

Effect of varying proportions of *L. buchneri* and *L. plantarum* in silage additives



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Introduction

- Many silage additives have been developed using heterofermentative bacteria to increase acetic acid concentration and aerobic stability
- Purely heterolactic fermentation can have disadvantages in terms of decreased silage quality characteristics.
- To date, it is unclear how well different traits of homo- and heterofermentative bacteria develop when varying proportions of *Lentilactobacillus buchneri* (*L. buchneri*) and very competitive *Lactiplantibacillus plantarum* (*L. plantarum*).
- The aim of this study was to determine an ideal combination of *L. buchneri* and *L. plantarum* to improve silage quality and aerobic stability.

Methods

- Silage trail in glass jars was conducted over 90 days
- Ensiling material: perennial ryegrass (*Lolium perenne*) and oat-grass (*Arrhenatherum elatius*); dry matter (DM) content: 39%; sugar concentration: 14.1% DM

Table 1. Treatment groups with different proportions of *L. buchneri* and *L. plantarum*.

Species	Group					
	1/Control (n = 5)	2 (n = 5)	3 (n = 5)	4 (n = 5)	5 (n = 5)	6 (n = 3)
<i>L. buchneri</i> [%]	-	94	85	70	50	0
<i>L. plantarum</i> [%]	-	6	15	30	50	100

- Epiphytic population: lactic acid bacteria: $5.3 \cdot 10^7$ CFU/g fresh matter (FM); yeasts $4.1 \cdot 10^7$ CFU/g FM.
- All silage additives (groups 2-6) were applied at a concentration of $3 \cdot 10^5$ CFU/g FM (Table 1).

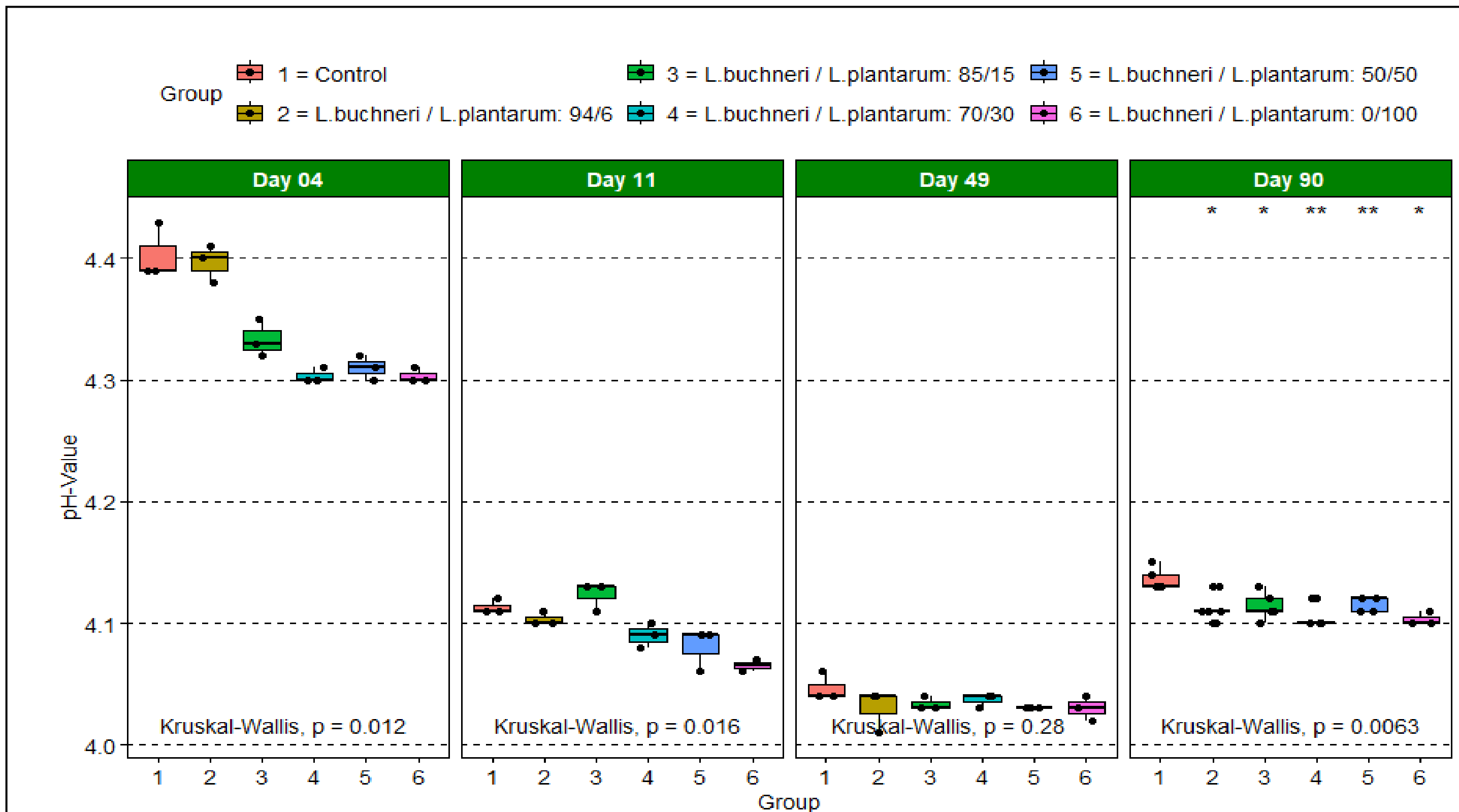


Figure 1. pH-values on days 4 (n = 3 per group), 11 (n = 3 per group), 49 (n = 3 per group), and 90 (n = 5 per group in groups 1-5, n = 3 in group 6) of a silage trail. Groups 2 to 5 vary in proportions of *L. buchneri* and *L. plantarum*, e.g. group 2 was inoculated with 94% *L. buchneri* and 6% *L. plantarum* at $3 \cdot 10^5$ CFU/g FM. Control group without additives. * p < 0.05; ** p < 0.01

Table 2. Mean (standard deviation) of fermentation characteristics of grass silage on day 90 after ensiling including 6 treatment groups (1 = control group without additives, and groups 2 to 6 with varying proportions of *L. buchneri*/*L. plantarum*: 2 = 94%/6%, 3 = 85%/15%, 4 = 70%/30%, 5 = 50%/50%, 6 = 0%/100%). Differences among groups were assessed using Kruskal-Wallis Test. Differences between treated groups and the control group were established using Mann-Whitney-U Test (* p < 0.05).

Fermentation characteristics	Group						P-Value
	1/ Control (n = 5)	2 (n = 5)	3 (n = 5)	4 (n = 5)	5 (n = 5)	6 (n = 3)	
Dry matter [g/kg]	377 (4)	368 (6)	364 (12)	367* (10)	358* (3)	368 (14)	0.036
Lactic acid [% DM]	9.99 (0.25)	10.04 (0.27)	10.02 (0.92)	9.96 (0.40)	10.21 (0.23)	10.07 (0.55)	0.8
Acetic acid [% DM]	1.68 (0.02)	2.17* (0.09)	2.08* (0.20)	1.90* (0.11)	1.75 (0.05)	1.49* (0.11)	<0.001
1,2-Propanediol [% DM]	0.07 (0.01)	0.33* (0.04)	0.29* (0.06)	0.21* (0.03)	0.18* (0.03)	0.10* (0.01)	<0.001
Ethanol [% DM]	0.41 (0.008)	0.41 (0.024)	0.40* (0.007)	0.39 (0.023)	0.35* (0.015)	0.35* (0.016)	0.001
NH3-N [% total N]	5.34 (0.14)	5.81 (0.69)	5.88* (0.23)	5.70* (0.14)	6.05* (0.10)	5.40 (0.43)	0.02
Aerobic stability [d]	12.6 (1.6)	16.5* (2.3)	15.5 (3.2)	11.3 (1.3)	10.0* (0.8)	10.4 (1.1)	0.002

Results

- On days 4 and 11, highest pH-values were observed in groups 1 to 3 and lowest values in group 6 (Figure 1).
- On day 90, silage additive treatments significantly affected pH-value (P = 0.002), concentrations of acetic acid (P < 0.001), 1,2-propanediol (P < 0.001), and ethanol (P = 0.001) as well as NH3-N of total N (%) (P = 0.02), and aerobic stability (P = 0.002, Table 2, Figure 2).
- Propionic acid and butyric acid were below detection limits.
- Acetic acid and ethanol concentrations were lowest in group 6 and highest in group 1 (ethanol) or 2 (acetic acid).

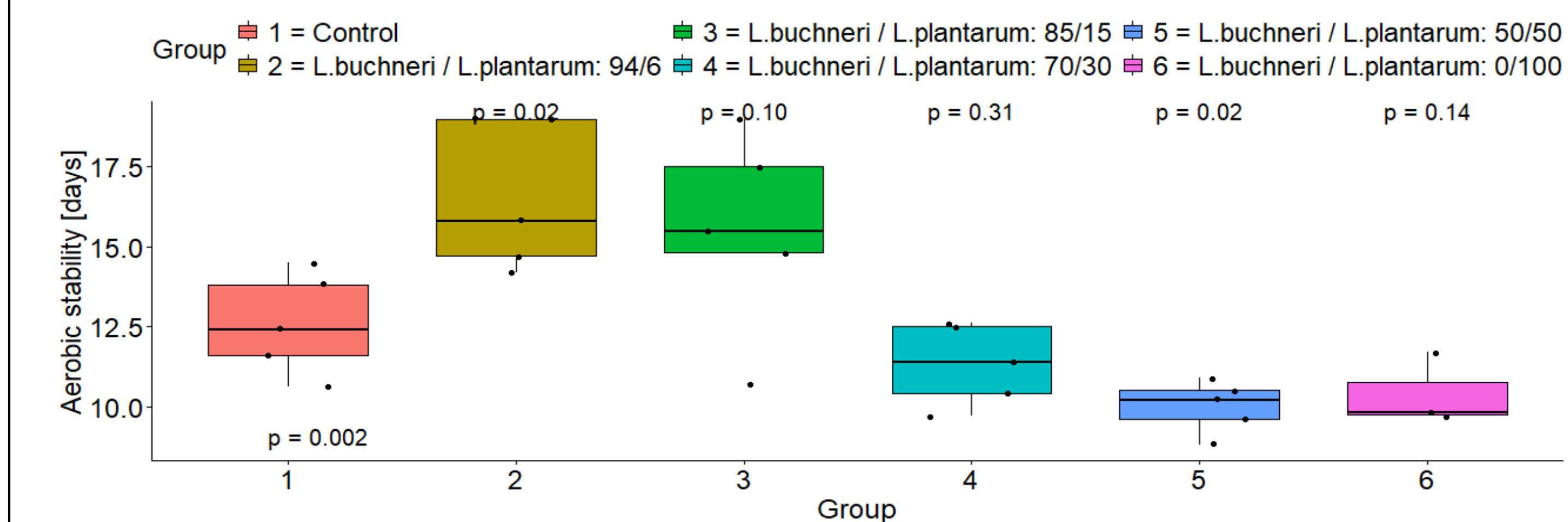


Figure 2. Aerobic stability 90 days after ensiling. Groups 2 to 6 vary in proportions of *L. buchneri* and *L. plantarum* e.g. group 2 was inoculated with 94% *L. buchneri* and 6% *L. plantarum*.

Conclusions

- Fermentation characteristics in silages inoculated with a mixture of homo- and heterofermentative bacteria can be directly influenced by proportions of bacterial species.
- A purely homofermentative silage additive has positive effects on silage quality in terms of pH-value, low acetic acid and ethanol concentrations, but no advantage in terms of aerobic stability.
- If an increased aerobic stability is desired, the effect of heterofermentative bacteria is determined by the proportions of bacterial species in the silage additive and the competitiveness of each species.