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Effect of okara levels on corn grain silage

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ABSTRACT - We ensiled different levels of okara and ground corn to evaluate the effects on the fermentative pattern, aerobic stability, and chemical composition of resulting silages. The experimental design was completely randomized with four replicates per treatment. The okara levels were (dry matter basis): control (without okara) and 200, 300, 400, and 500 g kg^{-1} okara, with four replicates per treatment. Control silage did not contain okara, but water was added to adjust the moisture content (400 g kg^{-1} as fed). Mixtures were ensiled in lab-scale silos and stored for 150 days. Compared with the control silage, okara inclusion linearly increased crude protein (from 89.1 to 251 g kg^{-1} DM), ether extract (from 39.6 to 136 g kg^{-1} DM), neutral detergent fiber (from 79.9 to 174 g kg^{-1} DM), acid detergent fiber (from 22.4 to 119 g kg^{-1} DM), and ash (from 12.2 to 32.4 g kg^{-1} DM), whereas decreased dry matter content and *in vitro* dry matter digestibility (from 830 to 730 g kg^{-1} DM). The use of okara linearly increased lactic acid concentration but also intensified secondary fermentation. On the other hand, aerobic stability of silages increased due to okara inclusion because of the higher amount of short-chain fatty acids, such as butyric and acetic acids, which accumulated during fermentation. Okara inclusion in corn grain silage must be conditioned to the dry matter content at ensiling, but must not exceed 200 g kg^{-1} on dry matter basis.

Keywords: ammonia nitrogen, butyric acid, digestibility, fermentation, soybean

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Introduction

Okara is the main byproduct of soymilk and tofu manufacturing process, presenting low commercial value, but good nutritional quality (O'Toole, 2004; Bowles and Demiate, 2006). Of each 1000 L of soymilk manufactured, approximately 250 kg of okara are produced. In this way, about 14 million tons of okara are produced annually worldwide (Choi et al., 2015).

During soymilk and tofu production, soybean grains are washed, macerated, and then ground and heated. Afterwards, the ground grains go through a filtration process that separates it in an aqueous extract (soymilk) and okara (Bowles and Demiate, 2006). Due to the soybean wet-grinding process, okara presents from 72 to 77% moisture; however, it contains 95% of the solid components of soybean (Perussello et al., 2012; Lee et al., 2019). Nevertheless, differently from other byproducts of soybean manufacturing (e.g., soybean meal, soybean hulls), the chemical composition of okara is variable and mainly influenced by soybean variety and extraction process (Redondo-Cuenca et al., 2008; Pauletto and Fogaça, 2012).

The crude protein (CP) content of okara ranges from 240 to 375 g kg^{-1} dry matter (DM), whereas ether extract (EE) content ranges from 93 to 223 g kg^{-1} DM (Jiménez-Escrig et al., 2008; Mateos-Aparicio et al., 2010a; Mateos-Aparicio et al., 2010b; Diaz-Vargas et al., 2016). Due to the high moisture and nutrient content, okara is extremely prone to spoilage, increasing drying costs and limiting its commercial use *in natura* (Redondo-Cuenca et al., 2008; Li et al., 2013). Therefore, ensiling is a promising alternative to preserve its quality.

Ensiling is natural acidification process through carbohydrate fermentation by lactic acid bacteria. However, excessive moisture (as observed in okara) leads to effluent production and clostridial fermentation (McDonald et al., 1991). In addition, the high CP content of okara increases the buffer capacity, and the high EE levels impair the development of lactic acid bacteria, hampering pH drop and adequate conservation (Rooke and Hatfield, 2003). An interesting alternative to reduce moisture content is the addition of dry grains (e.g., corn) to okara before ensiling, creating an easy mixture to ensile. In addition, the high-water level of okara might be used to rehydrate the corn grains, which could increase corn starch digestibility due proteolysis (Hoffman et al., 2011). However, there is a gap of information about the ideal ratio of okara and corn to ensile.

Therefore, we aimed to evaluate different levels of okara inclusion in corn grain silage on the chemical composition, fermentative pattern, and aerobic stability in the respective silages.

Material and Methods

The experiment was carried out in Maringá (23°25'38" S and 51°56'15" W), located in the state of Paraná, Brazil.

For ensiling, flint corn grains (*Zea mays*) were ground in a stationary grinder (10-mm sieve). Before ensiling, the DM content of okara and corn were estimated using a microwave oven, for further calculations of okara inclusion in corn grain silage, based on DM of both ingredients. The DM estimated by microwave oven was 880 g kg^{-1} DM for okara and 190 g kg^{-1} DM for corn. The treatments consisted of mixing different levels of okara to ground corn in the levels (DM basis): 0 (control) and 200, 300, 400, and 500 g kg^{-1} okara. The okara levels corresponded, in wet basis, to an inclusion of 534, 660, 751, and 819 g kg^{-1} as fed. For each okara level, four piles (14 kg of fresh matter each) of a mixture of okara and corn were prepared per treatment. Thus, the water contained in okara was used to rehydrate the ground corn. All treatments were inoculated with the starter cultures *Lactobacillus plantarum* MA 18/5U and *Propionibacterium acidipropionici* MA 26/4U (Lallemand Animal Nutrition) to achieve a theoretical dose of 1×10^5 cfu/g fresh matter. From each pile, an experimental PVC (polyvinyl chloride) silo (40 cm length × 20 cm diameter, 0.013 m^3) was filled (11 kg per silo), aiming to reach a compaction density of 900 kg fresh matter m^{-3}. For the treatment without okara (control), corn was rehydrated to reach a DM content of 600 g kg^{-1} as fed (moisture content of 400 g kg^{-1} as fed), inoculated and ensiled as aforementioned. All silos were sealed with white-on-black polyethylene film and stored for 150 days at room temperature (23.3±4 °C).

Before ensiling (Table 1) and at silo opening, sub-samples were taken (500 g) and dried in a forced-air oven at 55 °C for 72 h. Dried sub-samples were ground in a Willey mill (1 and 2 mm-sieves) for further analyses. Dry matter (105 $°C_{oven}$; method 967.03), CP (method 990.03), EE (method 920.39), and ash (method 942.05) were determined according to AOAC (1990); neutral detergent fiber, using thermostable alpha-amylase and ash inclusive (aNDF), and acid detergent fiber (ADF) were assessed according to Mertens (2002) and Van Soest (1963), respectively.

In vitro DM digestibility (IVDMD) was determined as proposed by Holden (1999), using the artificial rumen developed by Ankom® (Ankom Technology, Macedon, NY). The rumen fluid (*inoculum*) was collected from a cannulated Holstein steer (480±20 kg body weight [BW]) fed a total mixed ration containing (DM basis) corn silage (600 g kg^{-1}) and concentrate mixture (400 g kg^{-1}) during 15 days before fluid sampling. Multilayer polyethylene polyester cloth bags (F57 filter bag; Ankom Technology, Macedon, NY) were used for incubation (0.25 g per bag) of ground samples (2 mm) and placed in

Table 1 - Chemical composition of okara, corn grain, and mixtures before ensiling (g kg^{-1} DM unless stated)

Item	Okara	Corn grain	Okara level (g kg^{-1} DM)				
			0	200	300	400	500
Dry matter (g kg^{-1} as fed)	194	880	594	520	451	371	348
Organic matter	952	988	987	982	979	976	975
Crude protein	299	88.6	88.6	131	181	209	240
Neutral detergent fiber	277	79.9	132	133	159	163	176
Acid detergent fiber	176	26.5	26.5	44.8	53.5	79.6	90.8
Ether extract	181	39.5	40.6	66.5	94.9	111	136

digestion jars. The incubation was carried out for 24 h, after which, jars were removed from the chamber and bags rinsed with distilled water for cleaning.

The fermentation parameters were evaluated in a water extract prepared from each silo, by mixing 25 g fresh silage with 225 mL distilled water. The mixture was homogenized with an industrial blender (Model TA-02N; Skymsen, Brusque, SC, Brazil) during 1 min, and the extract was filtered with a cheesecloth. The pH in aqueous extracts was determined using a digital potentiometer (Digimed DM-22, São Paulo, Brazil). The supernatant (2 mL) was pipetted and stored in Eppendorf tubes at −20 °C for further analyses. Lactic acid concentration was determined by a colorimetric method (Pryce, 1969) in a MARCONI® Janway 6305 spectrophotometer, with λ = 565 nm. The ammonia content (NH_3-N) was determined according to Detmann et al. (2012). Alcohol content, esters, and volatile fatty acids were determined by gas chromatography equipped with a mass-spectrophotometry detector (GCMS QP 2010 plus, Shimadzu®, Kyoto, Japan) and capillary column (Stabilwax, Restek®, Bellefonte, USA; M, 0.25 mmø, 0.25 µm Crossbond Carbowax polyethylene glycol).

The aerobic stability trial was performed as described by Jobim et al. (2007). From each silo, 3 kg of loosely fresh silage were taken and placed in plastic buckets (20 L). Buckets were stored in a controlled temperature chamber during 168 h at 25 °C. The pH measurement was performed daily at 08.00 h according to Silva and Queiroz (2002) to evaluate aerobic deterioration intensity.

All Statistical analysis was performed using the MIXED procedure of SAS (Statistical Analysis System, version 9.0). The experimental design was completely randomized, evaluating control silage (no okara addition) and four okara levels, with four replicates per treatment, resulting in 20 silos. The mathematical model adopted for mathematical procedures was:

$$Y_{ij} = \mu + O_j + \varepsilon_{ij},$$

in which Y_{ij} = observation of the j-th treatment in the i-th observation, µ = overall mean, O_j = effect of okara level j, and ε_{ij} = random error associated with each observation Y_{ij}. Degrees of freedom for treatment were partitioned into two single degree of freedom orthogonal contrasts: linear effect and the quadratic effect of okara level. Contrasts were declared significant at P≤0.05. Coefficients of contrasts were generated using the IML procedure of SAS. For the linear contrast, the coefficients were −0.73, −0.21, +0.05, +0.31, and +0.57, whereas for the quadratic contrast, the coefficients were +0.49, −0.47, −0.46, −0.12, and 0.56.

Silage pH during aerobic exposure was analyzed as repeated measurements over time. The mathematical model adopted for mathematical procedures was:

$$Y_{ijk} = \mu + O_i + \delta_{ij} + T_k + (OT)_{ik} + \varepsilon_{ijk},$$

in which Y_{ijk} = pH value at k-th aerobic exposure period, in j-th silo and i-th okara level; µ = overall mean; O_i = fixed effect of okara level i; δ_{ij} = random effect of silo j in Okara level i; T_k = fixed effect of aerobic exposure period k; $(OT)_{ik}$ = interaction effect between okara level and aerobic exposure period; and ε_{ijk} = random error associated with each observation Y_{ijk}. Covariance structure was chosen by

considering the lowest Akaike Information Criterion (Littell et al., 1998). Structures of covariance tested included variance compounds (VC), compound symmetry (CS), first-order autoregressive (AR (1)), and unstructured (UN).

Results

A quadratic effect (P<0.01) was observed in pH values at silo opening due to okara inclusion (Table 2). Okara addition in silages linearly increased the contents of acetic (P<0.01), propionic (P<0.01), and valeric acids (P<0.01), ethanol (P<0.01), 2,3-butanediol (P<0.01), 1-propanol (P<0.01), methanol (P<0.01), ethyl acetate (P<0.01), 2-butanol (P<0.01), and propyl acetate (P<0.01). Okara addition linearly decreased the NH_3-N content in silages (P<0.01), and a quadratic behavior was observed for lactic acid (P<0.05), butyric acid (P<0.01), and acetone (P<0.05).

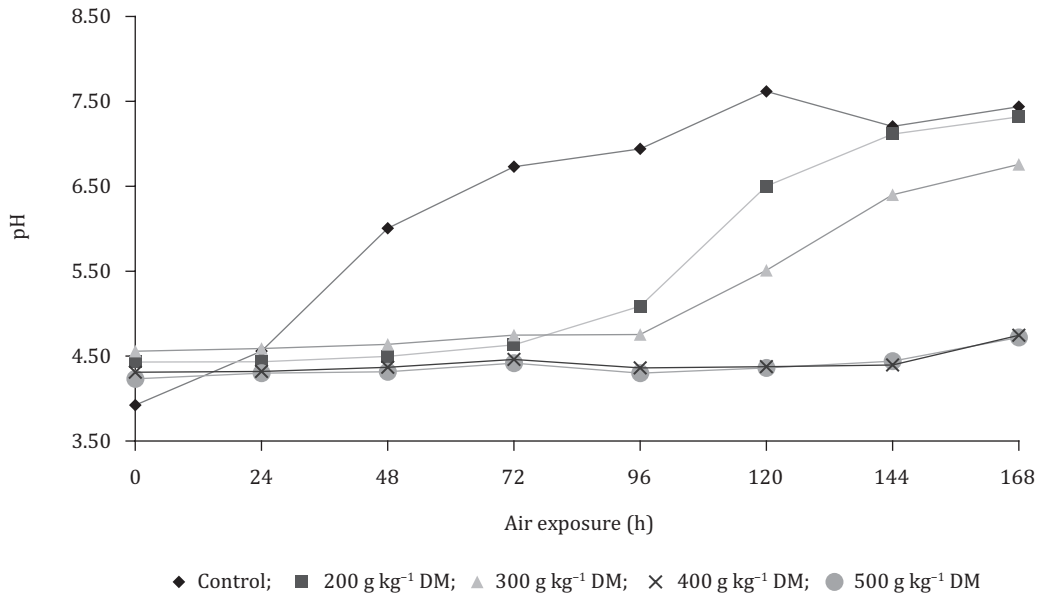
An interaction (P<0.01) between okara level and aerobic exposure was observed for silage pH during the aerobic stability trial (Figure 1). The corn grain silage without okara inclusion showed lower pH at the begging (0 h) of aerobic exposure; however, a rapid increase in pH was observed after 48 h of aerobic exposure. The silages containing 200 and 300 g kg^{-1} okara remained stable up to 96 and 120 h after exposure, respectively. In silages prepared with 400 and 500 g kg^{-1} okara, the pH slightly increased after 168 h of aerobic exposure.

The DM (P<0.01) content and IVDMD (P<0.01) linearly decreased (Table 3) as Okara level increased in silages. An opposite effect was observed for CP (P<0.01), EE (P<0.01), aNDF (P<0.01), and ADF (P<0.01) and ash (P<0.01), which presented a positive linear slope as okara inclusion increased.

Table 2 - pH values and fermentation profile of okara and corn grain-mixed silages

Item	Okara level (g kg^{-1} DM)					SEM	P-contrast[1]	
	0	200	300	400	500		L	Q
pH	3.92	4.43	4.56	4.31	4.23	0.10	0.05	<0.01
NH_3-N (g kg^{-1} N)	58.0	44.6	52.6	42.5	28.5	2.99	<0.01	0.07
Lactic acid (g kg^{-1} DM)	12.7	10.4	10.1	26.8	19.7	1.62	<0.01	0.01
Acetic acid (g kg^{-1} DM)	3.40	16.1	26.7	37.5	32.2	3.48	<0.01	0.20
Ethanol (g kg^{-1} DM)	1.90	1.30	3.60	6.60	6.90	0.81	<0.01	0.05
1,2-Propanediol (g kg^{-1} DM)	1.00	9.20	6.80	10.8	7.20	2.10	0.02	0.06
Butyric acid (g kg^{-1} DM)	0.80	0.40	3.60	6.10	24.8	2.89	<0.01	<0.01
2,3-Butanediol (g kg^{-1} DM)	0.20	0.90	2.90	4.80	4.20	0.48	<0.01	0.76
Propionic acid (g kg^{-1} DM)	0.10	1.30	6.00	11.9	9.80	1.31	<0.01	0.56
1-Propanol (mg kg^{-1} DM)	69.3	422	2419	5264	4643	6486	<0.01	0.30
Ethyl lactate (mg kg^{-1} DM)	42.5	8.75	9.50	26.8	17.0	8.07	0.10	0.03
Methanol (mg kg^{-1} DM)	17.0	41.5	94.0	131	125	12.0	<0.01	0.99
Isovaleric acid (mg kg^{-1} DM)	12.3	42.3	149	257	254	98.9	0.05	0.77
Ethyl acetate (mg kg^{-1} DM)	11.0	3.50	10.0	66.0	34.8	8.00	<0.01	0.19
Isobutyric acid (mg kg^{-1} DM)	10.0	46.5	111	169	191	60.3	0.02	0.76
Isopropyl alcohol (mg kg^{-1} DM)	9.50	10.0	39.0	162	467	112.9	0.01	0.07
Valeric acid (mg kg^{-1} DM)	7.25	25.3	305	615	2081	319	<0.01	0.01
Acetone (mg kg^{-1} DM)	6.25	29.5	59.8	32.8	42.8	6.96	<0.01	0.02
2-Butanol (mg kg^{-1} DM)	6.00	10.3	123	296	546	49.0	<0.01	<0.01
Propyl acetate (mg kg^{-1} DM)	1.00	3.75	23.8	119	53.3	14.1	<0.01	0.80

DM - dry matter; SEM - standard error of the mean.
[1] L - linear effect; Q - quadratic effect.



SEM = 0.332; P<0.01 for the interaction okara level × aerobic exposure.

Figure 1 - pH values during aerobic exposure in corn grain silages containing different okara levels.

Table 3 - Chemical composition of okara and corn grain-mixed silages (g kg^{-1} DM unless stated)

Item	Okara level (g kg^{-1} DM)					SEM	P-contrast[1]	
	0	200	300	400	500		L	Q
Dry matter (g kg^{-1} as fed)	594	508	411	344	293	11.9	<0.01	0.26
Crude protein	89.1	136	173	209	251	7.09	<0.01	0.04
Ether extract	39.6	66.5	94.9	111	136	4.60	<0.01	0.12
Neutral detergent fiber	79.9	105	123	150	174	3.77	<0.01	<0.01
Acid detergent fiber	22.4	41.1	70.1	104	119	4.82	<0.01	0.01
Ash	12.2	18.3	24.0	27.1	32.4	0.80	<0.01	0.16
IVDMD	830	754	763	753	730	10.3	<0.01	0.08

IVDMD - *in vitro* dry matter digestibility; SEM - standard error of the mean.
[1] L - linear effect; Q - quadratic effect.

Discussion

The fermentation profile was affected by okara addition, mainly because of the reduction in DM content. Lactic acid content in our trial was within normally observed in rehydrated corn grain silage (from 5 to 20 g kg^{-1} DM) (Morais et al., 2017; Kung Jr. et al., 2018). Lactic acid is a strong acid (pK_a 3.86) and mainly responsible for pH drop in silage. Since okara addition stimulated lactic acid synthesis, a reduction in pH values should be expected; however, an opposite behavior was observed. The increase in CP and ash contents (as observed in our trial) enhances buffer capacity in silage, which, coupled with high moisture might hamper the speed of pH drop, extending the fermentation process (Rooke and Hatfield, 2003). However, pH values at silo opening were within normally found in rehydrated grain silages (from 4.0 to 4.5) (Jobim et al., 2010; Tres et al., 2014; Kung Jr. et al., 2018). A rapid pH drop decreases the activity of spoilage microorganisms (e.g., enterobacteria, clostridia, bacilli, and fungi) and mitigates the negative effects of these microorganisms on the silage nutritional quality (Muck, 2010).

The butyric acid values found were higher than acceptable in rehydrated corn grain silage (below 1 g kg^{-1} DM) (Mahanna and Chase, 2003), indicating clostridial activity due to the high-moisture

levels in silage (Jobim and Nussio, 2013). Another evidence of clostridial fermentation was the increase in valeric and isovaleric acids and acetone contents (Pahlow et al., 2003; Rooke and Hatfield, 2003). Clostridia development is generally linked to high DM losses as well as synthesis of biogenic amines and even toxins (e.g., botulin toxin), reducing the silage hygienic quality (Pahlow et al., 2003; Scherer et al., 2015). On the other hand, ammonia synthesis was reduced due to okara inclusion, indicating a lower deamination activity in those silages (McDonald et al., 1991).

Acetic acid content was higher than lactic acid in our trial, showing a higher predominance of heterofermentative pathways in silages even with the inoculation of homofermentative bacteria. During the first stages of fermentation, enterobacteria play an important role in the acetic acid synthesis; however, other microorganisms such as heterofermentative bacteria can also produce acetic acid (McDonald et al., 1991). Moreover, *Lactobacillus buchneri*-like strains consume sugars and lactic acid, increasing acetic acid content in silage (Holzer et al., 2003). According to Li and Nishino (2011), low DM content and long storage periods (as observed in our trial) may intensify acetic acid formation; however, acetic acid is a strong antifungal compound, increasing aerobic stability during feed-out phase (McDonald et al., 1991; Danner et al., 2003).

A significant accumulation of 1,2-propanediol was observed in okara silage, also demonstrating activity of *Lactobacillus buchneri*-like strains in silages (Oude Elferink et al., 2001). In addition, *Lactobacillus diolivorans* is capable of converting 1,2-propanediol to similar equimolar amounts of 1-propanol and propionic acid (Krooneman et al., 2002). However, the concentration of propionic acid was greater than 1-propanol in our study, suggesting that propionic acid might have been formed by other microorganisms, such as clostridia, yeasts, and propionibacteria (McDonald et al., 1991; Rooke and Hatfield, 2003).

Okara inclusion increased the concentration of all alcohols normally found in silage. Ethanol is the main alcohol produced during silage fermentation and normally observed in high-moisture corn grain silage from 2 to 20 g kg^{-1} DM (Kung Jr. et al., 2018). Enterobacteria, heterolactic bacteria, and yeast produce ethanol during silage fermentation (Rotz and Muck, 1994; Kung Jr. et al., 2018). However, according to Kung Jr. et al. (2018), ethanol content above 30-40 g kg^{-1} DM may be associated with high yeast development. Other alcohols such as 1-propanol and 2-butanol are also produced during yeast development (Kung Jr. and Shaver, 2001; Pahlow et al., 2003). Ethanol synthesis usually increases in moist silages (Buchman-Smith et al., 2003). Besides the high losses associated with ethanol synthesis, ethanol is also extremely volatile, enhancing DM losses during feed-out phase (Rooke and Hatfield, 2003). Furthermore, the accumulation of 2,3 butanediol is also related to enterobacteria development, whereas methanol may be synthesized by clostridia or during pectin demetallation by plant enzymes (stimulated by low DM condition) (Hippe et al., 1992; Fall and Benson, 1996; Steidlová and Kalac, 2002; Rooke and Hatfield, 2003). In addition, alcohol formation is also linked to ester presence in silage (by abiotic esterification of carboxylic acids and alcohols under low pH conditions), as observed by higher contents of ethyl acetate (acetate plus ethanol) and propyl acetate (acetate plus n-propanol) in okara silages (Hangx et al., 2001; Weiss, 2017).

Rehydrated corn grain silage is highly prone to aerobic deterioration during feed out-phase (as observed in silage without okara) (Morais et al., 2017). According to Kung Jr. et al. (2018), silages with high butyric acid content (as observed in okara silages) are stable when exposed to air because of the strong antifungal characteristic of butyric acid. Moreover, other short-chain fatty acids are also related to lower spoilage during feed-out phase such as propionic acid and acetic acid (McDonald et al., 1991; Danner et al., 2003). However, this data must be interpreted with caution, since, besides beneficial organic compounds, (e.g., acetic acid), okara inclusion markedly increased other undesirable molecules (e.g., butyric acid, ethanol) associated with high DM losses and poorer hygienic quality. Aerobic deterioration is also dependent on the amount of soluble substrate (e.g., glucose, sucrose) not metabolized during fermentation. Therefore, an increase in secondary fermentation might reduce the amount of readily metabolizable substrate, constricting fungi development.

Dry matter content decreased in silages due to okara inclusion, representing a limitation to okara use. The higher CP, EE, aNDF, ADF, and ash values in silages were expected, since okara presented a higher content of these compounds compared with corn. On the other hand, the constituents of these fractions are not metabolized during fermentation (Rooke and Hatfield, 2003). Thus, an enhancement in these fractions might be related to a higher consumption of soluble substrate (as observed by the higher accumulation of volatile organic compounds) during fermentation (a concentration effect).

Per unit of nutrient, protein is the most expensive in ruminant nutrition. Therefore, enhancing CP content in diet through okara inclusion might be economically advantageous, once okara has a low commercial price. However, from another perspective, increasing CP in silage enhances silage buffer capacity, reducing pH drop and increasing spoilage, as observed in our trial (Rooke and Hatfield, 2003). The increase in aNDF content is related to lower DM intake by ruminants; conversely, enhancing ADF content normally reduces DM digestibility, as observed for IVDMD in this trial (Van Soest, 1994; Casler and Jung, 2006). In fact, IVDMD decreased by 9.7%, on average, due to okara use. In addition, high EE levels (above 70 g kg^{-1} DM) are linked to a reduction in the ruminal fiber digestion due to fat attachment to fiber as well as the impairment in microbial activity (Van Soest, 1994; NRC, 2001).

Conclusions

Addition of okara to rehydrated corn grain silage improves the crude protein and ether extract contents but reduces silage dry matter digestibility. Besides, the high moisture content in silages containing okara stimulates secondary fermentation and accumulation of undesirable organic molecules. Okara inclusion in corn grain silage must be conditioned to the dry matter content at ensiling but should not exceed 200 g kg^{-1} on dry matter basis.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: T.T. Tres and C.C. Jobim. Data curation: T.T. Tres, C.C. Jobim and J.L.P. Daniel. Formal analysis: T.T. Tres, A.V.I. Bueno, C.C. Jobim and J.L.P. Daniel. Funding acquisition: C.C. Jobim. Investigation: T.T. Tres, A.V.I. Bueno and V.C. Gritti. Methodology: T.T. Tres, C.C. Jobim and V.C. Gritti. Project administration: T.T. Tres and C.C. Jobim. Resources: C.C. Jobim. Software: T.T. Tres and J.L.P. Daniel. Supervision: T.T. Tres and C.C. Jobim. Validation: T.T. Tres and C.C. Jobim. Visualization: T.T. Tres, A.V.I. Bueno and C.C. Jobim. Writing-original draft: T.T. Tres, C.C. Jobim and V.C. Gritti. Writing-review & editing: T.T. Tres, A.V.I. Bueno, C.C. Jobim and J.L.P. Daniel.

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