agriculture

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The Effects of Brewer's Spent Yeast (BSY) Inclusion in Dairy Sheep's Diets on Ruminal Fermentation and Milk Quality Parameters

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Abstract: Brewer's spent yeast (BSY) is a byproduct of the beer industry, rich in proteins and bioactive compounds. The effects of BSY were investigated through a 6-week feeding trial on 30 lactating dairy Blackhead sheep (54.7 ± 5.66 kg, 177.34 days in milk) distributed under a completely randomised design in two groups: a control group (with soybean meal and sunflower meal as protein sources), and a BSY group, where soybean meal was totally replaced by BSY (5.4% inclusion, DM basis). The inclusion of BSY led to a 25% decrease in ruminal propionate and in the acetate/propionate ratio. BSY influenced the milk fatty acid profile by significantly decreasing the omega 6: omega 3 ratio and increasing the contents of caproic, capric, and lauric acids. Also, the inclusion of BSY was associated with an increase in the milk's total polyphenols. Consistently, determinations of the parameters of the milk fat's oxidative stability revealed a decrease in conjugated diene contents. Moreover, the mineral contents of milk were influenced, with an increase in Ca content noted. Overall, these results indicate that BSY represents an alternative feedstuff for ruminants' nutrition, which could have the potential to induce changes in ruminal fermentation and milk composition that are beneficial for consumers.

Keywords: BSY; dairy sheep; milk quality; milk oxidative status; rumen metabolism

Citation: Oancea, A.-G.; Dragomir, C.; Untea, A.; Saracila, M.; Turcu, R.; Cismileanu, A.; Boldea, I.; Radu, G.L. The Effects of Brewer's Spent Yeast (BSY) Inclusion in Dairy Sheep's Diets on Ruminal Fermentation and Milk Quality Parameters. *Agriculture* **2023**, *13*, 1605. https://doi.org/10.3390/agriculture13081605

Academic Editor: Federico Infascelli

Received: 7 July 2023
Revised: 28 July 2023
Accepted: 12 August 2023
Published: 14 August 2023

1. Introduction

Today, there is an increasing interest in a healthy and balanced diet, but the explosive population growth makes supplying the human diet with all necessary nutrients a challenge for the food industry [1]. Thus, it is important to develop several strategies aimed at improving foodstuffs' composition with several nutritive compounds that can help the proper functioning of the human body and prevent some diseases, in order to achieve the nutritive requirements for a healthy lifestyle [2]. The most important strategy used to influence animals' food products, leading to nutritionally enriched foodstuffs, is the animal feeding system, but the commonly used feed resources (e.g., soya) are limited and some of them are unavailable or expensive. Thus, the valorisation of several industry byproducts as raw materials for animals' nutrition represents a sustainable alternative [3–5]. Yeast is an important feed ingredient in ruminants' diets. Several studies have examined the

impact of including live yeast in ruminants' diets (e.g., *Saccharomyces cerevisiae*), obtaining contradictory results depending on the level of yeast inclusion. The addition of 10 g of live yeast in cows' diet enhanced their dry matter intake and milk output. However, no significant differences in these parameters were identified at a lower inclusion level (4.0 g/day) [6,7].

Also, brewery byproducts can be used as feedstuffs for ruminants. It has been reported that brewer's yeast, liquid brewer's yeast, or brewer's spent grain can be used as alternative feedstuffs to commonly used protein sources (such as soybeans), without affecting ingesta behaviour, gas kinetics, or ruminal fermentation [8–11]. Brewer's spent yeast (BSY) represents one of the major byproducts obtained from the brewery industry. BSY is generated from the fermentation of yeast that is no longer useful in the fermentation process and must be discarded. A small portion of it can be used to initiate a new fermentation cycle, but the majority of it represents the spent yeast (BSY) [12]. In the brewery process, BSY can be considered to be waste and disposed of by the brewers [13]. However, from the economical point of view, and considering its composition, BSY may have potential application in ruminants' nutrition, as an alternative feedstuff to commonly used protein sources [12]. In addition to being a protein source, the composition of BSY suggests that its inclusion in ruminants' diets may have positive effects, e.g., by influencing the ruminal environment. Because BSY is rich in α- and β-acids, it has potential inhibitory activity against hyper-ammonia bacteria and Gram-positive bacteria; therefore, the inclusion of BSY in ruminants' diets can reduce the ruminal concentration of ammonia and methane production, which is important from the environmental point of view [14]. Also, BSY may have effects on milk quality, considering its rich composition of bioactive compounds, such as vitamins, minerals, β-glucans, and phenolic compounds, known for their anti-inflammatory, antitumor, and antioxidant activities [12,15]. Despite its potential, little attention has been paid to the effects of BSY on ruminal fermentation parameters and milk quality. Moreover, very few publications are available in the literature that discuss the effects of the other inactivated yeasts in ruminants. Several studies have suggested that BSY or other live yeasts may have the potential to improve milk production and milk fat; however, the effects on milk minor constituents have not been fully elucidated [12,16–18]. The ability to change the minor constituents of the milk may be of interest, especially in the periods when the milk yield is decreasing, such as during late lactation.

The aim of this study was to investigate the effect of BSY's inclusion in late-lactation dairy sheep's diets on the parameters describing ruminal fermentation and several milk quality parameters, given that BSY can positively influence ruminal fermentation and increase milk's antioxidant capacity.

2. Materials and Methods

2.1. Animals, Diets, and Experimental Design

The feeding trial was performed according to legislation in this field (Directive 2010/63/EU) [19], and the procedures used were authorised by the Ethical Commission of the National Research and Development Institute for Biology and Animal Nutrition (INCDBNA), Balotesti, Romania. Brewer's spent yeast was obtained from a local company (Isalnita, Dolj County, Romania) as a byproduct from a large local brewery, inactivated and dried. The feeding trial was assessed using 30 multiparous lactating Teleorman Blackhead dairy sheep, in the late lactation period (177.34 days in milk), with an average live weight of 54.7 ± 5.66 kg, aged 3.64 ± 0.8 years, and with an average parity number of 2.9 ± 0.76. The sheep were distributed under a completely randomised design in two groups, kept in paddocks: a control group (15 sheep)—C (with soybean meal and sunflower meal as protein sources), and a BSY group (15 sheep), where soybean meal (SBM) was replaced with BSY (5.4% inclusion in diet, dry matter basis (DM)). The average milk production for the C group was 0.36 L, while that for the BSY group was 0.34 L, with no significant difference between groups. The experimental period started in mid-August and lasted 6 weeks (ingesta and milk records), after 2 weeks of dietary adaptation; rumen and milk

samples were taken separately in the final week of the trial. Dairy sheep were fed in order to meet their nutritional demands, which were assessed based on their weight, physiological status, and predicted milk yield, and they had continuous access to fresh water.

The proximate chemical composition of BSY and the replaced feedstuff, SBM, is presented in Table 1.

Table 1. Chemical composition of SBM and BSY.

Feedstuff	Crude Protein (%)	Crude Fat (%)	Cellulose (%)	Ash (%)
SBM [1]	44.23	0.36	5.46	5.87
BSY [2]	38.82	0.62	0.19	5.96

[1] Soybean meal; [2] brewer's spent yeast.

The two diets (C and BSY) were formulated to be isoenergetic and isonitrogenous, using the French feeding system [20]. The dietary ingredients were fed in limited amounts in order to minimise the differences between the diets in terms of energy and protein supplies (no refusals were recorded).

Because SBM has greater protein content than SBY, a proportion of corn grains was replaced with wheat grains in order to ensure that the diets were isonitrogenous.

Thus, the only differences between the control and BSY groups could be attributed to the specifics of BSY, including its active substances. The structure of the diets is presented in Table 2.

Table 2. Experimental diets and their nutrient supply.

Ingredients in Concentrate (as Fed, %)	Control Group	BSY Group
Maize	50	33
Wheat	23	40
Wheat bran	3	3
Sunflower meal	10	10
Soybean meal	10	0
BSY in concentrate	0	10
Calcium carbonate	2	2
Sodium chloride	1	1
Mineral–vitamin supplement	1	1
Intake (kg/day/head)		
Concentrate	1.40	1.40
Hay grass (cock's foot)	1.60	1.60
Nutrients in total diet (g/day)		
DM [1], as fed	2580	2591
EE [2]	82.15	81.37
Starch	635.68	615.07
MFU [3]	2.04	2.03
IDPE [4]	223.93	222.01
IDPN [5]	223.12	222.72

[1] Dry matter, [2] ether extract, [3] milk feed units, [4] intestinally digestible protein allowed by rumen-available energy, and [5] intestinally digestible protein allowed by rumen-available nitrogen; dietary parameters were calculated based on the French feeding system [20].

2.2. Samples Collection

Milk and ruminal fluid samples were collected individually on the same day, in the last week of the experiment, from all animals involved in the experiment.

Milk samples were collected on the same day and stored at −4 °C, until the complete analysis.

The rumen contents were collected 4 h after the morning feed, in sufficient quantities to ensure multiple analyses and replicates, using a flexible oesophageal tube (2 mm wall

thickness and 6 mm internal diameter) connected to a vacuum pump. After sampling, the ruminal fluid was filtered through four layers of gauze.

2.3. Proximate Chemical Composition of Diets and Milk Samples

The chemical composition of the SBM and BSY was determined through the Weende system. The Kjeldahl method was used to study the crude protein content. Soxhlet determination was used in order to determine the crude fat content. Crude ash was obtained using a Nabertherm Labotherm L15/11/P320 Comfort (Bremen, Germany) equipment. Crude fibre extraction was performed using the Raw Fiber Extractor FIWE 6 (Velp Scientifica, Via Stazione, Usmate, Italy). The methods used for the determination of the proximate chemical compositions of the SBM and BSY were performed following the methods described in [21].

The primary chemical composition of sheep milk (total protein, total fat, total caseins, lactose, and pH) was obtained by the FTIR method using a CombiScope FTIR 200 with a system consisting of a component analyser, a somatic cell count, and a sample handling (ISO 9622: 2013) [19].

2.4. Mineral Composition of the BSY and Milk Samples

Determination of the trace mineral (i.e., cooper, iron, manganese, zinc) contents of the BSY and milk samples was performed using the method described in [22]. A complexometric method using ethylenediaminetetraacetic acid (EDTA) as a titration agent in the presence of murexide was used to determine the calcium concentration. In order to study the phosphorus content, we used a colorimetric method with molybdovanadate and a Jasco V530 UV/VIS spectrophotometer (Japan Servo Co., Ltd.,Tokyo, Japan), as described in [22].

2.5. Hydrophilic Components of the Milk Samples

The total polyphenol contents of the BSY and milk samples were determined using the Folin–Ciocâlteu method, and antioxidant capacity was determined using the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) method [23].

2.6. Lipophilic Components of the Milk Samples

High-performance liquid chromatography (HPLC) was used in order to study the vitamin E contents of the BSY and milk samples, using a Finnigan Surveyor Plus chromatograph (Thermo-Electron Corporation, Waltham, MA, USA) with a DAD detector and a C18 column. The mobile phase consisted of 4% water and 96% methanol, and the elution of the analytes was performed with a 1 mL/min flow for 20 min, as described in [24]. The fatty acid profile of the milk samples was determined using gas chromatography with a PerkinElmer Clarus 500 gas chromatograph (Waltham, MA, USA) equipped with a chromatographic column with a high-polar stationary phase (60 m $\times$ 0.25 mm, 0.25 µm; TRACE TR-Fame, Thermo-Electron, Waltham, MA, USA) and a flame ionisation detector [25].

The extraction of lipids from the milk samples was performed using a modified Folch method [26]. The analysis of the parameters that describe the first phase of lipid oxidation (conjugated dienes (CD) and conjugated trienes (CT)) was performed using a spectrophotometric method, as described in [23]. The thiobarbituric-acid-reactive substances (TBARS) values were obtained using a determination method specific to milk samples, following the method described in [25], with 5 mL of milk sample, 20% trichloroacetic acid (TCA), and 0.8% thiobarbituric acid (TBA), by recording the specific absorbances for milk lipids' oxidative products at 450 nm (saturated aldehydes), 495 nm (dienals), and 532 nm (malondialdehyde (MDA)).

2.7. Determination of the Ruminal Parameters

The pH of ruminal fluid was measured immediately after sampling, using a Dostmann Electronic GmbH pH meter (Wertheim-Reicholzheim, Germany) [27]. The ruminal ammonia-N concentration was determined using an electrode with a permeable ammonia membrane [27]. Then, 10 mL of ruminal fluid was preserved with 2.5 mL phosphoric

acid and kept at −20 °C until measurement of the volatile fatty acids (VFAs). The VFA profile of the ruminal fluid was determined using gas chromatography with a Varian gas chromatograph system, equipped with an Elite-FFAP capillary column (30 m length, inner diameter 320 µm, film thickness 0.25 µm), hydrogen carrier gas flow of 1.5 mL/per min, injector temperature of 250 °C, and a flame ionisation detector, as described in [24]. The contents of VFAs were expressed as mmol/L and calculated using a calibration curve obtained using a standard mix (CRM47975 from Supelco, Bellefonte, PA, USA) [28].

2.8. Statistical Analysis

Data were checked for normality using the Kolmogorov–Smirnov statistical test. T-test analysis (version 16, Minitab® Statistical Software) was used to perform statistical analyses for all of the parameters describing ruminal fermentation and milk quality, with the exception of the TBARS method, which was statistically analysed using bifactorial (storage time and diet) analysis of variance (ANOVA) (version 16, Minitab® Statistical Software). The differences between mean values were considered significant at $p < 0.05$. Graphs were obtained using GraphPad Prism (9.3.0) software (San Diego, CA, USA). Pearson's correlation coefficient test between the primary chemical composition of milk, milk fatty acids, milk minerals, antioxidant compounds, and oxidation products of milk lipids was presented as correlation heatmap, where yellow colours correspond to positive correlation coefficients and dark purple colours correspond to negative correlation coefficients.

3. Results

3.1. Chemical Composition of the BSY

It is worth mentioning that, in addition to its high protein content, the studied BSY can be an important source of minerals and plant secondary metabolites (Table 3).

Table 3. Composition of minerals and antioxidant potential of BSY.

Parameter	BSY [1]
Composition of minerals	
Cooper (µg/g)	1.97
Iron (µg/g)	106.43
Manganese (µg/g)	4.05
Zinc (µg/g)	73.01
Calcium (%)	0.38
Phosphorus (%)	1.21
Antioxidant potential	
TPC [2] (mg/g gallium eq.)	2.77
Vitamin E [3] (mg/kg)	125.69
TAC [4] (mM Trolox eq.)	13.26

[1] Brewer's spent yeast, [2] total polyphenol content, expressed as gallium acid equivalents, [3] vitamin E composition, expressed as mg/kg, and [4] total antioxidant capacity, expressed as Trolox equivalents.

Our analyses indicated that BSY can be an important source of minerals, with high contents of iron (106.43 µg/g iron) and zinc (73.01 µg/g). Moreover, from the point of view of antioxidant potential, we should highlight the high concentration of vitamin E (125.69 mg/kg) and the remarkable antioxidant capacity (13.26 mM Trolox equivalents) of the studied BSY.

3.2. The Effects of BSY on the Parameters of Ruminal Fermentation

The results for the ruminal pH value, ammonia content, and total volatile fatty acid (VFA) contents of ruminal fluid in the C and BSY groups are presented in Table 4.

Table 4. The effects of BSY inclusion on pH level, ammonia content, and total volatiles fatty acids of ruminal fluid.

Parameter	Control Group	BSY Group	SEM	*p*
pH	6.29	6.10	0.084	0.264
Ammonia N (mg/dL)	18.60	23.00	11.515	0.060
Acetate (mmol/L)	51.36	53.79	2.600	0.781
Propionate (mmol/L)	29.48 ^{A}	22.09 ^{B}	1.803	0.019
Butyrate (mmol/L)	6.68	9.44	0.889	0.091
Total VFAs ^{1} (mmol/L)	93.15	87.40	4.690	0.394
Acetate/propionate	1.83 ^{B}	2.52 ^{A}	0.219	0.006

^{1} Volatile fatty acids; $^{A, B}$ means within a row with no common superscript differ significantly ($p < 0.05$).

The ruminal pH value registered at 4 h post-feeding was not affected by the treatments. The ammonia concentration and total VFA concentration were not influenced by the inclusion of BSY. The inclusion of BSY was associated with a change in the relative proportions of the VFAs. A statistically significant decrease in the propionate concentration ($p = 0.019$) and a tendency to increase ($p = 0.091$) in the case of butyric acid concentration were observed in the BSY group. Consequently, the acetate: propionate ratio significantly increased ($p = 0.006$) in the BSY group.

3.3. The Effects of BSY Inclusion on Milk Composition

The effects of BSY on the primary chemical composition of sheep milk are presented in Table 5. The inclusion of BSY did not significantly influence the primary chemical composition of milk; however, a tendency to increase in the milk fat content was observed in the BSY group.

Table 5. The effects of the BSY's inclusion on the primary chemical composition of milk.

Parameter	Control Group	BSY Group	SEM	*p*
Fat %	6.88	7.41	0.649	0.577
Protein %	7.39	7.51	0.230	0.721
Caseins (g/L)	60.53	63.58	3.290	0.527
Solids %	19.21	20.25	0.985	0.476
Lactose%	4.03	4.10	0.151	0.755
pH	6.70	6.65	0.021	0.113

On the other hand, the inclusion of BSY induced significant changes in the milk's fatty acid profile; the data are presented in Table 6.

The inclusion of BSY resulted in a significant increase in saturated fatty acid contents ($p = 0.006$) and a significant decrease in monounsaturated and polyunsaturated fatty acid contents. There were also substantial decreases in total Ω6 fatty acids ($p = 0.001$) and the Ω6/Ω3 ratio ($p = 0.006$). Moreover, the inclusion of BSY induced a significantly increase in the contents of the short-chain fatty acids (SCFAs) (e.g., caproic acid, $p = 0.017$) and some of the medium-chain fatty acids (e.g., capric acid and lauric acid, $p = 0.012$ and $p = 0.029$, respectively).

The results obtained for the milk's mineral contents are presented in Table 7.

The BSY group had greater calcium concentrations than the control group, and the difference was statistically significant ($p = 0.001$). The BSY, on the other hand, had no effect on the phosphorus and zinc concentrations in milk.

The parameters that are relevant to the antioxidant potential of milk are presented in Table 8.

Table 6. The effects of BSY's inclusion on the fatty acid profile of milk.

g FAME/100 g Total FAME		Control Group	BSY Group	SEM	*p*
C 4:0	Butyric acid	0.047	0.079	0.013	0.129
C 6:0	Caproic acid	1.266 ^{B}	1.622 ^{A}	0.089	0.017
C 8:0	Caprylic acid	2.306	2.533	0.109	0.171
C 9:0	Nonanoic acid	0.239	0.285	0.029	0.283
C 10:0	Capric acid	9.934 ^{B}	11.169 ^{A}	0.284	0.012
C 11:0	Undecanoic acid	0.762	0.650	0.068	0.268
C 12:0	Lauric acid	8.055 ^{B}	9.347 ^{A}	0.358	0.029
C 13:0	Tridecanoic acid	0.405	0.349	0.036	0.301
C 14:0	Myristic acid	16.132 ^{B}	17.853 ^{A}	0.394	0.012
C 14:1	Myristoleic acid	1.306	1.406	0.114	0.547
C 15:0	Pentadecanoic acid	0.369 ^{A}	0.257 ^{B}	0.032	0.033
C 15:1	Pentadecenoic acid	2.660 ^{A}	1.964 ^{B}	0.179	0.021
C 16:0	Palmitic acid	28.500	30.388	0.638	0.063
C 16:1	Palmitoleic acid	3.599	3.211	0.259	0.316
C 17:0	Heptadecanoic acid	0.923	0.773	0.094	0.286
C 17:1	Heptadecenoic acid	0.487	0.430	0.042	0.357
C 18:0	Stearic acid	2.135	1.386	0.271	0.080
C 18:1n9c	Oleic cis acid	14.183 ^{A}	10.673 ^{B}	0.776	0.010
C 18:2n6t	Linoleic trans acid	0.270 ^{A}	0.136 ^{B}	0.030	0.010
C 18:2n6c	Linoleic cis acid	3.427	2.861	0.280	0.183
C20:0	Arachidic acid	0.169 ^{A}	0.130 ^{B}	0.012	0.045
C 18:3n6	Linolenic gamma acid	0.024 ^{A}	0.004 ^{B}	0.005	0.020
C 18:3n3	Linolenic alfa acid	0.361	0.341	0.030	0.648
CLA (c9, t11)	Conjugated Linoleic acid	0.509 ^{A}	0.264 ^{B}	0.036	0.001
C 20:2n6	Eicosadienoic acid	0.113 ^{A}	0.048 ^{B}	0.012	0.003
C 20:3n6	Eicosatrienoic acid	0.092	0.148	0.034	0.280
C 20:3n3	Eicosatrienoic acid	0.066	0.068	0.011	0.881
C 20:4n6	Arachidonic acid	0.245	0.180	0.022	0.064
ΣSFA ^{1} %		71.11 ^{B}	77.12 ^{A}	1.143	0.006
ΣMUFA ^{2} %		22.31 ^{A}	17.40 ^{B}	0.790	0.002
ΣPUFA ^{3} %		5.18 ^{A}	3.72 ^{B}	0.235	0.001
Ω3 ^{4} %		0.42	0.41	0.031	0.861
Ω6 ^{5} %		4.76 ^{A}	3.31 ^{B}	0.219	0.001
Ω6/Ω3 ^{6}		11.49 ^{A}	8.33 ^{B}	0.652	0.006

^{1} Saturated fatty acids, ^{2} monounsaturated fatty acids, ^{3} polyunsaturated fatty acids, ^{4} omega 3 polyunsaturated fatty acids, ^{5} omega 6 polyunsaturated fatty acids, ^{6} omega 6: omega 3 ratio; $^{A, B}$ means within a row with no common superscript differ significantly ($p < 0.05$).

Table 7. The effects of BSY's inclusion on the mineral profile of milk.

Parameter	Control Group	BSY Group	SEM	*p*
Calcium (%)	1.063 ^{B}	1.182 ^{A}	0.016	0.001
Phosphorus (%)	0.905	0.868	0.037	0.518
Zinc (ppm)	21.21	20.91	1.583	0.897

$^{A, B}$ Means within a row with no common superscript differ significantly ($p < 0.05$).

The inclusion of BSY led to a significant increase in the milk's total polyphenol content ($p = 0.004$). However, the milk's antioxidant capacity and vitamin E content were not significantly influenced by the inclusion of BSY.

The milk's oxidative stability was assessed through the quantification of the major oxidation products of the milk fatty acids' degradation, using the TBARS method, adapted for milk samples. The results of the TBARS determination after defrosting and after 24 h of storage in both the C and BSY groups are presented in Table 9.

Table 8. The effects of BSY's inclusion on the antioxidant capacity, total polyphenols, and vitamin E content in milk.

Parameter	Control Group	BSY Group	SEM	*p*
Total polyphenols (mg/g GAE [1] equivalent)	1.556 [B]	2.383 [A]	0.126	0.004
Antioxidant capacity (mM Trolox equivalent)	2.724	3.005	0.117	0.127
Vitamin E (mg/L raw milk)	8.619	8.724	0.180	0.691

[1] Gallium acid equivalents; [A, B] means within a row with no common superscript differ significantly ($p < 0.05$).

Table 9. The effects of BSY's inclusion on the thiobarbituric-acid-reactive substances method parameters of the milk.

Parameter	A450 [1]	A495 [2]	A532 [3]	MDA [4] µg/L
		Defrosting time		
Control group	0.442	0.252	0.218	238.5
BSY group	0.443	0.270	0.239	241.8
		24 h of storage time		
Control group	0.464	0.301	0.241	266.3
BSY group	0.476	0.305	0.245	233.9
		Effects		
		Treatment		
Control group	0.453	0.276	0.229	252.4
BSY group	0.459	0.387	0.242	237.8
SEM	0.011	0.011	0.010	11.22
		Time		
0 h	0.442	0.261 [B]	0.228	240.1
24 h	0.470	0.303 [A]	0.243	250.1
SEM	0.011	0.011	0.010	11.25
		p-Value		
Diet	0.695	0.472	0.388	0.372
Time	0.095	0.013	0.290	0.537
Diet*time [5]	0.733	0.638	0.534	0.278

[1, 2, 3] Absorbances read at 450, 495, and 532 nm; [4] malondialdehyde concentration expressed as µg/L; [5] The interactions between storage time and diet factors, [A, B] means within a row with no common superscript differ significantly ($p < 0.05$).

Overall, the inclusion of BSY did not induce significant changes in the TBARS parameters, nor at defrosting time or after 24 h of storage at room temperature, with the exception of absorbance read at 495 nm (specifically for MUFA oxidation products), which was significantly increased by increasing the storage time.

This implies that the inclusion of BSY had no significant influence on the secondary lipid oxidation products. On the other hand, fatty acids' oxidation is a complex process that occurs in different stages; therefore, in order to correctly appreciate the oxidation status of the milk, it is important to quantify the conjugated dienes and trienes as indicators of the first oxidation phase of fatty acids (i.e., primary lipid oxidation products). The results are presented in Figure 1a,b.

As shown in Figure 1a, BSY induced a significant decrease ($p = 0.004$) in the conjugated diene contents in milk; however, the conjugated triene contents in milk tended to decrease as a result of the inclusion of BSY (Figure 1b).

Figure 2 presents the Pearson's correlation coefficients as a correlation heatmap between the primary chemical composition of milk, antioxidant compounds, total antioxidant capacity of milk, milk fatty acids, milk minerals, and oxidation products of milk lipids.

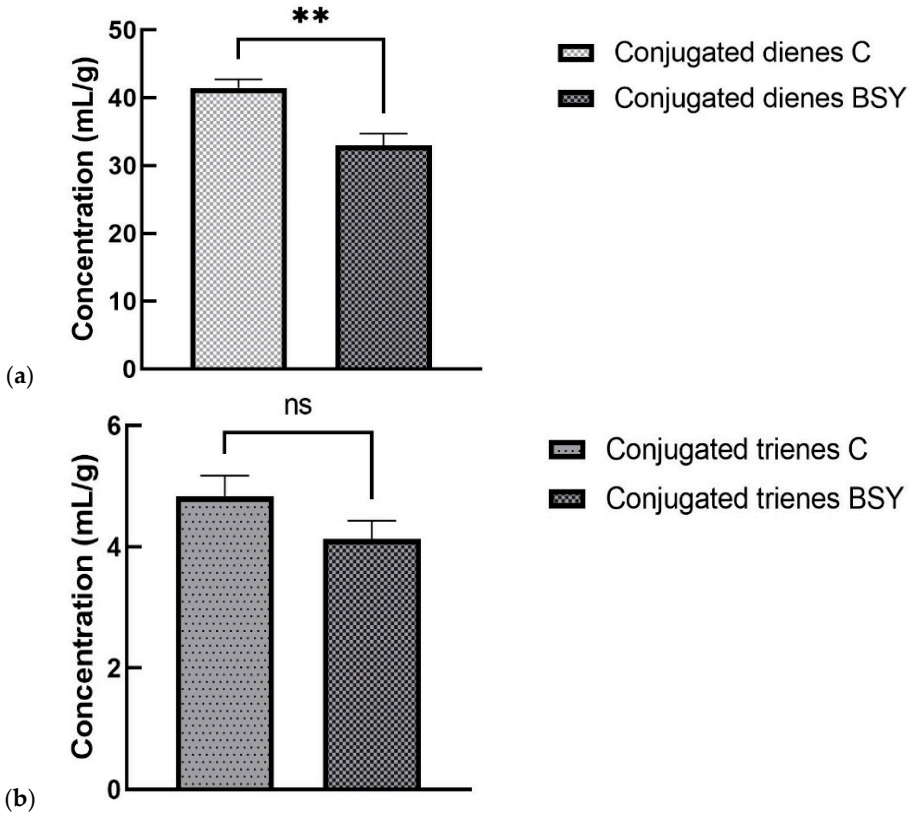

Figure 1. The concentration of conjugated dienes (**a**) and conjugated trienes (**b**) in the control (C) and brewer's spent yeast (BSY) groups. The results are presented as means ± SEM; ** represents significant differences between means ($p < 0.05$), and "ns" represents no significant differences between means.

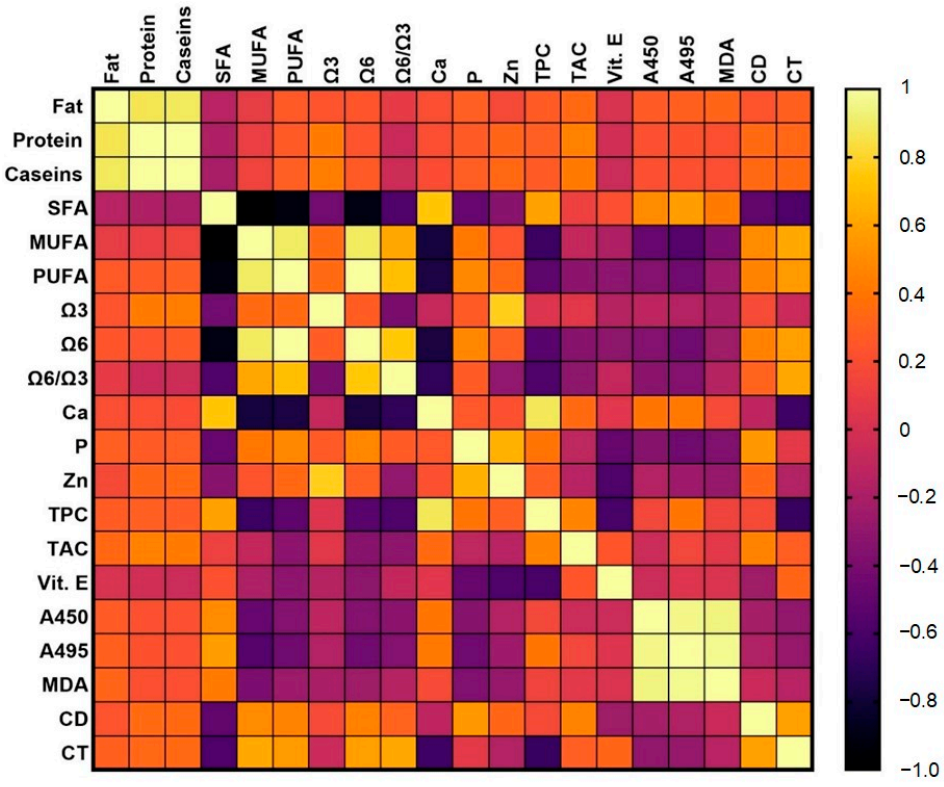

Figure 2. Heatmap presenting the Pearson's correlations between the primary chemical composition of milk, fatty acid profile of milk, milk minerals, antioxidant compounds, and milk lipids' oxidation

products. Abbreviations: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids, Ω3, omega 3 fatty acids; Ω6, omega 6 fatty acids; Vit. E, vitamin E content; TPC, total polyphenols content; TAC, total antioxidant capacity; A450 and A495, specific absorbances for saturated aldehydes and dienals, respectively; MDA, malondialdehyde concentration; CD, conjugated dienes; CT, conjugated trienes.

A negative correlation between milk antioxidant compounds (i.e., vitamin E and TAC) and secondary oxidation products was observed, while the total polyphenols content was negatively correlated with primary oxidation products ($r = -0.893$; $r = -0.913$). Unsaturated fatty acids (MUFA, PUFA) were negatively correlated with saturated fatty acids ($r = -0.996$; $r = -0.968$) and with Ca content ($r = -0.997$; $r = -0.910$). Positive correlations ($r > 0.5$) were found between unsaturated fatty acid contents, Ω3/Ω6 fatty acid contents, Ω6:Ω3 ratio, and *p* content. Unsaturated fatty acids, especially the omega 3 and omega 6 fractions, were positively correlated with secondary milk lipids' degradation parameters. Degradation parameters were positively correlated within their own classes (intraclass, primary, and secondary oxidation parameters) but negatively correlated between those two categories (i.e., interclass). The primary chemical composition of milk was not correlated with any of the degradation parameters, but they were strongly correlated with one another.

4. Discussion

The diversity of the processing technologies, the variability of the raw materials, etc., induce a great variability in the byproducts in terms of characteristics that are relevant from a nutritional point of view. Brewery is no exception; therefore, it is important to consider the nutritional particularities of the studied byproduct.

The crude protein content of the BSY used in the experiment was below the range of the values reported in the literature (45–60%) [12], suggesting particularities of the processing technology. Indeed, the chemical composition of BSY may vary based on diversity of yeast strains used for pitching, the characteristics of the growth medium, the wort gravity and density, etc. [29]. The data regarding the protein content of BSY used in our experiment were closer to the data obtained for the SBM (44.23%). However, it was higher than the protein content of the concentrates commonly used in ruminants' nutrition, such as sunflower or rapeseed cakes (27.9–33.7% crude protein), [30] or other minor oilseeds cakes such as linseed or safflower cakes (32–33% crude protein) [31]. This places BSY between this group and soybean meal, which opens the possibility of replacing the latter in some animal categories that have intermediate protein requirements. The resemblance to the soybean meal is also given by its low fat content (0.36%). For the BSY used in our experiment, the determined level was within the range of 0.02–6.5%, reported in the literature [32–34].

Also, the trace mineral concentrations of the BSY were within the ranges of the data presented in the literature [29,30,32,34], with the exception of zinc, whose concentration was lower than the values reported in other studies [32]. The data regarding the mineral composition of BSY presented in the literature are contradictory and dependent on the technological flux of beer production. The determination of antioxidant potential (antioxidant capacity, total polyphenols, and vitamin E content) confirmed that the studied BSY is an important source of bioactive compounds, showing remarkable antioxidant potential, with a total polyphenols content of 2.77 mg/g gallium acid equivalents, a vitamin E content of 125.69 mg/kg, and an antioxidant capacity of 13.26 mM Trolox equivalents.

Ruminal pH value was not affected by the treatments, being close to the "safe zone" from the point of view of the rumen's normal activity (fibre degradation, microbial protein synthesis, etc.). Our results suggest that BSY can influence the rumen-level digestion by acting on both nitrogen and energy metabolism. Thus, the sheep in the BSY group tended to had a higher level of ruminal ammonia (+23%), which stands in contrast to the fact that

active substances from BSY may actually inhibit the rumen's degradation of proteins, e.g., by decreasing the population of hyper-ammonia-producing bacteria [35,36].

Compared to the control group, the inclusion of BSY considerably influenced the profile of the primary VFAs, leading to a lower propionate concentration, a tendency towards a higher butyrate concentration, and a higher acetate: propionate ratio. Brewery byproducts, including BSY, presumably contain active substances from hop cones [14,35,36]. Various studies have also highlighted the capacity of yeasts to absorb the polyphenols [37] and α- and β-acids from hops during fermentation [14]. However, our results are in contrast to those of [38], who reported that ruminal bacteria produced less acetate in the presence of hop beta-acids. The inclusion of BSY is known to decrease the production of methane during in vitro incubations with ruminal microorganisms [8]; this is related to changes (e.g., in the ruminal populations) that also influence the VFA profiles.

Our results suggest that BSY has the potential to influence processes involved in ruminal methanogenesis (via carbohydrate metabolism, as evidenced by changes in the volatile fatty acid profile, e.g., an increase in the acetate: propionate ratio), but additional research, such as assessing microbial populations, will be required to confirm this finding [35].

In our study, BSY did not significantly influence the primary chemical composition of milk. In both groups, the protein content was at the upper limit of the range reported in the literature for various sheep breeds [39]. Very few data about the effects of BSY on milk composition are presented in the literature; therefore, we also compared our results with those for other yeast-based ingredients, inactivated or not. Studies on the use of BSY slurry or *Saccharomyces cerevisiae* in the diets of ruminants (e.g., cows and sheep) have reported significant increases in the milk fat content by improving the composition of several fatty acids, such as C18:3 [18,40]. Data obtained by other authors [41] revealed an increase in the milk protein content after *Saccharomyces cerevisiae* supplementation in dairy cows, but these results are less comparable, considering that BSY contains inactivated yeast. Other milk parameters, such as total casein content, lactose content, and pH, were not affected by the inclusion of BSY according to data presented in the literature [42–44].

The fact that BSY, in our study, did not significantly influence the primary chemical composition of milk is consistent with the fact that the level of BSY dietary inclusion was limited, and that the diets were designed to be isonitrogenous and isoenergetic.

The fatty acid profiles were also analysed to better observe the effects of BSY on fat quality. The inclusion of BSY induced a significant increase in saturated fatty acid (SFA) contents and a significant decrease in monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) contents. In the literature, there are no data on the effects of BSY on milk's FA profile. Several studies have reported that *Saccharomyces cerevisiae* supplementation did not significantly change the fatty acid profile of cow milk, but unlike BSY they used live yeasts and, consequently, a lower inclusion rate [45,46]. Also, the mechanisms involved in their influence on digestion are different.

On the other hand, the inclusion of BSY induced significant decreases in milk's contents of Ω6 fatty acids, which contributed to the significant decrease in the Ω6/Ω3 ratio. These are important for consumers' health, as it is known that a low Ω6/Ω3 ratio in the consumed lipids may help prevent some autoimmune, inflammatory, and cardiovascular diseases [47].

The fact that the inclusion of BSY induced a significant increase in SCFA (caproic acid) and MCFA (capric and lauric acids) contents may have potential beneficial implications for human health. Various studies have reported that SCFAs and MCFAs are better sources of energy for humans, compared with long-chain fatty acids, due to their smaller molecules and to the pathways of their transport through the organism [48]. Other studies have suggested that SCFAs and MCFAs may play a potential protective role against the growth of pathogens and can exert anti-inflammatory activity [49]. Moreover, recent studies have highlighted that SCFAs can have beneficial effects in the context of cardiovascular diseases such as coronary artery calcification [50].

Considering the particularities of BSY, the milk samples were also compared from the point of view of fine constituents that can influence the quality of milk and can improve

human health. Among the noticeable effects of the BSY was the modification of the levels of some fine constituents of milk. The inclusion of BSY induced a significant increase in calcium content, which is important from the point of view of consumer health. Some authors even consider Ca to be a "super-nutrient" due to its capacity to reduce the risk of developing diseases such as osteoporosis and hypertension, while recent studies have revealed its potential to reduce the risk of developing colon cancer [51].

In terms of milk's antioxidant potential, the inclusion of BSY was linked to an increase in total polyphenol content; this is important from the perspective of milk quality and consumer health, given the polyphenols' ability to block or delay lipid oxidation [52]. The increased in milk's total polyphenol content can be explained by the capacity of BSY to absorb the bioactive compounds (such as polyphenols) from wort during the brewery fermentation process. This opens the possibility to transfer these bioactive compounds, through animal feeding, into the final products, such as milk [37].

On the other hand, the antioxidant capacity of the milk was not significantly influenced by the inclusion of BSY. There are no reports in the literature on the effects of the inclusion of BSY on milk's antioxidant capacity; however, in a study performed on sows' colostrum and milk, a significant increase in milk's antioxidant status was observed when *Saccharomyces cerevisiae* was included in their diet [53].

Vitamin E is another antioxidant compound that can be found in milk, and it is important due to its powerful capacity to inhibit peroxidation [54]; however, in our study, its concentration was not significantly changed by the inclusion of BSY. It is known that BSY is not a rich source of vitamin E, but the antioxidant compounds could influence the absorption and deposition of vitamin E [55].

The antioxidant capacity of milk is considered to be a priority subject, considering that fatty acids' oxidation is one of the most important processes that can lead to changes in milk's flavour and nutritional quality [56]. Studies on the effects of BSY on TBARS parameters are still lacking; however, the same trend was observed in sows' colostrum and milk with the inclusion of a yeast extract [57]. After performing the TBARS analysis adapted for milk samples [25], it was observed that BSY did not influence the TBARS parameters, with the exception of the absorbance read at 495 nm (specifically for saturated aldehydes), which was significantly increased.

The process of fatty acids' oxidation occurs in different stages; therefore, it is important to quantify the primary (conjugated dienes and conjugated trienes) and secondary (TBARS) degradation products. Our results suggested that BSY significantly decreased the contents of conjugated dienes, but the contents of conjugated trienes were not influenced by the inclusion of BSY. Very few publications can be found regarding the degradation products of milk obtained from ruminants fed diets including BSY; however, in one study performed on dairy cows to investigate the effects of the inclusion of malt bagasse and selenium-enriched *Saccharomyces cerevisiae* on animals' performance, it was observed that the contents of conjugated dienes were significantly increased in the experimental group, in contrast with data presented in our study [16].

The heatmap results (Figure 2) confirmed that the presence of antioxidant compounds in milk can slow down the lipids' oxidation processes. The same pattern of negative correlations was presented between both parameters of lipid oxidation and the antioxidant potential of milk (secondary degradation parameters with antioxidant capacity and vitamin E content, and primary degradation products with total polyphenols content), as also reported by other studies [58]. The contents of Ω6 fatty acids were positively correlated with all milk lipid degradation parameters, reflecting that a decrease in Ω6 fatty acids (influenced by the inclusion of BSY) can slow down the milk lipids' oxidation process by decreasing the contents of primary oxidation products of milk lipids (i.e., conjugated dienes).

The primary chemical composition of milk was not correlated with any of the milk lipid oxidation products, but the contents of milk protein and caseins were positively correlated with the milk's total antioxidant capacity. Indeed, the scientific literature confirms the

correlation between antioxidant capacity and milk protein content, as it is also known that proteins can protect the molecules of bioactive compounds, such as polyphenols, and can enhance the antioxidant activity [59]. Moreover, it is known that several peptides present in milk, such as casein fractions, are directly related to the antioxidant capacity of milk, due to their antioxidant potential [60].

5. Conclusions

The results showed that BSY can replace soybean meal (5.4% of DM intake) in the diets of lactating sheep without significantly influencing the production performances, such as milk yield and proximate milk composition.

On the other hand, the BSY significantly influences several ruminal parameters, such as decreasing the propionate content and increasing the acetate: propionate ratio, presumably due to its contents of active substances. Also, the inclusion of BSY had a significant impact on the milk fat quality. Our findings suggest that BSY has the potential to slow down the fatty acid oxidation process in milk, which is consistent with its relevant contents of antioxidant-containing substances.

Overall, the importance of BSY as an alternative feedstuff lies not only in its capacity to substitute classical protein sources, but also in its contents of bioactive substances, which can influence the ruminal fermentation and induce changes in some milk constituents that are beneficial for consumers' health. However, attention should be paid to its side effect of increasing the milk's contents of SFAs.

Author Contributions: Conceptualisation, A.U., C.D., A.-G.O. and I.B.; methodology, A.U., C.D., A.-G.O. and I.B.; formal analysis, A.U., A.-G.O., M.S., R.T. and A.C.; writing—original draft preparation, A.U., A.-G.O. and C.D.; writing—review and editing, A.-G.O., A.U., C.D. and G.L.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research was financially supported by the PN 23-20 0101 National Research Project, and the National Research Development Projects to Finance Excellence 8 PFE/2021, granted by the Ministry of Research, Innovation and Digitalization (MCID).

Institutional Review Board Statement: The animal study protocol was approved by the Ethics Committee of the National Research and Development Institute for Biology and Animal Nutrition (no. 52/30.07.2014) for studies involving animals. The experiment followed the rules of Romanian Law 43/2014 for the handling and protection of animals used for experimental purposes, as well as Directive 2010/63/EU on the protection of animals used for scientific purposes.

Data Availability Statement: All data are contained within the article.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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