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ORIGINAL ARTICLE

A meta-analysis on the effectiveness of homofermentative and heterofermentative lactic acid bacteria for corn silage

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Abstract

Aims: This meta-analysis aims to assess the effect of lactic acid bacteria (LAB) inoculation on fermentation parameters, microbiological composition and aerobic stability of corn silage.
Methods and Results: Databases (PubMed, ScienceDirect and Scopus) were searched from 1980 to 2017. The criteria for inclusion were: randomized and controlled experiments using corn silage and published in peer-reviewed journals. The meta-analysis showed that LAB supplementation increased pH, acetate and propionate concentrations, and decreased acid detergent fibre, water-soluble carbohydrates and ammoniacal nitrogen (NH_3-N) compared to controls in the pooled raw mean difference random effect model. In addition, inoculation reduced counts of yeasts and moulds, increased LAB counts and markedly improved aerobic stability in corn silage. However, results indicated that the effect of inoculants may differ depending on the administration of homofermentative or heterofermentative LAB.
Conclusions: For the development of functional bacterial inoculants, both types of LAB should be used.
Significance and Impact of the Study: To our knowledge, this is the first meta-analysis to compare the application of homofermentative and heterofermentative LAB for corn silage.

Introduction

Ensiling is an effective method with a long history of use in the preservation of forage crops for livestock (Dunière *et al.* 2013). Although silage fermentation may occur naturally under anaerobic conditions due to the presence of native bacteria in plants, the speed and efficiency of the fermentation is variable. Therefore, inoculants containing selected strains of lactic acid bacteria (LAB) have been developed in order to reduce the influence of epiphytic micro-organisms on the outcome of ensiling forages and to standardize the fermentation (Muck and Kung 1997).

Corn (*Zea mays* L.) silage is the most widely foraged fibre source for feeding ruminants because it is easily digested and highly nutritious (Li and Nishino 2011; Klopfenstein *et al.* 2013). In many parts of the world, commercial corn silage inoculants are available; they may contain homofermentative LAB or both (Tabacco *et al.* 2011; Sadeghi *et al.* 2012). Inoculants comprising homofermentative LAB ensure a rapid and efficient fermentation of water-soluble carbohydrates (WSC) into organic acids, induce a fast decrease in pH and improve silage preservation while minimizing nutrient and energy losses (Weinberg *et al.* 1993). The homofermentative inoculants are intended to minimize the activity of other micro-organisms early in fermentation such as the enterobacteria and bacilli (Muck 2010). Yet, in these fermentations, the homofermentative LAB inoculants produce

mainly lactic acid, which can serve as a substrate for lactate-assimilating micro-organisms upon exposure to air (Filya *et al.* 2006). Furthermore, only small amounts of volatile fatty acids, which inhibit the growth of yeasts and moulds, are produced (Wohlt 1989). Micro-organisms causing spoilage decrease the nutrient value of silage and may have harmful effects on animal health and performance (McDonald *et al.* 1991). Among the homofermentative LAB most frequently used are *Enterococcus faecium*, *Pediococcus acidilactici*, *Pediococcus pentosaceus*, *Lactobacillus plantarum* and *Lactobacillus acidophilus* (Weinberg *et al.* 2004). Conversely, heterofermentative LAB, which are recognized to grow in latter phases of fermentation, produce a higher concentration of acetic acid that inhibits yeasts and moulds, thereby improving the aerobic stability of silage (Hu *et al.* 2009; Santos *et al.* 2016). This make heterofermentative LAB, with *Lactobacillus buchneri* being the most common species used, attractive microorganisms for the production of silages. Dual-purpose inoculants containing homofermentative and heterofermentative LAB have been developed to overcome the limitations of inoculants containing either type of bacteria alone (Comino *et al.* 2014). However, no agreement has been reached yet as to whether corn silage inoculants are effective in improving the quality of silages. Although many authors reported promising effects of LAB inoculation on fermentation patterns (Queiroz *et al.* 2012; Ferraretto *et al.* 2015), responses to silage inoculants could be influenced by several factors including duration of ensiling, application rate of LAB inoculant, LAB species and other ensilage management practices (Addah *et al.* 2011; Mohammadzadeh *et al.* 2012).

Such disputes and increasing information published on the subject need to be reviewed and treated with statistical techniques that allow a quantitative assessment of results obtained to date. Hence, it is extremely relevant to carry out a meta-analysis. The latter can estimate the magnitude of factors affecting the response, reduce multiple biases inherent in traditional checks and must clearly state the criteria used in the selection and evaluation of scientific papers selected for the topic under review (Zimmermann *et al.* 2016).

Consequently, our purpose was to conduct a meta-analysis to assess the effect of homofermentative and heterofermentative LAB inoculation on fermentation parameters, microbiological composition and aerobic stability of corn silage.

Materials and methods

Criteria for study selection

Pubmed, ScienceDirect and Scopus databases were consulted for articles restricted by language. The studies included in this meta-analysis were selected only if they were research articles with randomized and controlled experiments using corn silage, and results were published in peer-reviewed journals between 1980 and 18 May 2017. To evaluate effects of applying LAB inoculants on fermentation parameters, microbiological composition and aerobic stability of corn silage, peer-reviewed papers were retrieved using the terms 'silage', 'corn', 'maize' and 'inoculant'. Studies must have examined uninoculated and inoculated treatment groups, held treatments comprising only LAB and reported response variables with measures of variance. Assorted reviews, duplicate reports, experiments that used different forages species and a number of studies that evaluated other additives were excluded. The term 'study' refers to a scientific article, which can involve one or more experiments.

Outcomes and definitions

Supplementation with LAB was analysed as a tool which may improve fermentation parameters, microbiological composition and aerobic stability of corn silage. Data concerning response variables correspond to the whole trial. When the study included more than one inoculant, or when different doses of the same inoculant were used, each inoculated group was compared with the uninoculated group separately.

Data extraction

Information on study design, the number of replicates, means and variances were extracted from each research report. The following response variables were inferred: pH, percentage of dry matter (DM), DM recovery, neutral detergent fibre (NDF), acid detergent fibre (ADF), ammoniacal nitrogen (NH_3-N) (% of total N), lignin, acid detergent insoluble nitrogen (ADIN), crude protein (CP), WSC, ethanol, lactate, acetate, propionate and butyrate, *in vitro* DM digestibility-48 h (IVDMD-48h), counts of LAB, yeasts, moulds and clostridia (CFU per g), and aerobic stability (h). For each study, the methodology used to achieve the results was evaluated. However, no scores were used to exclude studies (Lean *et al.* 2009).

Statistical analysis

Statistical analysis used Comprehensive Meta Analysis ver. 2.2 (2011). Due to continuous variables being analysed, results were presented as raw mean differences between the inoculant treatment and controls with 95% confidence intervals using a random effects model. In this model, the true effect could vary from experiment to experiment; between-experiment variability (true

heterogeneity) as well as sampling error are included (Borenstein *et al.* 2009). To account for variation in precision across studies, the inverse of the squared standard error of each treatment mean was used as a factor in the weight statement of the model.

A meta-regression analysis was performed to explore the sources of heterogeneity in the treatment effects. In the meta-regression, the covariates are at the level of the study rather than the level of the subject (as in the primary studies) and the dependent variable is the effect size in the studies rather than subject scores. To test the impact of covariates for statistical significance, it is important to quantify the magnitude of their relationship with effect size. For this purpose, we can use an index based on the percent reduction in true variance, analogous to the R^2 index used with primary studies (Borenstein *et al.* 2009). Meta-regression allowed assessing the relationship between year of publication, application rate of LAB inoculant and duration of the studies as covariates, and silage quality and preservation as outcome variables. Unusually high rates of inoculation (close to or higher than 1×10^{10} CFU per g of fresh material) were observed in certain works (Baytok *et al.* 2005; Khorvash *et al.* 2006; Bíro *et al.* 2009; Erickson *et al.* 2012; Rodríguez *et al.* 2016). Considering that commercial freeze-dried products may commonly contain up to $1–5 \times 10^{10}$ CFU per g, trying to achieve such a high rate may be technologically challenging in practice, as freeze-dried powders must be dissolved in water to be sprayed on the chopped fresh material. Additionally, a rate $\geq 10^7$ CFU per g may be not economically viable in most cases (Oliveira *et al.* 2017). As a result, the referenced reports were not considered for application rate of LAB.

A priori subgroup analyses were planned depending on factors that could potentially influence the magnitude of the treatment: (i) For the purpose of grouping the newest articles, we used the last 10 years (before 2008 *vs* after 2008) as a prespecified cut-off; (ii) Type of inoculum (monostrain *vs* multistrain); (iii) Among monostrain inoculums, type of LAB (homofermentative *vs* heterofermentative); (iv) LAB species used (with *L. buchneri*, with *L. plantarum* and with *P. acidilactici*), (v) Enzymatic additives addition (with enzymes *vs* without enzymes); (vi) Study duration (from 0 to 60 days *vs* more than 60 days); and (vii) silo type (laboratory or farm scale).

Heterogeneity among studies was assessed using the DerSimonian and the Laird test (Q-statistic). The degree of heterogeneity was quantified with the inconsistency index (I^2-statistic; Higgins and Thompson 2002). The goal of a meta-analysis is not only simply to report the mean effect size but also to report how the effect sizes in the various studies are dispersed about the mean. I^2 is a measure about the extent of inconsistency of findings across studies in the meta-analysis, and reflects the extent to which confidence intervals from the different studies overlap with each other (Borenstein *et al.* 2009).

An adjusted rank correlation test using the Egger method (Egger *et al.* 1997) and the Begg test (Begg and Mazumdar 1994) were used to assess publication bias. It was considered that there was bias if both statistical methods were significant ($P < 0{\cdot}01$). If there was any evidence of publication bias, the 'trim' and 'fill' method (Duval and Tweedie 2000) was applied to estimate the quantity and magnitude of missing studies and resultant unbiased effect size.

Results

Excluded studies

The literature search yielded 1902 scientific papers on corn silage inoculants. Of the studies identified at the beginning of the meta-analysis, 1727 were excluded on the basis of publication type: articles involving other forages species ($n = 365$), other additives ($n = 139$), or both of them ($n = 211$), reviews ($n = 166$), duplicate reports ($n = 190$), wrong topics ($n = 620$) and studies using inoculants for other purposes ($n = 36$) were disregarded. Seventy-one experiments which passed initial screening were rejected due to a lack of statistical information for conducting a meta-analysis ($n = 25$), studies conducted to assess the impact of certain pathogens like *Escherichia coli* ($n = 2$), papers using simulation models ($n = 11$), no full-text articles ($n = 19$), no English, Portuguese or Spanish language full articles ($n = 5$), and studies that analysed the efficacy of symbiotics ($n = 1$), yeasts or propionic acid bacteria ($n = 8$) (Fig. 1).

Overview of included studies

This meta-analysis included 104 articles (276 studies) assessing the effects of using LAB as inoculants on corn silage. Most of the research papers reviewed did not assess the LAB inoculants' effect over all the parameters under study. Consequently, the number of studies included in the meta-analysis differed in each variable considered (Table 1). Of the screened studies, 107 were conducted before 2008 and the remaining 169 after 2008. One hundred and forty-five studies included monostrain inoculum and 131 included multistrain LAB. Among monostrain inoculums, 50 studies were carried out using homofermentative LAB, whereas 86 utilized heterofermentative LAB. A total of 148 studies used *L. buchneri* (89 alone and 59 in combination with other LAB), 140 used *L. plantarum* (40 alone and 100 in combination with other LAB) and 24 used *P. acidilactici* with other

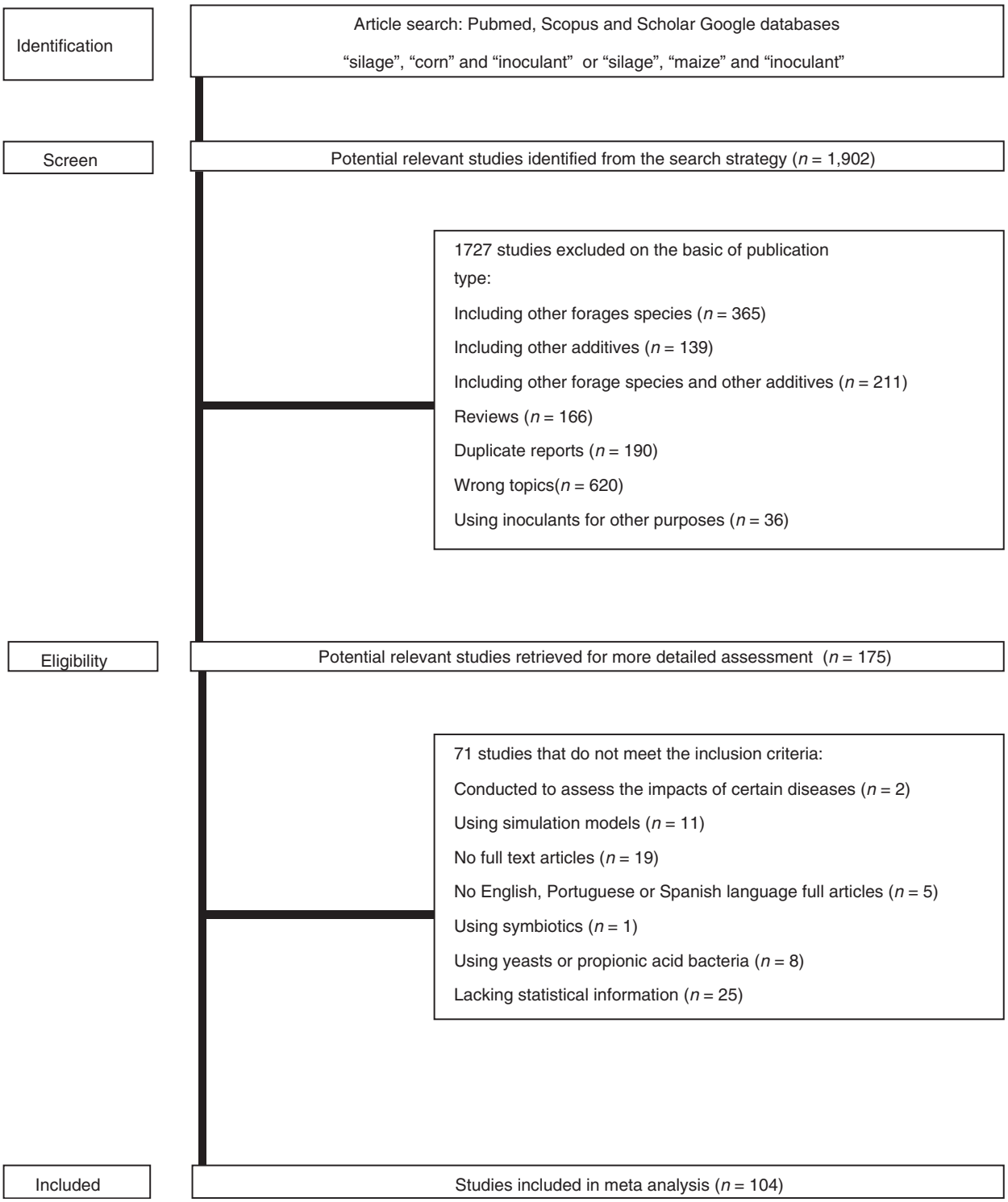

Figure 1 Study selection flow chart.

bacteria. Inoculants were administrated with enzymes (69) or without enzymes (205). Studies were conducted from 0 to 60 days (67), or for more than 60 days old (190). Two hundred and twenty-eight studies were carried out in laboratory-scale silos (glass and polyethylene bottles, plastic pouches and 20-l plastic buckets) (228),

Table 1 Effect of LAB inoculants on fermentation parameters, microbiological composition and aerobic stability of corn silage (% of DM, unless otherwise stated)

Response variable	Inoculated mean (SE)	Control mean (SE)	*n*	RMD	Lower limit	Upper limit	*P*-value	I^2 (%)
pH	3·86 ± 0·077	3·82 ± 0·077	248	0·041	0·020	0·062	**<0·001**	99·399
DM	34·39 ± 0·559	34·43 ± 0·567	204	−0·112	−0·246	0·021	0·100	98·141
DM recovery	94·55 ± 2·912	94·88 ± 3·093	60	−0·200	−0·583	0·184	0·308	81·891
NDF	48·89 ± 0·892	48·78 ± 0·899	142	0·211	−0·335	0·758	0·449	98·922
ADF	26·89 ± 0·668	27 ± 0·678	119	−0·230	−0·355	−0·106	**<0·001**	96·358
NH_3-N (% of total N)	5·70 ± 0·522	5·71 ± 0·528	106	−0·102	−0·157	−0·047	**<0·001**	99·741
Lignin	7·67 ± 0·611	7·56 ± 0·612	13	0·007	−0·610	0·623	0·983	81·599
ADIN (% of *N*)	16·07 ± 0·934	15·98 ± 0·907	10	0·353	−0·171	0·878	0·186	3·391
CP	8·08 ± 0·229	8·08 ± 0·226	139	0·014	−0·053	0·081	0·684	97·955
WSC	2·5 ± 0·182	2·87 ± 0·185	130	−0·411	−0·567	−0·256	**<0·001**	89·309
Ethanol	1·27 ± 0·096	1·13 ± 0·097	155	0·138	−0·016	0·291	0·078	99·672
Lactate	4·74 ± 0·286	4·8 ± 0·286	251	−0·068	−0·326	0·190	0·605	99·905
Acetate	2·18 ± 0·141	1·53 ± 0·139	236	0·658	0·535	0·781	**<0·001**	99·854
Propionate	0·19 ± 0·023	0·15 ± 0·023	71	0·038	0·020	0·055	**<0·001**	95·802
Butyrate	0·11 ± 0·035	0·11 ± 0·033	50	−0·005	−0·025	0·016	0·646	94·538
IVDMD–48 h	62·72 ± 1·488	63·02 ± 1·489	24	−0·263	−3·282	2·757	0·865	99·827
LAB (log CFU per g)	7·27 ± 0·248	6·40 ± 0·256	70	0·802	0·617	0·988	**<0·001**	99·692
Yeast (log CFU per g)	3·12 ± 0·533	4·17 ± 0·532	122	−1·034	−1·326	−0·742	**<0·001**	99·962
Mould (log CFU per g)	2·47 ± 0·49	2·81 ± 0·48	84	−0·199	−0·285	−0·113	**<0·001**	80·309
Clostridia (log CFU per g)	2·92 ± 0·232	2·96 ± 0·292	5	−0·104	−0·558	0·350	0·654	86·596
Aerobic stability (h)	150·38 ± 13·356	85·32 ± 13·825	94	66·518	33·587	99·449	**<0·001**	99·956

n = number of trials; RMD = raw mean difference between the inoculant treatment and control. Significant differences (*P* value) are highlighted in bold.

while 48 studies were executed in big drums or horizontal silos.

Corn silage parameters

The effects of LAB inoculation on silage quality across studies are shown in Table 1. In the pooled estimate, inoculation with LAB decreased silage ADF and WSC concentration ($P < 0·001$), but increased pH ($P < 0·001$) compared to controls in the pooled raw mean difference random effect model. In addition, LAB inoculation reduced NH_3-N concentration ($P < 0·001$) but did not affect DM ($P = 0·100$), DM recovery ($P = 0·308$), NDF ($P = 0·449$), lignin ($P = 0·983$), ADIN ($P = 0·186$), CP ($P = 0·684$) or IVDMD-48 h ($P = 0·865$). Acetate and propionate acid concentrations were increased ($P < 0·001$), whereas no effects were observed on ethanol ($P = 0·078$), lactate ($P = 0·605$) or butyrate ($P = 0·646$). Furthermore, LAB inoculation reduced ($P < 0·001$) the counts of yeasts and moulds and augmented ($P < 0·001$) LAB counts but did not affect clostridia ($P = 0·654$). Finally, LAB inoculation markedly increased aerobic stability ($P < 0·001$) in the pool estimate.

Significant heterogeneity (I^2 statistic >50%) was observed across all silage quality response variables, except for ADIN ($I^2 = 3·391\%$). Hence, subgroups were evaluated in order to identify sources of variability. In accordance with the subgroup analysis, inoculation increased pH ($P < 0·001$) in studies conducted before 2008, in laboratory-scale silos, in the absence of enzymes or with a monostrain inoculum. Among the group of monostrain inoculums, pH was significantly increased by inoculation of heterofermentative LAB ($P < 0·001$). Taking into account the duration of the experiments, the subgroup analysis indicated an increase in pH when they were more than 60 days old ($P < 0·001$; Table 2). LAB inoculant supplementation significantly decreased ADF in studies that used monostrain inoculants and when experiments were more than 60 days old ($P < 0·001$). Actually, ADF reduction was greater in studies less than 60 days old. However, there were no statistical differences ($P = 0·253$), which were primarily attributed to the fewer comparisons evaluated. Considering the silo type, the beneficial impact was observed in those experiments in which inoculants were included in farm-scale silos ($P < 0·001$) (Table 2).

Inoculants did not reduce NH_3-N ($P = 0·455$) when the experiments were carried out before 2008. However, analysing the experiments performed after 2008, a beneficial effect was observed ($P < 0·001$). NH_3-N accumulation was also reduced by multistrain LAB and, among the group of monostrain inoculums, by homofermentative bacteria ($P < 0·001$). Similarly, NH_3-N was significantly reduced when LAB were inoculated in farm-scale silos

Table 2 Subgroup analysis comparing the effects of silage inoculation with LAB on fermentation parameters, microbiological composition and aerobic stability of corn silage. There were no studies conducted with enzymes or for less than 60 days old for Clostridia

Response variables	Before 2008	After 2008	Mono-strain	Multi-strain	With *L. buchneri*	Without *L. buchneri*	With *L. plantarum*
pH	0·09 ± 0·028	0·01 ± 0·009	0·09 ± 0·015	−0·01 ± 0·010	0·09 ± 0·023	−0·01 ± 0·009	−0·01 ± 0·009
DM	0·11 ± 0·088	−0·21 ± 0·084	−0·003 ± 0·077	−0·24 ± 0·120	−0·09 ± 0·106	−0·15 ± 0·081	−0·10 ± 0·100
DM recovery	−0·29 ± 0·458	−0·11 ± 0·236	−0·48 ± 0·200	0·43 ± 0·399	−0·14 ± 0·241	−0·34 ± 0·364	−0·40 ± 0·273
NDF	−0·75 ± 0·284	0·56 ± 0·341	−0·09 ± 0·446	0·54 ± 0·247	0·98 ± 0·459	−0·36 ± 0·228	0·52 ± 0·216
ADF	−0·49 ± 0·207	−0·16 ± 0·071	−0·39 ± 0·084	−0·01 ± 0·132	0·03 ± 0·083	−0·39 ± 0·172	0·06 ± 0·161
NH_3-N (% of total N)	−0·004 ± 0·005	−0·12 ± 0·033	−0·07 ± 0·036	−0·15 ± 0·030	−0·06 ± 0·041	−0·15 ± 0·034	−0·08 ± 0·023
Lignin	0·78 ± 0·700	−0·59 ± 0·267	−0·57 ± 0·299	0·33 ± 0·513	−0·17 ± 0·152	0·16 ± 0·534	0·33 ± 0·513
ADIN (% of N)	0·57 ± 0·328	−0·12 ± 0·709	−1·00 ± 0·804	0·53 ± 0·267	−1·00 ± 0·804	0·53 ± 0·267	0·51 ± 0·274
CP	−0·06 ± 0·041	0·04 ± 0·041	−0·04 ± 0·067	0·02 ± 0·018	−0·01 ± 0·058	0·05 ± 0·046	0·02 ± 0·042
WSC	−0·53 ± 0·140	−0·32 ± 0·091	−0·51 ± 0·119	−0·30 ± 0·100	−0·39 ± 0·103	−0·45 ± 0·123	−0·36 ± 0·094
Ethanol	0·12 ± 0·055	0·15 ± 0·138	0·19 ± 0·127	0·06 ± 0·037	0·16 ± 0·131	0·10 ± 0·038	0·06 ± 0·036
Lactate	−0·24 ± 0·263	0·06 ± 0·090	−0·43 ± 0·253	0·30 ± 0·070	−0·46 ± 0·100	0·40 ± 0·189	0·53 ± 0·210
Acetate	0·90 ± 0·146	0·49 ± 0·046	0·91 ± 0·110	0·38 ± 0·030	1·21 ± 0·110	−0·02 ± 0·028	0·14 ± 0·026
Propionate	0·17 ± 0·062	0·01 ± 0·007	0·07 ± 0·019	0·01 ± 0·008	0·07 ± 0·014	−0·01 ± 0·008	0·01 ± 0·007
Butyrate	−0·01 ± 0·006	−0·002 ± 0·013	−0·02 ± 0·015	0·01 ± 0·009	−0·01 ± 0·015	−0·003 ± 0·005	0·01 ± 0·007
IVDMD–48 h	0·70 ± 0·563	−0·31 ± 1·588	0·11 ± 2·404	0·01 ± 0·310	−0·34 ± 0·602	−0·01 ± 1·862	−1·14 ± 1·759
LAB (log CFU per g)	0·73 ± 0·151	0·87 ± 0·172	0·86 ± 0·119	0·69 ± 0·115	0·86 ± 0·094	0·68 ± 0·117	0·70 ± 0·104
Yeast (log CFU per g)	−0·82 ± 0·223	−1·13 ± 0·124	−1·22 ± 0·179	−0·67 ± 0·155	−1·41 ± 0·099	0·18 ± 0·162	0·05 ± 0·142
Mould (log CFU per g)	−0·03 ± 0·027	−0·29 ± 0·062	−0·26 ± 0·060	−0·13 ± 0·064	−0·24 ± 0·053	−0·08 ± 0·041	−0·13 ± 0·061
Clostridia (log CFU per g)	−0·46 ± 0·224	0·09 ± 0·100	0·09 ± 0·098	−0·60 ± 0·081	0·14 ± 0·141	−0·20 ± 0·267	0·04 ± 0·141
Aerobic stability (h)	108·60 ± 76·877	33·31 ± 3·812	90·27 ± 27·618	26·44 ± 3·863	91·62 ± 26·018	1·54 ± 7·316	4·08 ± 2·481

($P < 0·001$) and when studies were more than 60 days old ($P = 0·003$). NH_3-N reduction was greater in studies less than 60 days old. Nevertheless, no significant effects were observed ($P = 0·059$), which was due to the lower number of comparisons found (Table 2). Inoculation with LAB reduced ($P < 0·001$) WSC concentrations, except in farm-scale silos ($P = 0·989$). Even so, since the number of experiments that used farm-scale silos was relatively small ($n = 18$), the effects must be interpreted with caution (Table 2).

Regarding organic acids, acetate concentrations were significantly increased in all conditions ($P < 0·001$), except when homofermentative LAB were applied ($P = 0·093$). Propionate was significantly increased in studies conducted before 2008 and by inoculation of heterofermentative LAB ($P < 0·001$). It was also increased ($P < 0·001$) when LAB was inoculated in laboratory-scale silos, in the absence of enzymes or with a monostrain inoculum. In view of the duration of the experiments, the subgroup analysis indicated an increase in propionate when they were more than 60 days old ($P < 0·001$; Table 2).

With respect to microbiological composition, LAB counts increased ($P < 0·001$), whereas yeasts and moulds counts were reduced ($P < 0·001$) when inoculants were administered, independently of the factors that could influence the treatment magnitude. However, no significant effects on moulds were evident in studies before 2008 ($P = 0·322$) and in those less than 60 days old ($P = 0·267$) (Table 2).

Lastly, aerobic stability was found to be higher in supplemented corn silage ($P < 0·001$), independently of the subgroup analysis. Nevertheless, inoculants had no effects when homofermentative LAB were used ($P = 0·758$) (Table 2).

The data obtained in this study showed crucial differences depending on the administration of homofermentative or heterofermentative LAB. Type of LAB was the most consistent factor influencing the silage quality response (Figs 2 and 3). For example, pH was increased by heterofermentative LAB inoculation ($P < 0·001$), but it tended to be reduced in silos with homofermentative LAB supplementation ($P = 0·056$) (Fig. 2). DM recovery was significantly reduced by heterofermentative LAB ($P < 0·001$), while no effects were observed with homofermentative bacteria addition ($P = 0·430$) (Fig. 2). Furthermore, inoculation with homofermentative LAB diminished NH_3-N ($P < 0·001$), but heterofermentative LAB did not affect this response variable ($P = 0·847$) (Fig. 2). As expected, the WSC concentrations were reduced when both types of LAB were added to the silos ($P = 0·007$ and $P < 0·001$ respectively) (Fig. 2). With respect to organic acids, lactate concentrations were increase by homofermentative LAB ($P = 0·002$) and reduced by heterofermentative LAB ($P < 0·001$) (Fig. 3). Inoculation with heterofermentative LAB increased acetate and propionate concentrations ($P < 0·001$) (Fig. 3). Nevertheless, acetate and propionate concentrations were not significantly influenced by homofermentative LAB ($P = 0·093$ and $P = 0·608$ respectively; Fig. 3).

Without *L. plantarum*	With *P. acidilactici*	Without *P. acidilactici*	With enzymes	Without enzymes	Less than 60 days old	More than 60 days old	Laboratory scale	Farm scale
0·09 ± 0·023	−0·01 ± 0·028	0·05 ± 0·012	−0·001 ± 0·015	0·06 ± 0·013	0·01 ± 0·014	0·05 ± 0·016	0·06 ± 0·014	−0·03 ± 0·012
−0·12 ± 0·098	−0·39 ± 0·195	−0·09 ± 0·074	−0·40 ± 0·112	0·03 ± 0·090	−0·16 ± 0·110	−0·10 ± 0·091	−0·06 ± 0·043	−0·19 ± 0·268
−0·14 ± 0·279	3·35 ± 0·683	−0·39 ± 0·193	0·21 ± 0·211	−0·73 ± 0·367	−3·98 ± 1·900	−0·03 ± 0·194	−0·23 ± 0·199	−0·54 ± 1·623
−0·19 ± 0·528	−0·70 ± 0·359	0·42 ± 0·321	0·43 ± 0·596	0·13 ± 0·216	0·35 ± 0·341	0·28 ± 0·368	0·39 ± 0·334	−0·38 ± 0·445
−0·36 ± 0·081	−0·97 ± 0·242	−0·15 ± 0·067	−0·15 ± 0·089	−0·11 ± 0·185	−0·27 ± 0·235	−0·25 ± 0·071	−0·04 ± 0·068	−0·62 ± 0·309
−0·10 ± 0·035	−0·23 ± 0·068	−0·09 ± 0·029	−0·10 ± 0·020	−0·14 ± 0·040	−0·13 ± 0·068	−0·09 ± 0·031	−0·003 ± 0·010	−0·32 ± 0·044
−0·57 ± 0·299	0·53 ± 0·639	−0·04 ± 0·335	−0·16 ± 0·146	0·27 ± 0·670	−0·02 ± 1·836	0·01 ± 0·324	0·16 ± 0·534	−0·17 ± 0·152
−0·26 ± 0·914	0·70 ± 0·282	−0·93 ± 0·574	0·41 ± 0·378	−0·29 ± 0·673	0·83 ± 0·396	0·07 ± 0·329	0·22 ± 0·379	0·28 ± 0·626
−0·01 ± 0·061	0·01 ± 0·019	0·02 ± 0·049	0·003 ± 0·029	0·004 ± 0·058	0·16 ± 0·063	−0·02 ± 0·052	−0·02 ± 0·025	0·12 ± 0·089
−0·48 ± 0·133	−0·32 ± 0·221	−0·42 ± 0·084	−0·35 ± 0·135	−0·44 ± 0·098	−0·41 ± 0·135	−0·42 ± 0·096	−0·47 ± 0·088	−0·002 ± 0·135
0·20 ± 0·135	0·14 ± 0·188	0·14 ± 0·081	0·03 ± 0·056	0·17 ± 0·098	0·57 ± 0·460	0·06 ± 0·036	0·13 ± 0·085	0·17 ± 0·079
−0·66 ± 0·124	0·41 ± 0·251	−0·11 ± 0·137	−0·01 ± 0·227	−0·09 ± 0·153	0·21 ± 0·180	−0·15 ± 0·189	−0·12 ± 0·164	0·24 ± 0·241
1·16 ± 0·113	0·20 ± 0·083	0·69 ± 0·070	0·95 ± 0·070	0·60 ± 0·078	0·53 ± 0·145	0·73 ± 0·074	0·68 ± 0·075	0·54 ± 0·105
0·07 ± 0·018	−0·01 ± 0·021	0·04 ± 0·010	−0·01 ± 0·021	0·05 ± 0·011	−0·02 ± 0·010	0·05 ± 0·011	0·05 ± 0·011	−0·01 ± 0·017
−0·02 ± 0·016	−0·01 ± 0·013	−0·004 ± 0·011	−0·001 ± 0·010	−0·01 ± 0·011	−0·03 ± 0·029	−0·001 ± 0·012	−0·01 ± 0·012	0·004 ± 0·009
1·81 ± 0·879	0·16 ± 0·625	−0·32 ± 1·718	1·49 ± 1·042	−1·02 ± 2·045	2·48 ± 0·992	−1·11 ± 1·573	−0·72 ± 1·643	3·18 ± 1·503
0·86 ± 0·101	0·52 ± 0·337	0·83 ± 0·099	1·42 ± 0·329	0·67 ± 0·105	0·56 ± 0·174	0·97 ± 0·219	0·88 ± 0·106	0·50 ± 0·263
−1·51 ± 0·098	−1·05 ± 0·803	−1·03 ± 0·152	−1·84 ± 0·262	−0·72 ± 0·176	−1·18 ± 0·353	−0·85 ± 0·088	−1·08 ± 0·157	−0·58 ± 0·310
−0·26 ± 0·067	−0·78 ± 0·490	−0·19 ± 0·044	−0·50 ± 0·055	−0·17 ± 0·045	−0·04 ± 0·032	−0·25 ± 0·055	−0·18 ± 0·045	−0·31 ± 0·127
−0·15 ± 0·303	−0·60 ± 0·081	0·09 ± 0·098	–	–	–	–	0·09 ± 0·100	−0·46 ± 0·224
106·25 ± 26·529	338·00 ± 33·234	63·64 ± 16·889	70·17 ± 7·149	58·32 ± 21·059	36·04 ± 10·254	68·98 ± 24·948	71·69 ± 17·916	13·53 ± 4·446

Meanwhile, ethanol concentrations were high with homofermentative and heterofermentative LAB addition ($P = 0{\cdot}011$ and $P < 0{\cdot}001$ respectively) (Fig. 3). All LAB types increased LAB and decreased mould counts ($P < 0{\cdot}001$) (Fig. 3). In spite of the counts of yeasts being lower with heterofermentative LAB ($P < 0{\cdot}001$), they were augmented when homofermentative LAB were applied ($P = 0{\cdot}005$) (Fig. 3). The addition of heterofermentative LAB significantly improved aerobic stability ($P < 0{\cdot}001$); however, homofermentative LAB had no effects on this parameter ($P = 0{\cdot}758$) (Fig. 3). Finally, regardless of the presence (or absence) of statistical differences, inoculation with homofermentative or heterofermentative micro-organisms showed an opposite behaviour in the following response variables: pH, DM, NDF, NH_3-N, CP, lactate, acetate, IVDMD-48 h, yeast counts and aerobic stability (Figs 2 and 3).

Considering the LAB species included in the inoculants, *L. buchneri* was able to induce a reduction in WSC concentrations, yeasts and moulds ($P < 0{\cdot}001$) and an increase in pH ($P = 0{\cdot}007$), acetate, propionate and LAB counts ($P < 0{\cdot}001$). However, the absence of this micro-organism also produced an increase of LAB in the treated group, and there were no significant differences ($P = 0{\cdot}224$). Additionally, the inclusion of *L. buchneri* induced a positive effect on aerobic stability ($P < 0{\cdot}001$). No effects were observed on this parameter when this species was not included ($P = 0{\cdot}834$). Conversely, a reduction in ADF and NH_3-N was visualized in the inoculated group in the absence of this micro-organism ($P = 0{\cdot}025$ and $P < 0{\cdot}001$ respectively) (Table 2).

An increase in pH ($P = 0{\cdot}009$) and a decrease in ADF, yeasts and aerobic stability was observed in the inoculated group in the absence of *L. plantarum* ($P < 0{\cdot}001$). The effect of inoculant supplementation on acetate was higher when *L. plantarum* was not included in the formulation than when this species was included ($P < 0{\cdot}001$). WSC diminish both in the presence and in the absence of *L. plantarum* in the inoculated group, and there were no significant differences ($P = 0{\cdot}474$). Similarly, the impact on NH_3-N, LAB and mould counts remained unchanged in those experiments irrespective of using *L. plantarum* as a strain, and there were no relevant variations ($P = 0{\cdot}556$, $P = 0{\cdot}282$ and $P = 0{\cdot}156$ respectively) (Table 2).

A significant increase in pH and propionate, and a decrease in WSC, was observed in the inoculated group in the absence of *P. acidilactici* ($P < 0{\cdot}001$). The effect of applying inoculants on ADF and aerobic stability remained in those experiments using *P. acidilactici* as a strain or not ($P < 0{\cdot}001$). However, inoculation with *P. acidilactici* reduced ADF to a greater extent than inoculation in the absence of this species ($P < 0{\cdot}001$). Acetate concentrations increased and NH_3-N decreased both in the presence and in the absence of *P. acidilactici* in the inoculated group ($P < 0{\cdot}001$). Nevertheless, since the number of experiments that made use of this micro-organism was relatively small, the effects must be interpreted with caution. There were also very few studies

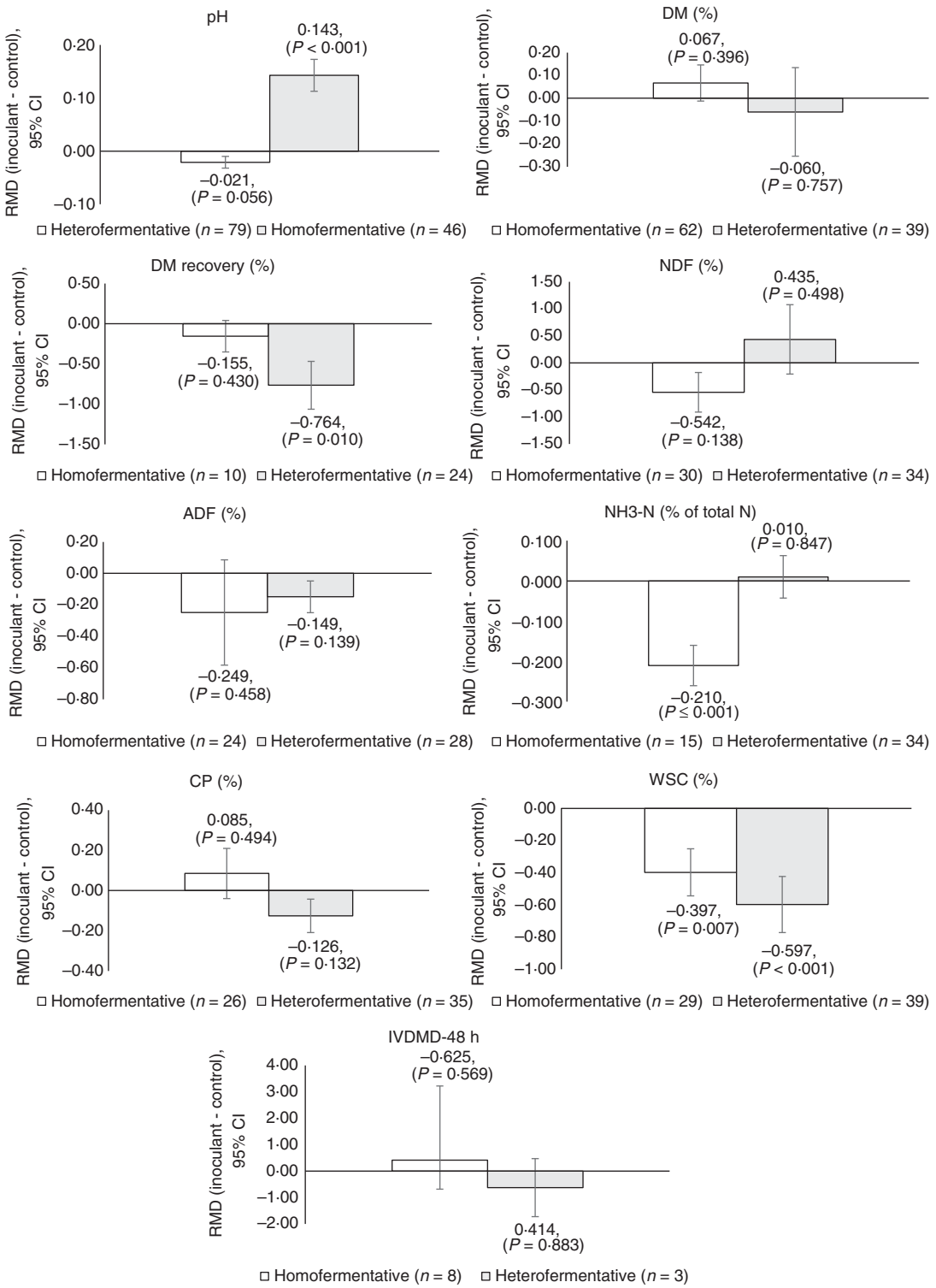

Figure 2 Subgroup analysis (subgroup = LAB type) of the effects of silage inoculation with LAB on fermentation parameters of corn silage. RMD = raw mean difference between the inoculant treatment and controls. White columns indicate homofermentative LAB treatment, grey columns indicate heterofermentative LAB treatment and the bars (within the columns) indicate the SE. Among monostrain inoculums, there were few comparisons for lignin (n = 3) and ADIN (n = 1).

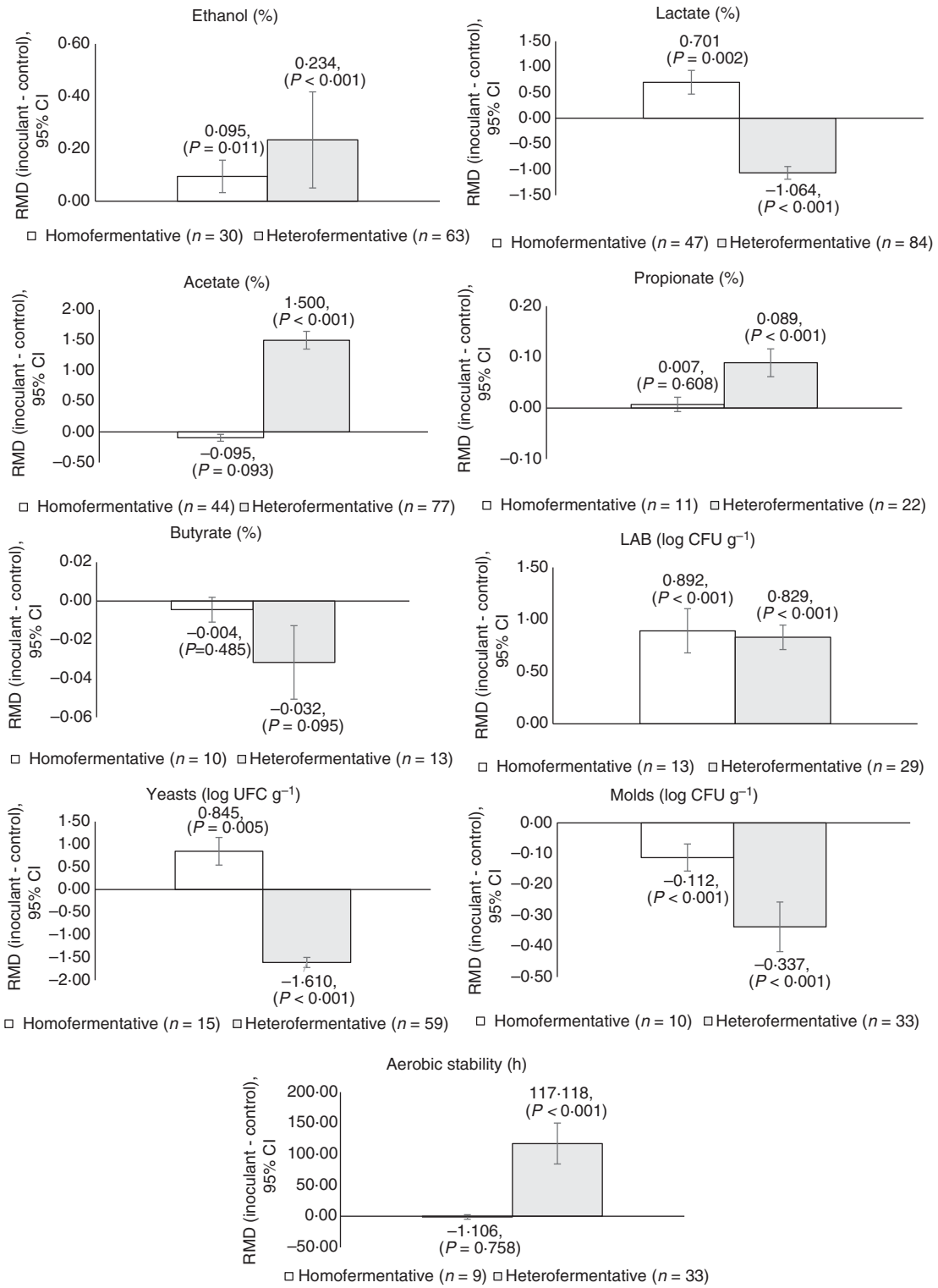

Figure 3 Subgroup analysis (subgroup = LAB type) of the effects of silage inoculation with LAB on ethanol, short-chain fatty acids, microbiological composition and aerobic stability of corn silage. RMD = raw mean difference between the inoculant treatment and controls. White columns indicate homofermentative LAB treatment, grey columns indicate heterofermentative LAB treatment and the bars (within the columns) indicate the SE. Among monostrain inoculums, there were few comparisons for Clostridia (n = 3).

comparing LAB ($n = 6$), yeasts ($n = 4$), mould counts ($n = 2$) and aerobic stability ($n = 1$) that included *P. acidilactici* (Table 2).

Based on the results from the meta-regression analysis, interactions were observed between the year of publication and pH ($P < 0{\cdot}001$), lignin ($P = 0{\cdot}010$), aerobic stability ($P = 0{\cdot}006$), NH_3-N ($P < 0{\cdot}001$), propionate ($P < 0{\cdot}001$) and clostridia ($P < 0{\cdot}001$). Moreover, the duration of the studies was associated with ADF ($P = 0{\cdot}003$), NH_3-N ($P < 0{\cdot}001$), CP ($P = 0{\cdot}017$), WSC ($P = 0{\cdot}048$) and LAB ($P = 0{\cdot}007$). Finally, the application rate of LAB was associated with pH ($P < 0{\cdot}001$), WSC ($P = 0{\cdot}0139$), acetate ($P = 0{\cdot}0211$), counts of LAB ($P = 0{\cdot}0346$), number of yeasts ($P = 0{\cdot}002$) and aerobic stability ($P < 0{\cdot}001$) in the meta-regression (Table 3).

A significant publication bias was observed for the response variables associated with pH and butyrate as confirmed by Begg's test ($P < 0{\cdot}001$) and Egger's test ($P < 0{\cdot}001$). The application of the Duval and Tweedie's trim and fill methods allowed for the identification of 111 and 18 studies trimmed and the adjusted value was −0·11509 (95% CI −0·13748 to −0·0927) for pH and −0·04129 (95% CI −0·06124 to −0·02134) for butyrate. In the remaining response variables, there were no evidences of publication bias (Table 4).

Discussion

This quantitative meta-analysis of data from randomized controlled experiments showed that the use of LAB inoculants increased pH in the pooled estimate. However, among monostrain inoculums, pH was significantly increased only by heterofermentative LAB inoculation. After an initial drop in pH, a moderately higher pH in silage treated with *L. buchneri* is a common finding (Kleinschmit and Kung 2006) because of the conversion

Table 3 Summary of random weighted meta-regression analysis for independent variables (year of publication, application rate of LAB and duration of studies) that influenced the effects between inoculated and uninoculated treatments for corn silage quality parameters (% of DM, unless otherwise stated)

Response variable	Meta-regression parameters											
	Year of publication				Application rate of LAB				Duration of studies			
	Intercept*	Slope	*P*-value	I^2 (%)	Intercept	Slope	*P*-value	I^2 (%)	Intercept	Slope	*P*-value	I^2 (%)
pH	11·4389	−0·0057	**0·0003**	99·08	−0·2784	0·0598	**<0·001**	99·43	0·0414	0·0001	0·6907	99·19
DM	35·4934	−0·0177	0·1647	98·09	−0·5208	0·0744	0·5982	98·26	0·0351	−0·0012	0·164	95·93
DM recovery	101·0655	−0·0505	0·1916	80·17	3·3609	−0·6583	0·3014	82·20	−0·1669	−0·0002	0·8659	82·79
NDF	−143·9995	0·0718	0·1298	98·92	1·5195	−0·2115	0·7028	99·08	0·1972	0·0011	0·7225	98·56
ADF	−4·5212	0·0021	0·8139	96·32	−0·5903	0·0799	0·4256	96·69	−0·4577	0·0033	**0·003**	95·92
NH_3-N (% of total N)	24·3495	−0·0122	**0·0369**	99·7	−0·1256	0·0047	0·9347	99·77	−0·2523	0·0015	**<0·001**	93·97
Lignin	267·984	−0·1333	**0·0095**	75·26	−5·7204	1·0241	0·8782	76·54	−0·974	0·0094	0·2612	79·13
ADIN (% of N)	94·8804	−0·0472	0·1011	<0·001	−8·8701	1·8377	0·6856	30·04	1·3081	−0·0144	0·2062	<0·001
CP	−13·3202	0·0066	0·2431	97·81	0·3995	−0·0730	0·4206	98·04	0·1463	−0·0012	**0·017**	98·12
WSC	−44·6442	0·022	0·1949	89·18	1·9331	−0·4311	**0·0139**	89·22	−0·7107	0·002	**0·0475**	88·84
Ethanol	−21·9936	0·011	0·5047	99·67	0·8509	−0·1293	0·2895	99·68	0·2795	−0·0011	0·2762	99·68
Lactate	−61·9618	0·0308	0·2061	99·9	1·3462	−0·2668	0·3219	99·91	−0·0542	−0·0005	0·812	99·91
Acetate	36·2972	−0·0177	0·096	99·85	−0·6357	0·2442	**0·0211**	99·86	0·4326	0·0019	0·0518	99·86
Propionate	13·9538	−0·0069	**<0·001**	95·84	−0·1218	0·0296	0·0643	95·86	0·0137	0·0003	0·1445	96·19
Butyrate	−1·0976	0·0005	0·7333	94·46	0·0546	−0·0109	0·5364	94·97	−0·03	0·0002	0·1725	94·57
IVDMD–48h	−44·2026	0·0218	0·9584	99·81	−2·1915	0·3418	0·8899	99·84	1·9176	−0·0236	0·5047	99·49
LAB (log CFU per g)	−23·9828	0·0123	0·436	99·68	−1·0060	0·3309	**0·0346**	99·70	0·2871	0·0048	**0·0067**	99·71
Yeast (log CFU per g)	−14·1867	0·0065	0·8441	99·96	3·0179	−0·7464	**0·0002**	99·96	−0·8791	−0·0011	0·6403	99·96
Mould (log CFU per g)	33·5868	−0·0168	0·1248	79·93	0·5532	−0·1414	0·0844	80·37	−0·3067	0·0009	0·1529	80·35
Clostridia (log CFU per g)	−69·3027	0·0345	**<0·001**	<0·001	−0·5982	0·1115	0·8302	<0·001	–	–	–	–
Aerobic stability (h)	20091·0728	−9·9639	**0·0064**	99·95	−267·2567	62·1793	**<0·001**	99·96	69·5926	−0·0218	0·9192	99·96

*Intercept: constant in the model. Significant differences (*P* value) are highlighted in bold.

Table 4 Results of publication bias detection (% of DM, unless otherwise stated)

Response variable	Fail-safe *N**	Begg and Mazumdar test	Egger's regression test	
			Intercept	*P*-value
pH	111	<0·001	6·77347	<0·001
DM	7	0·06217	−0·29806	0·62623
DM recovery	0	0·58335	−0·14287	0·69527
NDF	0	0·39526	−0·61631	0·54767
ADF	0	0·54581	−0·69145	0·17233
NH_3-N (% of total N)	5	0·37495	1·3999	0·49609
Lignin	3	1	1·52682	0·21937
ADIN (% of *N*)	0	1	−0·62585	0·30669
CP	0	0·02631	−0·38717	0·56296
WSC	24	0·88161	0·90366	0·43727
Ethanol	0	0·57225	−70·83795	0·02801
Lactate	0	<0·001	−2·7427	0·22603
Acetate	4	<0·001	2·9993	0·13913
Propionate	1	0·06926	0·77496	0·35075
Butyrate	18	0·00085	2·53536	0·00614
IVDMD–48h	13	0·00269	2·46659	0·69031
LAB (log CFU per g)	5	0·09736	0·77723	0·75979
Yeast (log CFU per g)	0	0·00112	0·57457	0·90885
Mould (log CFU per g)	0	0·01258	−0·60145	0·06392
Clostridia (log CFU per g)	3	1	2·15344	0·38609
Aerobic stability (h)	0	<0·001	−0·24485	0·9682

*Number of studies required to reverse the effects are calculated on the condition of $P = 0{\cdot}05$.

of lactic acid into acetic acid with CO_2 production (Oude Elferink *et al.* 2001). Driehuis *et al.* (2001) reported an increase in pH taking place during the storage phase because of the high metabolic activity of *L. buchneri* in these silages. The increase in studies using monostrains was probably because most of the trials involved employed *L. buchneri*. pH also increased with storage time since some LAB strains are capable of using lactic acid in anaerobic conditions when glucose becomes a limiting substrate for their metabolism (Lindgren *et al.* 1990).

In the present work, inoculation decreased ADF but did not affect NDF in the pool estimate. NDF and ADF are important quality parameters of silage. High contents can adversely affect quality and decrease digestibility. Temel *et al.* (2015) pointed that NDF and ADF are undesired structures in fodder crops. Degradation of cell wall content during fermentation may be considered positive to the process for providing soluble carbohydrates to fermentative micro-organisms and raising silage intake by animals (Junges *et al.* 2013).

The levels of residual WSC were substantially reduced in inoculated silages compared to that found in the controls. Increasing the inoculation rate of LAB further lessened WSC concentrations. Lower WSC content of inoculated silages may be a result of the higher microbial population and fermentation activity (Hassanat *et al.* 2007). Although WSC are substrates for lactate-assimilating yeasts and can induce the growth of deleterious micro-organisms (Filya and Sucu 2007), high residual WSC concentrations are desirable because they reflect a more efficient fermentation in the silo and indicate greater availability of energy-yielding substrates for ruminal microbes (Arriola *et al.* 2011). In agreement with the previously mentioned results, Rooke and Hatfield (2003) cited several studies in which the cell wall fraction was markedly decreased by hydrolases and stated that hydrolases are more likely to improve silage quality when the WSC concentration is low. The amount of NH_3-N in the inoculated group was lower compared with the control group. Among monostrain inoculums, NH_3-N was only reduced by homofermentative LAB inoculation. It has been described that inoculation reduced proteolytic activity, amino acid deamination and decarboxylation during ensiling and resulted in improved efficiency of silage protein utilization and reduced N losses (Charmley 2001; Scherer *et al.* 2015). According to McDonald *et al.* (1991), this effect arose as a result of the pH reduction with inoculation, which inhibits protein degradation in silages.

The results in our meta-analysis provide evidence that acetic and propionic values were greater in treated silages than in untreated ones, whereas no differences were observed in ethanol or lactic and butyric acids in the pool estimate. However, among monostrain inoculums, acetate and propionate concentrations were significantly increased only when heterofermentative LAB were applied. Hence, the fermentation of lactic acid by heterolactic LAB may be responsible for the higher production of acetic and propionic acids in treated silages. In addition, increases in acetic and propionic acid concentrations during long storage periods may indicate sustained activities of heterofermentative LAB species (Herrmann *et al.* 2011).

All treated silages had numerically higher numbers of LAB and lower counts of yeasts and moulds than the control in the pool estimate. Among the monostrain subgroups, yeast counts where only diminished by heterofermentative micro-organisms. The main factor responsible for the inhibition of yeasts and moulds was probably the increased concentrations of acetic and propionic acid produced by heterofermentative LAB, as these acids act on the metabolism of spoilage micro-organisms (Muck 2010). Their effective antimitotic activity is considered a key factor for this beneficial effect. However, shifting fermentation to a more heterolactic pathway increased DM

losses (Comino *et al.* 2014). For this reason, practical recommendations in the field have suggested a desirable lactic: acetic acids ratio higher than 3 : 1 (Kung and Stokes 2001). Associated with these lower number of yeasts and moulds was an improvement in aerobic exposition. In this respect, higher application rates of LAB increased acetate, reduced yeasts counts and improved aerobic stability. Enhanced aerobic stability is in line with the effects of inoculation with heterofermentative LAB, especially *L. buchneri* (Kleinschmit and Kung 2006; Kristensen *et al.* 2010). However, aerobic spoilage could be initiated by *Acetobacter* rather than yeasts, as they would most likely be immune to the acetic acid. Failure of inoculants designed to improve aerobic stability via the inhibition of yeasts in some trials could be due to this reason (Kung 2009).

In the studies summarized in this analysis, *L. buchneri*, *L. plantarum* and *P. acidilactici* were mostly administered as a multistrain inoculum due to the synergistic effects when bacteria are applied together. Accordingly, the infrequent use of individual inoculants (except for *L. buchneri*) may have limited our ability to detect LAB species-related impacts on measures of silage quality. Microbial additives based on classical homofermentative bacteria have been used to improve the efficiency of silage fermentations (Kung *et al.* 2003). Among homofermentative bacteria, *P. acidilactici* would rapidly decrease pH to 5 and dominate the early stages of ensilage, while *L. plantarum* would then further decrease the pH (Fitzsimons *et al.* 1992). However, using these types of organisms has sometimes made the silages less stable when they are exposed to air (Lynch *et al.* 2012) because there is less production of organic acids with strong antifungal characteristics. Since then, addition of heterofermentative bacteria like *L. buchneri* improves aerobic stability through the production of acetic and propionic acids (Mari *et al.* 2009). Conversely, treating silages with this organism has led to DM losses in corn silage (Wilkinson and Davies 2013). The undesirable characteristics of each type of microbial additive may be overthrown by combining several strains with different mechanisms of action that target different phases and aspects of the fermentation (Reich and Kung 2010).

Our meta-analysis showed that homofermentative and heterofermentative LAB exhibit different effects on silage quality. Regardless of the fact that there are several types of LAB inoculants, which have different purposes, corn silage fermentation is a complex process and a number of factors may explain why microbiological additives are not always effective: viability and storage conditions of the inoculants, crop characteristics (stage of maturity, moisture content, length of chop) and other ensilage management practices could be mentioned among the aforesaid factors. The lack of effects of LAB inoculation on some fermentation parameters could also be attributed to sufficient WSC concentrations for the fermentation and high epiphytic LAB populations in corn silage, which overcome any response associated with bacteria in the inoculant (Bolsen *et al.* 1992). The efficiency of fermentation of inoculated forages depends on the interactions of the microbial species in the inoculant with epiphytic microorganisms and chemical components within the forage (Muck 2010). Silage inoculants must produce their desired end-products in sufficient concentrations to affect specific epiphytic microbial populations (Kung 2009). Muck (2010) suggested that when an inoculant is added at a rate that is at least 10% of the epiphytic population, the inoculant always improves fermentation and when the inoculant is applied at less than 1% of the epiphytic population, the inoculant produces no significant changes in fermentation. However, factors not always described could affect how silages ferment when they are inoculated with LAB, and such factors need to be thoroughly assessed because they may explain the differences between the studies reviewed.

To our knowledge, this is the first meta-analysis to compare the application of homofermentative and heterofermentative LAB for corn silage. The addition of bio-inoculants significantly improved some fermentation characteristics. Reductions in ADF and NH_3-N accumulation were the most important benefits for the attainment of silage quality. LAB additives also enhanced microbiological composition and markedly improved aerobic stability. The findings of this meta-analysis suggest that the effect of inoculants may differ depending on the administration of homofermentative or heterofermentative LAB. Therefore, for the development of functional bacterial inoculants, both types of LAB should be involved, and further studies are necessary in order to identify proper silage inoculant combinations.

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Conflict of Interest

No conflict of interest declared.

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