Paper ID: 895
Theme 2. Grassland production and utilization
Sub-theme 2.1. Quality, production, conservation and utilisation

Selenium enrichment of laboratory scale silos using lactic acid bacteria inoculum

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Keywords: Bio-available, Lactic acid bacteria, Nano-Se, Se Silage, Silage inoculants

Introduction

Selenium (Se) is a trace element essential for normal cellular function, which has been linked with reduced risk of cancer, cardiovascular disease, cognitive decline and thyroid disease in humans. Se deficiency in livestock is associated with white muscle disease, retained placenta, ill-thrift and mastitis. Where Se status or bioavailability from the soil for plants is poor, livestock rely on supplemental Se in their diets either as sodium selenite (inorganic form) or seleno-yeast (organic form). As lactic acid bacteria have been shown to incorporate Se as either organic or elemental (Nano-Se) (Eszenyi *et al*., 2011) there may be potential to use silage inoculant bacteria to improve the Se status of feed to provide Se requirements to livestock. In a previous study (Fleming *et al*., 2015) LAB isolates were screened for their ability to convert inorganic sodium selenite into Nano-Se and organic-Se (predominately Selenocysteine). Based on this ability and reduced retardation on growth properties when the Se was added, three LAB were selected for the current study to determine their potential as silage inoculants to increase bioavailable forms of Se (Nano and organic) in silage.

Materials and Methods

Three isolates were selected from the results of Fleming *et al*. (2015). A – *Lactobacillus brevis* DSMZ; B – *Lactobacillus plantarum* LF1; C – *Lactobacillus plantarum*

There were 3 treatments for each of the three isolates (A,B,C) each set up in triplicate at each opening time (destructive sampling at: 1, 2, 8, 30 and 90 days) in addition there was a negative control with no inoculum. This gave 150 mini silos. The treatments were:

- Isolate LAB (A, B or C) with no Se enrichment (LAB)
- Isolate LAB (A, B or C) grown in Se enriched media (787.7 mg Na_2SeO_3/L MRS) (Se LAB)
- Isolate LAB (A, B or C) with Se added (787.7 mg Na_2SeO_3/L MRS) at ensiling (LAB + Se)
- Control with no inoculant (Con)

The LAB culture medium contained 4×10^{12} CFU/L and the inoculum was added to silage at an application of LAB 1×10^{12} per ton fresh weight (FW). The inoculum contained 90 mg Se for 1 tonne silage FW to equate to livestock requirements (ca. 30 ug/kg DM) based on mean silage intake and DM. Permanent pasture ca. 0.5 tonnes FW was cut with a plot harvester (Haldrop 1500, J. Haldrop, Logstor, Denmark) and layed out on top of silage big bale wrap to wilt for 24 h. The grass was then passed through a forage harvester. Kilner® jars (1 litre, Lakeland Ltd., Windermere, Cumbria, UK) were used as mini silos and pre-weighed before filled with ca. 1 kg FW and sealed.

At day 1, 2, 8, 30 and 90 the relevant jars (n = 30) were opened. On the day of opening a fresh sample of silage was taken and a water extract performed to determine pH. Ammonia Nitrogen (NH_3-N), volatile fatty acid (VFA) and lactic acid analysis on the silage samples was performed by NIRS (Sciantec Ltd., UK). For total Se 0.1g of freeze-dried silage was weighed into an acid washed Teflon microwave digestion vessel, and 3 mL HNO_3 added. This was heated for 15mins at 120 ^oC, then allowed to cool before 1 mL H_2O_2 was added and the samples heated for a further 15 mins. The digest was then transferred to hot plate digestion vessels containing 12.5 mL HCl and heated at 120^oC for 1 h, before being transferred to 50 mL volumetric flasks and diluted to volume with de-ionised water and analysed by UV-HG-AFS. The system uses 0.7% m/v $NaBH_4$ in 0.1M NaOH as the reductant and 10% v/v HCl as reagent blank. A pre-reductant solution (50% v/v HCl with 5% m/v KBr) is used to reduce Se (VI) to Se (IV). A one-step enzymatic extraction with protease XIV was applied for Se speciation. An aliquot of freeze-dried sample (0.1 g) was accurately weighed into a 15 mL clean polypropylene centrifuge tube followed by the addition of 20 mg enzyme and 8 mL phosphate buffer (60 mM, pH 7.4). The samples were then capped tightly and put on an automatic shaker for 24 h at room temperature. After proteolysis, the

samples were centrifuged for 20 min. The supernatants filtered by 0.45 μm PTFE syringe filter and transferred into a clean vial. These solutions were then analysed by HPLC-UV-HG-AFS**.**

The results were first analysed by a simple t-test comparing each treatment against the Con for differences in fermentation parameters. For the ability to increase Nano and Organic Se in silage a repeated measure ANOVA was used with isolate (A+B+C) * treatment (LAB+LABSe+LAB+Se) with open day as the time course. The results in Table 1 refer to an ANOVA performed on the day 90 samples.

Results and Discussion:

Final fermentation parameters at the day 90 opening are shown in Table 1. The control silage (Con) was poor in quality with high pH, VFA and NH_3-N with negligible lactic acid detected. The addition of the three inoculants (A, B or C) significantly improved the quality of the silage reducing pH, VFA, NH_3-N and increasing lactic acid content. Previous studies has shown that the addition of sodium selenite reduced the growth of LAB (Fleming *et al.*, 2015), however this did not result in a reduction in their ability to act as silage inoculants with no difference between LAB, LABSe or LAB+Se treatments in terms of fermentation characteristics. Total Se, Organic-Se and Inorganic-Se were significantly higher for LABSe and LAB+Se than Con and LAB with no difference between isolates A, B or C.

Table 1: Mini silo fermentation characteristics and Se status at day 90

	Con	**LAB A**	**LAB B**	**LAB C**	**LAB Se A**	**LAB Se B**	**LAB Se C**	**LAB+ Se A**	**LAB+ Se B**	**LAB+ Se C**	**Sed**	**P**
pH	4.90	3.60	3.80	3.69	3.71	3.81	3.69	3.83	3.71	3.83	0.067	***
VFA (g/kg)	14.7	6.60	1.86	1.90	1.59	2.02	1.90	3.19	3.14	1.38	2.280	***
Lactic acid (%)	-	1.50	1.78	2.04	1.98	1.66	1.98	1.74	1.54	1.84	0.373	***
NH_3-N (g/kg)	13.7	7.70	4.77	4.93	4.50	4.80	4.57	5.63	5.47	4.37	1.627	***
Total Se (ug/kg)	19.3	5.9	9.4	13.0	81.9	85.5	98.6	94.6	73.8	102	3.30	***
Inorganic Se (ng/kg)	17.9	15.1	12.5	17.6	69.7	63.4	64.3	91.9	46.1	75.8	22.4	***
Organic Se (ng/kg)	28.1	29.5	29.9	35.1	136	81.3	133.6	86.0	87.8	115	18.0	***

Conclusion

The addition of sodium selenite either into the growth media of LAB (LABSe) or applied at inoculation of grass silage (LAB + Se) did not interfere with the ability of the LAB to act as a silage inoculant with no difference shown in silage fermentation characteristic between LAB with no Se added. The addition of sodium selenite either to the LAB growth medium or at inoculation resulted in the conversion of sodium selenite into Nano and Organic-Se (Nano ca. 10^3 higher than organic), as previously shown in culture experiments (Fleming *et al.*, 2015). There was no difference shown between LAB isolates A, B and C. There is potential to develop silage inoculants to increase the bioavailable form of Se (Nano and organic) to livestock through conversion of inorganic forms during ensiling.

References

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Acknowledgement

This work was part of the Development of silage inoculants to improve mineral composition of animal products 'Silage' project co-funded by the Technology Strategy Board (TSB) and Biological and Biotechnology Scientific Research Council (BBSRC) with industrial support from Silage Solutions Ltd and Kelvin Cave Ltd.