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Full Length Research Paper

Effects of different bacterial inoculants on the fermentation and aerobic stability of whole-plant corn silage

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This study was conducted to evaluate the effects of bacterial inoculants on the fermentation and aerobic stability of whole-plant corn silage using different combinations of *Lactobacillus plantarum*, *Pediococcus acidilactici*, *Propionibacterium acidipropionici*, and *Lactobacillus buchneri*. Inoculation of silage with a combination of *L. plantarum* and *P. acidilactici* increased (P < 0.05) lactate concentration and lactate-to-acetate ratio but decreased (P < 0.05) pH, acetate and NH_3-N concentrations, and yeast count. Inoculation of silage with a combination of *L. plantarum* and *P. acidipropionici* had no effect on pH or concentrations of lactate, acetate, total organic acid, and NH_3-N, but negatively influenced aerobic stability. Inoculation of silage with *L. buchneri* increased (P < 0.05) pH and acetate concentration but decreased (P < 0.05) lactate and water-soluble carbohydrate concentrations. The pH value increased slowly following exposure to air. Yeast and mold did not multiply during the experimental period, and aerobic stability improved greatly. The results indicate that inoculation of silage with *L. buchneri* improved the fermentation and aerobic stability of whole-plant corn silage.

Key words: Bacterial inoculant, whole-plant corn silage, fermentation, aerobic stability.

INTRODUCTION

Whole-plant corn is a good crop for silage and has become the main feed source of dairy cows. However, aerobic corruption has been one of the main problems that limits the use and storage of this kind of silage. Inoculation with different bacterial inoculants is an effective way to improve silage quality. Inoculation of silage with a homofermentative *Lactobacillus* can quickly reduce pH and restrain microbial activity for long storage, but aerobic stability is negatively influenced (Contreras-Govea and Muck, 2006).

In contrast, inoculation of silage with a homo-fermentative *Lactobacillus* also can increase acetic acid concentration, decrease lactic acid production, reduce the lactate-to-acetate (L:A) ratio, and decrease dry matter (DM) recovery in the feed (Kleinschmit and Kung Jr., 2006). Our study was conducted to evaluate the effects of bacterial inoculants on the fermentation and aerobic stability of whole-plant corn silage using different combinations of *Lactobacillus plantarum, Pediococcus acidilactici, Propionibacterium acidipropionici*, and *Lactobacillus buchneri.*

MATERIALS AND METHODS

Bacterial inoculants

L. plantarum (MA18/5U), *P. acidilactici* (MA18/5M), *P. acidipropionici* (MA26/4U), and *L. buchneri* (NCIMB 40788) were obtained from Lallemand Animal Nutrition.

Experimental design

Four treatments were used to evaluate the effects of different bacterial inoculants and their combinations on the fermentation and

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aerobic stability of silage. The experimental treatments were as follows:

1. Control: silage without bacterial inoculants;
2. LP1 treatment: silage inoculated with a combination of *Lactobacillus plantarum* (5×10^5 CFU/g of fresh forage) and *P. acidilactici* (1.7×10^5 CFU/g);
3. LP2 treatment: silage inoculated with a combination of *Lactobacillus plantarum* (5×10^5 CFU/g) and *P. acidipropionici* (5×10^5 CFU/g); and
4. B treatment: silage inoculated with *L. buchneri* (5×10^5 CFU/g) alone.

Manufacture of whole-plant corn silage

Whole corn plants (Nongda 108) were harvested at the waxen maturity stage and dried overnight to reduce water content to 70%. The dried plants were then chopped to a length of 1 to 2 cm. The frozen inoculant powders were dissolved in water to make inoculant solutions for each treatment. To initiate the fermentation treatment, the microbial inoculant solution was sprayed evenly onto the dried plant particles and mixed thoroughly. The inoculant(s)-silage mixture was placed into a 2-L glass jar, squeezed and compacted, covered with a lid, and sealed. The silage jar was then maintained in a storage room without direct light exposure and fermented at room temperature. Thirteen glass jars were used for each treatment.

Analyses of silage quality and chemical components

Three jars per treatment were subsampled 90 days after ensiling for chemical and microbial analyses.

Quantification of yeast and mold

The culture dish coating method was performed in an ultraclean operating hood. Briefly, a 25-g sample of fresh silage was blended in 225 mL of sterile saline (0.85%, w/v) for 30 min. The water extract was filtered through four layers of cheesecloth and serially diluted (10, 10^2, 10^3, and 10^4 times). The diluted water extracts were poured onto Thayer-Martin agarose culture medium with 0.1 g/L Chloromycetin, distributed evenly, and cultured for 72 h at 30°C. The numbers of yeast or mold colonies were counted.

Measurement of pH

A 10-g sample of fresh silage was blended in 90 ml of deionized water for 5 min and filtered through four layers of cheesecloth. The pH of the water extracts was measured using a Mettler Toledo FE20/EL20 pH meter (Mettler-Toledo Inc; Columbus, OH, USA).

Determination of lactate, VFA, and NH_3-N

A 25-g sample of fresh silage was blended with 225 mL of deionized water and acidified to pH<2 with 50 µl of 50% (v/v) H_2SO_4. After 24 h at 4°C, the water extract was filtered through quantitative filter paper and stored at -20°C until analysis of lactate, acetate, propionate, butyrate, and NH_3-N. Briefly, lactate and VFA were determined using an ion chromatograph (DIONEX-2500) (Dionex; Sunnyvale, CA, USA) equipped with an AS11 ion chromatography column, AS50 automatic sampler, and electrical conductivity detector. The mobile phase was produced by an EG50 leachate (1% KOH, w/v) online generator. NH_3-N concentration was determined as described by Weatherburn (1967).

Chemical analyses

Samples from each treatment were air-dried, ground, and used for chemical analyses. DM was determined according to the Association of Official Analytical Chemistry (AOAC) International guidelines (1995a). Neutral detergent fiber (NDF) was determined as described by Van Soest et al. (1991). Acid detergent fiber (ADF) was determined according to AOAC International guidelines (1995b). Water-soluble carbohydrates (WSC) were determined according to Thomas (1977).

Measurement of aerobic stability

To determine the aerobic stability of silage inoculated with the different bacterial inoculants, pH variation, yeast and mold content, and temperature were measured after 90 days of ensiling. The pH value and yeast and mold contents were measured every other day for 10 days. Silage temperature was measured every 4 h until it reached 24°C (that is, 2°C above room temperature, 22°C). All silage samples were sterilized with ultraviolet treatment before the experiments (Muck, 2002; Nishino et al., 2003).

Data analysis and statistics

Data were analyzed by one-way analysis of variance (ANOVA) using the general linear models (GLM) procedure (SAS Institute Inc.; Cary, NC, USA). Differences were declared significant at $P < 0.05$.

RESULTS

Effects of different bacterial inoculants on pH and fermentation products in whole-plant corn silage

The effects of different bacterial inoculants on silage fermentation products are shown in Table 1. The pH value was reduced ($P < 0.05$) in the silage inoculated with LP1 but was unchanged ($P > 0.05$) in the silage inoculated with LP2. The pH value in the silage inoculated with B was higher ($P < 0.05$) than the pH value in the control and the other two treatments. Lactate concentration in the silage inoculated with LP1 was higher ($P < 0.01$) than lactate concentrations in the control and the other two treatments. Lactate concentration in the silage inoculated with LP2 was unchanged ($P > 0.05$). Lactate concentration in the silage inoculated with B was lower ($P < 0.01$) than lactate concentrations in the control and the other two treatments. Acetate concentration was reduced ($P < 0.05$) in the silage inoculated with LP1 and was unchanged ($P > 0.05$) in the silage inoculated with LP2. Interestingly, acetate concentration in the silage inoculated with B was higher ($P < 0.05$) than acetate concentrations in the control and the other two treatments. Inoculation of silage with the different kinds of microbial inoculants had no effect ($P > 0.05$) on total organic acid and NH_3-N concentrations. However, NH_3-N concentration in the silage inoculated

Table 1. Effects of different bacterial inoculants on fermentation products in whole-plant corn silage.

Parameter	Control	*L. plantarum* + *P. acidilactici*	*L. plantarum* + *P. acidipropionici*	*L. buchneri*
pH	3.70 ± 0.01^{b}	3.67 ± 0.02^{c}	3.69 ± 0.01^{b,c}	3.75 ± 0.01^{a}
Lactate (%)	7.62 ± 0.08^{b}	8.05 ± 0.10^{a}	7.72 ± 0.08^{b}	6.18 ± 0.17^{c}
Acetate (%)	1.81 ± 0.10^{b}	1.58 ± 0.03^{c}	1.66 ± 0.07^{b,c}	3.55 ± 0.06^{a}
Volatile fatty acids (%)	9.43 ± 0.18	9.63 ± 0.05	9.38 ± 0.14	9.73 ± 0.16
NH_3-N (%)	0.073 ± 0.002^{a,b}	0.070 ± 0.002^{b}	0.072 ± 0.002^{a,b}	0.075 ± 0.002^{a}

Data presented as means ± SEM. Statistical significance values between each group were P > 0.05 for a-a, b-b, c-c; P < 0.05 for a-b, b-c; and P < 0.01 for a-c.

Table 2. Effects of different bacterial inoculants on yeast and mold counts in whole-plant corn silage.

Parameter	Control	*L. plantarum* + *P. acidilactici*	*L. plantarum* + *P. acidipropionici*	*L. buchneri*
Yeast (CFU g^{-1} FM)	4.49	3.82	4.53	<2
Mold (CFU g^{-1} FM)	<2	<2	<2	<2

Table 3. Effects of different bacterial inoculants on chemical component contents in whole-plant corn silage.

Parameter	Control	*L. plantarum* + *P. acidilactici*	*L. plantarum* + *P. acidipropionici*	*L. buchneri*
DM (%)	25.54 ± 0.58	24.33 ± 0.20	25.50 ± 0.05	25.75 ± 0.04
NDF (%DM)	46.11 ± 1.55	46.74 ± 1.34	48.23 ± 1.57	48.40 ± 1.37
ADF (%DM)	27.12 ± 0.32	27.18 ± 0.50	27.51 ± 0.15	27.71 ± 0.39
WSC (%DM)	1.08 ± 0.02^{a}	1.07 ± 0.04^{a}	1.01 ± 0.07^{a}	0.35 ± 0.02^{b}

Data presented as mean ± SEM. Statistical significance was P < 0.05 for a-b. Dm: Dry matter; NDF: neutral detergent fiber; ADF: acid detergent fiber; WSC: water-soluble carbohydrates.

with B was higher (P < 0.05) than NH_3-N concentration in the silage inoculated with LP1. The effects of the different bacterial inoculants on yeast and mold counts are shown in Table 2. No mold was detected in any of the treatments. No yeast was detected in the silage inoculated with B. Yeast count in the silage inoculated with LP1 was lower than yeast counts in the control and the LP2 treatment (Table 2).

Effects of different bacterial inoculants on the chemical composition of whole-plant corn silage

The chemical compositions of silage inoculated with different bacterial inoculants are shown in Table 3. The different bacterial inoculants had no effect (P > 0.05) on DM, NDF, and ADF contents in the silage. However, WSC content in the silage inoculated with B was lower (P < 0.05) than WSC contents in the control and the other two treatments.

Effects of different bacterial inoculants on the aerobic stability of silage

Silage corruption could be determined by changes in pH during air exposure. As shown in Figure 1, pH increased rapidly after 24 h of air exposure in the control and the LP1 and LP2 treatments. By the end of the experiment (that is 10 days of air exposure), pH had increased to approximately 8.0. In contrast, there was no change in pH in the silage inoculated with B. The effects of the different bacterial inoculants on yeast and mold production in the silage during air exposure are presented in Figures 2 and 3. Except for the B treatment, yeast counts (approximately 10^5) increased dramatically during the first 96 h of air exposure for the control and the LP1 and LP2 treatments. After 96 h, counts were stable for the remainder of the experiment (up to 10 days).

Similarly, mold counts increased after 114 h of air exposure in the control and the LP1 and LP2 treatments and continued to rise until the end of the experiment.

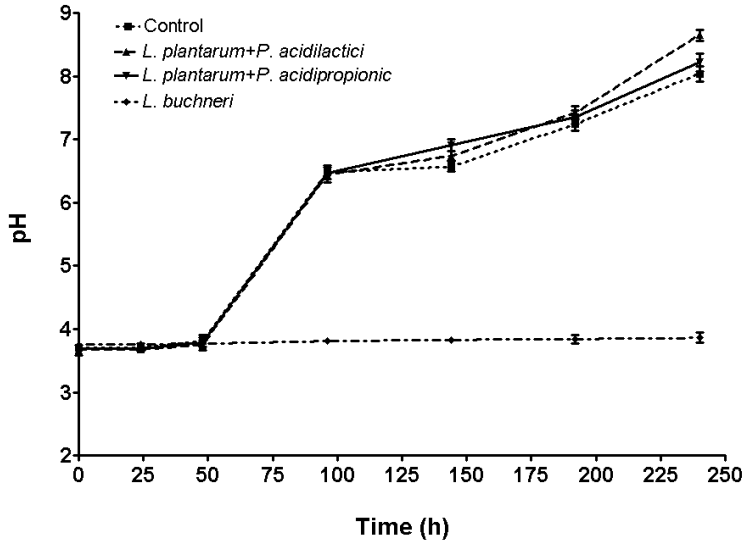

Figure 1. Variations in pH in whole-plant corn silage inoculated with different bacterial inoculants during air exposure.

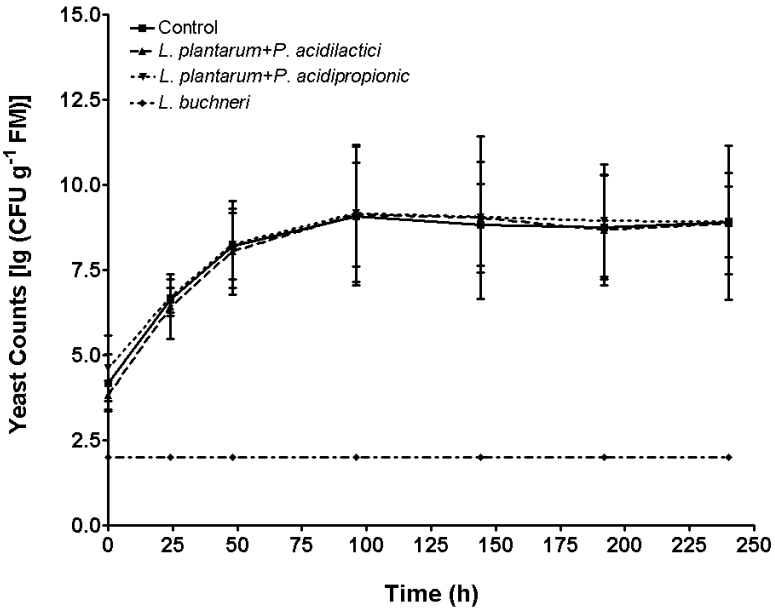

Figure 2. Effects of different bacterial inoculants on yeast counts in whole-plant corn silage during storage.

Interestingly, no mold was detected with the B treatment during the 10 days of the experiment. The time needed to increase temperature to 2°C above ambient temperature is important for determining aerobic stability in silage. In this study, aerobic stability was increased dramatically from 48 to 480 h in the silage inoculated with B (Table 4).

DISCUSSION

Aerobic stability (defined as the length of time that silage quality is maintained without molding or degenerating after exposure to air) is an important measurement for evaluating silage quality. In general, aerobic stability is important for maintaining silage quality. In addition to air, yeast content in silage is one of the factors that controls aerobic stability. Normally, silage with high yeast levels spoils rapidly when exposed to air, and silage with low yeast levels remains stable longer. Many products that claim to improve silage quality are now available. Currently, homofermentative lactic acid bacterial inoculants are widely used to improve fermentation and

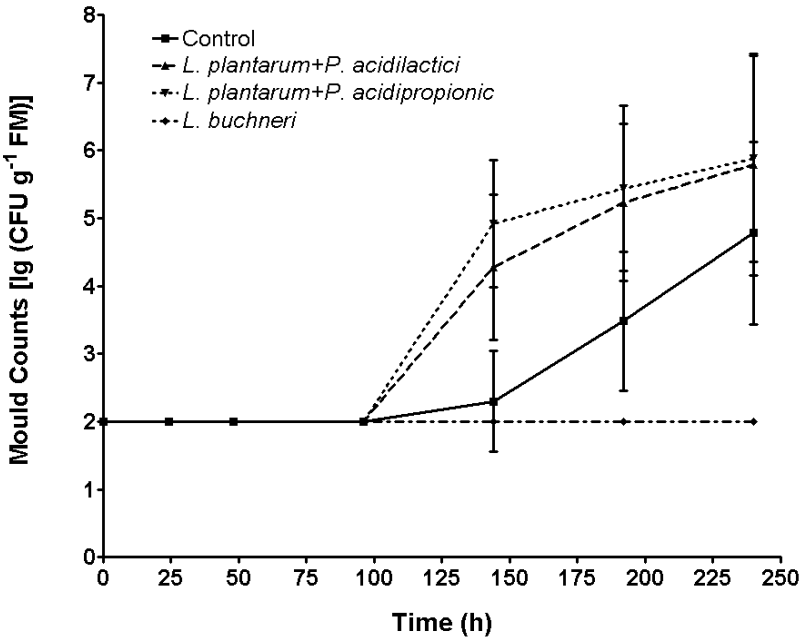

Figure 3. Effects of different bacterial inoculants on mold counts in whole-plant corn silage during storage.

increase DM and energy recovery of silage. However, most of these inoculants are less effective at inhibiting yeast and mold growth. The current study was conducted to evaluate the effects of bacterial inoculants on the fermentation and aerobic stability of whole-plant corn silage using different combinations of *L. plantarum*, *P. acidilactici*, *P. acidipropionici* and *L. buchneri*.

Inhibition of bacteria through lowered pH and decreased fungal growth through the elimination of oxygen during silage fermentation are the key factors in the production of high-quality and aerobically stable silage. The addition of homofermentative lactic acid bacteria to forage can enhance fermentation by increasing the concentration of lactic acid and reducing pH. Recent studies showed that the inoculation of silages with *L. plantarum* strain MTD1 improved DM intake and milk yield (Kung et al., 1977). However, inoculating silages with homofermentative lactic acid bacteria such as *L. plantarum* has not produced aerobic stability (Kung et al., 1991; Sanderson, 1993). Muck and Kung (1997) reported that inoculation improved aerobic stability in fewer than 30% of the studies conducted during the early 1990s. The two main reasons for this finding are as follows:

1. Relatively acid-tolerant yeasts, which assimilate lactate, are primarily responsible for silage spoilage following air exposure; and
2. Lactic acid, by itself, is not an effective antimycotic agent (Woolford, 1975; Moon, 1983).

In our study, bacterial inoculation with a combination of two strains of *L. plantarum and P. acidilactici* (LP1) or *L. plantarum and P. acidipropionici* (LP2) did not significantly affect L:A ratios and, thus, did not promote homolactic fermentation. Other indicators of improved homolactic fermentation such as reduced NH_3-N concentration and less residual WSC were also unaffected. These results suggest that the combined inoculation with these bacteria did not sufficiently affect dominant fermentation by epiphytic lactobacilli. Accordingly, no significant improvement or reduction in silage aerobic stability was detected in these experimental treatments compared with the control.

However, the current study found that the silage inoculated with B had the lowest lactate concentration and the highest acetate concentration (L:A ratio = 1.74) and the lowest pH during air exposure compared with the other treatments. According to Kleinschmit and Kung (2006), corn, green grass, and grain inoculated with *L. buchneri* degraded L:A ratios during fermentation, which decreases lactate concentration and increases acetate concentration and, hence, stabilizes silage pH during air exposure. Consistent with that report, we determined an 18.9% decrease in lactate concentration, a 96% increase in acetate concentration, and a relatively stable pH during 240 h of air exposure in the silage inoculated with B. To investigate pH, yeast and mold contents, and temperature changes when the silages were exposed to air, a second set of silages was sampled and analyzed periodically in addition to the first set of silages used for chemical content analyses. After 10 days of air exposure, pH and yeast and mold counts in the silage inoculated with B were lower ($P < 0.05$) than pH and yeast and mold

counts in the control and the LP1 and LP2 treatments. With the exception of the silage treated with *L. buchneri*, all silages were markedly spoiled within 2 to 3 days of air exposure. After 4 days of air exposure, yeast contents in the control and the LP1 and LP2 treatments increased significantly, and after 6 days of exposure, mold contents increased.

These observations suggest that there was no significant improvement in aerobic stability in the silages inoculated with LP1 and LP2. Unlike in the LP1 and LP2 treatments, yeast and mold contents remained below the detection limit during the 10-day experiment. Furthermore, the control and the LP1 and LP2 silages spoiled 2 to 3 days after air exposure. This time was extended to 20 days in the silage treated with *L. buchneri*. Because yeast fermentation is the main source of heat generation in silage following air exposure (Woolford, 1990), this dramatic increase in stability is likely because yeast growth was drastically inhibited by *L. buchneri* inoculants. Reportedly, acetate is the best yeast inhibitor during fermentation (Danner et al., 2003). We also detected a significantly high level of acetate in the silage inoculated with *L. buchneri.*

In summary, *L. buchneri* inoculation increased acetate production, stabilized pH, and inhibited yeast and mold growth in silage, which dramatically increased aerobic stability. Silage inoculated with *L. buchneri* is more resistant to heating at feed-out (exposure to air) compared with untreated silage and silage inoculated with LP1 or LP2. Our study demonstrated that silage inoculated with *L. buchneri* has the potential to dramatically improve aerobic stability in ensiled feeds and may significantly reduce feed waste induced by heating and molding of feeds during air exposure.

REFERENCES

AOAC International (1995a). Official Method of Analysis. 16th Ed. Association of Official Analytical Chemistry, Arlington, Virginia (United States of America). Method, pp.15-930

AOAC International (1995b). Official Method of Analysis. 16th Ed. Association of Official Analytical Chemistry, Arlington, Virginia (United States of America). Method, pp. 18-973.

Contreras-Govea F, Muck R (2006). Microbial inoculants for silage. Focus on Forage. 8(4): 1-4.

Danner H, Holzer M, Mayrhuber E, Braun R (2003). Acetic acid increases stability of silage under aerobic conditions. Appl. Environ. Microbiol., 69: 562-567.

Kleinschmit DH, Kung Jr. L (2006). A meta-analysis of the effects of Lactobacillus buchneri on the fermentation and aerobic stability of corn and grass and small-grain silage. J. Dairy Sci., 89: 4005-4013.

Kung Jr. L, Muck RE (1997). Animal response to silage additives. Silage: Field to Feedbunk, NRAES-99. NRAES, Ithaca, New York (United States of America), pp. 200-210

Kung Jr. L, Tung RS, Maciorowski K (1991). Effect of a microbial inoculant and/or glycopeptide antibiotic on fermentation and aerobic stability of wilted alfalfa silage. Anim. Feed Sci. Tech., 35: 37-48.

Moon NJ (1983). Inhibition of the growth of acid tolerant yeasts by acetate, lactate and propionate, and their synergistic mixture. J. Appl. Bact., 55: 453-460.

Muck RE (2002). Effects of corn silage inoculants on aerobic stability. Written for presentation at the 2002 ASAE Annual International Meeting. CIGR XVth World Congress. Chicago, Illinois, USA, July, pp. 28-31.

Muck RE, Kung Jr. L (1997). Effects of silage additives on ensiling. Silage: Field to Feedbunk, NRAES-99. NRAES, Ithaca, New York (United States of America), pp. 187-199.

Nishino N, Yoshida M, Shiota H, Sakaguchi E (2003). Accumulation of 1, 2-propanediol and enhancement of aerobic stability in whole crop maize silage inoculated with Lactobacillus buchneri. J. Appl. Microbiol., 94: 800-807.

Sanderson MA (1993). Aerobic stability and in vitro fiber digestibility of microbially inoculated corn and sorghum silages. J. Anim. Sci., 75: 505-514.

Thomas TA (1977). An automated procedure for the determination of soluble carbohydrates in herbage. J. Sci. Food Agric., 28: 639-642.

van Soest PJ, Robertson JB, Lewis BA (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74: 3583-3597.

Weatherburn MW (1967). Phenol-hypochlorite reaction for determinations of ammonia. Anal. Chem., 39: 971-974.

Woolford MK (1990). A review: The detrimental effects of air on silage. J. Appl. Bacteriol., 68: 101-112.

Woolford MK (1975). Microbiological screening of the straight chain fatty acids (C1-C12) as potential silage additives. J. Sci. Food Agric., 26: 219-228.