Fermentative profile, microbial and chemical characteristics and aerobic stability of whole crop soybean silage affected by the stage of growth and inoculation with lactic acid bacteria

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Introduction Finding new sources of home-grown protein is crucial for the future profitability and environmental sustainability of the dairy sector in Italy (Borreani et al. 2013). Soybean meal is the most frequently used source of protein in dairy cow diets, but its overseas origin poses many threats to the economic and environmental impact of milk production. In Italy, soybean is often double cropped with winter cereal, but an autumn harvest is, in most cases very difficult, due to difficulties in reaching the right maturity stages for grain harvesting (Undersander et al. 2007). Directly harvesting whole crop soybean and conserving it through ensiling could partially overcome these adverse effects and offer opportunities to increase the amount of on-farm produced protein (Mustafa and Seguin 2003). The aim of the study was to investigate the effects of stage of maturity and lactic acid bacteria (LAB) inoculant application on the fermentation quality, microbial and chemical characteristics of soybean grain harvested and ensiled as a direct-cut whole crop in the Po plain, northern Italy.

Material and Methods The research was conducted at the Research Centre of the University of Turin on loamy-sand soil. Two soybean varieties (VR) with low trypsin inhibitor activity (Ascasubi, VR1, medium-tall plant size, and Aires, VR2, low size plant, SIS, San Lazzaro di Savena, Bologna, Italy) were sown at the end of May (after wheat was harvested as whole-crop for ensilage) and then harvested at two stages of growth (R4-5, complete pod development and R7, early plant maturity). The whole crop was chopped to a theoretical length of 10 mm, using a self-propelled forage harvester (Claas Jaguar 960, equipped with a direct disc whole-crop header). The chopped materials were sampled for analyses and then ensiled as untreated (C) or treated (T) with a mixture of LAB (*Lactobacillus plantarum*, *Pediococcus acidilactici*, and *L. casei*, at an inoculation rate of $3x10^5$ cfu/g of wet weight), in 20-l laboratory silos, which were weighed and kept indoors for about 200 days. After 200 days, the silos were weighed, opened and silage aerobic stability (AS) was determined by monitoring the temperature increases and defined as the number of hours (h) the silage remained stable before rising more than 2°C above room temperature. The DM yield and grain-to-whole-plant ratio was determined at harvesting. The whole-crop was analysed prior to ensiling to establish the water activity, pH, buffering capacity (BC), water-soluble carbohydrates (WSC), starch, microbial counts (yeast, mould and LAB), ash, ether extract (EE), neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin, crude protein (CP, Dumas nitrogen 6.25), and in vitro NDF digestibility after 48 h (NDF-D). The silages were analysed, by means of HPLC, to establish the fermentative profiles, pH, ammonia, microbial counts (yeast, mould and LAB), ash, EE, NDF, ADF, lignin, CP and NDF-D. The significance of variety (VR), stage of growth (S) and LAB inoculation (T) effects were analysed via analysis of variance.

Results and Discussion The pre-ensiling characteristics of the two VR are reported in Table 1. The VR1 had lower WSC, starch, CP, nitrate and NDF-D and higher ash, NDF, ADF and water activity than VR2. The stage of growth affected almost all the measured characteristics, with the advanced stage of maturity showing a greater DM yield, grain to plant ratio, CP, NDF, ADF, lignin and lower BC, water activity, WSC, and starch and ash contents. All the silages, except the C silage of VR1 at the R4-5 stage, were well preserved. A huge fermentation activity, which led to high lactic and acetic acid contents, especially at the early stage of growth, was observed. The use of LAB inoculum reduced the risk of butyric fermentation, but failed to prevent it in VR1 at a lower DM content (Table 2).

Conclusion Directly ensiling whole crop soybean has been shown to be a feasible conservation option. The LAB inoculum reduced, but did not totally prevent butyric fermentation at lower DM contents. Harvesting at the R7 stage of growth increases the DM yields. Moreover, fermentation was found to be good, even without LAB inoculation.

Table 1. Pre-ensiling characteristics of two soybean crops at the R4-5 and R7 stages of growth.

Item	VR1		VR2		SE	*VR*	*S*	*VR·S*
	R4-5	R7	R4-5	R7				
DM yield (t ha^{-1})	5.28	5.61	4.86	5.33	0.066	**	*	NS
Grain/plant ratio	0.21	0.42	0.20	0.49	0.001	NS	***	NS
DM content (%)	26.7	37.4	22.0	30.9	0.002	***	***	NS
pH	6.34	6.05	6.13	6.14	0.029	NS	***	***
BC (meq kg^{-1} DM)	310	215	313	286	12.4	NS	*	NS
Water activity (a_w)	0.991	0.989	0.995	0.992	0.001	**	*	NS
Nitrate (mg/kg DM)	<100	<100	666	802	-	-	-	-
WSC (% DM)	3.7	3.6	7.6	5.4	0.160	***	**	*
Starch (% DM)	5.0	1.4	8.1	1.2	0.061	***	***	***
Crude protein (% DM)	16.7	18.4	20.8	25.0	0.607	**	*	NS
Ash (% DM)	11.6	9.2	8.0	7.4	0.087	***	***	**
Ether extract (% DM)	4.7	5.9	3.1	6.8	0.057	*	***	***
NDF (% DM)	43.3	47.0	35.4	39.3	0.544	***	**	NS
ADF (%DM)	35.6	38.3	26.7	30.6	0.483	***	**	NS
Lignin (% DM)	7.6	8.9	5.7	9.1	0.218	NS	***	NS
NDF-D (% NDF)	47.2	46.1	52.6	53.5	0.285	***	NS	NS
Yeast (cfu/g silage)	6.51	7.31	6.46	6.73	0.085	NS	*	NS
LAB (cfu/g silage)	7.92	9.16	8.31	8.64	0.095	NS	**	*

VR = variety effect; S = stage of growth effect.

Table 2. Fermentative, chemical and microbial characteristics of two untreated and LAB inoculated soybean silages harvested at the R4-5 and R7 stages of growth.

	VR1				VR2								
	R4-5		R7		R4-5		R7						
Item	C	T	C	T	C	T	C	T	*SE*	*VR*	*S*	*T*	*VR·S*
DM content (%)	22.9	23.9	34.1	36.2	20.5	20.9	27.2	28.1	0.154	***	***	*	***
pH	5.04	4.69	4.68	4.52	4.23	4.19	5.15	5.07	0.021	NS	***	**	***
Crude protein (% DM)	18.9	18.7	20.0	20.3	21.3	21.1	24.4	24.3	0.176	***	***	NS	***
NH_3 (% total N)	12.6	10.9	5.5	5.6	12.5	12.5	16.2	14.8	0.271	***	**	NS	***
Ash (% DM)	13.4	13.2	9.5	9.6	8.5	9.0	8.0	8.3	0.094	***	**	NS	***
NDF (% DM)	46.7	45.8	50.6	47.0	36.8	37.4	39.3	38.7	0.270	***	**	*	NS
ADF (% DM)	40.5	39.6	41.9	38.9	31.3	30.8	33.6	33.0	0.168	***	**	**	*
Lignin (% DM)	8.1	8.4	9.0	9.2	6.3	6.0	6.4	6.7	0.093	***	**	NS	NS
NDF-D (% NDF)	45.7	46.8	46.2	47.0	52.9	52.1	51.5	49.7	0.466	***	NS	NS	NS
Lactic ac. (g/kg DM)	14.7	43.4	37.7	54.4	116.3	122.1	17.7	25.2	2.808	***	***	NS	***
Acetic ac. (g/kg DM)	54.2	63.1	46.3	37.7	70.3	67.6	70.9	69.6	1.230	***	**	NS	**
Propionic ac (g/kg DM)	11.3	8.7	3.8	3.2	10.4	10.1	4.9	5.4	0.211	*	***	NS	NS
Butyric ac. (g/kg DM)	38.7	11.3	0.0	0.0	0.0	0.0	1.9	0.1	1.564	**	**	*	**
Ethanol (g/kg DM)	22.0	21.7	10.0	9.4	22.3	21.9	35.3	36.6	0.363	***	NS	NS	***
Weight loss (% DM)	12.2	8.3	4.5	4.6	7.7	7.4	8.5	8.5	0.371	NS	**	NS	***
Yeast (log_{10} cfu/g)	2.28	2.42	1.73	1.40	1.71	1.61	1.91	1.73	0.086	NS	NS	NS	NS
LAB (log_{10} cfu/g)	7.43	7.29	7.36	6.82	6.84	6.52	7.30	6.89	0.295	NS	NS	NS	NS
Aerobic stability (h)	>336	238	>336	>336	306	284	234	300	-	-	-	-	-

S = stage of growth effect; T = LAB inoculum effect; the VR·T, S·T, and VR·S·T interactions were not significant.

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