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Effect of invasive slug populations (*Arion vulgaris*) on grass silage I. Fermentation quality, *in-silo* losses and aerobic stability

Åshild Taksdal Randby[a,*], Kristine Gismervik[b], Arild Andersen[c], Ida Skaar[d]

[a] Norwegian University of Life Sciences, POB 5003, N-1432 Ås, Norway
[b] Norwegian Veterinary Institute, Pb 5695 Sluppen, 7485 Trondheim, Norway
[c] Norwegian Institute for Agricultural and Environmental Research, Fr. A. Dahlsvei 20, 1430 Ås, Norway
[d] Norwegian Veterinary Institute, Pb 750 Sentrum, 0106 Oslo, Norway

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ABSTRACT

This study aimed to explore how invasive slug populations of *Arion vulgaris* influence fermentation quality, *in-silo* losses and aerobic stability of grass silage, and the efficiency of silage additives and wilting to improve the quality of silages from slug contaminated crops. The effect of four levels, including control, of a slug contaminated grass crop, was evaluated in laboratory scale. The crop used was wilted to two dry matter (DM) levels: low (253 g DM/kg) and high (372 g DM/kg). Adult slugs were applied to the low DM crop corresponding to 5 (low level), 10 (medium) and 20 (high level) 7-g sized *A. vulgaris* per m^2 in an assumed harvested regrowth yield of 2.5 ton DM per ha. For the high DM crop, the applied slug levels corresponded to 6 (low level), 12 (medium) and 24 (high level) slugs per m^2.

At low DM level, the effect of four additive treatments, control (C), inoculation with *Lactobacillus plantarum* (LP), a formic, propionic and benzoic acid mixture (ACID) and a chemical additive containing benzoic acid, $NaNO_2$, hexamethylenetetramine and propionic acid (CHEM) were tested. Increasing slug contamination gave increasing quality reductions both in silages containing 253 and 372 g DM/kg. Compared with untreated silage, LP-treatment did not improve silage fermentation quality of contaminated crops. Treatment with ACID and CHEM, however, considerably improved the quality of heavily contaminated silages. The much higher crude protein concentration in slugs compared to grass crop made slugs a more “difficult-to-ensile” material. Wilting of the harvested crop to 372 g DM/kg was not sufficient to control silage fermentation of slug contaminated crop. With contamination levels from 138 to 553 g fresh slug weight/kg crop DM, efficient silage additives were able to ensure acceptable fermentation quality of grass silages.

Abbreviations: ACID, formic, propionic and benzoic acid mixture; AA, acetic acid; BA, butyric acid; BC, buffering capacity; C, control; CHEM, chemical additive with benzoic acid, $NaNO_2$ hexamethylenetetramine and propionic acid; CP, crude protein; DM, dry matter; FA, formic acid; FC, Fermentability coefficient; LA, lactic acid; LAB, lactic acid bacteria; LP, *Lactobacillus plantarum* inoculation; N, nitrogen; NADPH, Nicotinamide adenine dinucleotide phosphate; PA, propionic acid; WSC, water soluble carbohydrates.

* Corresponding author. Tel.: +47 67232689; fax: +47 64965101.
E-mail addresses: ashild.randby@nmbu.no (Å.T. Randby), stine.gismervik@vetinst.no (K. Gismervik), arild.andersen@nmbu.no (A. Andersen), ida.skaar@vetinst.no (I. Skaar).

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1. Introduction

Extensive invasions by the slugs *Arion vulgaris* (in name confusions also referred to as *Arion lusitanicus*) onto farmland have recently been reported in northern Europe during wet summers (Spörndly and Haaga, 2010). The slugs originate from a relatively dry climate in South France, where it operates in low densities, but have been wide spread in Europe the last decades. Adult slugs weigh around 10 g in August, with variation from 3 to 27 g within and between locations (Briner and Frank, 1998a). Dense populations of more than 50 slugs per square meter have been reported from wildflower strips and meadows (Briner and Frank, 1998b). As a consequence, high amounts of slugs might contaminate grass silage and cause a potential threat to animal feed quality.

Silage is the main form of preserved grass and other forages offered cattle and sheep in Europe and North America. Anaerobic conditions and rapid lowering of pH by lactic acid fermentation are essential to obtain high quality silage. Forage composition (dry matter (DM), soluble sugars and nitrate, buffering capacity and lactic acid bacteria (LAB) content) together with the silage-making technique determines the success of the conservation process and thereby the feed quality (Driehuis and Oude Elferink, 2000). In order to control the silage fermentation process, a large number of additives (generally assigned either fermentation stimulants or inhibitors) are commercially available. Still, the use of silage additives varies a lot among countries. In Norway, for instance, approximately 3/4 of silages are additive treated (Wilkinson and Toivonen, 2003), tower and bunker silages to a greater extent than round bale silages. The ensiling process is very complex and may be hard to control especially with low DM grass harvested under suboptimal conditions like rainy weather. Under such conditions are also slugs being active, and Swedish farmers experienced unspecific symptoms in cattle offered slug contaminated silage harvested during the wet summer in 2007. Addressing this problem, Spörndly and Haaga (2010) studied slug contaminated silage, untreated or treated with an acidic additive. They used juvenile slugs of 0.64 g in the primary growth, in relevant contamination levels for early summer, and concluded that silage quality was not severely affected, possibly due to high levels of LAB detected in the slugs. Slugs increase their weight considerably from early to late summer. It was therefore necessary to investigate slug contaminated silage also from the regrowth harvest. This study aimed to explore how invasive slug populations of *A. vulgaris* influence fermentation quality, *in-silo* losses and aerobic stability of grass silage and the efficiency of silage additives and wilting to control silage fermentation of slug contaminated crops. Microbiological aspects of the same silages are reported and discussed elsewhere (Gismervik et al., 2014).

2. Materials and methods

2.1. Experimental design

The effect on silage quality of four levels, including control, of a slug contaminated grass crop, was evaluated in a laboratory scale ensiling study. The crop used was wilted to two different DM levels: low (253 g DM/kg) and high (372 g DM/kg). At low DM level, the effect of four additive treatments, including control, was tested in a 4 × 4 factorial arrangement with slug contamination using three replicates (altogether 48 silages). At high DM level, the four levels of slug contamination were tested with three replicates without any additive treatment (12 silages).

2.2. Crop for ensiling

The harvested crop was from the first regrowth of a fifth year organic meadow at the Norwegian University of Life Sciences, Ås, Norway. The sward was initially sown with 15 kg/ha of timothy (*Phleum pratense*) and 7 kg/ha of red clover (*Trifolium pratense*), but contained additionally a variety of herbs, legumes and weeds when harvested. In 2012 the sward was fertilized with 30 ton per ha of diluted cattle manure on 2 May, and, following the primary growth harvest, with 15 ton per ha on 15 June. It started raining on 16 June, and total precipitation until the regrowth harvest was 245 mm. The crop was mown 13 August 2012 at 12 h, and wilted in the field for 2 h. Crop was filled into netting bags that allowed air to pass through for further wilting. The bags were stored indoors at 12–15 °C overnight, because showers were expected. Low DM crop was ensiled on 14 August. Crop for high DM silage was further wilted outdoors in the sun, spread on a trailer. The trailer was moved indoors overnight. High DM crop was ensiled on 15 August. Crops were chopped to approximately 30–50 mm length. The grass crop was not naturally contaminated by slugs.

2.3. Slugs for ensiling

A. vulgaris were collected on four locations in South-East Norway (Horten municipality). All slugs were collected by night at about 15 °C, 672 slugs on 12–13 and 13–14 of August (used for low DM silages) and 343 slugs on 14–15 of August (used for high DM silages). The slugs were kept in plastic boxes (36 × 27 × 17 cm), 53–61 slugs from the same location in each box. Average weight of the slugs per box was calculated by dividing grams by number, and overall mean weight for slugs collected were 7.0 g (low DM) and 6.1 g (high DM silages). To prevent the slugs from emptying their intestine, they were offered fresh plant leaves (*Taraxacum officinale*, *Dactylis glomerata*, *Rumex longifolius*, *Trifolium repens* and *T. pratense*) from the locations they were collected. Slugs were kept at 18–19 °C until ensiled.