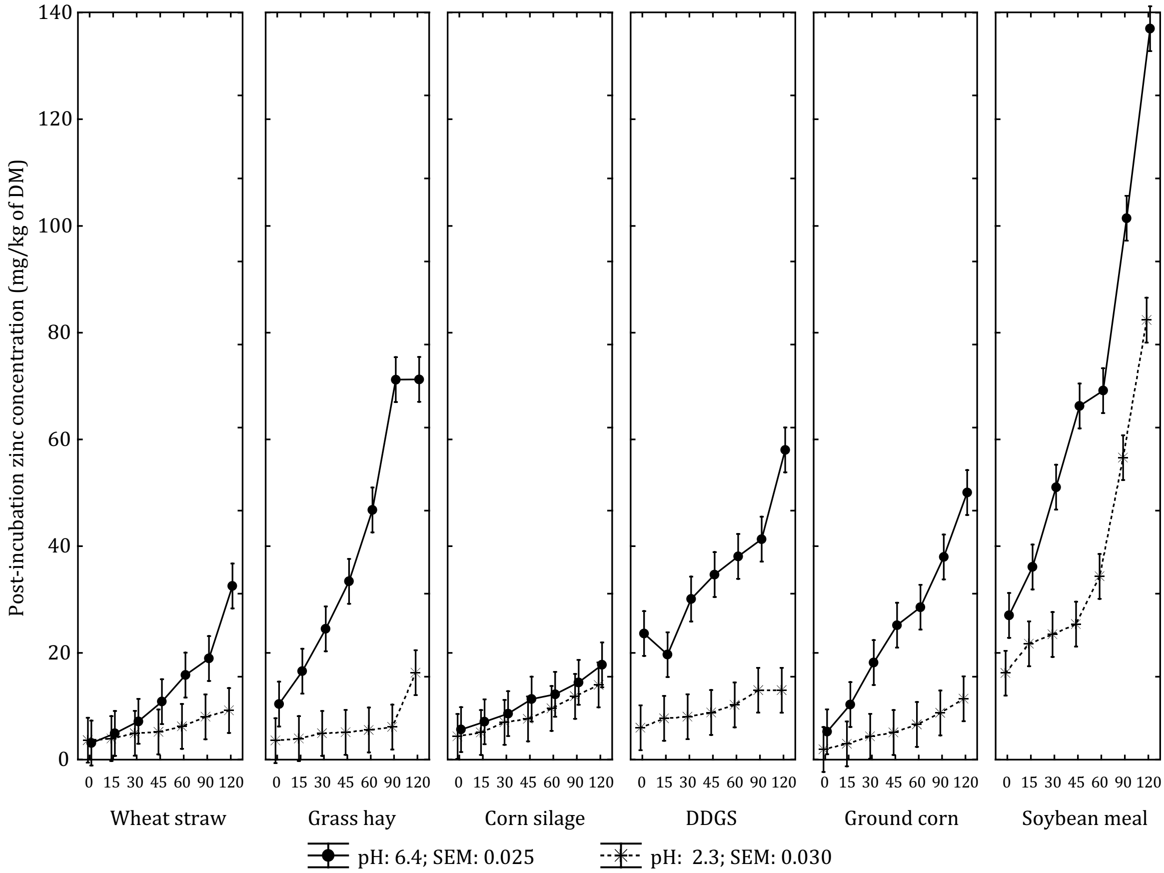
For Cu remaining in the indigestible residue, the cubic model was the best fit for wheat straw (pH: 6.4), grass hay (pH: 6.4), DDGS (pH: 2.3 and 6.4), and ground corn (pH: 2.3 and 6.4). The quadratic model was the best fit to wheat straw (pH: 2.3), grass hay (pH: 2.3), corn silage (pH: 2.3 and 6.4), and soybean meal (pH: 2.3 and 64).

For Zn remaining in the indigestible residue, the quadratic model was the best fit for wheat straw (pH: 6.4), grass hay (pH: 6.4), corn silage (pH: 2.3 and 6.4), DDGS (pH: 2.3), ground corn (pH: 2.3 and 6.4), and soybean meal (pH: 6.4). The linear model was the best fit for wheat straw (pH: 2.3), grass hay (pH: 2.3), DDGS (pH: 6.4), and soybean meal (pH: 2.3).



DDGS - dried distillers grains with solubles; DM - dry matter; SEM - standard error of the mean.
Vertical bars denote 0.95 confidence limits.

Figure 1 - Copper concentration remaining in the indigestible residue after 48-h incubation in water in relation to copper supplementation and pH.



DDGS - dried distillers grains with solubles; DM - dry matter; SEM - standard error of the mean.
Vertical bars denote 0.95 confidence limits.

Figure 2 - Zinc concentration remaining in the indigestible residue after 48-h incubation in water in relation to zinc supplementation and pH.

Therefore, the feedstuffs were compared within each pH by 95% confidence interval of the regression parameters. If two or more feedstuffs had an intersection between 95% confidence limits to all the estimated parameters, the regression equations of these feedstuffs were considered similar (in this way, the amount of Cu and Zn that stay bound to the feedstuffs – coefficient of mineral binding – was considered similar).

Based on the comparison of confidence intervals, grass hay (pH: 6.4) and DDGS (pH: 2.3) had similar cubic regression models for Cu. For Zn, wheat straw (pH: 2.3) and grass hay (pH: 2.3) had similar linear models as did the quadratic models for corn silage (pH: 6.4) and DDGS (pH: 2.3). All other models/equations were considered different, so the coefficient of mineral binding was distinct between all remaining feedstuffs in both pH conditions (pH values 2.3 and 6.4; Tables 2 and 3).

4. Discussion

Mature roughages, such as wheat straw and grass hay, can bind mineral and feed with lower metallic cations retention (e.g., in relation to ground corn), but when the pH is reduced, the mineral release is also less when compared with ground corn. The lower retention of Cu in the mature roughages suggests a very selective retention, which must occur at optimum sites binding to metallic cations. Therefore, the supramolecular structure formed by lignin, cellulose, and hemicellulose in this roughage type should limit the accessibility of ions to the electron pair donor sites. It is possible that the supramolecular structural flexibility of this lignocellulosic arrangement is very low, precluding a type of “induced

Table 2 - Model estimated parameters and 95% confidence limits for determining the impact of feedstuff and pH on copper content of indigestible residue after *in vitro* incubation

Effect	Feedstuff	pH	Estimate	95% confidence limit		Comparison
Quadratic models						
Beta 0	Wheat straw	2.3	0.5308	0.3931	0.6685	d
Beta 0	Grass hay	2.3	0.7914	0.6537	0.9291	d
Beta 0	Corn silage	2.3	1.1829	1.0452	1.3206	c
Beta 0	Corn silage	6.4	1.3331	1.1954	1.4708	c
Beta 0	Soybean meal	2.3	2.7727	2.635	2.9103	b
Beta 0	Soybean meal	6.4	3.2757	3.138	3.4134	a
Beta 1	Wheat straw	2.3	0.05791	0.04093	0.07488	bc
Beta 1	Grass hay	2.3	0.06973	0.05275	0.0867	abc
Beta 1	Corn silage	2.3	0.1015	0.08452	0.1185	a
Beta 1	Corn silage	6.4	0.08724	0.07026	0.1042	ab
Beta 1	Soybean meal	2.3	0.07248	0.05551	0.08945	abc
Beta 1	Soybean meal	6.4	0.05255	0.03557	0.06952	c
Beta 2	Wheat straw	2.3	−0.00052	−0.00093	−0.00011	a
Beta 2	Grass hay	2.3	−0.00044	−0.00085	−0.00003	a
Beta 2	Corn silage	2.3	−0.00146	−0.00186	−0.00105	b
Beta 2	Corn silage	6.4	−0.00097	−0.00137	−0.00056	ab
Beta 2	Soybean meal	2.3	−0.00077	−0.00118	−0.00037	ab
Beta 2	Soybean meal	6.4	−0.00057	−0.00097	−0.00016	a
Cubic models						
Beta 0	Wheat straw	6.4	0.2763	0.1327	0.4198	d
Beta 0	Grass hay	6.4	1.5255	1.3819	1.669	b
Beta 0	DDGS	2.3	1.7149	1.5714	1.8585	b
Beta 0	DDGS	6.4	2.1022	1.9587	2.2457	a
Beta 0	Ground corn	2.3	0.502	0.3584	0.6455	d
Beta 0	Ground corn	6.4	1.0904	0.9469	1.234	c
Beta 1	Wheat straw	6.4	0.1138	0.07914	0.1484	b
Beta 1	Grass hay	6.4	0.113	0.07837	0.1476	b
Beta 1	DDGS	2.3	0.08109	0.04647	0.1157	b
Beta 1	DDGS	6.4	0.08911	0.05448	0.1237	b
Beta 1	Ground corn	2.3	0.1407	0.1061	0.1753	b
Beta 1	Ground corn	6.4	0.2274	0.1928	0.262	a
Beta 2	Wheat straw	6.4	−0.00404	−0.00624	−0.00185	A
Beta 2	Grass hay	6.4	−0.00484	−0.00704	−0.00264	A
Beta 2	DDGS	2.3	−0.00249	−0.00468	−0.00029	A
Beta 2	DDGS	6.4	−0.00316	−0.00535	−0.00096	A
Beta 2	Ground corn	2.3	−0.00336	−0.00555	−0.00116	A
Beta 2	Ground corn	6.4	−0.00768	−0.00987	−0.00548	B
Beta 3	Wheat straw	6.4	0.000063	0.000026	0.0001	A
Beta 3	Grass hay	6.4	0.000076	0.000039	0.000112	A
Beta 3	DDGS	2.3	0.000024	−0.00001	0.000061	A
Beta 3	DDGS	6.4	0.000045	7.95E−06	0.000081	A
Beta 3	Ground corn	2.3	0.000033	−3.7E−06	0.00007	A
Beta 3	Ground corn	6.4	0.000092	0.000055	0.000129	A

DDGS - dried distillers grains with solubles.
Estimates of parameters followed by the same letters have intersection confidence intervals.

Table 3 - Model estimated parameters and 95% confidence limits for determining the impact of feedstuff and pH on zinc content of indigestible residue after *in vitro* incubation

Effect	Feedstuff	pH	Estimate	95% confidence limit		Comparison
Linear models						
Beta 0	Wheat straw	2.3	1.2289	1.1455	1.3122	C
Beta 0	Grass hay	2.3	1.1308	1.0474	1.2141	C
Beta 0	DDGS	6.4	2.6427	2.5594	2.726	A
Beta 0	Soybean meal	2.3	2.4312	2.3479	2.5145	B
Beta 1	Wheat straw	2.3	0.006763	0.005474	0.008052	B
Beta 1	Grass hay	2.3	0.008875	0.007586	0.01016	Ab
Beta 1	DDGS	6.4	0.005791	0.004502	0.00708	B
Beta 1	Soybean meal	2.3	0.009343	0.008054	0.01063	A
Quadratic models						
Beta 0	Wheat straw	6.4	1.089	1.0084	1.1695	E
Beta 0	Grass hay	6.4	2.0756	1.9951	2.1562	B
Beta 0	Corn silage	2.3	1.3535	1.2729	1.434	D
Beta 0	Corn silage	6.4	1.593	1.5125	1.6735	C
Beta 0	DDGS	2.3	1.637	1.5564	1.7175	C
Beta 0	Ground corn	2.3	0.6674	0.5868	0.7479	F
Beta 0	Ground corn	6.4	1.606	1.5255	1.6866	C
Beta 0	Soybean meal	6.4	2.8183	2.7378	2.8988	A
Beta 1	Wheat straw	6.4	0.02628	0.02297	0.02959	A
Beta 1	Grass hay	6.4	0.02538	0.02207	0.02869	A
Beta 1	Corn silage	2.3	0.01356	0.01025	0.01687	B
Beta 1	Corn silage	6.4	0.01353	0.01022	0.01683	B
Beta 1	DDGS	2.3	0.0096	0.006291	0.01291	B
Beta 1	Ground corn	2.3	0.02315	0.01984	0.02646	B
Beta 1	Ground corn	6.4	0.03045	0.02714	0.03376	A
Beta 1	Soybean meal	6.4	0.01415	0.01084	0.01746	B
Beta 2	Wheat straw	6.4	−0.00009	−0.00012	−0.00007	B
Beta 2	Grass hay	6.4	−0.00011	−0.00014	−0.00009	B
Beta 2	Corn silage	2.3	−0.00005	−0.00007	−0.00002	Ab
Beta 2	Corn silage	6.4	−0.00005	−0.00008	−0.00002	Ab
Beta 2	DDGS	2.3	−0.00004	−0.00006	−9.03E−06	A
Beta 2	Ground corn	2.3	−0.00009	−0.00012	−0.00007	B
Beta 2	Ground corn	6.4	−0.00015	−0.00017	−0.00012	B
Beta 2	Soybean meal	6.4	−0.00005	−0.00007	−0.00002	Ab

DDGS - dried distillers grains with solubles.
Estimates of parameters followed by the same letters have intersecting confidence intervals.

fit", which, in principle, should be generated by contact between these biomolecules and the metallic cations (Moreira et al., 2013).

However, the extreme selectivity of the Cu cation fixed in lignocellulosic net indicates that these selected cations can be maintained in a very stable form, even with subsequent chemical environment perturbation, such as the drastic transition for pH of 6.4 to pH of 2.3. It is important to note that the divalent Cu cation (Cu^{2+}) usually maintains the electron configuration of $3d^9$, which allows the formation of a hexacoordinated metallic complex (not only a tetracoordinate configuration), presenting six potential coordination sites to maintain the Cu cation in an optimum coordination site. Indeed, the d^9 Cu(II) ion is usually found in a tetragonal octahedral coordination environment, with four short equatorial bonds and another one or two longer axial bonds, although complexes with other structures are known, including tetrahedral, square planar, and trigonal bipyramidal geometry (Conry, 2005).

Copper did show a binding affinity for ground corn at pH 6.4. However, when the pH was reduced to 2.3, the Cu cation release was significant. The reason for this response is that the basic sites of starch are possibly more accessible to the electrophilic attack of the metallic cations and that metallic cations can be more easily displaced by the greater number of protons present at a pH 2.3.

Based on these findings, it is possible to infer that the stability of the chemical bonds involving the Cu cation and lignocellulose was similar to the one between Cu cation and starch. However, the electron donor sites of starch must be more accessible to the electrophilic attack by Cu cations, making the retention of the metallic cations at pH 6.4 higher in starch when compared with cellulose, hemicellulose, and lignin.

Indeed, the basic sites of starch can be associated with a higher structural flexibility, lower supramolecular compaction, and higher exposure of the respective sites, when compared with the lignocellulose. However, when this interaction occurs in a more acidic medium, Cu cation release is high, most likely due to the same factors that allow for the higher metallic retention by starch (higher structural flexibility, lower supramolecular compaction, and higher exposure of the binding sites) (Conry, 2005), thus making the acidification impacts much more effective at the sites coordinated by the Cu cation in this carbohydrate. In other words, we can infer that an intense competition between the hydrogen ions (H^+) of the acidic medium and Cu cations (Cu^{2+}) takes place by the electronic pair donor sites, such as oxygen atoms of carbohydrates, to starch and to lignocellulose, but it is possible that the basic sites are more influenced by the chemical compounds of the respective medium.

Soybean meal at pH 6.4 had greater Cu in the indigestible fraction when compared with soybean meal at pH 2.3. However, the release of bound Cu at pH 2.3 was minimal. The substantial presence of protein seems to be a possible factor to explain this result. The proteins can present other electron pair donor sites, such as sulphur atoms, which can occur in the lateral chain of some amino acid residues (Nelson and Cox, 2008). In fact, the great structural flexibility of the protein, the chemical variability of the lateral chains, including the different stereochemical properties (spatial sizes and arrangements) and basicity of electron donor groups, produce an environment that makes Cu cations to be more likely to become bound at a pH value of 6.4. In more acidic conditions, the cations would tend to favor the metallic cation release. The Cu cation is maintained in the supramolecular complex net of the respective biomolecules due to the great stability of the ligations and interactions formed between metallic cations, proteins, and other biomolecules present in soybean meal.

The feedstuff DDGS has an intermediate response in relation to the one observed between roughages and concentrates. The DDGS is the byproduct of the fermentation of corn (low starch content and a different fiber composition than that of roughages). This fact can be explained by considering the different affinities to bind to metallic cations between the respective components.

In the case of Zn (Zn^{2+}), with the exception of corn silage, all the other feedstuffs did increase Zn release when incubated at pH 2.3. We hypothesize that this response occurred due to the lower coordination number of Zn^{2+} when compared with Cu^{2+}. Zinc presents an electronic configuration $3d^{10}$, implying that its usual coordination number 4 has a tetracoordinate center that is different from Cu, which has coordination numbers of 6 and 4. Moreover, an electronic configuration $3d^9$ tends to favor a more intense electronic donation in the covalent coordinate bond when compared with a $3d^{10}$ center (Nelson and Cox, 2008). Therefore, the fixation of the metallic cation in a favorable pH value and its consequent retention in an, *a priori*, unfavorable pH must be more significant for the Cu cation when compared with the Zn cation.

The present data demonstrate that the Zn cation has a lower fixation in feedstuffs compared with Cu. However, the fixation of the Zn cation was greater in corn silage than Cu, indicating that corn silage has a more selective chemical system for the fixation of Zn cation. Thus, in spite of the more selective ligation of the Zn cation (which has a more electronically saturated center and frequently has a lower coordination number or "secondary valence"), when this bond occurs, it tends to be more stable in a more restrictive and isolated chemical environment (with less available coordination sites).

In comparison, wheat straw and grass hay have high concentrations of mature fiber (cellulose, hemicellulose, and lignin), little starch, and a low CP. Corn silage has intermediate fiber and starch concentrations and low CP. The DDGS feedstuff has almost no starch and contains fiber from the hulls of the corn kernel, which is different from the fiber contained in stems or leaves of forage plants. Furthermore, DDGS has an intermediate CP concentration, with a similar amino acid profile to that of ground corn. Ground corn has a high starch concentration and low fiber and CP concentrations. Soybean meal has a high CP and low fiber and starch concentrations (Table 1).

It is relevant to notice that more fibrous feeds can retain minerals by trapping them in the cell wall matrix. Indeed, in the fiber structure, there are micro spaces between the micro fibrils, which can be filled by matrix polysaccharides (Ghodrat et al., 2017). In this way, the interaction between fibrous feeds and metallic cations is constituted by strong chemical bonds as well as weak chemical interactions, such as van der Waals ones.

It is important to note that in the case of concentrate feedstuffs incubated at pH 2.3, there may be a significant release of Cu and Zn cations, namely, these minerals could solubilize in the first portion of the small intestine and, therefore, have a greater chance of being absorbed. Also, it can be argued that the digestion of starch and protein in the rumen and the small intestine can release these cations. However, the ruminal fermentability and digesta residence time in the rumen and lower gastrointestinal tract (GIT) can vary with feed processing, ration composition, animal age, and physiological status of the animal. In addition, Cu and Zn from the source with low solubility in the rumen appear to be less strongly bound to the solid rumen digestion than a source of Cu and Zn with high solubility (Caldera et al., 2019).

Some authors reported increases in the binding of Cu and Zn cations, within the GIT, reducing mineral availability as a function of the increased flow of undigested fiber fractions (Faulkner et al., 2017). However, it is important to register that the study by Faulkner et al. (2017) was not focused on the GIT of ruminants. In any case, the chemical interactions between metallic cations and biological macromolecules must be very similar in the GIT of ruminants and non-ruminants.

Fiber, starch, and protein have different chemical affinities for metallic cations, which can be influenced by the presence of different types of Lewis bases, structural flexibility, chemical compaction, supramolecular interactions, etc. Furthermore, it is interesting to note that the fixation of metallic cations in fiber and protein can also be influenced by anions (e.g., phosphate and sulfate) forming the respective salts of metallic complexes. This reinforces that the interaction between metallic cations and the several biomolecules generates a substantial mineral matter loss, which could be observed in studies focused on the loss of total ash in the feces.

Finally, the formation of the metallic cluster is greater for roughages than for feedstuff concentrates. However, the breakdown of this cluster in a pH that simulates the abomasum is greater in feedstuff concentrates than in roughages. This was a pilot study that demonstrated that it is possible to bind metal cations (Cu and Zn) with other components of the diet under pH conditions similar to those of the rumen and abomasum. *In situ* incubation studies are required for further clarification on these formed bonds.

5. Conclusions

Copper and zinc bind to other dietary constituents at a pH similar to that of the rumen, regardless the type of feedstuffs (concentrate or roughage). However, in a pH condition similar to that of the abomasum, part of these bonds is broken. In these pH conditions, there is greater breakdown of bonds in concentrate feedstuffs than in roughages, regardless of the mineral analyzed. Comparing these minerals, zinc forms weaker bonds than copper.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: F.P. Leonel, L.M. Moreira and T.E. Engle. Formal analysis: F.P. Leonel, D. Zanetti and R.S. Gomes. Investigation: F.P. Leonel. Methodology: F.P. Leonel. Project administration: F.P. Leonel. Writing-review & editing: R.S. Gomes.

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