

ADDITIVES FOR GRAIN SILAGES: A REVIEW

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ABSTRACT

Microbial inoculants have been used as a tool to improve the fermentation and aerobic stability (AS) of high moisture grain silages. To access the effects of additives in high moisture corn silages (HMCS), thirty-five scientific papers were reviewed. Other six scientific papers were used to investigate changes in winter cereal grain silages (HMWCS). Application of chemical additives in HMCS preserved WSC due to inhibition of fermentation. Yeast growth was efficiently controlled, reducing ethanol production and linearly increasing AS. The HMWCS treated with chemicals showed a marked reduction in fungal growth and in ethanol formation, and a higher AS. The inoculation of HMCS with homolactic bacteria decreased silage pH by 0.26 unit and decreased proteolysis, but did not promote AS. The HMCS inoculated with heterofermentative strains had lower WSC and higher content of weak acids with antifungal properties, reducing mold and yeast counts and increasing AS. Maximum improvement in AS was achieved when heterofermentative bacteria were applied at 4.67×10^5 cfu.g⁻¹ ($P < 0.01$, $R^2 = 0.50$). The combination of homo and heterofermentative bacteria in HMCS ensured a lower pH and decreased yeast counts and ethanol production, whereas AS was not changed. Since fermentative losses were usually low, we conclude that the use of chemical additives and heterofermentative bacteria are justified to improve AS of high moisture grain silages.

Key words: high moisture corn; winter cereals; microbial additives; chemical additives; aerobic stability

INTRODUCTION

Ensiling is an efficient strategy for grain storing and processing. Improvements on the nutritive value and the lower costs compared to other processing methods has stimulated the use of high moisture grain silages (HMGS). The typical lower field/harvesting losses accompanied by early harvesting are considered side advantages, which may increase farming efficiency. Insect and rodent damages typically observed in dry grains are also reduced by adopting HMGS. Additionally, HMGS allows the use of homegrown, traceable (source-verified) feedstuffs instead of purchased concentrates. However, it will constraint the cash crop at farm level because of the wet storage.

To exploit the benefits of HMGS, a proper management is mandatory to minimize fermentative losses and prevent the aerobic deterioration. A number

of studies have accessed the effects of silage additives on HMGS, however, to our knowledge, a systematic analysis of these data has not been conducted.

This review is focused on high moisture corn (HMCS) and winter cereals (HMWCS; barley, wheat and triticale) silages. The objective of this meta-analysis was to address the effect of chemical and microbial additives on the conservation of HMCS and HMWCS.

MATERIAL AND METHODS

Two data sets based on a literature review were compiled from scientific papers that reported treatment means. Silages made from whole or processed grains were considered and the minimal storage period adopted was 30 days. To analyze the effect of applying additives on winter cereal silages (barley, wheat and triticale),

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the data set included 3 referred journal articles (Adesogan *et al.*, 2003; Mathison *et al.*, 1989; Pieper *et al.*, 2011) and 3 abstracts (Davies *et al.*, 2009; Seppala *et al.*, 2015; Stacey *et al.*, 2009). The small number of publications found was due to the rare use of grain silages when compared to the production of whole plant silages.

The data set used to study high moisture corn silages was composed of 24 referred journal articles (Biro *et al.*, 2006; 2009; Canibe *et al.*, 2013; Da Silva, T. *et al.*, 2015; Dawson *et al.*, 1998; Doležal and Zeman, 2005; Dutton and Otterby, 1971; Ferrareto *et al.*, 2015; Flores-Galarza *et al.*, 1985; Gálík *et al.*, 2007; 2008; Ítavo *et al.*, 2006; 2009; Jobim *et al.*, 2008; Kung *et al.*, 2004; 2007; Loučka, 2010; Morais *et al.*, 2012; Prigge *et al.*, 1976; Pys *et al.*, 2009; Reis *et al.*, 2008; Taylor and Kung, 2002; Wardynski *et al.*, 1993); one technical note (Basso *et al.*, 2012) and 10 abstracts published in international scientific meetings on forage conservation (Auerbach *et al.*, 2015; Coudure *et al.*, 2012; da Silva N. *et al.*, 2015; Davies *et al.*, 2009; Doležal *et al.*, 2014; Gallo *et al.*, 2015; Mlynar *et al.*, 2006; Pys and Kowalski, 2014; Pys *et al.*, 2010; Revello-Chion *et al.*, 2012).

Data sets of corn and winter cereals were analyzed separately. A minimal of four treatment means from at least two articles was the prerequisite for keep the dependent variable into the data set. Data were analyzed using the mixed procedure of SAS (Littell *et al.*, 1996). The model included a fixed effect of treatment (control or additive) and random effect of experiment, due to the variations across experimental protocols that would contribute to study effects in these comparisons (St-Pierre, 2001).

Because the current knowledge indicates divergent responses for types of silage additives, they were sorted into different classes: “Homolactic” (homolactic bacteria), “Hetero” (heterofermentative bacteria), “Combo” (Homolactic plus heterofermentative bacteria) and “Chemical” (chemical additives). Despite of the scarcity of data for wheat, barley and triticale silages, only the consequences of heterofermentative bacteria and chemical additives in winter cereals silages were presented.

RESULTS AND DISCUSSION

Untreated HMCS

Survey data from untreated HMCS showed a wide variation in moisture content (range of 232 g.kg⁻¹ to 397 g.kg⁻¹) and an average storage time of 91 ± 54 d. Benton *et al.* (2005) reported increases in both total *in situ* dry matter digestibility (ISDMD) and degraded intake protein (DIP) when moisture level was increased in both high moisture corn (240 or 300 g.kg⁻¹) and reconstituted corn (280 or 350 g.kg⁻¹), with major variations in ISDMD

and DIP occurring during the first 28 d after ensiling. Taylor and Kung (2002) showed fermentative changes in HMCS over the storage period. The control silages showed most WSC consumption during the first 14 d, culminating in pH values below 4.0. At 49 d, the highest N-NH₃ value was recorded, which would be an indication of proteolysis caused by amino acid deamination (Oshima and McDonald, 1978). Specifically, Hoffman *et al.* (2011) working with different corn hybrids, found marked production of lactic acid and quick pH drop in the first 15 d of fermentation of a given hybrid without additives; however, for a second untreated hybrid, lactate quantification was possible only at day 30, besides the slow and gradual pH drop. For both hybrids, N-NH₃ and soluble protein levels increased with time for up to 240 d.

Untreated HMCS can be characterized by a moderate fermentation when compared to whole plant corn silage. In this review, the average production of acids (lactic acid + acetic acid) was 20.0 ± 11.8 g.kg DM⁻¹, with pH values varying between 3.73 and 4.95. These silages pointed a significant ethanol production (until 28.4 g.kg DM⁻¹), which would be an indication of the yeast metabolism (counts from 3.22 to 6.7 cfu.g FM⁻¹).

A wide range of moisture content was obtained in HMWCS (range of 256 to 461 g.kg⁻¹) and silages were stored for 88 ± 30 d. Previous reports relate the importance of moisture in silages as described by Pieper *et al.* (2011), regarding the fermentation profile of triticale, barley and wheat silages at 250 g.kg⁻¹ or 350 g.kg⁻¹ of moisture. In silages with higher moisture levels, pH declined within 3 days regardless *L. plantarum* inoculation. Treated low moisture silages had a pH decline after 10 d of storage, however pH of untreated grains remained unchanged. Lactic acid, propionic acid, acetic acid and NH₃ concentrations were also influenced ($P < 0.01$) by moisture content, showing a better pattern in silages with higher levels of moisture.

Winter cereals have a higher concentration of soluble carbohydrates in their composition, compared to corn. This greater availability of fermentable substrates may affect the effectiveness of additives during fermentation and feed-out phases. In this survey the average content of residual WSC observed in HMWCS without additives was 46.5 ± 28.0 g.kg DM⁻¹ and the pH values ranged from 3.85 to 5.90. The average concentration of lactic acid plus acetic acid was 21.1 ± 13.2 g.kg DM⁻¹, and the ethanol content ranged from 3.2 to 19.4 g.kg DM⁻¹.

Additives to HMCS

Chemical additives

Chemical additives reported in the reviewed articles included in the final data set are shown

in Table 1, with the respective application rates as reported by the authors. While some compounds were used alone (pure compounds or solutions), most treatments were based on mixtures of chemical substances.

High moisture grain silages with or without chemical additives displayed a large variation in chemical composition, DM loss, microbial counts and aerobic stability, indicating that the data set was broad, representative and covered a large part of the practically wide range of HMCS (Table 2). As expected, HMCS treated with chemical additives revealed a significant fermentation inhibition as indicated by the higher content of WSC and lower content of fermentation end-products, especially lactic acid.

Chemical additives were also effective in preventing DM losses, which explains the higher DM content of silages containing chemicals. Changes in variables such as ash, CP, N-NH₃ and pH reflect the formulations of chemicals added to the silages. The presence of minerals in the chemicals altered the ash content of silages and the presence of nitrogenous compounds affected CP and N-NH₃ concentration, in addition to the inhibitory effects on the microorganisms, preventing the pH drop.

A noticeable response achieved with chemical additives was the higher aerobic stability of silages, since spoiling microorganisms such as yeasts were markedly decreased. Higher stability associated with lower nutrient oxidation upon air exposure is a reasonable justification to recommend chemical additives for HMCS.

Table 1: Description of chemical additives used in the meta-analysis

Additive	Application rate
Ammonia	1.1 % to 2.3 %
Ammonium isobutyrate	2 %
Diammonium phosphate	4.6 %
Formic acid	3 L.t ⁻¹ to 4 L.t ⁻¹
Sulfur dioxide	1.3 % to 1.7 %
Urea	0.4 to 2 %
Urea solution	50 L.t ⁻¹
Acetic acid, isobutyric acid	8 L.t ⁻¹
Ammonium formate, propionate, ethyl benzoate and benzoate	4 L.t ⁻¹
Ammonium propionate, sodium propionate, acetic acid, benzoic acid and sorbic acid	0.1 % to 0.2 %
Formic acid, ammonium formate, propionic acid, benzoic acid	4 L.t ⁻¹
Formic acid, ammonium formate, propionic acid, benzoic acid and ethylbenzoate	6 L.t ⁻¹
Formic acid (42.5 %), formic ammonia (30.3 %) and propionic acid (10 %)	6 L.t ⁻¹
Formic acid (55 %), propionic acid (20 %), ammonium formate (4.3 %) and potassium sorbate (2.5 %)	4 L.t ⁻¹
Formic acid (55 %), propionic acid (5 %) and ammonium formate (24 %)	4 L.t ⁻¹ to 4.5 L.t ⁻¹
Formic acid (55 %), propionic acid (5 %) and ammonium formate (24 %) plus ammonium propionate	4.5 L.t ⁻¹
Propionic acid (80 %) and acetic acid (20 %)	1.5 %
Propionic acid and formic acid	3.5 kg.t ⁻¹
Propionic acid-based additive: ammonium and sodium propionate, ethoxyquin, BHA, and BHT	0.1 to 0.2 %
Propionic acid (50 %) and formic acid (50 %)	3 L.t ⁻¹
Propionic acid (90 %), ammonium propionate (4 %) and 1,2-propanediol (4 %)	3 L.t ⁻¹
Propionic acid, ammonium propionate, sodium benzoate, potassium sorbate	1.5 to 3 L.t ⁻¹
Propionic, acetic, benzoic and sorbic acids, sodium and ammonium hydroxide, methylparaben and propylparaben (Liquid mold inhibitor, 82 % acid content)	0.1 %
Propionic acid, formic acid, benzoic acid and calcium formate	3.4 kg.t ⁻¹
Propionic acid (37 %), sodium benzoate (14 %) and sodium propionate (11 %)	5 L.t ⁻¹
Sodium benzoate (22.9 %) and sodium propionate (8.3%)	3 L.t ⁻¹ to 6 L.t ⁻¹
Sodium benzoate (5 to 50 %), potassium sorbate (5 to 35 %) and sodium nitrite < 5 %	2 L.t ⁻¹ to 6 L.t ⁻¹
Sodium benzoate, sodium azide and calcium formate	3.5 kg.t ⁻¹
Potassium sorbate, sodium benzoate, ammonium propionate	1 L.t ⁻¹ to 2 L.t ⁻¹

Table 2: Data set of high moisture corn silages treated without or with chemical additives and their effects on silage quality

Item	Data set					Treatment effect		SEM	P
	n ¹	Mean	SD	Minimum	Maximum	Control	Chemical		
DM, g.kg ⁻¹	62	661.8	38.2	598.0	748.0	657.8	665.8	6.88	0.02
Ash, g.kg DM ⁻¹	26	15.2	1.49	13.3	19.1	14.6	15.8	0.38	0.01
NDF, g.kg DM ⁻¹	24	99.7	6.79	79.2	107.0	99.5	99.9	2.00	0.59
ADF, g.kg DM ⁻¹	24	39.7	12.3	22.8	57.4	39.8	39.7	3.64	0.85
Hemicellulose, g.kg DM ⁻¹	24	59.9	11.9	42.4	76.2	59.7	60.2	3.50	0.35
Starch, g.kg DM ⁻¹	40	712.9	51.8	593.0	796.7	711.5	714.3	11.7	0.44
Ether extract, g.kg DM ⁻¹	14	43.3	8.33	34.8	66.2	45.5	41.1	3.15	0.36
CP, g.kg DM ⁻¹	36	95.8	29.9	57.3	202.0	88.9	102.8	6.95	0.04
N-NH ₃ , g.kg DM ⁻¹	40	0.54	1.07	0.00	5.00	0.33	0.76	0.24	0.21
Soluble protein, g.kg CP ⁻¹	6	520.1	21.7	476.7	531.8	531.8	508.3	11.3	0.28
WSC, g.kg DM ⁻¹	20	9.84	3.97	1.00	15.2	8.34	11.3	1.19	< 0.01
pH	74	4.41	0.72	3.70	8.30	4.31	4.51	0.12	0.08
Lactic acid, g.kg DM ⁻¹	78	12.4	7.09	0.20	26.5	14.0	10.8	1.11	< 0.01
Acetic acid, g.kg DM ⁻¹	78	4.89	2.84	0.00	16.0	5.20	4.57	0.46	0.15
Propionic acid, g.kg DM ⁻¹	64	1.02	2.52	0.00	18.3	0.16	1.89	0.42	< 0.01
Butyric acid, g.kg DM ⁻¹	24	0.23	0.26	0.00	0.70	0.27	0.18	0.07	0.23
Ethanol, g.kg DM ⁻¹	58	6.91	9.05	0.00	44.0	9.50	4.32	1.62	< 0.01
Lactic:Acetic ratio	78	3.79	3.33	0.00	11.3	3.82	3.77	0.58	0.88
LAB, log cfu.g ⁻¹	6	2.25	0.23	2.00	2.45	2.45	2.04	0.03	0.01
Yeasts, log cfu.g ⁻¹	20	3.35	1.19	0.57	4.69	4.06	2.65	0.31	< 0.01
DM losses ² , g.kg ⁻¹	10	14.2	11.9	5.8	41.0	16.7	11.8	5.50	0.11
Aerobic stability, h	52	125	112	21	500	59	190	18	< 0.01

¹Number of means, ²Fermentative losses.

Microbial additives

Nowadays, there is enough knowledge indicating divergent responses for homolactic and heterolactic microbial inoculants (Kung *et al.*, 2003). Thus, homolactic, heterofermentative (including species of *Propionibacteria*) and combinations of homolactic and heterofermentative bacteria were evaluated separately. The microbial species used as silage inoculants are described in Table 3.

Homolactic bacteria are recognized for their efficiency in producing lactic acid, which is a strong acid (pKa = 3.86) capable to quickly drop the pH decreasing fermentative losses. On the other hand, heterofermentative bacteria are skilled in ferment sugars (pentoses and hexoses) into other products besides lactic acid, for instance acetic and propionic acids. These weak acids are good antifungal agents able to promote aerobic stability in silages (Moon, 1983).

Homolactic bacteria

Chemical composition, DM loss, microbial counts and aerobic stability of HMCS with or without homolactic inoculants are shown in Table 4. Nutrient compositions of HMCS were quite similar. Silages treated with the homolactic inoculants showed higher protein content and reduced ammonia content mainly due to the inhibition of proteolysis.

Although the database did not provide quantification of LAB, there was a trend towards greater use of soluble carbohydrates in the inoculated silages. As a consequence of the typical metabolism of added bacteria, the lactic acid content was higher in silages inoculated with homolactic bacteria, and this difference promotes significant changes in pH. The DM losses have been numerically lower in inoculated silages, however, both control and treated silages had shown low fermentative losses. Low concentrations of other organic acids indicated that fermentation profile was generally shortly interrupted.

Table 3: Microorganisms used as silage inoculants in the current meta-analysis

Bacterium	Inoculation rate (cfu.g ⁻¹ as fed)
<i>Lactobacillus buchneri</i>	5×10^4 to 5×10^6
<i>Lactobacillus fermentum</i>	1×10^5
<i>Lactobacillus plantarum</i>	5×10^4 to 1×10^7
<i>Leuconostoc mesenteroides</i>	1×10^5
<i>Propionibacterium acidipropionici</i>	1×10^7
<i>Propionibacterium freudenreichii</i>	1×10^7
<i>L. buchneri</i> and <i>L. plantarum</i>	2.5×10^5 to 6×10^5
<i>L. buchneri</i> and <i>P. pentosaceus</i>	7.5×10^5 to 9×10^5
<i>L. plantarum</i> and <i>P. acidipropionici</i>	1.5×10^5 to 3×10^5
<i>L. plantarum</i> and <i>P. freudenreichii</i>	1×10^5 to 1×10^7
<i>L. rhamnosus</i> and <i>E. faecium</i>	1×10^5 to 5×10^5
<i>P. pentosaceus</i> and <i>P. freudenreichii</i>	1.2×10^5 to 2.4×10^5
<i>L. buchneri</i> , <i>L. plantarum</i> and <i>E. faecium</i>	5×10^6
<i>L. plantarum</i> , <i>E. faecium</i> , and <i>P. acidilactici</i>	1.5×10^5 to 2×10^6
<i>L. plantarum</i> , <i>P. pentosaceus</i> and <i>P. acidipropionici</i>	1.5×10^5
<i>L. plantarum</i> , <i>L. bulgaricus</i> and <i>L. acidophilus</i>	1×10^5
<i>L. plantarum</i> , <i>L. casei</i> , <i>E. faecium</i> and <i>P. pentosaceus</i>	5×10^4
<i>L. buchneri</i> , <i>L. plantarum</i> , <i>E. faecium</i> , <i>L. casei</i> , and <i>P. pentosaceus</i>	1.5×10^5
<i>L. buchneri</i> , <i>L. plantarum</i> , <i>L. brevis</i> , <i>L. rhamnosus</i> and <i>P. pentosaceus</i>	2.5×10^5

Table 4: Data set of high moisture corn silages treated without or with homolactic inoculants and their effects on silage quality

Item	Data set					Treatment effect		SEM	P
	n ¹	Mean	SD	Minimum	Maximum	Control	Homolactic		
DM, g.kg ⁻¹	28	669.3	54.9	595.0	768.0	665.9	672.5	14.9	0.13
Ether extract, g.kg DM ⁻¹	6	34.5	3.37	29.3	38.4	32.3	36.7	1.54	0.18
CP, g.kg DM ⁻¹	20	85.7	11.1	66.0	97.6	84.5	86.9	3.57	0.03
N-NH ₃ , g.kg DM ⁻¹	18	0.26	0.17	0.10	0.70	0.20	0.19	0.04	0.06
Soluble protein, g.kg CP ⁻¹	4	296.3	119.1	225.0	473.0	225.0	367.5	74.6	0.41
WSC, g.kg DM ⁻¹	8	26.5	10.8	12.3	37.4	29.1	23.9	5.62	0.12
pH	24	4.29	0.48	3.88	5.65	4.42	4.16	0.14	0.02
Lactic acid, g.kg DM ⁻¹	16	24.5	17.4	8.80	69.3	21.4	27.6	6.25	0.10
Acetic acid, g.kg DM ⁻¹	16	9.13	9.14	1.10	28.7	9.85	8.41	3.33	0.40
Propionic acid, g.kg DM ⁻¹	10	0.77	0.61	0.00	1.50	1.00	0.54	0.27	0.27
Ethanol, g.kg DM ⁻¹	12	7.85	9.66	2.70	28.5	8.23	7.47	4.13	0.22
Lactic:Acetic ratio	16	3.96	2.42	1.64	9.74	3.02	4.89	0.81	0.12
Yeasts, log cfu.g ⁻¹	6	4.77	0.73	3.92	5.67	4.76	4.78	0.47	0.95
DM losses ² , g.kg ⁻¹	10	17.4	24.2	4.60	68.0	18.3	16.5	11.5	0.42
Aerobic stability, h	4	118	27	96	156	138	98	13	0.27

¹Number of means, ²Fermentative losses.

Unsurprisingly, homolactic inoculants were less effective in controlling aerobic deterioration, since lactic acid has a typical weak antifungal property (Moon, 1983). The influx of air into the silage mass has negative effects on silage quality, especially in HMGS due to its

high content of nutrients, low moisture, and because it ferments more slowly and less extensively compared to typical forage crop silages (Taylor and Kung, 2002). Nutrient losses and excessive production of heat by microbial spoliation result in lower feed quality and

may result in poor animal performance (Hoffman and Ocker, 1997; Salvo *et al.*, 2015). This makes the use of exclusively homolactic microorganisms inappropriate for HMCS.

Heterofermentative bacteria

The characteristics of HMCS treated or not with heterofermentative inoculants are presented in Table 5. Overall quality of HMCS was typical for well-preserved silages, although DM and WSC contents, which are key factors for silage fermentation, showed a wide range.

Indeed, the production of antifungal compounds (e.g., acetic and propionic acids) by heterofermentative bacteria was an effective way for decreasing yeast

and fungi population (Honing and Woolford, 1980) and largely improved the aerobic stability of HMCS. Silages inoculated with heterofermentative strains had lower WSC, indicating higher fermentative activity. *Lactobacillus buchneri*, a typical heterofermentative bacteria, has a predominant metabolic pathway leading to accumulation of acetic acid, whereas lactic acid concentration and pH, in general, remains similar to control silages. Furthermore, heterofermentative strains increase propionic acid as well, which might be produced either by the addition of *Propionibacterium* spp or by the degradation of 1,2-propanediol (Krooneman *et al.*, 2002) resulted from *L. buchneri* metabolism.

Table 5: Data set of high moisture corn silages treated without or with heterofermentative inoculants and their effects on silage quality

Item	Data set					Treatment effect		SEM	P
	n ¹	Mean	SD	Minimum	Maximum	Control	Hetero		
DM, g.kg ⁻¹	74	698.9	37.4	629.5	738.0	700.7	697.1	6.19	0.01
Ash, g.kg DM ⁻¹	10	14.0	0.92	12.7	15.6	14.0	13.9	0.44	0.73
NDF, g.kg DM ⁻¹	26	78.2	17.3	55.3	106.5	78.3	78.1	4.89	0.85
ADF, g.kg DM ⁻¹	26	26.9	13.7	10.4	56.8	26.9	26.9	3.87	0.92
Hemicellulose, g.kg DM ⁻¹	26	51.3	10.7	38.6	74.3	51.5	51.1	3.02	0.78
Starch, g.kg DM ⁻¹	12	740.2	38.0	687.0	794.0	739.8	740.6	16.3	0.85
CP, g.kg DM ⁻¹	32	94.9	11.6	72.3	109.6	95.3	94.5	2.95	0.48
N-NH ₃ , g.kg DM ⁻¹	64	0.24	0.21	0.03	0.81	0.23	0.26	0.04	0.02
WSC, g.kg DM ⁻¹	38	1.89	2.51	0.10	10.8	2.38	1.41	0.57	< 0.01
pH	66	4.22	0.43	3.73	5.65	4.24	4.20	0.07	0.39
Lactic acid, g.kg DM ⁻¹	66	13.5	10.6	1.40	39.0	13.8	13.2	1.87	0.17
Acetic acid, g.kg DM ⁻¹	66	5.75	4.21	0.40	27.1	4.06	7.44	0.68	< 0.01
Propionic acid, g.kg DM ⁻¹	36	0.37	0.67	0.00	3.50	0.10	0.65	0.14	0.01
Butyric acid, g.kg DM ⁻¹	8	0.16	0.24	0.00	0.60	0.23	0.10	0.12	0.19
Ethanol, g.kg DM ⁻¹	46	5.87	4.92	1.20	18.0	6.07	5.67	1.04	0.25
Lactic:Acetic ratio	66	3.11	2.95	0.48	10.9	3.71	2.51	0.51	< 0.01
LAB, log cfu.g ⁻¹	6	7.88	0.54	7.11	8.46	7.63	8.13	0.30	0.34
Yeasts, log cfu.g ⁻¹	46	4.24	1.34	1.34	6.70	4.83	3.65	0.24	< 0.01
Molds, log cfu.g ⁻¹	30	3.65	1.98	1.10	7.29	3.95	3.36	0.51	< 0.01
DM losses ² , g.kg ⁻¹	16	30.1	10.4	7.50	41.0	27.2	33.0	3.65	0.07
Aerobic stability, h	72	129.0	114	20.0	450	70	188	16	< 0.01

¹Number of means, ²Fermentative losses.

Combination of homo- and hetero-fermentative bacteria

Chemical composition and fermentative characteristics of silages treated with both homo- and heterofermentative inoculants are presented in Table 6. Combo inoculants led to silages with higher protein content, whereas ammonia concentration tended ($P = 0.11$) to be lower, as observed for homolactic inoculants. According to Ferraretto *et al.* (2015), inoculation had no effect on CP of silages added or not with homolactic or heterofermentative bacteria; instead, CP values remained similar even with protease addition. In this review changes in the protein fraction were observed suggesting the importance of further studies aiming to investigate the influence of different microbial strains as their fermentative routes on silage proteolysis.

Silages treated with combo inoculants showed lower NDF content, which can be attributed to a higher

hemicellulose disappearance ($P = 0.10$). It should be also noted that in the current meta-analysis, the inoculation with heterofermentative bacteria did not change the fibrous components of HMCS, most probably because none of the strains tested provided ferulic acid esterase activity.

The fermentation profile observed in silages treated with combo inoculants blended features from both homo- and hetero-fermentative bacteria. Inoculated silages had a greater consumption of soluble carbohydrates. In despite of the lower pH value attributed to the action of homolactic bacteria, treated silages had similar concentrations of lactic acid and higher levels of acetic and propionic acids than control silages. In turn, the presence of weak acids with antifungal properties reduced yeast counts and ethanol concentrations. Silages included in this data set generally

Table 6: Data set of high moisture corn silages treated without or with combinations of homo- and hetero-fermentative bacteria and their effects on silage quality

Item	Data set					Treatment effect		SEM	P
	n ¹	Mean	SD	Minimum	Maximum	Control	Combo		
DM, g.kg ⁻¹	52	685.3	47.8	596.3	739.0	686.2	684.3	9.46	0.17
Ash, g.kg DM ⁻¹	28	14.0	0.71	12.7	15.5	13.8	14.1	0.19	0.12
NDF, g.kg DM ⁻¹	8	100.1	5.3	91.1	107.0	101.6	98.5	2.72	0.02
ADF, g.kg DM ⁻¹	8	40.9	13.9	27.3	56.8	41.2	40.6	7.51	0.18
Hemicellulose, g.kg DM ⁻¹	8	59.2	14.8	39.7	74.3	60.4	57.9	7.95	0.10
Starch, g.kg DM ⁻¹	30	718.7	31.7	679.0	794.0	719.6	717.8	8.32	0.50
Ether extract, g.kg DM ⁻¹	8	38.2	3.83	33.9	44.2	38.2	38.2	2.07	1.00
CP, g.kg DM ⁻¹	36	82.4	8.98	72.3	97.2	81.8	83.1	2.14	0.05
N-NH ₃ , g.kg DM ⁻¹	44	0.20	0.21	0.00	0.80	0.23	0.18	0.04	0.11
WSC, g.kg DM ⁻¹	10	4.05	3.94	0.10	10.8	4.86	3.24	1.83	0.04
pH	52	4.21	0.41	3.73	5.65	4.29	4.13	0.08	0.03
Lactic acid, g.kg DM ⁻¹	44	15.3	6.81	3.90	25.1	15.3	15.4	1.47	0.90
Acetic acid, g.kg DM ⁻¹	44	5.77	3.14	1.50	14.2	5.08	6.47	0.66	0.03
Propionic acid, g.kg DM ⁻¹	18	0.37	0.47	0.00	1.38	0.16	0.59	0.14	0.04
Butyric acid, g.kg DM ⁻¹	14	0.19	0.19	0.00	0.62	0.23	0.16	0.07	0.47
Ethanol, g.kg DM ⁻¹	42	3.52	3.02	0.90	15.6	4.06	2.98	0.66	<0.01
Lactic:Acetic ratio	42	3.18	2.10	0.14	8.63	3.11	3.25	0.46	0.78
Yeasts, log cfu.g ⁻¹	10	3.61	1.13	2.00	5.67	4.37	2.84	0.37	0.03
Molds, log cfu.g ⁻¹	18	1.83	0.58	1.09	2.90	1.82	1.83	0.20	0.94
DM losses ² , g.kg ⁻¹	24	27.8	9.96	4.00	42.2	27.3	28.3	2.94	0.56
Aerobic stability, h	28	216	117	35	427	194	237	31	0.22

¹Number of means, ²Fermentative losses.

were stored for longer than 60 days (85 % of evaluated averages). Therefore, it is likely that assimilation of lactic acid by *L. buchneri* strains occurred throughout the fermentation process, giving rise to organic acids derived from the heterofermentative pathways. Puzzling, fermentative losses and aerobic stability were not altered.

Optimal dose of additives for improving aerobic stability to HMCS

In the current data set, aerobic stability was the most important response improved by additive utilization. Heterofermentative bacteria and chemical additives successfully enhanced aerobic stability of HMCS. For recommending an optimal application rate, a broken-line regression model was fitted to the data set.

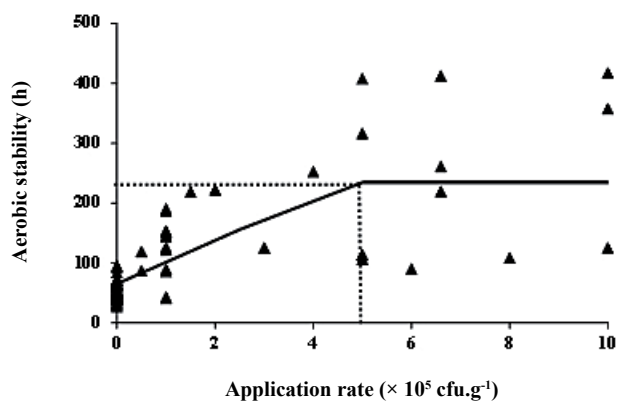


Fig. 1: Aerobic stability of HMCS according to inoculation rate of heterofermentative bacteria. If Dose $\leq 4.67 \times 10^5$ cfu.g⁻¹, aerobic stability = $64.4 + 28.3 \times \text{Dose}$ (g.kg⁻¹); otherwise, aerobic stability = 235 h. $P < 0.01$, $R^2 = 0.50$, RMSE = 47.88.

For heterolactic bacteria, treatment effectiveness was achieved when bacteria was applied up to the optimal dose of 4.67×10^5 cfu.g FM⁻¹ (Figure 1). It is important to highlight the inoculation rates of microbial inoculants. In the study reported by Taylor and Kung (2002), the inoculation of HMCS stored for 92 d with a low dose of *L. buchneri* (1×10^5 cfu.g⁻¹) did not enhance the aerobic stability.

In contrast, application rates $\geq 5 \times 10^5$ cfu.g⁻¹ improved the aerobic stability more than six-fold compared with untreated HMCS stored for the same period. However, in silages stored for 166 d, *L. buchneri* improved the aerobic stability even at 1×10^5 cfu.g⁻¹. Additionally to the inoculation rate, extending the length of storage is a potential practice to improve the aerobic stability and nutritive value of HMGS (Taylor and Kung, 2002; Hoffman *et al.*, 2011; Der Bedrosian *et al.*, 2012).

For chemical additives, the aerobic stability increased linearly within the studied range of application rates (Figure 2). Inhibition of spoiling microorganisms (e.g., yeasts and molds) requires a minimum acid concentration in silage aqueous fraction. Organic acid concentrations between 12.5 and 30 g.kg⁻¹ of water may be required to control spoiling microorganisms in feedstuffs with high DM content (Collins, 1995).

In the present data set, 1.0 to 4.0 g.kg⁻¹ are the most frequent range of application rate of chemicals. Probably, the cost:benefit ratio issue and negative effect on animal responses might be plausible justifications for these lower application rates. The lack of data for higher dosages focusing on aerobic stability in HMCS also contributes to this trend. Extrapolations should be avoided for silages with high moisture content (i.e., whole plant silages) because they typically contain higher levels of fermentation end-products.

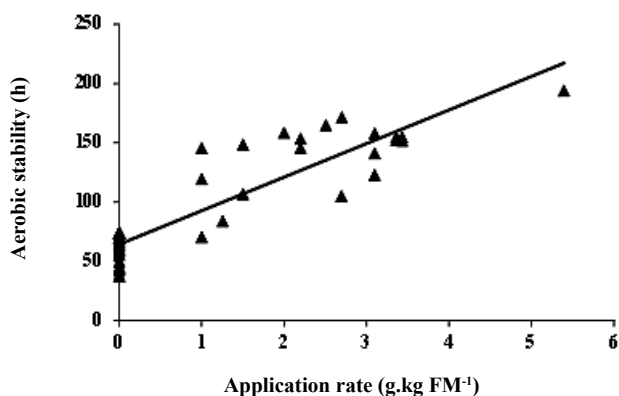


Fig. 2: Aerobic stability of HMGS according to chemical additive dosage. Aerobic stability = $64.4 + 28.3 \times \text{Dose}$ (g.kg⁻¹). $P < 0.01$, $R^2 = 0.81$, RMSE = 20.14.

Additives to HMWCS**Chemical additives**

The changes imposed on HMWCS by adding chemical additives are shown in Table 7. The starch disappearance in silages treated with additives may have been favored by chemical solubilization of the protein matrix. However, the agents promoting such breakage are not easily identifiable, since the chemical compositions are diversified.

Crude protein, N-NH₃ and pH were directly influenced by applying chemical additives and their composition. Nitrogenous compounds added to silages certainly contributed to increase CP and N-NH₃ concentrations. The pH was further increased (5.59)

when silages were treated with chemical additives. This response is relevant because a proper acidification is mandatory to control pathogenic microorganisms. If silage does not reach low and stable pH (< 4.6), clostridial activity can be encouraged (Pitt *et al.*, 1990). However, this review does not allow further conclusions about clostridium growth risk, since the concentration of butyric acid and spore counts were not accessed.

The lower concentration of lactic acid in the treated silages suggests that the fermentation process was inhibited. The chemicals were efficient in controlling molds and yeasts growth, reducing the formation of ethanol. The antifungal activity has also been proven effective at the feedout phase, increasing the aerobic stability of the grains.

Table 7: Data set of high moisture winter cereal silages treated without or with chemical additives and their effects on silage quality

Item	Data set					Treatment effect		SEM	P
	n ¹	Mean	SD	Minimum	Maximum	Control	Chemical		
DM, g.kg ⁻¹	52	641.6	67.4	527.0	756.0	639.6	643.6	13.3	0.35
ADF, g.kg DM ⁻¹	10	63.8	11.9	46.3	82.0	62.3	65.3	5.59	0.61
Starch, g.kg DM ⁻¹	40	608.7	34.1	532.0	679.8	614.3	603.1	7.62	0.07
CP, g.kg DM ⁻¹	48	118.8	29.8	94.6	221.0	111.7	125.9	5.96	0.02
N-NH ₃ , g.kg DM ⁻¹	12	0.325	0.23	0.20	0.90	0.28	0.37	0.09	0.04
WSC, g.kg DM ⁻¹	42	45.5	25.3	16.8	100.0	43.9	47.1	5.57	0.57
pH	52	5.16	1.44	3.80	9.20	4.73	5.59	0.27	0.01
Lactic acid, g.kg DM ⁻¹	52	11.0	10.5	0.88	40.0	12.6	9.41	2.05	0.07
Acetic acid, g.kg DM ⁻¹	52	3.55	3.70	0.10	21.7	3.16	3.95	0.73	0.35
Ethanol, g.kg DM ⁻¹	50	8.70	5.91	0.02	20.8	12.0	5.45	0.99	< 0.01
Lactic:Acetic ratio	52	4.59	4.15	0.37	21.6	4.50	4.68	0.82	0.86
Yeasts, log cfu.g ⁻¹	10	4.88	1.95	1.50	6.80	6.44	3.32	0.50	0.01
Molds, log cfu.g ⁻¹	10	4.40	2.34	1.70	6.90	5.86	2.94	0.84	0.03
DM losses ² , g.kg ⁻¹	4	31.5	15.2	16.0	45.0	33.0	30.0	13.0	0.37
Aerobic stability, h	6	223	78	87	301	165	281	29	0.10

¹Number of means, ²Fermentative losses.

Heterofermentative bacteria

The chemical composition, pH and fermentation end products of HMWCS treated with heterofermentative bacteria inoculants are shown in Table 8. The lower DM content of treated silages may be associated with the heterofermentative pattern, evidenced by the increase in acetic acid production. Despite losses have not been measured, the metabolic pathway of acetic acid production leads to carbon losses, which may explain the lower DM content of treated silages. Numerically, residual WSC

content was lower in inoculated silages, corroborating with higher microbial activity in these silages.

Even with an increase in acetic acid levels, ethanol production was similar among silages. The final content of lactic acid was similar, but the pH of the inoculated silages was lower, highlighting that winter cereals have enough substrate for an efficient acidification of the mass. Anaerobic assimilation of lactic acid performed by *L. buchneri* can also explain the similar content of this acid in the silages (Oude-Elferink *et al.*, 2001).

Table 8: Data set of high moisture winter cereal silages treated without or with heterofermentative inoculants and their effects on silage quality

Item	Data set					Treatment effect		SEM	P
	n ¹	Mean	SD	Minimum	Maximum	Control	Hetero		
DM, g.kg ⁻¹	18	584.2	66.5	499.0	744.0	618.6	549.8	19.4	0.02
Starch, g.kg DM ⁻¹	18	623.2	43.7	537.0	680.8	631.4	615.0	14.7	0.22
CP, g.kg DM ⁻¹	18	101.2	4.8	93.8	110.0	100.1	102.4	1.59	0.08
WSC, g.kg DM ⁻¹	18	46.6	27.9	15.0	100.0	53.6	39.5	9.26	0.17
pH	18	4.37	0.49	4.00	5.90	4.57	4.17	0.15	0.06
Lactic acid, g.kg DM ⁻¹	18	13.4	5.86	1.80	19.7	12.7	14.1	2.00	0.50
Acetic acid, g.kg DM ⁻¹	18	9.10	10.2	1.00	33.6	3.66	14.5	2.93	0.02
Ethanol, g.kg DM ⁻¹	18	10.4	4.48	5.29	19.4	11.3	9.55	1.51	0.27
Lactic:Acetic ratio	18	2.21	1.48	0.35	6.00	3.06	1.36	0.41	0.02

¹Number of means.

CONCLUSION

Control of fermentative losses is not a concern in properly made high moisture grain silages. Therefore, use of additives is justified if aerobic stability is improved. Additives based on chemical or heterofermentative bacteria proven to be effective in preventing aerobic deterioration at the same magnitude. The aerobic stability of high moisture corn silages was linearly increased with the application rate of chemical additives, whereas the optimal dose of heterofermentative bacteria was 4.67×10^5 cfu.g FM⁻¹.

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