Review

Effect of Different Additives on the Quality of Rehydrated Corn Grain Silage: A Systematic Review

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Abstract: This review aimed to analyze the effects of additives in the process of producing silage from rehydrated corn grains for ruminants. Control treatment studies used in this analysis was corn grain rehydrated with water only. To be included in the review, the studies needed to follow standardized criteria, including the absence of additives in the control treatment and the evaluation of the silage in beef animals, such as cattle, goats, and sheep. A total of seven publications, published between 2014 and 2022, were included in the final dataset. To compare the results, the PROC ANOVA of SAS was used, which included a random effect of comparison within the study, performing a paired comparison. It was observed that the use of additives did not influence the chemical composition, pH, organic acid and ethanol content, microbial population, fermentative losses, aerobic stability, and dry matter in vitro digestibility of rehydrated corn grain silage (p >0.05). Based on this information, it is concluded that the use of additives in rehydrated corn grain silage did not show major differences on silage quality. It is important, however, to evaluate the effects of additives in silages on animal performance.

Keywords: Additives; Corn grains; Digestibility; Silage

1. Introduction

Livestock production in tropical regions, including Brazil, faces challenges in grain availability due to climatic conditions that affect production. To circumvent this situation, it is possible to adopt strategies to increase animal production efficiency [1]. Among these strategies, rehydration and ensiling of corn grains have significant advantages, such as reducing storage costs and improving grain utilization efficiency [2,3 ,4].

The predominant cultivation of hard corn hybrids in Brazil, with higher proportion of vitreous endosperm [5], is negatively related to starch digestibility [6], making ensiling an advantageous practice in terms of storage management and nutritive value [7]. In addition, ensiling reduces insect and rodent damage normally seen in dry grains and increases starch digestibility [8].

In ruminant feeding, when ensiling corn grain silage, it is necessary to observe some crucial points to ensure the final quality of the product. During crop development, the soluble carbohydrates present in the grains are polymerized into starch in the endosperm, which results in small amounts

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of easily fermentable carbohydrates [9], which are the main substrates for the growth of lactic acid bacteria. These bacteria are responsible for the rapid acidification of the ensiled mass [10]. In addition, corn grain silages are more likely to suffer aerobic deterioration due to the starch content in it, Kleinschmit et al. [11] and reduced animal voluntary intake, especially in hot climates. To minimize these negative effects and improve silage quality, it is common to use additives that increase the content of soluble carbohydrates, reduce fermentation losses, and increase aerobic stability during the ensiling process.

Thus, the objective of this study is to perform a systematic review of the scientific literature to investigate the effects of different additives in the ensiling of rehydrated or reconstituted corn grains, evaluating the quality of the silage and its impact on animal performance. The relevance of this investigation is highlighted due to the importance of these grains in animal production. This review hypothesizes that the addition of additives when ensiling of rehydrated or reconstituted corn grains can promote improvements in silage quality, besides increasing starch digestibility and reducing fermentative and aerobic losses. This improvement in silage quality can result in increased animal performance, presenting a viable alternative for the use of these grains in feeding high-producing ruminants.

2. Materials and Methods

2.1. Dataset

This systematic review was carried out according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [12]. The PICO strategy followed the PRESS guidelines statement [13] and defined the population as beef cattle, sheep, and goats, the intervention as the evaluation of the effect of additives on silage quality and animal performance, the control as silages without additives, and the outcomes as the most suitable additives to improve silage quality.

Database searches were performed between January and June 2022, based on title and abstract, and with language refinement, including articles in English and Portuguese. The following databases were used for literature search: Web of Science, Wiley Online Library, Scielo, and Science Direct. After the search, a total of 119 publications were found using the terms: “Reconstituted corn grain silage” or “Rehydrated corn grain silage” or “Silagem de grãos de milho reidratados” or “Silagem de grãos de milho reconstituídos”. Only articles that met the predetermined inclusion criteria were included in the systematic review.

For inclusion, studies needed to have the following standardized criteria: (1) one of the treatments did not include any additives in the silage (silage quality dataset); and if it evaluated silage in ruminant feed, (2) included treatments comprising only beef cattle, goats, and sheep.

2.2. Data Mining

After conducting the search of publications in the databases, the articles were forwarded to the bibliographic reference management software Mendeley®, which helped in the elimination of duplicate articles and in the organization of the abstracts. No studies were identified evaluating rehydrated corn silage as feed for beef cattle, goats, and sheep. After the refinement process, the remaining 20 articles were tabulated in Excel® file to be evaluated in the screening process according to the following information: a) Author, b) Journal, c) Year, d) Title, e) Meet the selection criteria; d) Does not meet the selection criteria, e) Reason why the article does not meet the selection criteria.

Based on the inclusion criteria, 7 peer-reviewed publications were sorted by: first author, publication reference, and additive used; number of replications, standard error of the mean (SEM), and the following variables were extracted from rehydrated corn grain silage on the control (silage without additives) and treated (silage with additives) response: dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) concentrations, pH, lactic acid, acetate, propionate, butyrate, and ethanol, counts of lactic acid bacteria (LAB), yeast, and molds (cfu/g DM), effluent losses, gas losses, DM recovery, aerobic stability (h), and DM in vitro digestibility.

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A flow chart explaining the study identification and selection process to analyze the effects of additives on the quality of rehydrated corn grain silage is shown in Figure 1.

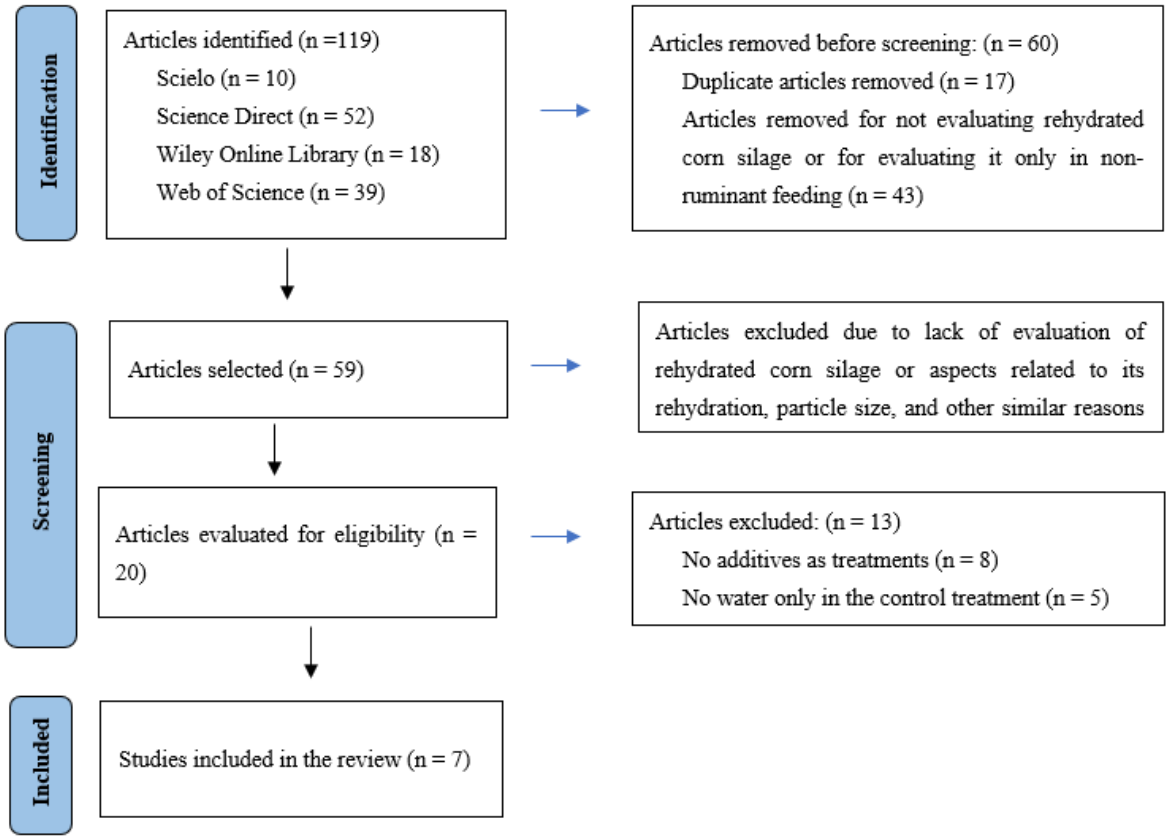

Figure 1. Flowchart showing the inclusion criteria for the selection of studies used to conduct the systematic review on the effects of additives on the quality of rehydrated corn grain silage.

2.3. Statistical Analysis

Data were analyzed using the SAS® statistical software (Statistical Analysis System, version 9.4). The results of the parameters evaluated were compared using analysis of variance (ANOVA) with a significance level of 5%. The model included a random effect of paired comparison within the study and a fixed effect of the treatments without additives and with additives. The mean, standard error of the mean, minimum and maximum value were calculated for each treatment through the MEANS procedure.

3. Results

The study was carried out based on a sample of seven articles published between the years 2014 and 2022, as shown in Table 1. The studies found addressed the effects of additives on chemical composition, fermentation losses, and aerobic stability of silage. Two of the studies evaluated dry matter in vitro digestibility.

Table 1. Articles selected from the databases.

ID	Reference	Title	Evaluated parameters
1	Menezes et al.[32]	Effects of different moist orange pulp inclusions in the corn grain rehydration for silage production on chemical composition, fermentation, aerobic stability, microbiological profile, and losses	Chemical composition, fermentation parameters, aerobic

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			stability and DM in vitro digestibility
2	Ferraretto et al.[15]	Effect of ensiling time on fermentation profile and ruminal in vitro starch digestibility in rehydrated corn with or without varied concentrations of wet brewers grains	Chemical composition
3	Rezende et al.[22].	Rehydration of corn grain with acid whey improves the silage quality	Chemical composition, fermentation parameters, and aerobic stability
4	Souza et al.[23]	Effect of rehydration with whey and inoculation with *Lactobacillus plantarum* and *Propionibacterium acidipropionici* on the chemical composition, microbiological dynamics, and fermentative losses of corn grain silage	Chemical composition and fermentation parameters
5	Cruz et al.[24]	Fermentative losses and chemical composition and in vitro digestibility of corn grain silage rehydrated with water or acid whey combined with bacterial-enzymatic inoculant	Chemical composition, fermentation parameters and DM in vitro digestibility
6	Silva et al.[9]	Fermentation and aerobic stability of rehydrated corn grain silage treated with different doses of *Lactobacillus buchneri* or a combination of *Lactobacillus plantarum* and *Pediococcus acidilactici*	Chemical composition, fermentation parameters, and aerobic stability
7	Jungues et al.[19]	Short communication: Influence of various proteolytic sources during fermentation of reconstituted corn grain silages	Chemical composition and fermentation parameters

The articles were published in four different scientific journals, namely: Journal of Dairy Science (three articles), Semina: Agricultural Sciences (two articles), Animal Feed Science and Technology (one article), and Animal Science Journal (one article). It is important to note that some additives in this review may fall into more than one category, as shown in Table 2.

Table 2. Description of additives added to rehydrated corn grain silages.

Category	Additives	Classification[1]
By-product	Wet orange pulp	Fermentation stimulants / nutrients
	Wet brewery waste	Nutrients
By-product	Milk whey	Fermentation stimulants / nutrients
Bacterial inoculant	*Lactobacillus plantarum*	Fermentation stimulants
	Pediococcus	Fermentation stimulants
	Lactobacillus buchneri	Fermentation stimulants / Aerobic spoilage inhibitors
	Enterococcus faecium	Fermentation stimulants
	Pediococcus acidilactici	Fermentation stimulants
	Propionibacteriu acidipropionici	Fermentation stimulants
Enzymatic Inoculant	Cellulase and hemi-cellulase	Fermentation stimulants
Antimycotic agent	Natamycin	Fermentation inhibitors
Irradiation	Gama irradiation	Fermentation inhibitors

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Chemical compound	Lactic acid	Fermentation stimulants
	Acetic acid	Fermentation inhibitors / Aerobic spoilage inhibitors
	Ethanol	Fermentation inhibitors

[1] McDonald et al. [10].

Using the additives did not influence the chemical composition of the silage (p>0.05) (Table 3). Regarding dry matter (DM) content, a value of 658 g kg^{-1} was found in silages without additive and 644 g kg^{-1} in silages with additive. It is worth noting that the fermentative process of these silages can affect the protein (CP) and neutral detergent fiber (NDF) content. However, although not significant in this review, some additives can influence these processes more.

Table 3. Chemical composition of rehydrated corn grain silage with and without additives.

Item		Rehydrated corn grain silage		n[1]	SEM[2]	*P*-value[3]
		Without additive	With additive			
Chemical composition (g kg^{-1} DM)						
Dry matter	**Mean**	**658**	**644**	**39**	**8.91**	**0.27**
	Minimum	586	564			
	Maximum	700	695			
Crude protein	**Mean**	**90.3**	**93.0**	**39**	**3.67**	**0.48**
	Minimum	70.0	74.7			
	Maximum	101	118			
Neutral detergent fiber	**Mean**	**120**	**130**	**28**	**19.8**	**0.66**
	Minimum	61.2	56.9			
	Maximum	214	232			
Acid detergent fiber	**Mean**	**27.2**	**28.3**	**20**	**4.81**	**0.88**
	Minimum	11.4	5.4			
	Maximum	38.2	62.2			

[1] n= number of observations. [2] Standard error of the mean. [3] *P*-values significant at p<0.05. Means followed by the same letter in the row are not statistically different.

As a result, they observed that bacterial activity was the main contributor to proteolysis (60%), followed by corn grain enzymes (30%), while fungi and fermentation end products (organic acids) had only minor contributions (~5% each) during fermentation of rehydrated corn grain silage.

The pH values, organic acids and ethanol concentration, as well as microbial population counts of the rehydrated corn grain silage without and with additive are presented in Table 4.

Table 4. pH, organic acids and ethanol, and microbiology of rehydrated corn grain silage with and without additives.

Item		Rehydrated corn grain silage		n[1]	SEM[2]	*P*-value[3]
		Without additive	With additive			
pH	**Mean**	**4.09**	**4.25**	**28**	**0.20**	**0.42**
	Minimum	3.74	3.67			
	Maximum	4.94	5.66			
Organic acids and ethanol (g kg^{-1} DM)						
Lactic acid	**Mean**	**15.4**	**15.54**	**27**	**2.21**	**0.99**
	Minimum	9.07	0.90			
	Maximum	27.6	28.1			
Acetic acid	**Mean**	**2.27**	**4.47**	**27**	**1.74**	**0.26**
	Minimum	1.49	1.10			
	Maximum	3.60	16.2			
Propionic acid	**Mean**	**0.54**	**0.68**	**23**	**0.38**	**0.62**
	Minimum	0.03	0.01			

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	Maximum	1.10	1.51			
	Mean	**0.47**	**0.01**	**15**	**0.16**	**0.074**
Butyric acid	Minimum	0.01	0.00			
	Maximum	1.71	0.14			
	Mean	**6.53**	**5.57**	**15**	**2.08**	**0.66**
Ethanol	Minimum	5.25	0.30			
	Maximum	7.16	12.5			
Microbial population (log cfu g^{-1})						
	Mean	**5.03**	**4.74**	**12**	**0.95**	**0.50**
Lactic acid bacteria (LAB)	Minimum	3.70	2.00			
	Maximum	6.10	6.28			
	Mean	**3.49**	**2.47**	**8**	**0.40**	**0.13**
Yeasts	Minimum	4.02	2.00			
	Maximum	4.23	3.37			
	Mean	**3.54**	**3.23**	**12**	**0.67**	**0.65**
Molds	Minimum	2.39	2.00			
	Maximum	4.51	4.85			

[1] n= number of observations. [2] Standard error of the mean. [3] *P*-values significant at $p<0.05$. Means followed by the same letter in the row are not statistically different.

In addition, fermentative losses, and aerobic stability (AS) of the silages were not influenced by the use or non-use of the additives ($p>0.05$) (Table 5). Fermentative losses are usually not affected or are reduced due to the low moisture content of rehydrated corn grain silage. The DM in vitro digestibility (DMIVD) was not influenced by the use of additives ($p>0.05$).

Table 5. Fermentative losses, aerobic stability, and DM in vitro digestibility of rehydrated corn grain silage with and without additives.

Item		Rehydrated corn grain silage		n[1]	SEM[2]	*P*-value[3]
		Without additive	With additive			
	Mean	**2.36**	**3.05**	**8**	**1.07**	**0.55**
Effluent losses (kg/t[4])	Minimum	2.12	1.23			
	Maximum	2.33	5.70			
	Mean	**4.84**	**5.13**	**15**	**3.70**	**0.93**
Gas losses (%)	Minimum	1.11	1.31			
	Maximum	12.3	21.2			
	Mean	**965**	**976**	**24**	**7.48**	**0.14**
Dry matter recovery (g kg^{-1})	Minimum	941	936			
	Maximum	987	999			
	Mean	**96.2**	**98.9**	**23**	**42.7**	**0.95**
Aerobic stability (hours)	Minimum	36.0	25.5			
	Maximum	213	288			
	Mean	**875**	**839**	**8**	**48.1**	**0.77**
DM in vitro digestibility (g kg^{-1} DM)	Minimum	805	786			
	Maximum	911	909			

[1] n= number of observations. [2] Standard error of the mean. [3] *P*-values significant at $p<0.05$. Means followed by the same letter in the row are not statistically different. [4] Tons.

4. Discussion

Out of the seven studies, six were took place in Brazil and one in the United States. This higher number of studies developed in Brazil can be attributed to several factors, including the high cost of grain in recent years [14], and the traditional use of hard corn hybrids. These hybrids are usually used for their competitive agronomic characteristics in tropical conditions, such as resistance to insects in

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the field and during grain storage [15]. Because of these factors, rehydrated grain silage emerges as a viable alternative to increase starch digestibility, improving grain utilization efficiency in animal production.

In this review, additives were categorized into seven distinct categories: coproduct, by-product, antimycotic agent, chemical compound, irradiation, bacterial inoculant, and enzyme inoculant. McDonald et al. [10] classify silage additives into five main groups, which include fermentation stimulants, fermentation inhibitors, aerobic spoilage inhibitors, nutrients, and absorbents.

This practice is explained by the low content of water-soluble carbohydrates in corn grains [9] and the reduced activity of lactic acid bacteria (LAB), caused by the limitation of water activity and the stress to which the grains are subjected during natural drying in the field. This can compromise the fermentation capacity of rehydrated corn grains [16].

These results are expected for rehydrated corn grain silages, since it is common that these grains are rehydrated until they reach 35 to 40% moisture to ensure good fermentation. Importantly, moisture activity is necessary for the growth and reproduction of lactic acid bacteria, as mentioned by Hu et al. [17].

As for CP, it is possible that the use of additives changes the microbial profile of the silage, which would increase proteolysis of the hydrophobic protein matrix surrounding the starch granules, thus promoting greater starch digestibility [18]. Importantly, this increase in proteolysis can convert some of the true protein into soluble protein and N-NH_3. Organic acid-based chemical additives are the most commonly used to control microbial growth [3]. Although only one study was included in this review evaluating chemical additives in silages, Junges et al. [19] used these additives with antifungal and fermentation inhibitory functions only to estimate the relative contribution of corn grain enzymes, bacteria, fungi, and fermentation end products to protein solubilization during fermentation. In addition to additives, silage time is another factor that influences proteolysis in grains. According to Silva et al. [20], the intensity of proteolysis is higher at the beginning of the storage period, due to the higher concentration of lactic acid bacteria, but it is maintained until the opening of the silo.

These findings are relevant for the development of strategies that allow an adequate degree of proteolysis, especially for corn grains that present lower nutrient digestibility due to their high vitreousness or advanced maturity, without compromising the fermentative characteristics.

On the other hand, the NDF content of silage may be reduced due to acid hydrolysis of hemicelluloses [21], which results in increased availability of soluble substrates. Although it is not possible to compare NDF content before and after ensiling in this review because there is no data on the chemical composition of the mass before ensiling, previous studies such as that of Rezende et al. [22] indicate greater reductions of NDF in silage when whey is used to rehydrate corn grains when compared to water. However, Sousa et al. [23] and Cruz et al. [24] did not find the same effect of whey in comparison to water, which makes it difficult to evaluate the intrinsic effect of this additive in reducing the NDF content of silage.

During the proper fermentative process in rehydrated corn grain silages, lactic fermentation occurs, which generates mainly lactic acid and, to a lesser extent, acetic acid, from carbohydrates present [25]. As a result, the pH is reduced to a level where undesirable fermentation is prevented. The increased clostridium population results in the utilization of desired fermentation products such as lactic acid and sugars, proteins, and amino acids to form butyric acid and subsequently amines, amides, and ammonia [26].

Therefore, measurements of pH, concentrations of organic acids, alcohols and N-NH_3, and quantification of microbial populations are most commonly used to assess silage fermentation [25]. In this review, it was not possible to include the N-NH_3 concentration of silages since the data obtained in the studies presented different ways of quantification. However, it is important to note that N-NH_3 is an accurate indicator of proteolysis during grain ensiling [27]. Overall, both silages showed adequate fermentation, shown mainly by the lower pH and higher lactic acid content.

The mean pH of the silages is within or very close to the desirable standards for good fermentation, which should be between 3.8 - 4.2[10]. The concentrations of lactic acid averaged 15.4

g kg^{-1} DM. Although differences were found in the classifications of the additives used in rehydrated corn grain silages, it is important to note that analysis of the effect of these additives on the silage microbiota is difficult to perform due to the scarcity of studies that have performed this analysis.

The diversity of microorganisms in silage is altered according to the characteristics of the forage crop, with a succession of genera and species as the conditions of the environment change [28]. Among these phyla are lactic acid bacteria and propionic acid bacteria, which are considered desirable during ensiling. On the other hand, undesirable microorganisms such as enterobacteria, molds, and yeasts, perform activities that are detrimental during the ensiling process because they compete with lactic acid bacteria in the fermentation of sugar [29]. Despite the different classifications of additives used in rehydrated corn grain silages, it has not been possible to clearly evaluate the effect of these additives on the silage microbiota due to the small number of studies that have performed this analysis.

AS, on the other hand, can be improved when undesirable microorganisms are inhibited with appropriate additives after silo opening [30], as is the case with the bacterial inoculant *Lactobacillus buchneri* [9]. This occurs due to the increased production of acetic acid, resulting in inhibitory effects observed by yeast and mold populations after silo opening [31]. The increase in AS may also occur with increasing storage time, due to the gradual accumulation of fermentation products with antifungal properties, such as acetic acid and propionic acid [20].

Probably the lack of effect can be explained by the small number of studies that performed this analysis. It is important to note that, due to the fermentation process, the digestibility of these silages can be improved by the higher content of proteolysis in the grains, however, digestibility can be decreased by increasing the hydrolysis of hemicellulose, which decreases the content of NDF and consequently increases the content of ADF of the silage.

Finally, for rehydrated corn grain silages a minimum storage period of 52 days is recommended to maximize the protein matrix breakdown effects and increase digestibility [20].

5. Conclusions

The use of additives in corn silage is a promising practice that can provide significant benefits to silage fermentation, digestibility, and aerobic stability. Although the studies reviewed show that additives do not significantly affect the chemical composition of silage, they can influence the processes of proteolysis and fiber digestion. However, it is important to note that the effects of additives in rehydrated corn grain silages on animal performance need to be evaluated.

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