Animal 15 (2021) 100235

Contents lists available at ScienceDirect

Animal

The international journal of animal biosciences

Ensiled biomass of *Solaris* tobacco variety used as forage: chemical characteristics and effects on growth, welfare, and follow-up of Holstein heifers

A. Fatica [a,*], F. Fantuz [b], F. Di Lucia [c], M. Zuin [d], L. Borrelli [e], E. Salimei [a]

[a] *Dipartimento Agricoltura, Ambiente, Alimenti, Università degli Studi del Molise, via Francesco De Sanctis, 1, Campobasso 86100, Italy*
[b] *Scuola di Bioscienze e Medicina Veterinaria, Università degli Studi di Camerino, via Gentile III Da Varano, 62032 Camerino, Italy*
[c] *via Mazzini 11, 71010 Chieuti, Italy*
[d] *via D'Annunzio 5, 33170 Pordenone, Italy*
[e] *via Turno 47 D-E, 86021 Bojano, Italy*

ARTICLE INFO

Article history:
Received 11 July 2020
Revised 15 March 2021
Accepted 16 March 2021

Keywords:
Crop co-product
Growing cattle
Nicotiana tabacum L.
Ruminant nutrition
Silage

ABSTRACT

This study examined the use of an innovative tobacco variety, *Nicotiana tabacum* L., cv. *Solaris*, as forage. The whole plant biomass was ensiled, and the composition of SiloSolaris from bunker-silo and mini-silos was investigated. The effects of dietary inclusion of SiloSolaris on the growth, welfare, and nutritional profile of sixteen Holstein heifers, divided into two groups ($n = 8$), SiloSolaris (**SS**) and Control (**CTR**), were investigated. Heifers were group-fed diets with a 70:30 forage to concentrate ratio (on a DM basis). Both groups received 16.24 kg DM of concentrate mixture daily, including corn meal, wheat middlings and soybean meal. The CTR group was fed 39.43 kg DM of hay daily, and the SS group received 23.00 kg DM of the same hay and 12.69 kg DM SiloSolaris blended with the concentrate mixture. The feeding trial lasted eighty-one days with a thirty-six day adaptation phase. Data on forty-five days of diet administration are reported. At the end of the feeding trial, the plasma constituents of the heifers were studied. Moreover, heifers were monitored during a follow-up period, lasting up to 1 year after calving, for age at first insemination, age at first calving and daily milk yield. The SiloSolaris chemical composition showed an average DM content of 24.1 (±0.65) g/100 g. During ensiling, a decrease in CP and an increase in ammonia nitrogen contents were observed. The lactic acid content was variable (9.00 ± 2.66 g/100 g DM), while the acetic acid concentration was stable (4.27 ± 0.21 g/100 g DM). No butyric acid was detected in SiloSolaris, whose ammonia nitrogen content accounted for 15.7 (±1.86)% of the total nitrogen on average, and the mean pH value was 5.02 (±0.08). The SiloSolaris diet did not affect heifer growth performance. No differences were detected for body condition, fecal consistency, or locomotion scores. All the investigated plasma constituents were within or very close to the ranges reported for heifers; however, significant differences between the experimental groups were observed for triglycerides, cholesterol, albumin, and magnesium. The follow-up results did not differ between the experimental groups. These initial findings suggest that *Nicotiana tabacum* cv. *Solaris* is a promising ensiled forage for growing heifers that deserve to be further investigated.

Implications

This study reports the conservation of whole *Nicotiana tabacum* cultivar *Solaris* plants through ensiling (SiloSolaris), suggesting the added value of the multipurpose cultivar for use as forage. The reported effects of SiloSolaris introduction into the diet of Holstein heifers show that the consumption of ensiled *Solaris* biomass did not affect the investigated growth, welfare, and follow-up parameters. Although these first results need to be confirmed, they suggest an additional potential use for this energy cultivar with multiple co-products, which may contribute to the recovery of tobacco cultivation knowledge.

Introduction

During recent decades, agriculture has been characterized by several innovations aiming to satisfy the increased demands in

* Corresponding author.
E-mail address: a.fatica@studenti.unimol.it (A. Fatica).

https://doi.org/10.1016/j.animal.2021.100235
1751-7311/

the food and non-food sectors from the growing global population (Food and Agriculture Organization of the United Nations, 2019). Within this context, several supply chains cannot be considered sustainable due to their environmental impacts and to negative externalities arising from the human demand for food and non-food items (Notarnicola et al., 2015). Most of the research in agriculture is therefore focused on multipurpose crops according to the concept of a circular economy (FAO, 2019).

In this regard, a significant case has been made for *Nicotiana tabacum* L., a plant traditionally cultivated for the production of smoking products, but recently, an alternative use of tobacco has been proposed (Grisan et al., 2016; Poltronieri and D'Urso, 2016; Fatica et al., 2019). A new cultivar of *Nicotiana tabacum*, named *Solaris*, has been developed (international patent PCT/IB2007/053412) as a non-genetically modified energy plant for maximizing the production of oilseeds used as biofuel for transportation (Fogher, 2008; Grisan et al., 2016). Compared with smoking tobacco varieties, *Nicotiana tabacum* cv. *Solaris* contains a low level of nicotine and maximizes the production of inflorescences and seeds with reduced leaf growth (Fatica et al., 2019). In addition to its main feature as an energy cultivar, due to its short vegetative cycle, strong vegetative capacity, and great pedo-climatic adaptations, *Nicotiana tabacum* cv. *Solaris* has been suggested as a multifunctional plant for biomethane production, animal nutrition and for the food industry with a circular economy approach (Fatica et al., 2019). Advances of knowledge on this versatile plant could drive the development of management strategies based on the multiple destination of product (biofuel) and feed or non-food co-products, in a highly competitive supply chain, especially interesting in those areas traditionally suited to tobacco cultivation and now threatened by marginalization, abandonment and land degradation (Fatica et al., 2019).

The global dairy sector is currently under high pressure due to the variable feed costs and incomes from farmgate milk prices, which implies a minor consideration of heifer management compared to that of milking herds (Boulton et al., 2017). Heinrichs et al. (2013) reported that the costs for raising heifers were 15–20% of total dairy farm costs. Therefore, a way to reduce these costs could be to raise dairy heifers calving at a younger age without a negative effect on milk yield (Brown et al., 2005). Another strategy could be to decrease feed costs by using, for example, by-products, i.e. secondary products obtained after harvesting or processing principal commodities (Grasser et al., 1995), for partial replacement of grains and oilseeds (Bocquier and González-García, 2010). Ruminants can obtain a large amount of energy through the consumption of forage (Bocquier and González-García, 2010), so a good strategy may be also replacing traditional forage sources with other conserved forms that can provide the same energy and/or protein fraction.

As a further contribution to advances in knowledge on the emerging *Nicotiana tabacum* cv. *Solaris*, the present study has been addressed to its ensiled biomass, in terms of composition and potential use in ruminant nutrition.

The chemical composition of *Nicotiana tabacum* cv. *Solaris* has been therefore studied in ensiled samples aiming to evaluate the potential storage attitude of this plant. The use of the innovative tobacco silage was then tested in Holstein heifer diet in order to consider the possible introduction of this new forage in bovine rations, monitoring the effects on growth, welfare, and follow-up period.

Material and methods

The present on-field trial was performed at a private dairy farm, and the experimental protocol was in accordance with the European Commission guidelines (2010/63/EU) concerning the protection of animals used for experimental and other scientific purposes.

Whole plant ensiling process

Nicotiana tabacum cv. *Solaris* green biomass was harvested in fall at approximately 25% of DM, when the plants were in waxy ripening phase, i.e. d 150 after transplanting and d 60 after the seed collection. Briefly, after *Solaris* transplanting in May, seed harvesting (plant apex collection) occurred in August, and the whole plant green biomass was harvested in November. Whole plants were chopped to a 4 cm length by a corn shredder. The green chopped biomass of cv. *Solaris* (11 t) was ensiled in a dairy farm located in the Molise Region (Oratino municipality, Italy, 41°35′05.3″N 14°35′17.6″E) in a bunker-silo (6.5 × 3 × 1.1 m, max height) made of reinforced concrete walls on three sides and straw on the fourth side. During ensilage, a specific microbiological additive containing different strains of *Lactobacillus plantarum* (Pioneer 11F79) was sprayed on the green biomass after diluting 10 g of the product in approximately 35 L of water. At this stage, samples (n = 15, approximately 1 kg each) of inoculated biomass were collected in plastic bags (30 × 40 cm, as mini-silos) and stored under vacuum conditions at 8–9 °C. The biomass was appropriately pressed in the bunker-silo with a tractor to ensure an anaerobic environment and to maximize microbial activity. The ensiled biomass was then covered with black and white PVC cloth on which wood and metal weights were placed to ensure the adhesion of the cloth to the biomass surface, limiting contact with the air as much as possible (Borreani et al., 2018).

At d 0, 20, 35, 61 and 84 of ensiling, mini-silos were opened and samples collected and analyzed (in triplicate) to monitor their chemical and physico-chemical characteristics. After eighty-eight days of ensiling, in February, the bunker-silo containing the ensiled biomass of cv. *Solaris* was opened and cleaned. During the feeding trial, samples of SiloSolaris (n = 8) were subjected to chemical and physico-chemical analyses similar to mini-silo samples. The moisture, pH, CP, ether extract, ash, NDF, ADF, ADL, ammonia nitrogen ($N\text{-}NH_3$) and total alkaloid (expressed as nicotine) contents were determined for all samples (n = 23, n = 15 from mini-silos plus n = 8 from bunker-silo) by an accredited laboratory (Mérieux NutriSciences Corporation, Resana, TV, Italy), according to official methods (Association of Official Analytical Chemist, 2000). The $N\text{-}NH_3$ content was also calculated as a percentage of the total nitrogen.

Animals, diets, and experimental design

Sixteen growing Holstein heifers, 220–360 kg bodyweight and 8 to 16 months of age, were selected and divided into two homogeneous groups (Table 1), the SiloSolaris (**SS**) and Control (**CTR**) group.

The grouping procedure occurred a few days before the beginning of the trial to allow the animals to adapt to the environment. The animals of the two groups were kept on a concrete floor in two separate and adjacent pens of the same size, allowing contemporary access to the manger. Each group had unlimited access to

Table 1
Experimental heifer groups (n = 8) at d 0 (mean values ± SD).

Item	SS	CTR
Age, d	359.4 (±56.4)	349.1 (±57.3)
BW, kg	297.6 (±47.9)	289.1 (±43.5)

SS = SiloSolaris group; CTR = Control group.

fresh water by means of two different automatic drinkers positioned in each pen.

For both groups, isonitrogenous and isoenergetic diets (Table 2) were calculated according to the nutritional needs of the animals (National Research Council (NRC), 2001; Van Amburgh et al., 1998). Heifers were group-fed experimental diets characterized by a 70:30 forage to concentrate ratio on DM basis. Mixed hay consisted of a prevalence of *Trifolium* L. and *Lolium* L., and concentrate mixture was made by corn meal, wheat middlings and soybean meal. *Solaris* silage was blended with concentrates in the SS group diet (55% of the DM from forages, Table 2). For both groups, rations were administered into two daily meals at 0700 and 1730 h. For the first thirty-six days of the trial, all heifers were allowed to metabolically adapt to the diet, especially SS heifers, which were subjected to a gradual daily increase of the silage in the diet since they had never eaten any kind of silage before. Starting from the second week of the trial, the mixture of silage and feeds was offered in the manger separated from the hay to allow SS heifers to consume either *Solaris* silage and concentrates or hay, based on their preference. Including the adaptation phase (d 36), the feeding trial lasted eighty-one days. Following the adaptation phase, the experimental period lasted forty-five days and experimental data were collected and processed for this period.

Measurements and recordings

Before each meal (0700 and 1730 h), refusals were weighed and recorded as well as all feedstuffs successively administered to experimental groups. Refusals and feedstuffs administered were weekly sampled and analyzed for their DM contents (AOAC, 2000). The daily group DM intake was calculated as the DM difference between feedstuffs administered and refusals for each group.

At d 0, 36 and 81, all heifers were weighed and scored for body condition, fecal consistency and locomotion capacity according to the literature (Edmonson et al., 1989; Sprecher et al., 1997; Stallings, 1998; Hall, 2002). For each group, the average daily gain was calculated, and the estimated SS and CTR group feed conversion rate (**FCR**) has been calculated as the ratio between the average group DM intake and the group average daily gain (**ADG**).

At the end of the trial, heifers underwent blood sampling by jugular venipuncture using EDTA-treated vacuum tubes (BV Vacutainer® Collection Tubes) approximately 3 hours after the morning feeding. Samples were cooled on ice, centrifuged for 10 min at 1 880*g*, frozen in liquid nitrogen and stored at −80 °C. Analyses of plasma samples were carried out by Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise (Campobasso, Italy) following standardized analytical methods (IZSAM, 2018), i.e. for aspartate aminotransferase (**AST**), alanine aminotransferase (**ALT**), alkaline phosphatase (**ALP**) and gamma-glutamyl transferase (**GGT**) was applied the kinetic methodology, for glucose and urea nitrogen was applied the enzymatic methodology, for cholesterol and uric acid was used the enzymatic-colorimetric methodology, for triglycerides and creatinine was used the kinetic-enzymatic methodology, and for total protein, albumin, calcium, inorganic phosphorus, magnesium, and iron contents was applied the colorimetric methodology.

After the feeding trial, experimental heifers were raised according to the usual management and they were monitored during the follow-up period, lasting up to 1 year after calving, for the following parameters: age at first insemination, age at first calving and average daily milk yield (305 days of lactation). One CTR heifer was lost during the follow-up period because of a uterine malformation.

Table 2
Ingredients, calculated chemical composition and energy of the diet for the experimental heifer groups (*n* = 8).

Item	SS	CTR
Ingredients, kg DM/group per d		
SiloSolaris	12.69	–
Corn meal	7.04	7.04
Wheat middlings	7.04	7.04
Soybean meal (44% CP)	2.16	2.16
Mixed hay	23.00	39.43
Total group DM administered per d	51.93	55.67
Chemical composition (g/kg DM) and energy content (per kg DM)		
CP	150	140
Ether extract	50	30
Ash	90	90
NDF	390	420
ADF	250	270
ADL	50	30
UFL	0.81	0.80

SS = SiloSolaris group; CTR = Control group; UFL = feed unit for lactation (1 UFL = 7.12 MJ NEl/kg) (Sauvant et al., 2004).

Statistical analyses of results

Descriptive statistics were calculated for all the investigated variables (Microsoft© Excel© for Office 365 ProPlus Version 1903-11425.202429).

Data on the chemical components and pH of the silage from mini-silos were processed by analysis of variance (GLM). In case of significant effects ($P < 0.05$), differences among means were analyzed by least significant difference.

Because there was only one pen per treatment, group DM intake, refusal and FCR data were not subjected to statistical analysis and only descriptive statistics are reported. As animals were group-fed with no replication of pens within treatments, the investigators assumed no pen effect, and that errors within pens were independent, allowing for the heifer to serve as the experimental unit.

Individual data for BW, ADG, body condition, fecal, and locomotion score were processed by repeated measure analysis of variance, also considering the covariate effect of 'age at d 0'. Data on plasma constituents were processed by analysis of variance considering the covariate effect of 'age at d 81' (GLM, ANCOVA). Follow-up data were processed by analysis of variance considering the covariate effect of 'age at d 0' (GLM, ANCOVA). All statistical analyses were conducted with IBM SPSS Data Editor ver. 25.

Results

Chemical composition of Solaris ensiled biomass (SiloSolaris)

The results of the chemical analyses conducted on SiloSolaris samples from bunker-silo are reported in Table 3, and all data are expressed on a DM basis. Among the fermentation end products, no butyric acid was detected and the lactic acid content ranged from 1.35 g/100 g DM to 16.6 g/100 g DM. Moreover, N-NH_3 (Table 3) accounted in average for 15.7 (±1.86, SEM) % of the total N. Observed pH values averaged 5.02 (±0.08, SEM) and the total alkaloid content was on average 1.35 (±0.09, SEM) g nicotine/100 g DM.

Fig. 1 shows the chemical changes during the ensiling process of whole plant of cv. *Solaris*, monitored in mini-silos for 84 days. DM content ranged (minimum and maximum values) from 23.1 g/100 g to 27.6 g/100 g, while the organic matter content varied from 82.4 g/100 g DM to 86.3 g/100 g DM (Fig. 1a). CP content, ranging from 15.6 g/100 g DM to 19.4 g/100 g DM, showed a significant decline ($P < 0.01$) at the beginning of the ensiling process (Fig. 1b). The ether extract content varied from 10.4 g/100 g DM to 12.2 g/100 g DM, while the observed values of ash content ran-

Table 3
Chemical components of *Nicotiana tabacum* cv. *Solaris* silage (SiloSolaris) from bunker-silo (n = 8) administered to heifers.

Item	Mean	SEM	Minimum	Maximum
DM, g/100 g	24.1	0.65	21.4	25.9
Organic matter, g/100 g DM	87.5	0.22	86.6	88.2
CP, g/100 g DM	16.4	0.54	15.2	19.4
Ether extract, g/100 g DM	11.2	0.32	9.90	12.3
Ash, g/100 g DM	12.5	0.22	11.8	13.4
NDF, g/100 g DM	42.6	0.71	41.0	46.5
ADF, g/100 g DM	32.3	0.56	30.6	34.3
ADL, g/100 g DM	9.95	0.28	9.20	10.8
Starch, g/100 g DM	4.89	0.23	3.96	5.90
Lactic Acid, g/100 g DM	9.00	2.66	1.35	16.6
Acetic Acid, g/100 g DM	4.27	0.21	3.32	4.79
N-NH_3, g/100 g DM	0.41	0.04	0.28	0.56

N-NH_3 = ammonia nitrogen.

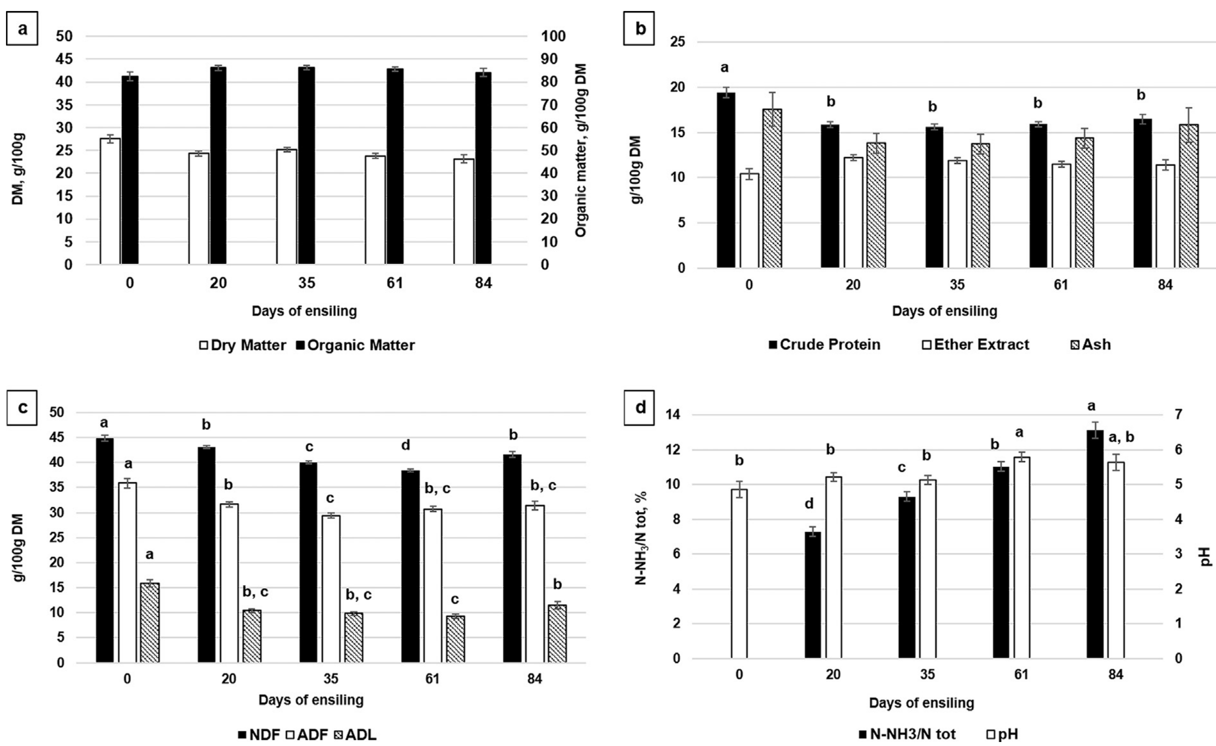

Fig. 1. Chemical components and pH (mean ± SEM) of *Solaris* silage during the ensiling process studied in mini-silos (n = 15): a) dry and organic matter; b) crude protein, ether extract and ash contents; c) NDF, ADF and ADL contents; d) ammonia nitrogen (N-NH_3) and pH. [a, b, c, d] means with different superscripts differ; $P < 0.05$.

ged between 13.7 g/100 g DM and 17.6 g/100 g DM (Fig. 1b). Fig. 1c displays the average values of the fiber fractions observed during the ensiling period, showing significant reduction in the cell wall ($P < 0.001$), ADF and ADL ($P < 0.01$) contents. Observed minimum and maximum values were 38.4 g/100 g DM and 44.9 g/100 g DM, 29.4 g/100 g DM and 35.9 g/100 g DM, 9.3 g/100 g DM and 15.9 g/100 g DM, for NDF, ADF and ADL, respectively (Fig. 1c). Finally, data on N-NH_3, not available for d 0, varied between 7.3% of total nitrogen (d 20) and 13.1% of total nitrogen (d 84), while pH values increased from 4.9 (d 0) to 5.8 (d 84). Significant differences were found for both N-NH_3 ($P < 0.001$) and pH ($P < 0.05$) (Fig. 1d).

DM intake and refusal, growth performance, welfare indices and follow-up

During the 45 days of experimental phase, the daily group DM intake ranged from 43.5 kg to 51.9 kg, and from 52.5 kg to 55.7 kg, for SS and CTR group, respectively. Group DM consumption averaged 48.5 (±0.31 SEM) kg/d and 54.9 (±0.12 SEM) kg/d for SS and CTR group, respectively. The observed group DM refusals averaged 2.22 (±0.22 SEM) kg/d, within a range from 0.00 kg to 5.82 kg/d, for SS diet, and was in average 0.74 (±0.11 SEM) kg/d, within a range 0.00–3.14 kg/d, for CTR diet.

The investigated individual growth performance indices, BW and ADG, were not affected by the dietary treatments ($P > 0.05$) (Table 4). Based on a group average daily bodyweight gain of 7.88 (±0.20, SD) kg for SS diet and 5.91 (±0.24, SD) kg for CTR diet, the estimated FCR was 6.16 kg DM/kg ADG for SS group and 9.29 kg DM/kg ADG for CTR group. Regarding the welfare indices reported in Table 4, the dietary treatment did not significantly affect ($P > 0.05$) both body condition and fecal consistency of heifers while the locomotion scores were always 1 (in a 1–5 scale) in both experimental groups.

As far as the reproductive traits are concerned, age at first insemination and at calving did not significantly differ ($P > 0.05$) between the two groups as well as the average daily milk yield (Table 4).

Table 4
BW, growth, welfare, and follow-up of experimental heifers.

Item	SS	CTR	SEM	*P-value*
BW (kg), d 36	337.4	332.1	11.8	0.456
BW (kg), d 81	374.1	354.1	11.6	0.456
ADG (kg/d), d 36–81	0.94	0.80	0.07	0.172
Body condition score, d 36	3.65	3.54	0.10	0.333
Body condition score, d 81	3.87	3.54	0.09	0.333
Fecal score, d 36	3.18	3.23	0.08	0.545
Fecal score, d 81	3.12	3.38	0.08	0.545
Age at first insemination (d)	627.3	630.5	37.2	0.964
Age at first calving (d)	863.1	846.3	6.96	0.237
Average milk yield (kg/d)	27.0	26.2	0.96	0.667

SS = SiloSolaris group; CTR = Control group; ADG = average daily gain.

Nutritional profile of heifers

As Table 5 displays, significant differences between the dietary treatments were observed for triglycerides and cholesterol ($P < 0.01$), magnesium ($P < 0.001$), and albumin ($P < 0.05$) plasma contents. However, all the investigated plasma constituents were within or very close to the range reported in the literature for heifers in the winter season (Kaneko et al., 2008; Stefani et al., 2011). More in detail, plasma constituent intervals (minimum - maximum) observed respectively for SS and CTR diet were 0.18–0.31 mmol/L and 0.14–0.21 mmol/L for triglycerides, 3.89–5.08 mmol/L and 2.20–4.35 mmol/L for cholesterol, 34–36 g/L and 28–34 g/L for albumin, and 0.99–1.05 mmol/L and 0.88–0.99 mmol/L for Mg.

Discussion

Chemical composition of SiloSolaris

The data reported in Table 3 show for the first time the chemical composition of ensiled whole plants of cv. *Solaris* biomass which have been mainly compared to data reported in literature for major silages traditionally used in Center-South Italy (Crovetto, 2006; Colombini et al., 2010), as well as in the selected farm. Most likely because of the adverse climatic conditions during the late harvesting (beginning of November), the average DM content was lower than values reported in the literature for corn (34.4 g/100 g) and alfalfa (61.0 g/100 g) silages but consistent with values observed in ensiled sorghum (24.9 g/100 g) (Crovetto, 2006; Colombini et al., 2010). On a DM basis, the SiloSolaris organic matter content was close to the values reported in the literature for alfalfa silage (87.7 g/100 g DM) (Crovetto, 2006), but lower than sorghum (89.0 g/100 g DM) and corn (95.8 g/100 g DM) silages (Crovetto, 2006; Colombini et al., 2010). The concentration of proteins in SiloSolaris was intermediate between those for sorghum and alfalfa silages (10.0 g/100 g DM and 21.0 g/100 g DM, respectively) and almost double the values reported for corn silage (8.20 g/100 g DM) (Crovetto, 2006; Colombini et al., 2010). During ensiling, a decrease in CP (Fig. 1b) was observed along with an increase in N-NH_3 expressed as % of total N (Fig. 1d), which values were very close to the range reported by Kung et al. (2018) for grass silage characterized by 25–35% of DM content. Bearing in mind the main characteristic of cv. *Solaris* as an energy plant, it is not surprising that SiloSolaris had a higher content of ether extract compared to the values of 2.0 g/100 g DM, 2.5 g/100 g DM and 7.7 g/100 g DM reported respectively for sorghum, corn and alfalfa silages (Crovetto, 2006; Colombini et al., 2010). The starch content observed in SiloSolaris samples was approximately five times lower than that in corn silage (32.3 g/100 g DM) (Crovetto, 2006; Colombini et al., 2010). Moreover, the ash content of SiloSolaris was remarkably higher than that of corn (4.8 g/100 g DM), alfalfa (7.5 g/100 g DM) and sorghum (9.4 g/100 g DM) silages (Crovetto, 2006; Colombini et al., 2010). Among the fiber fractions (Table 3 and Fig. 1c), SiloSolaris displayed NDF and ADF contents consistent with the values reported for alfalfa silage (42.7 g/100 g DM and 33.5 g/100 g DM, respectively for NDF and ADF) (Crovetto, 2006), but showing higher values of ADL, possibly

Table 5
Plasma constituents of experimental heifers and values reported in the literature for comparison.

Plasma parameters	Unit	SS	CTR	SEM	*P-value*	Kaneko et al. (2008)	Stefani et al. (2011) (95% confidence interval)
AST	U/L	88.0	88.5	3.35	0.906	78–132	51–89
ALT	U/L	34.4	34.6	1.51	0.959	11–40	13–35
ALP	U/L	182.9	184.3	15.3	0.950	0–488	113–268
GGT	U/L	11.8	13.4	1.72	0.536	6.1–17.4	10–23
Glucose	mmol/L	3.17	3.11	0.15	0.789	2.50–4.16	3.1–4.0
Cholesterol	mmol/L	4.51	3.64	0.16	0.002	2.07–3.11	1.93–4.33
Triglycerides	mmol/L	0.24	0.19	0.01	0.007	0–0.2	0.11–0.44
Total protein	g/L	68.0	68.0	1.14	0.968	67.4–74.6	61–81
Albumin	g/L	34.7	32.4	0.55	0.013	30.3–35.5	31–40
Creatinine	mmol/L	108.3	103.9	4.17	0.471	88.4–177	72–119
Urea N	mmol/L	10.3	9.54	0.42	0.243	7.10–10.6	–
Uric acid	mmol/L	58.5	57.5	2.05	0.727	0–119	–
Ca	mmol/L	2.58	2.55	0.02	0.405	2.43–3.10	2.27–2.67
P	mmol/L	2.68	2.54	0.07	0.184	1.81–2.10	1.53–2.44
Mg	mmol/L	1.03	0.93	0.01	<0.001	0.74–0.95	0.71–0.99
Fe	μmol/L	26.0	24.1	0.87	0.143	10.2–29.0	14.5–32.4

SS = SiloSolaris group; CTR = Control group; AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase; GGT = gamma-glutamyl transferase.

related to the late harvest of the biomass. As far as the fermentation end products are concerned, the average lactic acid content of SiloSolaris (Table 3) was consistent with the values reported for both legume and grass silages with a low DM content (Kung et al., 2018); however, the ample variability among samples should be highlighted. Relatively stable values were found for the acetic acid content, whose concentration on a DM basis was observed to be higher than those reported for corn, sorghum, and alfalfa silages (Crovetto, 2006). According to Kung et al. (2018), a high acetic acid concentration can be observed in wet silages but also when the ash content is high. No butyric acid was detected in SiloSolaris, and the N-NH_3 content was consistent with values reported for both sorghum (Crovetto, 2006) and high-moisture silages (Kung et al., 2018). The observed pH values were intermediate between sorghum and alfalfa silages (Crovetto, 2006). The previously mentioned SiloSolaris high protein and ash contents can be responsible for its high buffering capacity, according to Kung et al. (2018).

The total alkaloid content of SiloSolaris was lower than the values reported for some traditional smoking tobacco cultivars (Wang et al., 2008; Tassew and Chandravanshi, 2015; Fatica et al., 2019).

Effects of dietary treatments on growth, welfare, follow-up data and plasma constituents

The dietary treatment did not significantly affect individual heifers' bodyweight and ADG (Table 4), whose values were consistent with the optimal ADG before puberty as reviewed by Heinrichs et al. (2017). For descriptive purposes, the individual DM intake was calculated at 6.1 kg/d and 6.9 kg/d for SS and CTR diet, respectively. Because data on DM intake and FCR were not subjected to statistical analysis, the reported descriptive data need further investigation and cannot be considered conclusive.

Dietary treatment with SiloSolaris did not significantly affect the body condition of heifers, whose scores ranged from 3.5 to 4 (on a 1–5 scale) (Table 4). Lean et al. (2015) reported that the optimal body condition score for calving cows should be 3.25–3.5, while as reported by Petrovski (2015), the optimal score for dry cows and heifers is 4. According to El-Kasrawy et al. (2020), generally, the best body condition score of heifers at first artificial insemination should range between 2.5 and 4, for a positive effect on productive efficiency during lactation. Among the welfare indices, the evaluation of fecal consistency may provide information about the digestion of feeds and can allow for the diagnosis of early fermentation problems (Stallings, 1998). In this trial, for both groups of heifers, manure appeared thick and formed a solid pat two–three cm in height (Stallings, 1998), with scores ranging from 3 to 3.5 (on a 1–4 scale). The higher lipid intake by the SS group (5% DM) vs. the CTR group (3% DM) did not affect the fecal consistency. Furthermore, according to Sprecher et al. (1997), the deambulatory capacity of the heifers was normal, and all animals stood and walked with a level back posture.

Regarding the follow-up period, the investigated parameters were not significantly different in the experimental groups of heifers (Table 4). The average milk production was 27.0 L for SS vs. 26.2 L for CTR, and the age at first calving was 863.1 d vs. 846.3 d in the SS and CTR groups, respectively. The observed reproductive trait, reflecting a non-significant difference in age at first insemination, felt within the range reported in the literature for primiparous cows (Heinrichs et al., 2013). According to Krpalkova et al. (2017), the optimal age for the first calving in Holstein-Friesian heifers should be twenty-four months to maximize production and minimize rearing costs. However, the optimal age for first calving is always a specific trait of each animal population and management strategy (Cooke et al., 2013; Heinrichs et al., 2017).

Regarding the nutritional plasma profile (Table 5), although cholesterol, triglycerides, albumin and Mg were significantly different between the SS and CTR groups, the results were within or very close to the normal ranges reported in the literature (Kaneko et al., 2008; Stefani et al., 2011). The observed nutritional profile related to lipid metabolism could be associated with a higher intake of lipids in the SS group. Lipids have many functions, among which the most important are considered energy storage and cell membrane structure. Cholesterol is a precursor of steroid hormones, vitamin D, and bile acids and is a constituent of bile micelles (Kaneko et al., 2008). Triglycerides, instead, are the most important lipids with regard to energy storage, while phospholipids and cholesterol are important cell membrane constituents (Kaneko et al., 2008). Regarding the cholesterol plasma level, the observed values were within the range between 1.68 and 6.55 mmol/L reported by Van Saun (2008). The plasma albumin concentration is determined by the hepatic synthesis rate, which normally is in equilibrium with its degradation. However, the investigated indices for hepatic disorders, i.e. ALT and AST, as well as for the status of the biliary system, i.e. ALP and GGT, suggest the integrity of the hepatocytes in both groups (Kaneko et al., 2008). Regarding the plasma Mg content being higher in the SS group than in the CTR group, it must be considered that the normal range varies from 0.9 to 1.2 mmol/L provided that the influx into the cellular space is larger than the efflux (Martens et al., 2018). Mg, following K, is the second abundant intracellular cation (Kaneko et al., 2008) and is involved in enzymatic reactions after combining with enzyme/substrate systems (Martens et al., 2018). Furthermore, plasma Mg is influenced in a non-specific manner by catecholamines, insulin and the parathyroid hormone (Martens et al., 2018). Inorganic phosphorus plasma levels were unaffected by the dietary treatment, but the values were higher than those reported in literature (Table 5). However, Van Saun (2008) reported that plasma P ranged from 1.45 to 2.58 mmol/L since it is excreted from the body via the kidneys and thus may be elevated when the glomerular filtration rate is decreased (Kassel, 2015).

In conclusion, the present study demonstrates that the *Nicotiana tabacum* cv. *Solaris* biomass is suitable for ensiling. Furthermore, SiloSolaris administration did not affect heifers' growth performance and welfare status as well as the follow-up period on productive and reproductive traits. The observed plasma constituents were found always within or very close to the normal ranges; however, the reported high lipid content of *Solaris* silage could be responsible for the higher plasma triglycerides and cholesterol values in SiloSolaris than Control group.

Taken together, the results indicate that the inclusion of *Nicotiana tabacum* cv. *Solaris* ensiled biomass in the diet of growing heifers may be beneficial, suggesting a potential new use as valuable forage, as further co-product of this versatile energy plant. Although these initial results need to be confirmed, they suggest the multipurpose nature of *Nicotiana tabacum* cv. *Solaris* could add value to its cultivation in dedicated areas, also contributing to the recovery of tobacco production knowledge. Especially due to the generalized crisis of smoking tobacco sector, occurred during the last decades, the valorization of products obtained from this new cultivar would appear essential to local economy of the tobacco cultivation areas, representing a crucial step toward sustainable and green agriculture, with a circular economy approach capable of reducing the competition between food and feed products. At the same time, the chemical characteristics of ensiled cv. *Solaris* biomass suggest that its use could be also interesting in beef production as an ingredient of finishing diets, allowing to expand the productive chains in which *Nicotiana tabacum* cv. *Solaris* could be involved, besides its destination for energy, i.e. biofuel and biomethane, and as alternative protein source for animal nutrition, i.e. the co-product seed cake from oil extraction.

Ethics approval

The University of Molise Bioethics Committee approved the trial (No. II/23-8357).

Data and model availability statement

None of the data were deposited in an official repository. The data that support the study findings are available upon request.

Author ORCIDs

Antonella Fatica: https://orcid.org/0000-0003-0207-089X;
Francesco Fantuz: https://orcid.org/0000-0002-6464-4636;
Elisabetta Salimei: https://orcid.org/0000-0001-9945-1458.

Author contributions

A. Fatica: Conceptualization, Investigation, Formal analysis, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization. **F. Fantuz:** Conceptualization, Validation, Formal analysis, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization. **F. Di Lucia:** Investigation, Resources, Writing - Review & Editing. **M. Zuin:** Conceptualization, Resources. **L. Borrelli:** Investigation, Resources. **E. Salimei:** Conceptualization, Methodology, Data Curation, Writing - Original Draft, Writing - Review & Editing, Supervision, Project administration.

Declaration of interest

None.

Acknowledgments

The authors are grateful to Idroedil s.r.l. (Arma Di Taggia, Imperia, Italy) for providing *Nicotiana tabacum* L., cv. *Solaris* biomass and partially supporting the experiment.

Financial support statement

This research received no specific grant from any funding agency, commercial or not-for-profit section.

References

Association of Official Analytical Chemist (AOAC), 2000. Official Methods of Analysis. The Association of Official Analytical Chemists, Gaithersburg, MD, USA.
Bocquier, F., González-García, E., 2010. Sustainability of ruminant agriculture in the new context: feeding strategies and features of animal adaptability into the necessary holistic approach. Animal 4, 1258–1273. https://doi.org/10.1017/S1751731110001023.
Borreani, G., Tabacco, E., Schmidt, R.J., Holmes, B.J., Muck, R.E., 2018. Silage review: factors affecting dry matter and quality losses in silages. Journal of Dairy Science 101, 3952–3979. https://doi.org/10.3168/jds.2017-13837.
Boulton, A.C., Rushton, J., Wathes, D.C., 2017. An empirical analysis of the cost of rearing dairy heifers from birth to first calving and the time taken to repay these costs. Animal 11, 1372–1380. https://doi.org/10.1017/S1751731117000064.
Brown, E.G., Vandehaar, M.J., Daniels, K.M., Liesman, J.S., Chapin, L.T., Keisler, D.H., Nielsen, M.S.W., 2005. Effect of increasing energy and protein intake on body growth and carcass composition of heifer calves. Journal of Dairy Science 88, 585–594. https://doi.org/10.3168/jds.S0022-0302(05)72722-3.
Colombini, S., Rapetti, L., Colombo, D., Galassi, G., Crovetto, G.M., 2010. Brown midrib forage sorghum silage for the dairy cow: nutritive value and comparison with corn silage in the diet. Italian Journal of Animal Science 9, 273–277. https://doi.org/10.4081/ijas.2010.e53.
Cooke, J.S., Cheng, Z., Bourne, N.E., Wathes, D.C., 2013. Association between growth rates, age at first calving and subsequent fertility, milk production and survival in Holstein-Friesian heifers. Open Journal of Animal Science 3, 1–12. https://doi.org/10.4236/ojas.2013.31001.
Crovetto, G.M., 2006. Composizione chimica e valore nutritivo dei principali foraggi. Progetto "FORAGE" (n°688), Regione Lombardia. Retrieved on 04 April 2019 from http://www.aral.lom.it/public/upload/file/CONVEGNI/Convegno%20Forage/03_a_Crovetto.
Edmonson, A.J., Lean, I.J., Weaver, L.D., Farver, T., Webster, G., 1989. A body condition scoring chart for holstein dairy-cows. Journal of Dairy Science 72, 68–78. https://doi.org/10.3168/jds.S0022-0302(89)79081-0.
El-Kasrawy, N.I., Swelum, A.A., Abdel-Latif, M.A., Alsenosy, A. El-W, Beder, N.A., Alkahtani, S., Abdel-Daim, M.M., Abd El-Aziz, A.H., 2020. Efficacy of different drenching regimens of gluconeogenic precursors during transition period on body condition score, production, reproductive performance, subclinical ketosis and economics of dairy cows. Animals 10, 937. https://doi.org/10.3390/ani10060937.
Food and Agriculture Organization of the United Nations (FAO), 2019. Transforming Food and Agriculture to Achieve the SDGs: 20 interconnected actions to guide decision-makers. Rome, IT. Retrieved on 07 November 2019 from http://www.fao.org/3/ca1612en/ca1612en.pdf.
Fatica, A., Di Lucia, F., Marino, S., Alvino, A., Zuin, M., De Feijter, H., Brandt, B., Tommasini, S., Fantuz, F., Salimei, E., 2019. Study on analytical characteristics of *Nicotiana tabacum* L., cv. *Solaris* biomass for potential uses in nutrition and biomethane production. Scientific Reports 9, 16828. https://doi.org/10.1038/s41598-019-53237-8.
Fogher, C., 2008. Mutagenized tobacco plant as seed culture for the production of oil for energetic, Industrial and alimentary uses. European Patent Application Bulletin 2013/46 - Wo/2008/110876(PCT/IB2007/053412). Retrieved on 03 December 2019 from http://www.eapatis.com/getdocument.asp?Document=//patstorage/EapatisStorage/wo/wo209050/wo2008110876r3.
Grasser, L., Fadel, J., Garnett, I., DePeters, E., 1995. Quantity and economic importance of nine selected by-products used in California dairy rations. Journal of Dairy Science 78, 962–971. https://doi.org/10.3168/jds.S0022-0302(95)76711-X.
Grisan, S., Polizzotto, R., Raiola, P., Cristiani, S., Ventura, F., Di Lucia, F., Zuin, M., Tommasini, S., Morbidelli, R., Damiani, F., Pupilli, F., Bellucci, M., 2016. Alternative use of tobacco as a sustainable crop for seed oil, biofuel, and biomass. Agronomy for Sustainable Development 36, 1–8. https://doi.org/10.1007/s13593-016-0395-5.
Hall, M.B., 2002. Characteristics of manure: what do they mean? In: Proceedings of the Tri-State Dairy Nutrition Conference, 16-17 April 2002, Fort Wayne, Indiana, US, pp. 141–147.
Heinrichs, A.J., Jones, C.M., Gray, S.M., Heinrichs, P.A., Cornelisse, S.A., Goodling, R.C., 2013. Identifying efficient dairy heifer producers using production costs and data envelopment analysis. Journal of Dairy Science 96, 7355–7362.
Heinrichs, A.J., Zanton, G.I., Lascano, G.J., Jones, C.M., 2017. A 100-year review: a century of dairy heifer research. Journal of Dairy Science 100, 10173–10188. https://doi.org/10.3168/jds.2017-12998.
Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise (IZSAM), 2018. Standardized procedures IZS TE S5 B2.1.6 SOP001-Rev1-07 and IZS TE S5 B2.1.6 SOP003-Rev0-07. IZSAM, Campobasso, IT.
Kaneko, J.J., Harvey, J., Bruss, M., 2008. Appendix VIII Blood analyte reference values in large animals. In Clinical Biochemistry of Domestic Animals (ed. JJ Kaneko, J Harvey and M Bruss), sixth ed. Academic Press, San Diego, CA, USA, pp. 882–888.
Kassel, A., 2015. Bovine haematology and biochemistry. In Bovine Medicine (ed. PD Cockcroft), third ed. John Wiley & Sons Ltd, Oxford, UK, pp. 146–160.
Krpalkova, L., Syrucek, J., Kvapilik, J., Burdych, J., 2017. Analysis of milk production, age of first calving, calving interval and economic parameters in dairy cattle management. Mljekarstvo 67, 58–70. https://doi.org/10.15567/mljekarstvo.2017.0107.
Kung Jr., L., Shaver, R.D., Grant, R.J., Schmidt, R.J., 2018. Silage review: interpretation of chemical, microbial, and organoleptic components of silages. Journal of Dairy Science 101, 4020–4033. https://doi.org/10.3168/jds.2017-13909.
Lean, I.J., Rabiee, A.R., Moss, N., 2015. A hazards analysis critical control point approach to improving reproductive performance in lactating dairy cows. In Bovine Medicine (ed. PD Cockcroft), third ed. John Wiley & Sons, Ltd, Oxford, UK, pp. 450–464.
Martens, H., Leonhard-Marek, S., Röntgen, M., Stumpff, F., 2018. Magnesium homeostasis in cattle: absorption and excretion. Nutrition Research Reviews 31, 114–130. https://doi.org/10.1017/S0954422417000257.
National Research Council (NRC), 2001. Nutrient requirements of dairy cattle. 7th Revised ed. National Academy Press, Washington, DC, USA.
Notarnicola, B., Tassielli, G., Renzulli, P.A., Lo Giudice, A., 2015. Life Cycle Assessment in the Agri-food Sector: an overview of its key aspects, international initiatives, certification, labelling schemes and methodological issues. In: Notarnicola, B., Salomone, R., Petti, L., Renzulli, P.A., Roma, R., Cerutti, A.K. (Eds.), Life Cycle Assessment in the Agri-food Sector. Springer International Publishing, Cham, CH, pp. 1–56.
Petrovski, K., 2015. Cattle Signs. In Bovine Medicine (ed. PD Cockcroft), third ed. John Wiley & Sons Ltd, Oxford, UK, pp. 347–359.
Poltronieri, P., D'Urso, O.F., 2016. Tobacco seed oil for biofuels. In biotransformation of agricultural waste and by-products (ed. P Poltronieri and OF D'Urso). Elsevier, Amsterdam, NL, pp. 161–187. https://doi.org/10.1016/B978-0-12-803622-8.00006-9.
Sauvant, D., Chapoutot, P., Peyraud, J.L., Meschy, F., Doreau, B., 2004. Nutritional values for ruminants. In Tables of composition and nutritional value of feed materials: pigs, poultry, cattle, sheep, goats, rabbits, horses, and fish (ed. D Sauvant, JM Perez and G Tran). Wageningen Academic Publishers, The

Netherlands & INRA, Paris, France, pp. 43–52. https://doi.org/10.3920/978-90-8686-668-7.
Sprecher, D.J., Hostetler, D.E., Kaneene, J.B., 1997. A lameness scoring system that uses posture and gait to predict dairy cattle reproductive performance. Theriogenology 47, 1179–1187. https://doi.org/10.1016/s0093-691x(97)00098-8.
Stallings, C.C., 1998. Manure scoring as a management tool. Proceedings of the Western Canadian Dairy Seminar, Red Deer, Alberta. Retrieved on 15 November 2019 from https://wcds.ualberta.ca/wcds/wpcontent/uploads/sites/57/wcds_archive/Archive/1998/ch25.htm.
Stefani, A., Casu, A., Moro, L., 2011. Guida pratica all'interpretazione dei parametri di chimica-clinica nella bovina da latte. Istituto Zooprofilattico delle Venezie. Retrieved on 15 December 2019 from https://issuu.com/izsvenezie/docs/bovina_da_latte._guida_pratica_all_.
Tassew, Z., Chandravanshi, B.S., 2015. Levels of nicotine in Ethiopian tobacco leaves SpringerPlus 4, 649. https://doi.org/10.1186/s40064-015-1448-y.
Van Amburgh, M.E., Galton, D.M., Bauman, D.E., Everett, R.W., Fox, D.G., Chase, L.E., Erb, H.N., 1998. Effects of three prepubertal body growth rates on performance of holstein heifers during first lactation. Journal of Dairy Science 81, 527–538. https://doi.org/10.3168/jds.S0022-0302(98)75604-8.
Van Saun, R.J., 2008. Metabolic Profiling. In Current veterinary therapy: food animal practice, Fifth Volume (ed. DE Anderson and M Rings). Saunders Elsevier, St. Louis, MO, USA, pp. 153–162.
Wang, S.S., Shi, Q.M., Li, W.Q., Niu, J.F., Li, C.J., Zhang, F.S., 2008. Nicotine concentration in leaves of flue-cured tobacco plants as affected by removal of the shoot apex and lateral buds. Journal of Integrative Plant Biology 50, 958–964. https://doi.org/10.1111/j.1744-7909.2008.00684.x.