AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
FUNDING CYCLE 2020 – 2022

TITLE

Original: Legume hay with high bioactive compounds and organic selenium to improve the transition from pregnancy to lactation using sheep as animal model
Modified (see Interim report): Combination of chicory and selenium yeast to improve the transition from pregnancy to lactation using sheep as animal model

RESEARCH LEADER

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COOPERATORS

Serkan Ates and Mary Smallman (Animal and Rangeland Sciences) as co-PIs
Hunter Ford as PhD that carried out the experiment.
Daniella Hasan, as an undergraduate student that carried out part of the project.

SUMMARY/ABSTRACT

The transition from pregnancy to lactation is the most critical period in dairy animals due to large metabolic and immune changes that can affect their health and performance. Therefore, means to improve the transition from pregnancy to lactation in ruminants, especially the immune response, are in high demand. One of the nutrients with the major effect on improving the immune system is selenium (Se), especially in the organic form as Se-yeast. It is becoming evident that secondary metabolites present in plants can have an important effect on health and performance in lactating animals. Prior studies have shown effectiveness of improving animal health using either Se or bioactive compounds-rich forages alone on dairy ruminants; however, there are not studies where Se was combined with forages containing high amount of secondary metabolites with nutraceutical properties. Thus, our hypothesis is that the combination of organic Se with forages with high bioactive compounds improve the health and performance of sheep during the transition from pregnancy to lactation.

OBJECTIVES

Test the effect of feeding a combination of Se-yeast supplementation and forages containing high amount of bioactive compounds on the transition from pregnancy to lactation in dairy sheep.

PROCEDURES

Experimental procedures used in this study were approved by the Institutional Animal Care and Use Committees (IACUC) of Oregon State University (protocol# 2020-0122). Thirty-six pregnant Polypay ewes from the Oregon State University Sheep Center were moved into individual 5’x5’ pens at the HOGG Animal Metabolism Laboratory Building at Oregon State University campus. The pens were bedded with wood shavings until lambing and with wheat straw after lambing

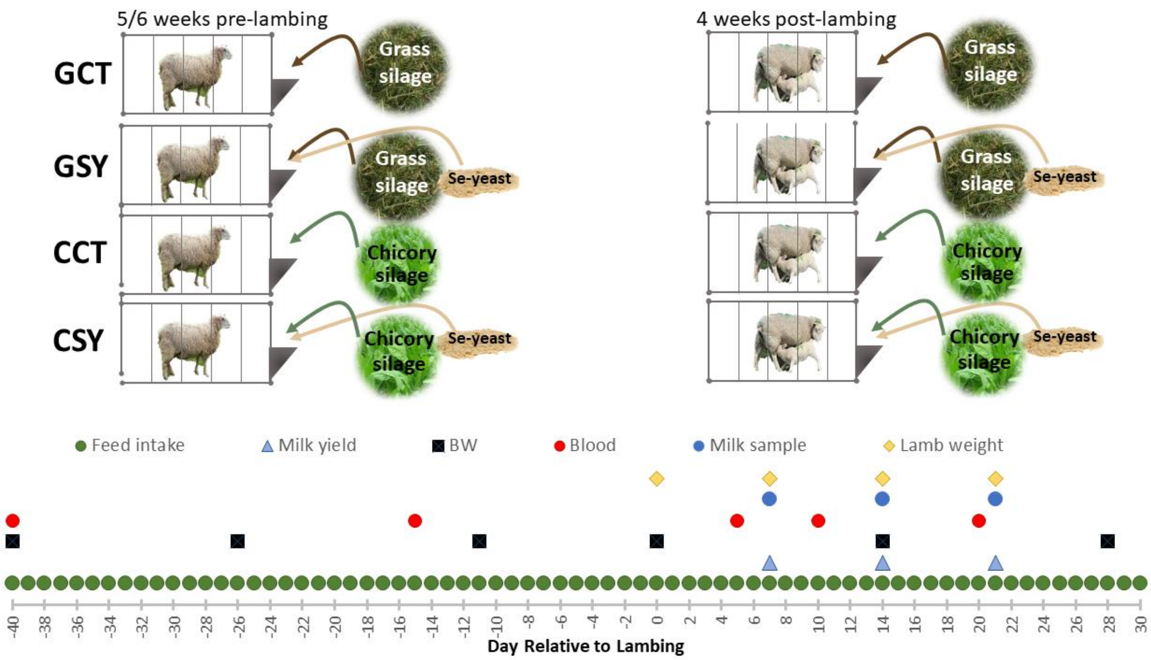

Figure 1. Experimental design. Pregnant Polypay sheep were fed with either grass silage or chicory silage and supplemented with Se yeast or and isonitrogenous and isoenergetic supplement of alfalfa pellet. Indicated in the lower quadrant is the timeline of the experiment with the various sample collection/assays during the trial. There were 9 ewes in each group.

and bedding was added as needed and replaced every 3-4 days. The ewes were randomized based on body weight (BW) into the various groups as indicated in **Figure 1**. Besides forages, sheep also received a mixed grain that was provided in a separate trough (**Figure 2**).

Figure 2. Set up of pens. Sheep ate silages from a trough and grain in a different trough. Water was provided by an automatic water trough.

The following measurements originally proposed were obtained:

- Individual feed intake (as dry matter) was measured by weighing the amount of feed provided twice a day and measuring the residuals the day after. The dry matter of the silage was measured every day using a microwave;
- Water intake was measured using a digital flow meter attached to an automatic waterer. Water was recorded every 3 days.
- Analysis in blood of the following items:
    - Phytochemicals (flavonoids and flavonoids conjugated) from the silages using LC MS/MS.
    - A complete cell blood count that included WBC and HMT were performed using a VET SCAN HM5 (Abaxis, USA) after lambing;
    - Level of Selenium in whole blood at the Keck Collaboratory for Plasma Spectrometry at Oregon State University;

- Level of the antioxidant markers reactive oxygen metabolites (ROM) and ferric reducing ability of the plasma (FRAP) and neutrophil activity marker myeloperoxidase (MPO).
- Milk yield was measured indirectly using the weigh-suckle-weigh method on days 7, 14 and 21 after lambing. Lambs were separated from their mothers within the individual pens by plastic netting that allowed for close contact, but no suckling (**Figure 3**). Lambs were separated for the first 3-hour period in the morning before being placed back with the ewe and allowed to suckle until finished (up to 15 min) in an attempt to empty the mammary glands of the majority of the milk. A milk sample was collected from both glands just before putting back the lamb. Following this suckling period lambs were separated again for 3 hours within the pens. After this 2nd three-hour separation period, lambs were weighed, put back in the pen with the ewe, and allowed to suckle until finished before being weighed again. The difference in lamb weights before and after the final suckling period was used as an estimate of 3-hour milk production by the ewe and extrapolated over a 24-hour period to estimate daily milk yield.
- Milk composition and somatic cells (SCC) were measured in the milk collected at 7, 14, and 21 d using a LactoScope™ FT-A and SomaScope Smart (Perkin Elmers, USA), respectively.
- Level of parasites measured in ewes at 30 day after lambing (end of experiment) and in lambs.

Figure 3. The separation of the lambs from the ewe was obtained by using a plastic net, so the lambs and the ewe could see each other but the lambs could not nurse.

For the statistical analysis, the data were checked for outliers using a Proc Reg of SAS and data with studentized-T>3.0 were removed. The analysis was done using GLIMMIX (SAS) to evaluate the effects of forage (F), Se-Yeast (Se), Time (T), and their interactions with ewe as random effect. Significance was declared at p≤0.05 and tendencies were declared when 0.05>p<0.1.

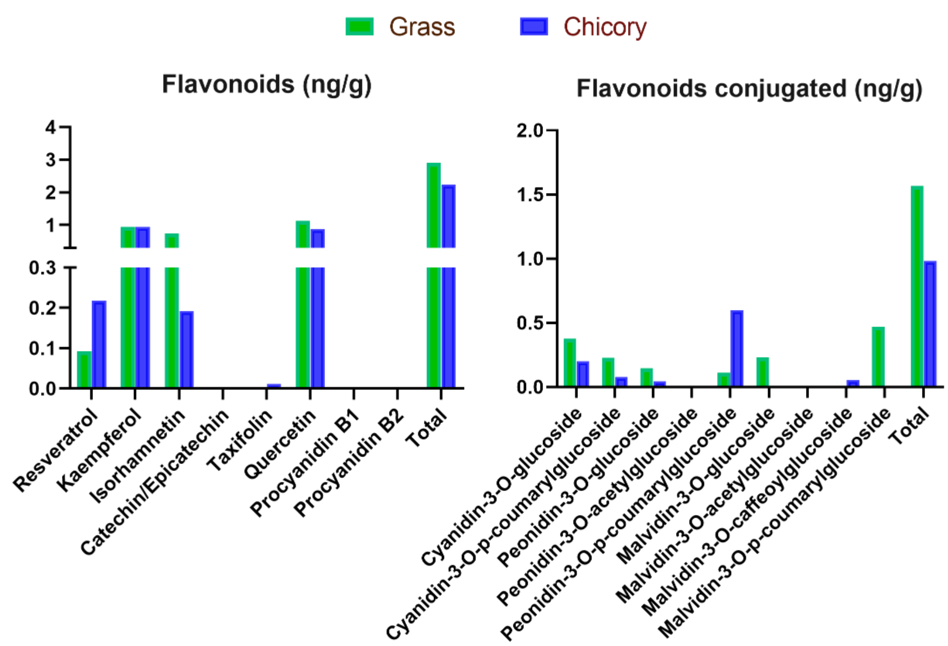

Figure 4. Secondary compounds determined in grass and chicory silages.

SIGNIFICANT ACCOMPLISHMENTS

The level of plant secondary compounds was not greater in chicory compared to grass silage (**Figure 4**). Of all the phytochemical measured, only resveratrol and Peonidin-3-O-p-coumarylglucoside were more abundant in chicory vs. grass silage.

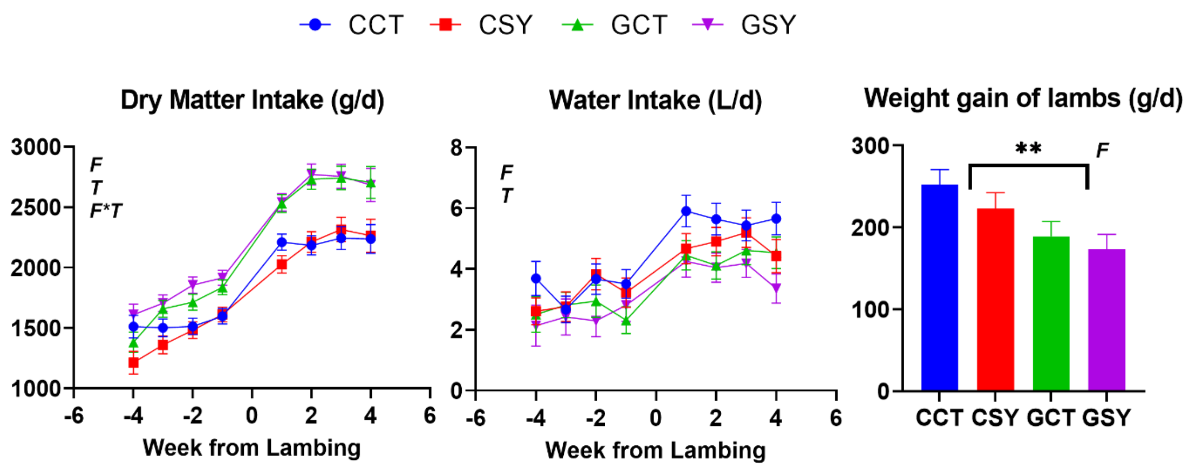

Figure 5. Feed intake, water intake, and average body weight gain of lambs fed chicory silage or grass and receiving supplement of Se-yeast. Letters in the graph denote significant (P<0.05) statistical effect of the silage (or forage, F), time (T), and their interaction (F*T).

Feed intake was lower while water intake was larger in sheep fed chicory silage compared to grass silage (**Figure 5**). Despite the lower feed intake, the body weight gain of the lambs was greater when nursing in ewes fed chicory vs. grass silage (**Figure 5**) and milk yield was not affected, resulting in a larger feed efficiency (**Figure 6**).

The various components of the milk (**Figure 6**) were also affected by the treatments, with a larger effect by the type of silage compared to SE supplementation, that had a very minor effect. Feeding chicory silage increased proportion of fat in milk, mostly driven by a larger uptake by the mammary gland of preformed fatty acids, likely coming from the diet. Feeding chicory silage however reduced the proportion of protein and lactose in milk reducing also the milk urea content but no effects were observed in milk somatic cells (SCC) (**Figure 6**). Se had not effect on those parameters, except increased SCC in milk at 7- and 21-day post-lambing. Those results were unexpected, especially considering data from a prior study where chicory was grazed by dairy cows (Wilson et al., 2020). The observed results may be due to the use of silage instead of fresh forages; however, chicory silage had an overall interesting effect on milk fat, the most commercially important component of milk. Contrary to the cow's study (Wilson et al., 2020), we did not observe a decrease of SCC by feeding chicory. Also, contrary to what expected (Piva et al., 1993), Se yeast did not affect any of the parameters, with a contrary effect observed in cows about SCC (Malbe et al., 1995).

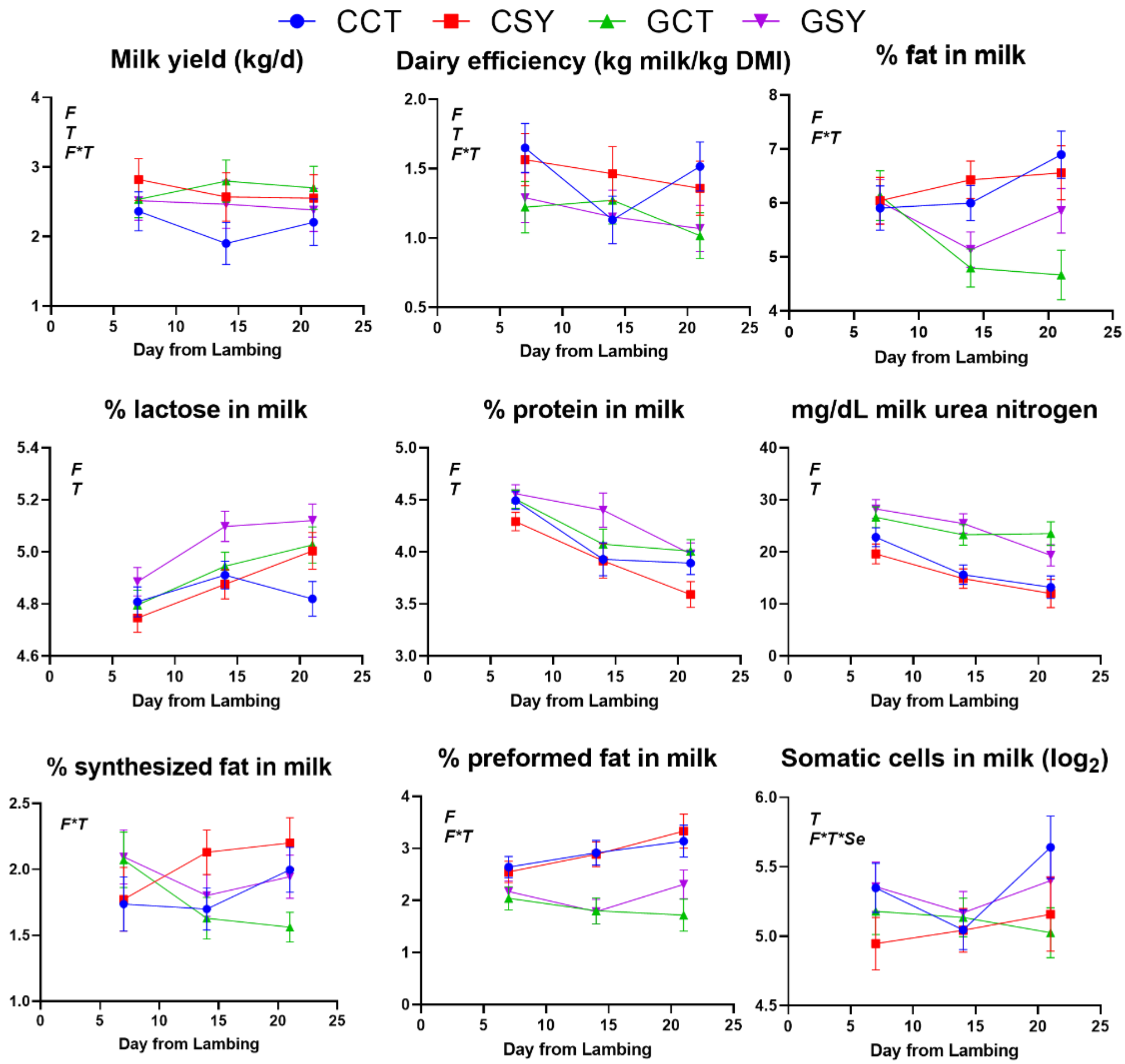

Figure 6. Milk yield, dairy efficiency, and milk components in sheep fed with chicory silage or grass and receiving supplement of Se-yeast. Letters in the graph denote significant (P<0.05) statistical effect of the silage (or forage, F), time (T), Selenium (Se), and their interaction (F*T or F*T*Se).

The complete blood cell count indicated no effect by either type of silage or Se supplementation on the composition of the immune system. However, the % hematocrit was higher when sheep were fed chicory vs. grass silage (**Table 1**). There was also a tendency for an interaction of type of silage and Se supplementation in the amount of neutrophil and monocyte, with a decrease by supplementation of Se when ewes were fed chicory but an increase when fed grass (**Table 1**).

Table 1. Complete blood cell counts in sheep fed chicory silage or grass and receiving supplement of Se-yeast. Letters in the graph denote significant (P<0.05) statistical effect of the silage (or forage, F), Selenium (Se), and their interaction (F*T or F*Se). Effect of time is omitted.

Parameter*	Unit	Group				*SEM*	*P-value*			
		CCT	CSY	GCT	GSY		*F*	*Se*	*F*Se*	*F*Se*T*
WBC	10^3 cells/ul	9.70	9.21	8.17	9.60	*1.05*	*0.273*	*0.359*	*0.065*	*0.950*
Lymphocyte	10^3 cells/ul	6.31	6.02	5.44	5.88	0.65	0.117	0.816	0.256	0.694
Monocyte	10^3 cells/ul	0.049	0.045	0.040	0.048	0.01	0.229	0.439	0.031	0.832
Neutrophil	10^3 cells/ul	3.34	3.15	2.69	3.68	0.60	0.832	0.181	0.047	0.522
Lymphocyte	%	65.5	66.3	67.0	62.4	*3.88*	*0.531*	*0.314*	*0.169*	*0.408*
Monocyte	%	0.51	0.49	0.49	0.50	*0.01*	*0.328*	*0.595*	*0.083*	*0.339*
Neutrophil	%	34.0	33.2	32.5	37.1	*3.87*	*0.529*	*0.313*	*0.166*	*0.406*
RBC	10^6 cells/ul	9.35	9.58	9.26	9.21	*0.33*	*0.117*	*0.553*	*0.352*	*0.429*
Hemoglobin	mmol/L	6.78	6.99	6.87	6.77	*0.27*	*0.606*	*0.695*	*0.240*	*0.465*
Hematocrit	%	30.7	31.3	29.9	29.9	*1.13*	*0.042*	*0.623*	*0.630*	*0.693*
Platelet	10^3 cells/ul	141.2	129.9	133.7	159.7	*29.1*	*0.443*	*0.610*	*0.201*	*0.977*

*WBC = white blood cells; RBC = Red Blood Cells

Despite a very minor effect on most parameters, the animals receiving the Se supplementation had higher concentration of Se in whole blood (**Figure 7**). However, this did not improve the activity of the immune system as measured by the activity of myeloperoxidase or the oxidative status of the animals, as observed by the ROM, as index of oxidative stress, and FRAP< as index of antioxidative status; however, the latter appear to indicate that ewes fed with grass silage did not benefit from supplementing Se while a benefit on the level of FRAP was observed when ewes were fed chicory silage (**Figure 7**).

We did not observe any effect of type of silage or Se supplementation of the amount of fecal parasites in ewes; surprisingly, we observed a lower level of fecal parasites in lambs from ewes fed with chicory vs. grass silage (**Figure 8**).

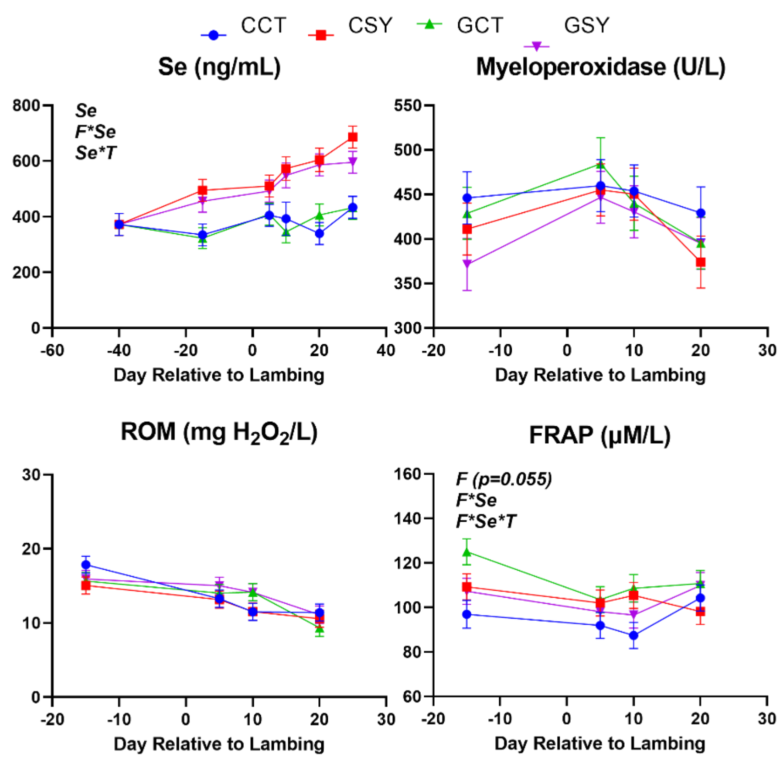

Figure 7. Level of Se in whole blood and level of myeloperoxidase, reactive oxygen species (ROM), and ferric reducing ability of the plasma (FRAP) in sheep fed chicory silage or grass and receiving supplement of Se-yeast. Letters in the graph denote significant (P<0.05) statistical effect of the silage (or forage, F), time (T), Selenium (Se), and their interaction (F*T, F*Se, or F*SeT*).

BENEFITS & IMPACT:

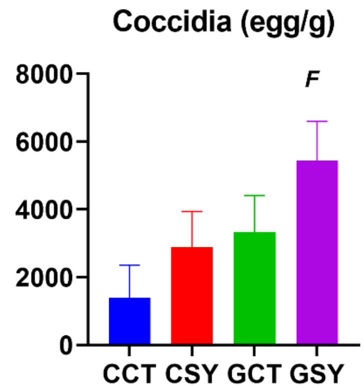

Figure 8. Level of coccidia in feces of lambs born from sheep fed with chicory silage or grass and receiving supplement of Se-yeast. Letters in the graph denote significant ($P<0.05$) statistical effect of the silage (or forage, F)

Our data only partly support the original hypothesis that a combination of organic Se with chicory silage improve the health and performance of sheep during the transition from pregnancy to lactation. The most striking result was the lack of difference in secondary compounds between grass and chicory silage. Our prior data clearly indicated higher amount of secondary compounds in chicory compared to grass (Ford et al., 2021). Thus, the similar amount of secondary compounds of chicory and grass is likely due to a decrease in secondary compounds during the process of silaging, although this was unexpected, according to prior studies (Kuppusamy et al., 2020; Huang et al., 2021).

Our data also suggest an almost complete lack of effect by supplementing organic Se. It is possible that our sheep had already an optimal amount of Se in the diet, as indicated by the high basal level of Se detected in whole blood (i.e., >300 ng/mL).

Despite the amount of phytochemical was similar to the grass silage, feeding chicory appeared to have had some beneficial effects on the animals. This was even more surprising considering the significant lower feed intake as consequence of the presence of large stems due to late harvest after the plants bolted (> 1.5 m). Despite this limitation, the animals fed chicory had larger milk fat and had a better feed efficiency. It is unclear what have caused such as effect, but we previously explained some of those same effect in grazing cows due to higher content of non-fiber carbohydrates in chicory compared to grass, that might have improved the efficiency of rumen fermentation (Ford et al., 2021).

The immune system and the antioxidative system were not affected by chicory silage. Feeding chicory silage also did not improve the status of parasites in the feces of the ewes. The above data agree with the lack of difference in the amount of phytochemicals between the two silages. However, those observation do not help to explain the improved parasitic status of the lambs.

Overall, our data provide some evidence that feeding chicory silage to ewes can be safe without any major detrimental effect, with even some positive effects, including an increase in milk fat. This can be relevant for the dairy industry.

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM:

Did not receive additional funding to complete the project

FUTURE FUNDING POSSIBILITIES:

We used the data generated by the present proposal to submit and successfully get an USDA Animal Health formula fund (Dr. Ates as PI and Dr. Bionaz as one of the co-PIs, project title: The effect of supplementing dried chicory roots rich in sesquiterpene lactones and condensed tannins on parasite loads and immune system of ewes). We used the data generated in the present experiment to write a proposal to be submitted to the USDA NIFA Organic Agriculture Research and Extension Initiative (proposal is ready for submission once the opportunity will open).

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