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Use of Fresh *Scotta* Whey as an Additive for Alfalfa Silage

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Abstract: *Scotta* is a dairy industry waste obtained by ricotta cheese production. Because of its high availability and its high lactose content, *scotta* could be used as an additive to improve fermentation characteristics of alfalfa silage. Silage samples were obtained for 2 years from an alfalfa second cut, collecting the forage at three growth stages: mid bud (MB), early flowering (EF), and late flowering (LF). After wilting the forage at 38% dry matter (DM), four *scotta* doses were added (0, 75, 150, and 300 g/kg fresh forage) and the main chemical and microbiological characteristics were evaluated after 3, 13, and 90 days of ensiling (DOE). The lowest pH (4.3) was recorded in the EF and LF growth stages, after 90 DOE and with the highest *scotta* dose (300 g/kg fresh forage). After 90 DOE, the concentration of the main spoilage microorganisms and clostridial spore loads was always negligible. The addition of *scotta* decreased pH and fiber fractions, increased the relative feed value, and had no effect on the crude protein concentration or the total digestible nutrients. Therefore, the *scotta* can be successfully used as an additive to improve the fermentation characteristics of alfalfa silage.

Keywords: alfalfa; silage additive; *scotta*; fermentative bacteria

1. Introduction

Legumes are difficult to ensile successfully without an additive, and this is especially true for alfalfa (*Medicago sativa* L.). McDonald [1] reported that the difficulty of ensiling legumes was attributable to three factors: i) legumes are highly buffered, ii) they tend to have a low water-soluble carbohydrate (WSC) content, and iii) they often have low dry matter (DM) content. As a result, it is difficult to quickly reduce the silage pH, minimize clostridia growth, proteolysis, and heterofermentation, and improve silage palatability. Excessive moisture interferes with the rapid proliferation of lactic acid-producing bacteria, leading to clostridia growth and effluent outflow [2,3]. In fact, it has been recommended that alfalfa should be ensiled with 30–40% DM [4]. Several works have reviewed the effect of wilting on dry matter losses, silage quality parameters, and animal performance [3,5,6].

The stage of alfalfa maturity at harvest significantly influences the concentration of nutrients, and the right harvesting date is thus very important [7–10]. Lloveras et al. [7] reported that cutting at full bloom stage increased the DM yield compared with cutting at the late bud stage. However, the crude protein (CP) decreases with the advancing of stage of maturity, and the structural carbohydrates increase [10]. Alfalfa shows a low level of WSC, and some authors have found that WSC decreases with advancing maturity [10,11]. It is therefore necessary to use some additives to increase the supply of available carbohydrate substrates for the growth of lactic acid bacteria, to inhibit the activity of aerobic bacteria, or to decrease the loss of WSC in the early stage of ensilage [12]. Treatments with alfalfa

silage additives, such as previously fermented juice [13,14], lactic acid bacteria [15,16], and sucrose [1], may improve the fermentation quality of alfalfa silage.

Ricotta cheese whey, also known as *scotta*, is a valuable source of the lactose (about 4% w/w) [17], so it could be used as a silage additive. To date, most of *scotta* is used as a supplement feed for livestock. In addition, the growing popularity of ricotta cheese in Europe means that the subsequent disposal of increasing amounts of *scotta* will be a problem. In Italy alone, *scotta* production amounts to about 0.5 Mt y^{-1} [17].

The aim of this research was to evaluate whether the *scotta* whey could be used as an additive to ameliorate the fermentation characteristics of alfalfa silage.

2. Materials and Methods

2.1. Fresh Forage and Silages

The research was carried out in 2014 and 2015 in a field at the Enrico Avanzi Interdepartmental Centre of Agro-Environmental Research (CIRAA) of the University of Pisa and in the laboratory at the Department of Veterinary Science of the University of Pisa, Italy. The treatments compared in this research consisted of three growth stages at harvest, four *scotta* doses, and three different opening times of the silages. Three replicates for each treatment were performed. The ensiled forage consisted of second cuts from two 3-year old stands of alfalfa (cv Messe) that were established in adjacent plots in 2012 and 2013. Forage was harvested with a sickle-bar mower at three growth stages: mid bud (MB), stage 3.5 of the Kalu and Fick [18] scale, early flower (EF), stage 5, and late flower (LF), stage 6. The yield obtained at each stage was determined by weighing the forage harvested from 1 m^2 in three replicates. The partitioning of DM in the different plant parts was determined immediately after cutting and separating leaves, stems, and inflorescences from 50 randomly selected stems. The remaining forage was wilted in the field for 24 h to a DM concentration of approximately 40%. Wilted forage was chopped into 2–3 cm pieces with a laboratory chopper and ensiled in laboratory mini-silos. The mini-silo consisted of 1 L-glass jars, packed at a density of 150 kg DM m^{-3} [19]. Just before ensiling, the *scotta* was applied at the rates of 0, 75, 150, and 300 g kg^{-1} fresh weight. The main *scotta* chemical parameters were pH 6.19, lactose 4.28%, lipids 0%, crude proteins 0.43%, and solids 5.60%. The jars were opened on 3, 13, and 90 days of ensiling (DOE) for chemical and microbiological analyses.

2.2. Chemical Analyses of the Silages

The pH was determined in the aqueous silage extract, using a pH meter (Eutech instruments pH 510, Thermo Fischer Scientific, Milan, Italy).

Plant parts and forage were oven dried for DM determination at 75 °C to constant weight. Forages were analyzed to determine crude protein (CP), ash, ether extract (EE), neutral-detergent fiber (NDF), acid-detergent fiber (ADF), and acid-detergent lignin (ADL), according to Martillotti's method [20]. Hemicellulose and cellulose were estimated as the difference between NDF and ADF and between ADF and ADL, respectively. Water-soluble carbohydrates (WSCs) were obtained using the Luff–Schoorl official method [21]. Lactic and monocarboxylic acids (acetic, propionic, and butyric) were determined by HPLC, according to Canale's method [22]. Total digestible nutrients (TDNs) were estimated, as suggested by National Rereach Council (2001). The relative feed value (RFV) was calculated to estimate fiber quality [23]. The ammonia nitrogen was determined according to the Wall and Gehrke's method [24].

The CP, NDF, and TDN yields per unit area were calculated by multiplying the yield per hectare and the CP, NDF, and TDN concentrations, respectively.

2.3. Microbiological Analyses of the Silages

For the quantitative microbiological analyses, 10 g of silage were suspended in 90 mL of sterile saline solution and homogenized; further serial dilutions were performed in sterile tubes. Bacteria

belonging to *Lactobacillus* spp. were enumerated on the Man–Rogosa–Sharpe (MRS) agar (Thermo Fisher Scientific, Milan, Italy) after 48 h at 37 °C in anaerobioses; Enterobacteriaceae on violet red bile glucose (VRBG) agar (Thermo Fisher Scientific, Milan, Italy) after 24 h at 37 °C in aerobiose; clostridia on reinforced clostridial medium (Thermo Fisher Scientific, Milan, Italy) after 48 h at 37 °C in anaerobiose; and yeasts and molds on yeast glucose chloramphenicol (YGC) agar (Thermo Fisher Scientific, Milan, Italy) after 5 days at 25 °C in aerobiose. The detection limit of the analysis was 2 log CFU/g. For the statistical analyses, all the values under this threshold were set to 1 log CFU/g. The presence/absence of *Listeria monocytogenes* in 10 g of silage was assessed as follows: A pre-enrichment step in Oxoid Novel Enrichment broth Listeria (Thermo Fisher Scientific, Milan, Italy) at 30 °C for 24 h, and then by subculturing a loopful of broth-culture onto Agar Listeria Ottaviani Agosti, incubated at 37 °C for 24 h.

2.4. Statistical Analysis

The results were subjected to ANOVA. To analyze the effects of stage of harvest on the yield characteristics of the alfalfa second cut, the main effect of year and growth stage at harvest and their interactions were tested. To analyze the effect of wilting on the chemical characteristics of the fresh forage, the main effects of year and wilting and growth stage at harvest and their interactions were tested. To analyze the effect of DOE and the *scotta* dose on the pH, WSC, lactic acid, volatile fatty acids, and the biological characteristics of the alfalfa silage, the main effects of year and DOE and growth stage at harvest and *scotta* dose and their interactions were tested. To analyze the effect of the *scotta* dose on the bromatological characteristics of the alfalfa silage, the main effects of year and growth stage at harvest and *scotta* dose and their interactions were tested. The combined analyses over years was conducted after verifying the homogeneity of error variance by the chi-square test. Regression analysis was performed to test the relationships between pH and *scotta* dose. Correlation analysis was performed to test the relationships between pH, WSC, lactic acid, and protein concentrations. CoStat statistical package vers. 6.451 (CoHort Software, Berkeley, CA, USA) was used, and, in all analyses, the year and treatments were considered as fixed effects. Significantly different means were separated at the 0.05 probability, level by Tukey's (HSD) test.

3. Results

Neither the year mean effect nor any of the interactions of year with other treatments were significant for any of the measured or calculated parameters. This was probably because the differences were very low between soil conditions (two adjacent plots) and between years: In the period of October–June, it rained 760 mm in 2014 and 742 mm in 2015 [25]. Accordingly, all data are presented as averaged over years.

3.1. Characteristics of Alfalfa Yield

The dry matter yield of the second cut of alfalfa increased from the first to the last harvest (+120%), reaching about 3.4 t ha^{-1} (Table 1). This increase was due to the increase in all the parts of the plant, in particular, the stems and inflorescences (data not shown).

Table 1. Forage dry matter, crude protein, neutral-detergent fiber (NDF), and total digestible nutrient (TDN) yield (kg ha^{-1}) of the second cut of alfalfa as affected by growth stages at harvest. Data are the mean of 2 years and three replicates.

Harvest	Forage	Crude Protein	NDF	TDN
Mid Bud	1548 c	329.6 b	538.2 c	991.8 c
Early Flower	2426 b	440.1 ab	945.4 b	1393.7 b
Late Flower	3374 a	540.7 a	1443.7 a	1993.1 a

Within a column, mean values followed by the same letter are not statistically different for $p \leq 0.05$.

The phenological stage also changed the proportion of dry matter in the plant organs. From the MB to LF stage, the percentage of leaves and stems decreased in relative value by about 27% and 14%, respectively, while that of the inflorescences increased by about 10 times (Figure 1).

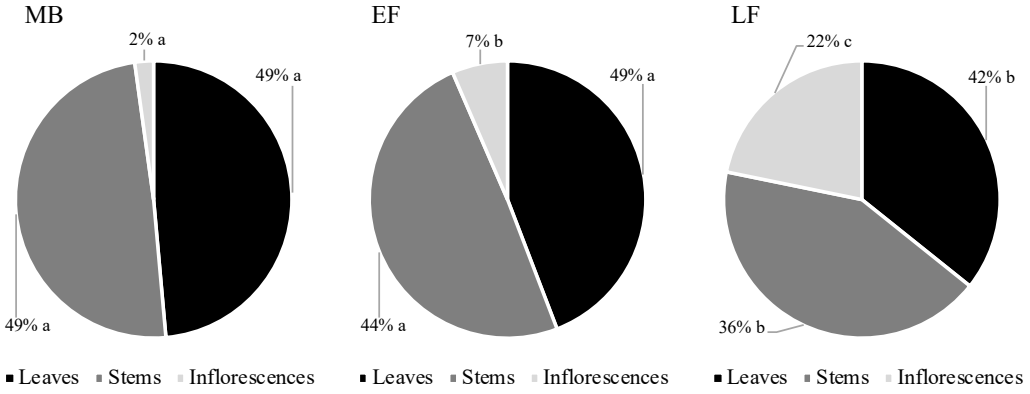

Figure 1. DM partitioning in leaves, stems, and inflorescences, as affected by the growth stage at the harvest mean effect. Mid bud (MB); early flowering (EF); late flowering (LF). Data are the mean of 2 years and three replicates. For each plant part, areas with the same letter are not statistically different for $p \leq 0.05$.

3.2. *Nutritional Characteristics of Alfalfa Forage as Affected by Growth Stage at Harvest and Wilting*

The dry matter concentration in the fresh forage increased from 21% in MB to about 27% in EF and LF stages (data not shown). Wilting increased these values to approximately 38% at all stages.

No statistically significant interaction was recorded between the growth stage at harvest and wilting for any of the characteristics examined, i.e., wilting similarly affected all characteristics in all three stages.

As a mean effect of the growth stage at harvest, the crude protein, ash, WSC, RFV, and TDN concentrations decreased from the MB to LF stage (Table 2), with the highest decrease recorded for the WSC (–4% in relative value). In contrast, the concentration of fiber fractions increased, with the highest increase in the ADL (+46%).

Table 2. Crude protein (CP), ether extract (EE), ash, neutral-detergent fiber (NDF), acid-detergent fiber (ADF), acid-detergent lignin (ADL), relative feed value (RFV), total digestible nutrients (TDN), and water-soluble carbohydrates (WSC) of the alfalfa forage prior to ensiling (% DM), as affected by growth stage at harvest. Data are the mean of 2 years, two wilting treatments, and three replicates.

Harvest	CP	EE	Ash	NDF	ADF	ADL	RFV	TDN	WSC
MB	21.3 a	1.6 a	8.8 a	34.7 b	24.1 c	5.6 c	190.5 a	64.0 a	6.3 a
EF	18.1 b	1.7 a	8.2 b	39.1 a	29.0 b	6.8 b	160.4 b	57.6 b	4.1 b
LF	16.1 c	1.7 a	7.4 c	42.8 a	31.6 a	8.2 a	140.1 c	59.0 b	2.9 c

Within a column, mean values followed by the same letter are not statistically different for $p \leq 0.05$.

Although the nutrient concentration decreased from MB to LF, at least for CP and TDN, the yield of CP, NDF, and TDN increased significantly by 64%, 168%, and 101%, respectively (Table 1): Therefore, the nutrient yield was a consequence of the greater sensitivity of the biomass accumulation than of the nutrient concentration to the growth stage.

Wilting, averaged over the three stages (Table 3), reduced the EE, NDF, and ADF concentrations, but increased the RFV (+18%).

Table 3. Ether extract, NDF, ADF, and RFV of the alfalfa forage prior to ensiling (% DM). Data are the mean of 2 years, three growth stages at harvest, and three replicates.

Wilting	EE	NDF	ADF	RFV
Unwilted	1.8 a	41.2 a	29.4 a	150.1 b
Wilted	1.5 b	36.4 b	27.1 b	177.3 a

Within a column, the mean values followed by the same letter are not statistically different for $p \leq 0.05$.

3.3. Fermentation Characteristics of Alfalfa Silage as Affected by DOE and Scotta Dose

Between the beginning and the end of the ensiling period, the weight of the jars decreased, although in a range of just 0.5–1.0% (data not shown).

The pH of the alfalfa silage was affected by the interaction between the DOE, stage of harvest, and *scotta* dose (Figure 2). Generally, the pH decreased as the *scotta* dose increased. This effect was more pronounced as more days elapsed since the ensiling. In addition, the *scotta* effect on lowering the pH was slight in the MB and LF stages (approximately −10%) and marked in the EF phase (–17%). However, using the highest *scotta* dose, the alfalfa silage pH reached 4.3 in both the EF and LF growth stages (Figure 2).

The WSC concentration, measured over the ensiling period, showed the highest values in the MB stage and the lowest in the EF and LF stages. In addition, averaged over the harvest and *scotta* doses, the WSC concentration decreased during the ensiling period, reaching the lowest value at 90 DOE. Finally, averaged over the DOE and stage of harvest, the WSC decreased with the increase in *scotta* dose (Table 4).

In contrast, the concentration of lactic acid was highest in the EF and LF stages, increasing with the ensiling period and with the *scotta* dose (Table 4).

The decrease in WSC with the ensiling period and the increase in lactic acid were all linked effects in line with the expected trend of lactic fermentations. In fact, throughout the ensiling period, a strong relationship was found between the WSC and lactic acid concentrations (Figure 3).

In the alfalfa silage, a low amount of N-NH_3 and butyric acid was found, which was not dependent on the treatments studied (Table 4). As the average of all treatments, N-NH_3 was 5.2% of the total nitrogen and butyric acid was 0.1%. In contrast, propionic acid was always lower than 0.01%.

Minor amounts of ethanol were also detected (at most 0.01%) which, among the treatments tested, were found to be exclusively dependent on the DOE (Table 4).

The lactic/acetic ratio was enhanced with the growth stage, the DOE, and the scotta dose, respectively, by 75%, 94%, and 58% in relative value (Table 4).

Table 4. WSC, lactic acid, acetic acid, butyric acid, propionic acid, ethanol, N-NH_3 (% of total N), and lactic/acetic ratio (% DM) of the alfalfa silage as affected by the mean effects of growth stage at harvest, days of ensiling (DOE), and *scotta* dose. Values are the mean of 2 years and three replicates.

Treatment	WSC	Lactic Acid	Acetic Acid	Butyric Acid	Propionic Acid	Ethanol	NH_3	Lactic/Acetic
				Harvest				
MB	2.61 a	0.54 b	0.27 c	0.11 a	<0.01	0.008 a	4.9 a	2.0 c
EF	1.09 b	1.56 a	0.57 a	0.11 a	<0.01	0.011 a	5.5 a	2.7 b
LF	1.36 b	1.62 a	0.47 b	0.13 a	<0.01	0.007 a	5.3 a	3.5 a
				DOE				
3	2.60 a	0.47 c	0.30 c	0.10 a	<0.01	0.007 b	5.4 a	1.6 b
13	1.58 b	1.47 b	0.43 b	0.13 a	<0.01	0.005 b	5.6 a	3.4 a
90	0.88 c	1.77 a	0.57 a	0.13 a	<0.01	0.014 a	4.7 a	3.1 a
				Scotta (g kg^{-1} Fresh Weight)				
0	2.00 a	0.94 c	0.40 a	0.11 a	<0.01	0.007 a	5.4 a	2.4 c
75	1.69 b	1.05 bc	0.46 a	0.12 a	<0.01	0.006 a	5.2 a	2.3 c
150	1.74 b	1.19 b	0.42 a	0.12 a	<0.01	0.013 a	5.1 a	2.8 b
300	1.33 c	1.77 a	0.47 a	0.13 a	<0.01	0.008 a	5.3 a	3.8 a

Within a column, the mean values followed by the same letter are not statistically different for $p \leq 0.05$.

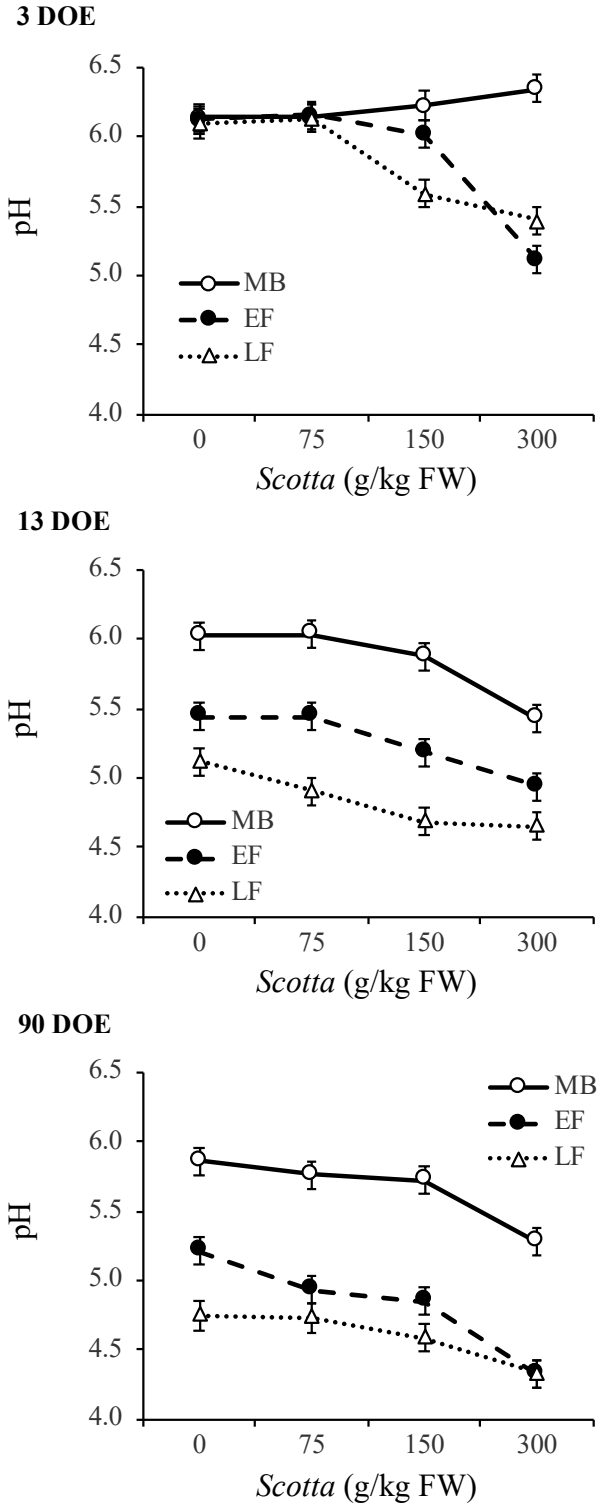

Figure 2. pH of the alfalfa silage as affected by days of ensiling (DOE), growth stage at harvest, and *scotta* dose. Vertical bars represent Least Significant Difference at $p \leq 0.05$.

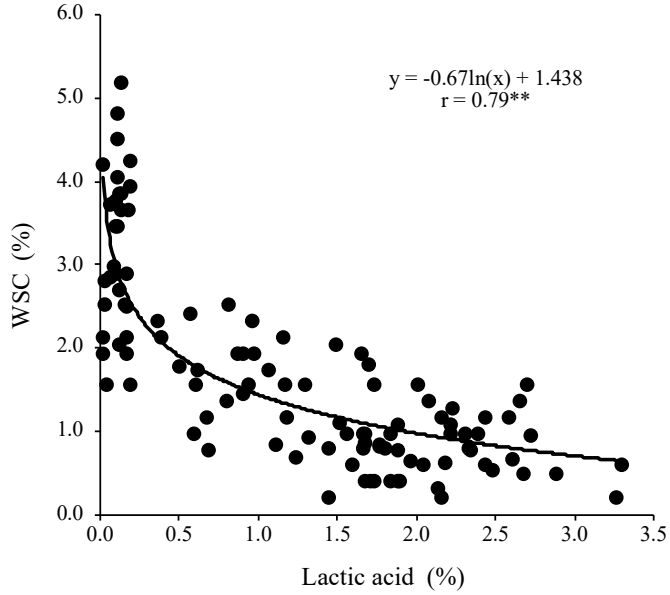

Figure 3. Relationship between WSC and lactic acid throughout the ensiling period and in all harvest stages. Each point represents a mean of 2 years (n = 108). ** = $p \leq 0.01$.

Before ensiling, the epiphytic bacterial concentrations showed different profiles: MB samples presented the highest bacterial loads, with 6.25, 5.61, 3.79, and 5.65 log (Colony Forming Unit/g) for mesophilic lactobacilli, Enterobacteriaceae, *Clostridium* spp., and molds, respectively. Compared to MB, the EF and LF samples had lower bacterial concentrations by about 39%, 5%, 74$, and 12%. Concerning clostridia, spores were not detectable in the EF and LF growth stages. Moreover, forages were all negative for *L. monocytogenes* and yeasts.

In silages, the dynamics of the targeted bacteria and molds were significantly affected by the interaction of the growth stage at harvest and the DOE (Figure 4). In general, the loads showed the highest value after 3 DOE and gradually decreased to the lowest value recorded after 90 DOE. At this point, only the *Lactobacillus* spp. counts were above the detection limit, with values higher than 5 log CFU/g, except for no detection of the LF samples.

The *scotta* dose, as the mean of years, growth stage at harvest, and DOE, had different effects on the different microorganisms (Figure 4): A slight reduction in enterobacteria and molds (–0.29 and –0.34 log CFU/g, respectively), a slight increase in *Clostridium* spp. (+0.43 log CFU/g), and no significant effect on *Lactobacillus* spp.

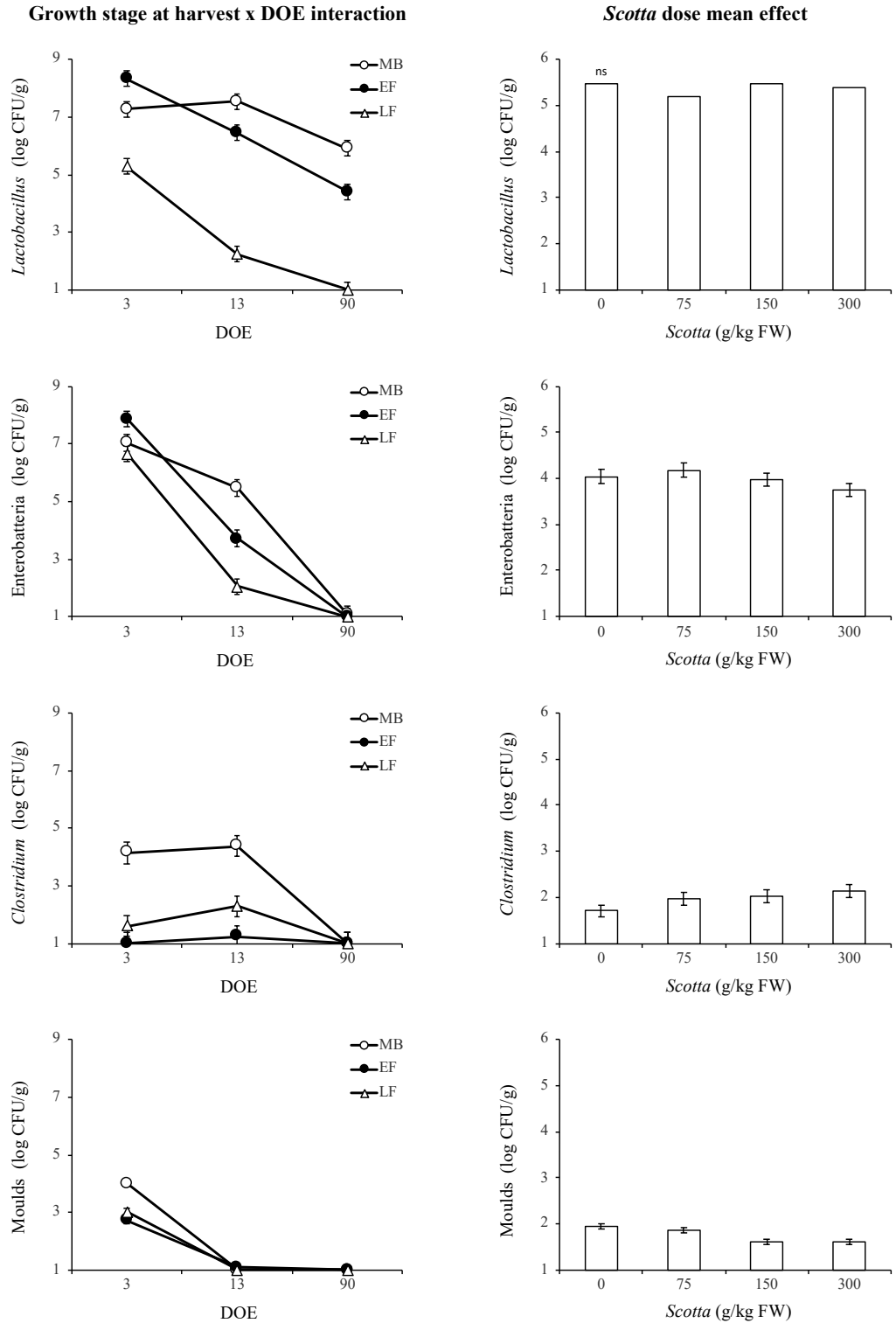

Figure 4. Mesophilic *Lactobacillus* spp., Enterobacteria, *Clostridium* spp., and molds, as affected by growth stage at harvest × DOE interaction (left) and by *scotta* dose mean effect (right). Vertical bars represent LSD at $p \leq 0.05$: When not indicated, error bar lies within the symbol. Not significant (NS).

3.4. Nutritional characteristics of Alfalfa Silage as Affected by Scotta Dose

After 90 DOE, the variations recorded in the nutritional characteristics of the forage were similar to those already observed in fresh forage. Thus, from MB to LF, crude protein, ash, RFV, and TDN decreased, while EE, NDF, ADF, and ADL increased (Table 5).

Table 5. Crude protein, ether extract, ash, NDF, ADF, ADL, RFV, and TDN after 90 DOE of ensiling (%DM), as affected by the mean effect of the growth stage at harvest. Data are the mean of 2 years, four *scotta* doses, and three replicates.

Harvest	CP	EE	Ash	NDF	ADF	ADL	RFV	TDN
MB	20.8 a	1.5 b	9.3 a	30.1 b	24.3 b	5.3 c	216.9 a	64.4 a
EF	17.4 b	2.1 a	8.7 b	36.8 a	30.5 a	6.6 b	165.6 b	61.8 b
LF	16.7 c	1.9 a	8.2 c	39.1 a	31.6 a	7.4 a	155.5 b	60.4 c

Within a column, the mean values followed by the same letter are not statistically different for $p \leq 0.05$.

The *scotta* reduced the DM concentration of the forage, which did not drop below 30% (Table 6). The *scotta* also increased the ash and RFV, while it decreased the NDF, the ADF, and the ADL with slight variations (<10% in relative value).

Table 6. DM concentration, CP, ash, NDF, ADF, ADL, RFV, and TDN of the alfalfa forage after 90 DOE (%DM), as affected by the mean effect of the *scotta* dose (g kg^{-1} FW). Data are the mean of 2 years, three growth stages at harvest, and three replicates.

Scotta	DM	CP	Ash	NDF	ADF	ADL	RFV	TDN
0	37.0 a	18.5 a	8.5 c	37.0 a	30.3 a	6.8 a	167.9 b	61.5 a
75	34.6 b	18.7 a	8.7 b	35.1 b	29.5 a	6.7 a	178.1 ab	62.3 a
150	33.1 c	18.3 a	8.9 a	34.5 b	27.9 b	6.1 b	183.2 a	62.6 a
300	29.6 d	17.7 a	9.0 a	34.6 b	27.5 b	6.2 b	188.1 a	62.6 a

Within a column, the mean values followed by the same letter are not statistically different for $p \leq 0.05$.

A negative linear regression was recorded between the *scotta* dose and the pH detected after 90 DOE: As the dose increased, the pH decreased linearly with different regression coefficients, depending on the growth stage. When the *scotta* was increased by 100 g kg^{-1} FW, the pH decreased by 0.19 units in MB, 0.29 units in EF, and 0.15 units in LF (Figure 5).

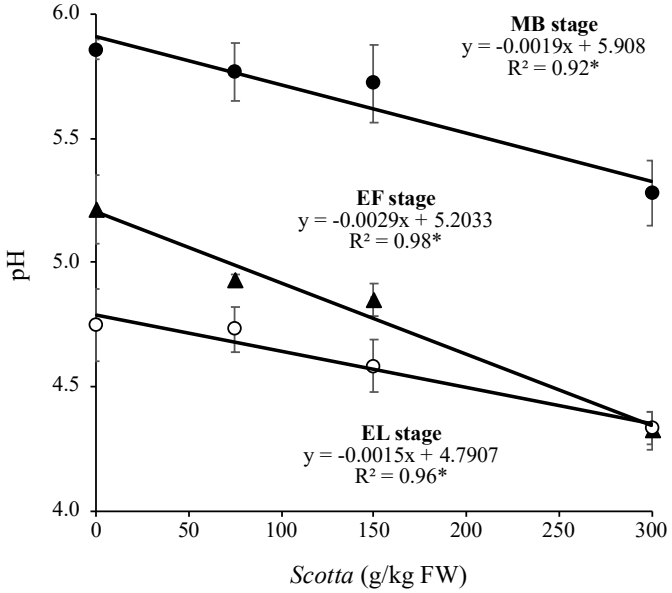

Figure 5. Relationship between pH at 90 DOE and *scotta* dose in MB, EF, and LF stages of harvest. Each point represents a mean of three replications and 2 years. Vertical lines represent SD of the mean. * = $p \leq 0.05$.

Considering the data collected at 90 DOE, regardless of the growth stage, the pH of the alfalfa silage correlated positively with the concentration of crude proteins and correlated negatively with the

concentration of lactic acid (Figure 6). When crude protein and lactic acid increased by one percentage point, the pH increased by 0.3 or decreased by 0.7 units, respectively.

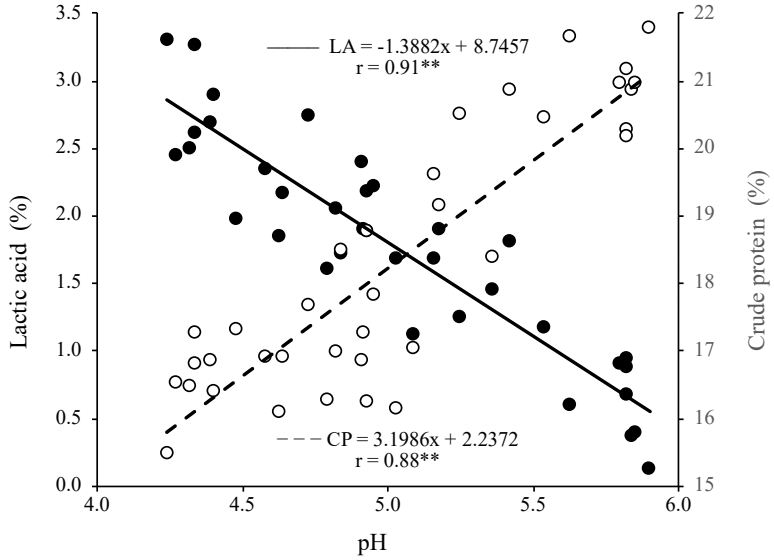

Figure 6. Relationship between pH at 90 DOE, lactic acid (LA, •) and crude protein concentration (CP, ○). Each point represents a mean of 2 years (n = 36). ** = $p \leq 0.01$.

4. Discussion

Our study was carried out on a second cut of a 3-year-old alfalfa meadow. The second cut was chosen because, in the cultivation environment, it shows fewer weeds and has the best quality [26]. The alfalfa yield of DM and nutrients increased from MB to LF, and the plants were proportionally richer in inflorescences and poorer in stems and leaves. This led to a decrease in protein concentration, WSC, RFV, and TDN and an increase in various fiber fractions, as also reported by other authors [27–30].

Before ensiling, the forage was subjected to wilting (up to 38% of DM), a treatment normally used to obtain a forage that does not cause effluents [31]. This treatment had limited consequences on the nutritional characteristics of the forage, with a slight reduction in some fiber fractions (hemicellulose and cellulose) and a slight increase in the RFV.

The pH of the alfalfa silage decreased as the ensiling progressed and was on average higher in the MB stage than in EF and in LF, i.e., the buffer capacity, which, in our study, was not measured, was probably the lowest in the EF and highest in the MB growth stages. In all growth stages, the addition of the *scotta* decreased the pH, which reached 5.27 in MB and 4.32 in EF and LF. The latter values were lower than those achieved by adding alfalfa forage with fresh whey (pH 4.67) [32], sucrose (4.58) [33], formic acid (4.36) [34], a chemical additive consisting of formic acid, propionic acid, formate ammonium, and benzoic acid (4.38), a bacterial inoculum (homofermentative and heterofermentative lactic bacteria) (4.47) [15], or a previously fermented alfalfa juice (4.53) [13].

Considering that, with the addition of the *scotta*, the DM concentration dropped from 38% to about 30%, the presence of effluents was prevented [1] and the forage was anaerobically stable, but only in the EF and LF phases and not in MB [35].

To the best of our knowledge, this research represents the first investigation evaluating the microbiological profile of experimental silage with added *scotta*.

Firstly, it is important to highlight the good microbiological quality achieved. Despite the amounts of *scotta* added, all the samples at 90 DOE showed a remarkable decrease in the main spoilage microorganisms, such as *Enterobacteriaceae*, molds, and clostridia. The latter are those responsible for the main problems in dairy production; however, at 90 DOE, the silages always presented values under the detection limit (<2 log spores/g) and thus can be considered of high quality [36]. As for the mesophilic lactobacilli, which are responsible for the desired fermentation, the addition of lactose from *scotta* did

not positively or negatively affect the concentration of cultivable microorganisms. It is possible that the microorganisms may have been less efficient in metabolizing lactose, compared to other carbon sources, such as arabinose, glucose, fructose, and xylose, which are typical of plant materials [37]. However, the increase in lactic acid in the silages suggested an active lactose metabolism. Probably, the good microbiological quality was also responsible for the low ammonia nitrogen concentration (about 5% of the total nitrogen), which was much lower than the maximum (about 12%) indicated for legume silages [38].

Secondly, the obtained data suggested that the floral stage played an important role in the microbial dynamics in the silage. In fact, the MB samples presented the highest microbial loads after 13 DOE for *Lactobacillus* spp., *Clostridium* spp., and Enterobacteriaceae. This was probably due to a shorter period of exposure to solar radiation, compared to the EF and LF samples, which could have affected the cultivability of the microorganisms [39].

The WSC concentration of alfalfa was different between the growth stages, as reported by Yari [10], and was higher in the MB stage than in EF and LF. In any case, the WSC decreased as the ensiling progressed, during which they were transformed into lactic acid, and consequently the pH decreased. However, the WSC concentration alone does not guarantee the success in ensiling the alfalfa. In the MB growth stage, the WSC concentration was the highest; however, the pH did not decrease much. Instead, the silage pH correlated strongly with the protein concentration, and the protein concentration was particularly high in the MB. Thus, the lowering of the pH may have been more hampered by the protein concentration, probably by increasing the silage buffer power [1], than favored by the WSC.

The addition of the *scotta* linearly lowered the silage pH in all harvest stages, reaching almost optimal values only in the EF and LF growth stages. The equations derived from the regression analysis can be used to estimate that to reach pH 4.2, which is considered as the target for the alfalfa silage conservation [40], about 350 g of *scotta* would be needed in the first growth stage and about 400 g in the second. Considering the EF growth stage, i.e., the stage at which a high quality forage was obtained, the addition of the *scotta* to reach pH 4.2 would lead to a decrease in DM concentration to 28%, while in the LF growth stage, i.e., the stage in which the high yield of nutrients was reached, the DM concentration dropped to 27%. In both cases, the forages would be in the stable silage area, with regard to the possible proliferation of clostridia, but there would be a low risk of effluent [41]. Wilting, which in our research led to about 38% DM, should therefore reach about 40% to prevent effluents.

The main changes in the nutritional characteristics of the forage from the beginning to the end of the ensiling were a slight reduction in crude proteins (<5% in relative value), an increase in the ether extract, a reduction in NDF and ADL (maximum 13% in relative value), a reduction in hemicelluloses (in 33–46%), and a slight increase in RFV (approximately +10%) and in TDN (approximately +3%). These variations were very similar to those obtained in other research on alfalfa ensiling carried out with different additives [42–44].

The ratio of lactic acid to acetic acid is used as a qualitative indicator of fermentation and is usually a good quality silage fermentation presents a ratio of about 2.5 to 3.0 [38]. With values higher than 3.0, silage can be aerobically unstable, while values lower than 2.5 can result in poor fermentation. In our research, the values were almost optimal in the EF and LF growth stages, although with the higher *scotta* dose, aerobic stability may be reduced.

5. Conclusions

Our results indicated that the addition of *scotta* whey as an additive to alfalfa silage had positive effects, which were highlighted by a decrease in pH, a slight reduction in the fiber fractions, and an increase in the relative feed value, while it had no effect on the crude protein concentration and on the total digestible nutrients. The microbiological profile was almost optimal, while the acidic profile seemed to indicate a low aerobic stability of the silage. The benefits were maximized if the *scotta* was used in the EF and LF growth stages, with rates no less than 300 g/kg of fresh forage.

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