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# Essential and toxic minerals content and fatty acid profile of colostrum in dairy sheep

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Simple Summary: Colostrum is gain attention of the scientific community thanks to its nutritional13and therapeutic capabilities. The aims of this study were to characterize the macro and micro composition of colostrum from Sarda dairy sheep, and to compare it with the composition of the mature14milk from the same breed.16

Abstract: Colostrum is the primary source for the acquisition of immunity in ruminant. It provides 17 the transfer of antibodies from the mother to the fetus, and it is the exclusive nutrient source of 18 newborn. The objectives of this study were i) to characterize the macro e micro composition of co-19 lostrum, ii) to analyze the antioxidant capacity, the fatty acid (FA) profile and the content of essential 20 and toxic element in colostrum, iii) and to compare FA profile of colostrum with that of mature milk. 21 For these purposes, colostrum and milk samples were collected from 10 animals arising from 8 22 sheep dairy farms distributed in North Sardinia (Italy). All colostrum samples were analyzed for 23 chemical composition, essential and toxic elements and FA profile. The comparison between milk 24 and colostrum FAs were assessed. The average of fat and protein (TP) concentration in colostrum 25 were 7.8% and 16%, respectively. Also, an average of 40 ± 20 g dm-3 was found for Immunoglobulin 26 G (IgG). As regard antioxidant capacity of colostrum, a high variation among samples from differ-27 ent farms was found for the 2, 2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) assay 28 which was  $30 \pm 10\%$  (Mean  $\pm$  SD). A high content of selenium (Se), zinc (Zn) and copper (Cu) was 29 found in colostrum (respectively 200, 2,5000 and 1,200 µg kg-1). A positive strong correlation be-30 tween TP and IgG was found (r = 91%). The colostrum IgG are correlated positively with Se and Zn, 31 because essential mineral for immune system. The FA profile evidenced, a higher content of me-32 dium- and long-chain fatty acids in the colostrum than mature milk, especially of ARA, EPA, DPA, 33 and DHA. This study provided new information about the colostrum quality of Sarda dairy sheep, 34 and it allowed to highlight the different fatty acid composition of fat in colostrum and mature milk. 35

Keywords: colostrum; essential minerals; fatty acid; immunoglobulins; antioxidant capacity; dairy 36 ewe 37

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# 1. Introduction

Colostrum in sheep provides immunoglobulins (Ig) that confer passive immunity to 40 the newborn lambs [1-3] because the syndesmocorial placenta prevents the transfer of 41 antibodies from the mother ewe to the fetus. Colostrum is also the exclusive nutrient 42 source of the newborn, as it is rich in fats, carbohydrates, and proteins. In addition, its 43 rather complex composition resulting in a vital source of micronutrients (vitamins and 44 minerals), antimicrobial (e.g., lactoferrin and lysozymes) and growth factors. Essential 45

Citation: Lastname, F.; Lastname, F.; Lastname, F. Title. *Animals* **2022**, *12*, x. https://doi.org/10.3390/xxxx

Academic Editor: Firstname Lastname

Received: date Accepted: date Published: date

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microelement in colostrum has also crucial role to avoid nutritional pathologies in suck-46 ling lambs as the myodegeneration disease known as white muscle disease due to sele-47 nium (Se) deficiency [4]. Beyond Se, other essential minerals like copper (Cu), manganese 48 (Mn) and zinc (Zn) have important antioxidant functions that are crucial in protecting the 49 integrity of cell membrane against oxidative stress. On the other hand, toxic minerals like 50 lead (Pb) and cadmium (Cd) could be found in colostrum as consequence of environmen-51 tal pollution or dietary contaminants. 52

The ruminant colostrum has recently received considerable interest because of their 53 potential nutritional and therapeutic effects also in humans [5-7], especially against in-54 flammatory intestinal diseases [6, 8]. Beyond its classical use, e.g. a farm colostrum bank 55 for lambs that have not been suckled from their mothers, aimed to reduce the use of anti-56 biotics in newborns [9], sheep colostrum can be utilized in human nutrition to make bev-57 erages or immunoglobulin-rich dietary supplements. Sheep colostrum has also been an 58 important resource for the nutrition of Mediterranean peoples; in some areas of Italy it 59 was used for the preparation of a fresh cream or a semihard cheese (Casada) a particular 60 ricotta recognized as a traditional agri-food product [10]. Currently, few data are available 61 on the nutritional components of sheep colostrum, such as trace elements content and 62 nutritional quality of fats. An adequate supply of microminerals to transitioning ewes 63 could significantly improve the composition and biological value of colostrum, which 64 could affect the health of newborn lambs. Moreover, it is known that organic supplements 65 of trace elements such as Zn, Cu, Mn, Se, and Fe fed in transitioning period significantly 66 improve immunity and reproduction in dairy animals. 67

The objective of this study was to characterize, through its variability at the farm 68 level, the gross composition, the Ig content, the amounts of essential trace elements as Se, 69 Cu, Mn, Zn, of toxic elements like Pb and Cd, of an allergenic oligoelement like Ni, of the 70 fatty acid profile and of the antioxidant potential of colostrum in Sarda dairy sheep. Fur-71 thermore, the last two parameters were compared with those measured in mature milk. 72

# 2. Materials and Methods

## 2.1. Ethical practices

The experiment was approved by the Ethics committee of the University of Sassari (Prot. n. 139652 03/11/2021 with the authorization of Ministero della Salute n° 676/2021-76 PR based on art. 31 D.lgs. 26/2014). 77

#### 2.2. Animals and Experimental Design

The survey was carried out in eight sheep dairy farms distributed in North Sardinia, 79 Italy, representative by size and breeding technique of the situation of dairy sheep indus-80 try in Sardinia and central Italy where the most important Italian sheep cheese, Pecorino 81 Romano PDO, is produced [11]. Twelve animals per farm, homogenous for age (3 years) 82 were randomly chosen for a total of 96 samples. Each ewe was identified with a medal 83 and was maintained within the flock. The lambing periods were concentrated in November. 85

## 2.3. Colostrum sampling

A colostrum sample was collected from each ewe, by manual milking, within 24 87 hours after lambing. Each colostrum sample has been divided in aliquots and stored for 88 the analysis. 89

### 2.4. Determination of IGg, protein, fat content and fatty acid profile

The Gamma-Globulin (IgG) content has been determined by using electrophoresis 91 MP (test method 13/023). 92

The total nitrogen (TN) was measured using Kjeldhal method [12], and the total pro-93 tein (TP) was calculated as TN x 6.38. Fat content was determined according to the Rose-94 Gottlieb [13]. 95

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Total antioxidant capacity was spectrophotometrically measured with the ferric ion 96 reducing antioxidant power (FRAP) and the 2, 2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical scavenging as described by Tsiplakou at al. [14]. 98

The fatty acid profile was determined in 6 samples for each farm, by gas-chromatog-99 raphy as detailed by Correddu et al. [15]. A gas-chromatograph Agilent model 7890 (Ag-100 ilent Technologies, Santa Clara, CA, USA), equipped with a 7693 Autosampler (Agilent 101 Technologies, Santa Clara, CA, USA) and a flame ionization detector (FID) operating to 102 225°C was used. The stationary phase was a CP-Sil 88 capillary column (100 m × 0.250 μm 103 i.d., 0.25 µn film thickness, Agilent Technologies, Santa Clara, CA, USA). Briefly, 1g of 104 colostrum was added 0.4 cm<sup>3</sup> of 25% of ammonia, 5 cm<sup>3</sup> of hexane and 1 cm<sup>3</sup> of ethyl 105 alcohol. The mixture was first vortexed for 2 min, and then centrifuged at 3000 rpm for 1 106 min, hence, after phase separation, the organic upper layer was separated by the aqueous 107 one. The whole extraction procedure was repeated again for two times, where the only 108 change being the nature of the extracting solution (i.e., 5 cm<sup>3</sup> of hexane and 1 cm<sup>3</sup> of ethyl 109 alcohol 95% in the second extraction, and 5 cm<sup>3</sup> of hexane in the third extraction). The 110 three organic extracts were combined and evaporated at 40°C at a reduced pressure until 111 the complete evaporation of the solvent. According to the FIL-IDF 1999 [16] standard pro-112 cedure, the fatty acid methyl esters (FAME) were prepared by means a base-catalyzed 113 transesterification. Hence, 25 mg of the lipid residue from evaporation was mixed with 1 114 cm<sup>3</sup> of hexane (containing 0.5 mg of internal standard) and 1 cm<sup>3</sup> of 2 mol dm<sup>-3</sup> methanolic 115 solution of KOH. The solution was vortexed for 2 minutes and centrifuged for 1 minutes 116 at 3000 rpm, hence 0.08 g of sodium hydrogensulfate monohydrate were added. Hence, 117 the supernatant used for the gas chromatographic analysis was obtained by the last cen-118 trifugation at 3000 rpm for 3 minutes. Individual FAMEs were identified by comparison 119 of the retention time of each analyte with that of a standard mixture of 37 components 120 FAME Mix (Supelco, Bellefonte, PA). The nonadecanoic acid (C19:0) methyl ester Sigma 121 Chemical Co., St. Louis, MO) was used as internal standard for FAME quantification. 122

The concentration of each fatty acid was expressed as g/100 g of FAME and the 123 groups of FA were also calculated. The nutritional properties were valuated as the ratio 124 between n-6 and n-3 and three indices, that are the atherogenic index (AI) and thrombo-125 genic index (TI) were calculated as reported [17]: AI =  $[12:0 + (4 \times 14:0) + 16:0] / [(PUFA) + (PUFA) + (PUFA$ 126 (MUFA)];  $TI = (14:0 + 16:0) / [(0.5 \times MUFA) + (0.5 \times n-6) + (3 \times n-3) + (n-3: n-6)];$  the 127 hypocholesterolemic to hypercholesterolemic ratio (h:H), calculated as [(sum of 18:1cis-9, 128 18:1cis-11, 18:2 n-6, 18:3 n-6,18:3 n-3, 20:3 n-6, 20:4 n-6, 20:5 n-3, 22:4 n-6, 22:5 n-3 and 22:6 129 n-3/(14:0 + 16:0)]. 130

The  $\Delta$ 9-desaturase indices (DI) were calculated according to Schennink et al. [18] to 131 evaluate the effect of the different diets on the capacity of desaturating SFA to  $\Delta$ 9- UFA: 132 C10 index =  $[C10:1/(C10:0 + C10:1)] \times 100; C14 index = [C14:1 cis-9/(C14:0 + C14:1 cis-9/(C14:0 + C14:1)] \times 100; C14 index = [C10:1/(C10:0 + C14:1)] \times 100; C10 index = [C10:1/(C10:0 + C10:1)] \times 100; C10 index = [C10:1/(C10:1)] \times 100; C10 index = [C10:1/(C1$ 133 9)] × 100; C16 index = [C16:1 cis-9/(C16:0 + C16:1 cis-9)] × 100; C18 index = [C18:1 cis-134 9/(C18:0 + C18:1 cis-9)] × 100; CLA index = [CLA cis-9,trans-11/(C18:1 trans-11 + CLA 135 cis-9,trans-11] × 100; total index = [(C10:1 + C14:1 cis-9 + C16:1 cis-9 + C18:1 ci 136 CLA cis-9,trans-11)/(C10:0 + C14:0 + C16:0 + C18:0 + C18:1 trans-11+ C10:1 + C14:1 cis-9 + 137 C16:1 cis-9 + C18:1 cis-9 + CLA cis-9,trans-11)] × 100. 138

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#### 2.5. Determination of oligoelements and toxic elements in colostrum

The determination of the total amount of Cd, Cu, Mn, Se, Ni, Pb, and Zn in sheep's 140 colostrum samples has been accomplished by means of a validated ICP-MS method. Sam-141 ples have been mineralized by means a Milestone Ethos Easy Labstation microwave oven 142 (Milestone, Sorisole, Italy) and mineralized solutions have been analyzed by means an 143 inductively coupled plasma mass spectrometry (ICP-MS) spectrometer model NexION 144 300X equipped with an autosampler model S10 (Perkin Elmer, Monza, Italy), running un-145 der the Windows 7 operating system. Ultrapure (Type 1) water (specific resistance  $\geq$  18 146  $M\Omega$ ) was used throughout the analytical procedure. The elemental standard solutions 147 were by Carlo Erba (Milan, Italy) for Cd, Cu, Mn, Pb, Ni, Se and Zn, (100 mg dm<sup>-3</sup> in 2% 148 aqueous HNO<sub>3</sub>. The 67% aqueous solution of HNO<sub>3</sub> and the 30% aqueous solution of H<sub>2</sub>O<sub>2</sub> 149 were both Ultrapure Normatom reagents (VWR International, Milan, Italy). The ERM-150 BD151 (Skimmed milk powder) and the IAEA-A-13 (Animal Blood) Certified Reference 151 Materials were by Merck, Milan, Italy and by IAEA, Vienna, Austria, respectively. The 152 NexION ICP-MS tuning solution (2% HNO<sub>3</sub> solution in water containing 1 µg dm<sup>-3</sup> each 153 of Be, Ce, Fe, In, Li, Mg, Pb, and U, code N8145051) and the NexION ICP-MS KED tuning 154 solution (1% HCI solution in water containing Co, 10 µg dm<sup>-3</sup> and Ce, 1 µg dm<sup>-3</sup>, code 155 N8145052) were both purchased from Perkin Elmer Italia (Monza, Italy). The mineraliza-156 tion procedure (Table S1), the instrumental settings (Table S2) and the validation param-157 eters (Table S3) have been reported in the Supplementary Material. 158

#### 2.6. Statistical Analysis

Differences in concentrations of the colostrum components, IgG, fatty acid profile 160 and minerals among the farms were analyzed with one-way ANOVA and compared by 161 Tukey test (SAS® software). Relationship among minerals, total protein, fatty acids and 162 immunoglobulin were also computed. The statistical model for the FA profile included 163 the fixed effects of type of secretion and farm, and the day of partum as random factor. 164

### 3. Results and Discussion

The TP content in the colostrum (from 7.1% to 29%, mean 14%) was not different 166 among farms (P=0.511). The high amounts of TP measured are due to the constant pres-167 ence of IgG (from 7.9 g dm<sup>-3</sup> to 105 g dm<sup>-3</sup>, mean 40 g dm<sup>-3</sup>). Also the concentration of IgG 168does not differ among farms (P = 0.379). The amounts of IgG here measured are consistent 169 with those previously measured in the same breed (unpublished data), and higher than 170 that reported in others dairy sheep as Lacaune and Friesian, 28.9 and 28.8 g dm-3 [19-20]. 171 Some research evidenced that lambs should intake an average of about 30 g of IgG in the 172 first 24 h from birth to acquire the proper passive immunity [20-22]. Applying this princi-173 ple, the lambs of this study should ingest 0.75 dm-3 of colostrum containing 40 g of IgG 174 dm<sup>-3</sup> to meet the immunity requirements. The antioxidant capacity of colostrum, meas-175 ured by ABTS evidenced a high variation among farms, probably related to the different 176 feeding techniques which may have determined passage of specific antioxidant sub-177 stances, as phenolic compounds, from feeds to colostrum. However, the FRAP assay was 178 not different among farms. The different results are likely because antioxidant assays can 179 target a specific compound (as FRAP), or the total antioxidant capacity (as ABTS) given 180 by the combined antioxidant capacities of all substances in a sample. 181

			Colostrum com- position		
Farm	TP (%)	IgG (g dm-3)	Fat (%)	FRAP (µmol ascor- bic acid/mL)	ABTS (% inhibi- tion)
Α	17	40	5.3c	3.5	20b
В	16	40	7.6abc	3.4	20b
С	14	30	7.7abc	nd	nd
D	15	40	9.6a	2.3	20b
Ε	15	40	6.9abc	2.4	20b
F	19	50	5.5bc	3.1	20b
G	14.5	40	9.1ab	2.2	40a
Н	16	40	10a	3.4	50a
Mean of all sam- ples	16	40	7.8	2.9	30
SD	5	20	3.2	1.4	10

**Table 1.** Total protein, fat and Immunoglobulin (IgG) contents and antioxidant properties of colos-182trum sampled in 8 farms.183

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Min	7.1	7.9	2.5	0.7	10
Max	29	105	18	8.5	58
Pvalue	0.511	0.379	< 0.001	0.120	< 0.001
SEM	0.54	2.20	0.36	0.15	1.53

Average amounts reported are rounded according to the number of significant digits of the relevant184standard deviation, while statistical tests have been accomplished on unrounded data. A-H = the185farms used in the survey; TP = Total Protein (TN x 6.38); IgG = immunoglobulin G; FRAP = Ferric186Reducing Antioxidant Power; ABTS = 2, 2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid); nd =not187determined.188

The contents of essential and toxic trace elements in colostrum are reported table 2, 190 whereas the correlation matrix of colostrum components was reported in Table 3. A wide 191 variation of minerals levels was found in colostrum collected from different farms. Mean 192 concentration of Se in sheep colostrum (from 100 µg kg-1 to 350 µg kg-1, mean 200 µg kg-1) 193 is greater than that found in colostrum of dairy cows [23] and goats [24] and markedly 194 higher than values observed in milk of the same bred (76.1 $\pm$ 40.6 µg kg<sup>-1</sup> [25]) and other 195 sheep breeds (28.4 $\pm$ 1.0 µg kg<sup>-1</sup> [26]). Selenium deficiency and the consequent risk of WMD 196 can be corrected by parenteral dosage of 0.1 mg of Se kg-1 BW, or by oral supplements, 197 ensuring a concentration of 0.3  $\mu$ g kg<sup>-1</sup>[4]. This recommended Se level for lambs could be 198 reached in our study by suckling 1.28 kg day<sup>-1</sup> of colostrum containing 234  $\mu$ g kg<sup>-1</sup> of Se. 199 On the other hand, the dosage of 0.4 µg kg<sup>-1</sup> of Se can be too high and cause acute symp-200 tomatology characterized by sialorrhea, prostration, and dyspnea [27]. It is reported that 201 Se has an important role in regulation the immunoglobulin and antioxidant capacity of 202 colostrum [28, 29]. This was also confirmed in this study where a significant positive cor-203 relation of Se and IgG has been observed (Table 3). 204

Also, the Zn content in sheep colostrum (from 5000 µg kg<sup>-1</sup> to 57000 µg kg<sup>-1</sup>, mean 205 25000 μg kg<sup>-1</sup>) is higher than reported in goats [24] and human [30] colostrum, and mark-206 edly higher than reported in sheep milk [9, 31]. Because of the role of Zn in immune func-207 tion and in teat keratin synthesis, it could reduce the susceptibility of the mammary gland 208 to mastitis, which is generally greatest exposed around the parturition [9]. However, the 209 correct concentration of Zn in colostrum and milk is crucial for lambs' growth as weight 210 gain is dramatically reduced in lambs when Zn supplements provided only 0.05 mg Zn 211 kg1 BW day-1, whereas providing 0.2 mg Zn kg1 BW day-1, a good rate of growth is ensured 212 [32]. 213

The Cu content in sheep's colostrum (from 130 µg kg-1 to 2800 µg kg-1, mean 1200 µg 214 kg<sup>-1</sup>) is almost 3 times higher than the Cu content reported in goat colostrum [24] and in 215 sheep milk (410 ppb [31]). Cu levels in sheep milk below 10 µg kg<sup>-1</sup> has been reported to 216 favor occurrence of swayback disease in newborn lambs [33], but values in colostrum 217 measured in this study are over 100fold higher than this critical limit. Like Se, also Mn, 218 Zn and Cu has been reported to improve immunoglobulins and antioxidant status of 219 dairy animals [34]. However, in our study IgG correlate positively only with Zn, whereas 220 a weaker negative relationship has been observed with Cu. No similar information has 221 been found, however the increase of Ig in blood of lambs supplemented with has been 222 reported [35]. 223

The mean concentrations of Pb and Cd heavy metals in colostrum are markedly 224 lower than the maximum limits indicated by the European Union for raw drinking milk 225 and baby foods which is set at 20  $\mu$ g kg<sup>-1</sup> and 10  $\mu$ g kg<sup>-1</sup>, respectively [36]. The intake by 226 ewes of toxic heavy metals could be thought contaminated feeds or soil in which animal 227 graze on pasture, with the consequent exposition of suckling lambs. 228

The concentration of nickel in sheep colostrum was below the value reported in milk 229 [37, 38] and below the permissible limit (0.43 mg dm<sup>-3</sup>) set by World Health Organization 230 (WHO). 231

**Table 2.** Concentration ( $\mu$ g kg<sup>-1</sup>) of essential and toxic elements in colostrum sampled in 8 farms.232

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			Elements				
Farm	Cd	Cu	Mn	Ni	Pb	Se	Zn
Α	$0.4^{ab}$	660 <sup>d</sup>	140 <sup>b</sup>	90 <sup>ab</sup>	10	300 <sup>ab</sup>	26000
В	0.8ª	1200 <sup>abc</sup>	70°	$110^{ab}$	12	200 <sup>ab</sup>	32000
С	$0.5^{ab}$	1600 <sup>a</sup>	240ª	80 <sup>b</sup>	7	200 <sup>ab</sup>	23000
D	0.6 <sup>ab</sup>	$880^{bcd}$	250ª	120 <sup>a</sup>	11	300 <sup>ab</sup>	23000
Ε	$0.4^{ab}$	1400 <sup>ab</sup>	$74^{bc}$	80 <sup>b</sup>	7	200 <sup>ab</sup>	18000
F	0.6 <sup>ab</sup>	670 <sup>cd</sup>	$74^{bc}$	80 <sup>b</sup>	7	100 <sup>b</sup>	27000
G	0.6 <sup>ab</sup>	1300 <sup>abc</sup>	$100^{bc}$	$110^{ab}$	9	350ª	25000
Н	0.3 <sup>b</sup>	1600ª	60 <sup>c</sup>	$100^{ab}$	11	200 <sup>ab</sup>	22000
mean	0.5	1200	130	100	9	200	25000
SD	0.3	600	90	30	5	200	11000
Min	0.1	130	33	39	3	20	5000
Max	1.5	2800	400	190	32	1000	57000
Pvalue	0.0083	<.0001	<.0001	0.0016	0.0417	0.0282	0.217

Average amounts reported are rounded according to the number of significant digits of the relevant234standard deviation, while statistical tests have been accomplished on unrounded data. Cd =235Cadmium; Cu= Copper; Mn =Manganese; Ni= Nickel; Pb = Lead; Se= Selenium; Zn =Zinc.236

The correlation matrix (Table 3) shown a positive strong correlation between immunoglobulin and TP. It is therefore evident that TP can be used as a reliable parameter to estimate the IgG content of colostrum in samples collected on the day of parturition. IgG significantly correlate with Se and Zn, because these two elements are essential for the immune system [39] but, even slightly, also with Ni. Conversely, negative correlations are observed between IgG and fat and, in a minor amount, between IgG and Cu. 238

A positive and significant correlation of ABTS and fat content and of FRAP and pro-244 tein and Ig contents. The ABTS assay measures the electron- or H-donating properties of 245 antioxidants, both hydrophilic and lipophilic. In accordance with our findings, whole 246 milk had the highest total antioxidant capacity measured by ABTS assays [40]. More spe-247 cifically, skimmed milk has a 6 % (ABTS) lower total antioxidant capacity than whole milk. 248 A significant higher total antioxidant capacity, as determined by ABTS assay, was also 249 found in cows' milk with 3% fat than cows' milk with 0,5-1,5 % fat and skimmed milk 250 [41]. These findings indicate that interference with lipids and lipophilic reactivity antiox-251 idants and lipid globule membrane proteins can affect the overall antioxidant capacity 252 and explains why the whole milk has a higher antioxidant potential rather than skimmed milk [42].

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**Table 3. -** Correlation matrix between trace elements and immunoglobulins, protein and fat content,269and antioxidant power of colostrum.270

	Se	Cu	Mn	Zn	Ni	Pb	Cd	ТР	IgG	Fat	FRAP
Cu	0.053	1									
Mn	0.220	-0.124	1								
Zn	0.469**	-0.115	0.114	1							
Ni	0.428**	-0.087	0.336**	0.486**	1						
Pb	0.127	-0.020	-0.003	0.135	0.433**	1					
Cd	0.041	0.017	-0.035	0.154	0.263*	0.059	1				
NT	0.492**	-0.208	0.113	0.680**	0.359**	0.039	0.051	1			
IgG	0.447**	-0.254*	-0.005	0.555**	0.250*	0.015	0.107	0.912**	1		
Fat	-177	0.247*	0.029	-0.063	0.058	0.014	-0.021	-0.331*	- 0.412**	1	
FRAP	0.135	-0.111	-0.038	0.310**	-0.028	-0.126	-0.058	0.285*	0.256*	-0.035	1
ABTS	0.051	0.39**	-0.219	-0.072	-0.048	0.087	-0.099	-0.063	-0.182	0.44**	-0.025

The fatty acid profile of colostrum is reported in Table 5. The FA of colostrum evi-273 denced that C16:0, C18:1c9, C14:0, and C18:0 are the most abundant fatty acids. The me-274 dium-chain fatty acids (MCFA) and the long chain fatty acids (LCFA) were higher, 275 whereas the short-chain FA (from C4:0 to C10:0) were markedly lower in colostrum than 276 in mature milk. The same pattern has been reported in colostrum of dairy cows compared 277 with mature milk [43]. The odd and branched-chain FAs were lower in colostrum than in 278 milk, and the same is observed for the content of C18:1 t11 and conjugated linoleic acid, 279 CLAc9t11 [44, 45]. This behavior is not in agreement with that observed in dairy cows, 280 where no differences (e.g., for C18.1 trans11) or a decrease of concentration (e.g., for C17:0; 281 CLAc9t11) passing from milk to colostrum has been observed [43]. 282

The long chain PUFA both n-6 and the n-3 family as ARA, EPA, DPA, and DHA were 283 higher in colostrum than in mature milk, probably because the specific higher require-284 ments for newborn lambs. In fact, arachidonic acid (AA) and DHA are essential and struc-285 tural constituents of cellular membranes and are mainly required for the growth and func-286 tion of the brain and nervous system [46, 47]. The ARA and DHA contents in colostrum 287 were 2-fold higher than that in the mature milk. This is important, as in newborn the abil-288 ity to elongate LA to ARA and ALA to DHA is very low; therefore, the high amounts of 289 LC-PUFA in colostrum could be of crucial advantage to lambs. 290

The fatty acid profile of colostrum compared to that of the mature milk indicates the 291 specific metabolism of ewe and udder during the transition period. In particular, the 292 higher content of FAs derived from body fat, the lower D9desaturase activity, and the 293 lower OBCFA due to reduced ruminal activity in the peripartum period are evidence of 294 the metabolism of animals after parturition. 295

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**Table 4.** Fat content and fatty acid profile of colostrum (g/100 g of FA) and mature milk sampled in2978 farms.298

	Туре	e		P value		
	Colostrum	Milk	SEM	Туре	Farm	
Fat, %	8	5.8	0.4922	0.0081	0.0021	
FA (% on Total FAs)						
C4:0	1.8	2.4	0.0656	<.0001	0.0017	
C6:0	0.8	2.0	0.0417	<.0001	0.0006	
C7:0	0.05	0.08	0.0048	0.0005	<.0001	
C8:0	0.6	2.1	0.0464	<.0001	0.0063	
C9:0	0.10	0.15	0.0095	0.0441	<.0001	
C10:0	1.8	7	0.1744	<.0001	0.0031	
C10:1	0.06	0.09	0.0052	0.0001	0.0226	
C11:0	0.12	0.32	0.0126	<.0001	0.0041	
C12:0	2.0	4.2	0.1112	<.0001	0.0097	

isoC13:0	0.01	0.02	0.0008	<.0001	0.0441
anteisoC13:0	0.03	0.04	0.0023	0.0064	0.0009
isoC14:0	0.06	0.12	0.0039	<.0001	0.3183
C14:0	11	11	0.4014	0.9561	0.0001
isoC15:0	0.18	0.31	0.0071	<.0001	0.0398
anteisoC15:0	0.20	0.54	0.0116	<.0001	0.1539
C14:1c9	0.4	0.17	0.0353	<.0001	<.0001
C15:0	0.6	1.1	0.0251	<.0001	0.0002
C15:1	0.03	0.08	0.003	<.0001	<.0001
isoC16:0	0.20	0.35	0.0083	<.0001	0.1687
C16:0	29	24	0.8182	<.0001	<.0001
C16:1t4	0.06	0.03	0.0031	<.0001	0.0182
C16:1t5	0.06	0.03	0.0032	<.0001	0.0144
C16:1t6-7	0.04	0.06	0.0016	<.0001	0.0776
isoC17:0	0.42	0.51	0.0132	<.0001	<.0001
C16:1t9	0.07	0.07	0.0055	0.3189	0.0002
C16:1t10	0.02	0.03	0.001	0.0005	<.0001
C16:1t11-t12	0.07	0.10	0.0046	<.0001	<.0001
C16:1c7	0.28	0.29	0.006	0.1557	0.0103
anteisoC17:0	0.47	0.48	0.0155	0.7088	<.0001
C16:1c9	1.6	0.8	0.1189	<.0001	0.0001
C16:1c10	0.04	0.05	0.002	<.0001	<.0001
C16:1c11	0.05	0.01	0.0047	<.0001	0.0053
3, 7, 11, 15-Tetramethyl-16:0	0.04	0.04	0.0015	0.7496	0.0004
C17:0	0.8	0.72	0.0264	0.0002	0.0004
7-methyl-hexadecyl-7-enoate	0.01	0.01	0.0008	0.0044	0.1594
isoC18:0	0.12	0.07	0.005	<.0001	0.0012
C17:1 c6-7	0.03	0.02	0.0016	0.2206	0.0314
C17:1c8	0.04	0.04	0.0023	0.3876	<.0001
C17:1c9	0.4	0.19	0.0148	<.0001	0.0006
C18:0	7	10	0.3547	<.0001	0.0162
C18:1t4	0.01	0.02	0.0008	<.0001	0.0029
C18:1t5	0.01	0.02	0.0011	<.0001	0.019
C18:1t6-8	0.19	0.24	0.0085	<.0001	0.0001
C18:1t9	0.21	0.25	0.009	0.0004	<.0001
C18:1t10	0.3	0.4	0.0273	<.0001	0.0367
C18:1t11	0.7	1.6	0.0752	<.0001	0.0059
C18:1t12	0.23	0.4	0.0162	<.0001	0.008
C18:1t13:t14	0.3	1.1	0.0391	<.0001	0.0306
C18:1c9	30	17	1.0669	<.0001	<.0001
C18:1c11	0.7	0.47	0.0181	<.0001	<.0001
C18:1c12	0.28	0.3	0.0156	0.0577	0.0022
	0.11	0.10	0.0042	0.1224	0.0086
	0.24	0.53	0.0193	<.0001	0.0011
	0.22	0.4	0.0139	<.0001	<.0001
	0.05	0.05	0.0022	0.0063	0.042
	0.02	0.06	0.0042	<.0001	0.0007
C18:2t9t12	0.02	0.02	0.0016	0.0577	0.0407
	0.4	0.5	0.0225	0.0062	<.0001
	0.10	0.12	0.0037	<.0001	0.0012
C10:2:00:13	0.17	0.22	0.0102	0.0054	<.0001 0.000 <b>2</b>
C10:209(12	0.12	0.15	0.0037	0.3376	0.0002
	0.03	0.04	0.0022	0.0163	0.0294
C10:217C12	0.03	0.03	0.0014	0.0095	0.1/45
C10:2011010	0.11	0.3	0.0201	<.0001 0.0001	<.0001
C10:2110	∠.3 0.07	2.1	0.1102	0.0001	<.0001 0 ⊑455
C10:2012c15	0.07	0.06	0.0025	U.2430	0.0000
	0.02	0.04	0.0025	<.0001	U.UUU8
C20:0	0.22	0.26	0.0078	<.0001	<.0001

Δ 7, 9 17:2	0.04	0.04	0.0019	0.3819	<.0001
C18:3n6	0.05	0.05	0.0037	0.0631	0.0044
C20:1c9	0.03	0.02	0.0012	<.0001	0.0002
C20:1c11	0.09	0.05	0.0032	<.0001	0.0022
C18:3n3	0.4	0.7	0.0349	<.0001	<.0001
CLAc9t11	0.7	0.8	0.0422	0.0043	<.0001
C20:1c15	0.03	0.03	0.0014	<.0001	<.0001
CLAt9c11/C21:0	0.05	0.10	0.0028	<.0001	0.0653
CLAt10c12	0.03	0.05	0.0031	<.0001	0.0003
CLAt12t14	0.01	0.04	0.0021	<.0001	0.0065
CLAt11t13	0.03	0.06	0.0034	<.0001	0.042
C20:2n9	0.01	0.04	0.002	<.0001	0.3913
CLAt9t11	0.02	0.03	0.002	<.0001	0.0098
C18:4n3	0.01	0.01	0.0005	0.0002	0.0229
C20:2n6	0.03	0.02	0.0011	<.0001	0.3811
C20:3n9	0.07	0.10	0.0051	<.0001	0.4713
C22:0	0.07	0.15	0.0048	<.0001	0.0035
C20:3n6	0.04	0.03	0.0014	<.0001	0.0006
10,14,17 C20:3	0.01	0.02	0.0007	<.0001	0.0035
C22:1n9	0.03	0.03	0.0027	0.0509	<.0001
C20:3n3	0.01	0.01	0.0007	0.4803	<.0001
C20:4n6 (ARA)	0.31	0.14	0.0116	<.0001	<.0001
C23:0	0.02	0.08	0.0025	<.0001	0.0252
C20:4n3	0.01	0.02	0.001	<.0001	0.0053
C22:2fi6	0.02	0.10	0.0043	<.0001	0.0022
EFA C24-0	0.07	0.05	0.0033	<.0001	<.0001
C24:0	0.03	0.07	0.0024	<.0001	0.0004
C22:5110	0.01	0.01	0.001	0.0004 < 0001	< 0001
C24;1015	0.02	0.02	0.001	<.0001	< 0001
C25:0	0.05	0.02	0.0023	< 0001	<.0001 0.3763
C26:0	0.01	0.02	0.0005	< 0001	0.0561
DPA	0.17	0.09	0.0025	< 0001	< 0001
DHA	0.06	0.03	0.0034	< 0001	<.0001
SCFA	5	14	0.2799	<.0001	0.0015
MCFA	48	46	1.3171	0.25	<.0001
LCFA	47	40	1.4586	0.0003	<.0001
SFA	58	69	1.1462	<.0001	<.0001
MUFA	37	25	1.0343	<.0001	<.0001
PUFA	6	6.1	0.1953	0.152	0.0103
UFA	42	31	1.1463	<.0001	<.0001
OCFA	1.7	2.4	0.0455	<.0001	0.0014
BCFA	1.7	2.4	0.0458	<.0001	0.0034
OBCFA	3.4	4.9	0.0769	<.0001	0.0023
PUFA6	3.0	2.5	0.1208	<.0001	<.0001
PUFA3	0.8	0.9	0.0464	0.0078	<.0001
n6/n3	4	3	0.3021	<.0001	<.0001
n3/n6	0.27	0.4	0.0213	<.0001	<.0001
CLA	0.8	1.1	0.0479	<.0001	<.0001
TFA	3.4	6	0.2146	<.0001	0.0012
TFA no VA	2.7	5	0.1519	<.0001	0.0013
AI	1.9	2.3	0.1329	0.0071	<.0001
TI	1.9	2.1	0.1237	0.1187	<.0001
h/H	0.9	0.60	0.049	<.0001	<.0001
DI C10:1	4	1.3	0.2513	<.0001	<.0001
DI C14:1	3	1.5	0.178	<.0001	0.0018
DI C16:1	5	2.9	0.2219	<.0001	0.0081
DI C18:1	80	63	0.7329	<.0001	0.1824
DI CLA	51	35	0.9838	<.0001	0.5566

Average amounts reported are rounded according to the number of significant digits of the relevant 299 standard deviation. SD = standard deviation;  $\Sigma FAs$  = sim of all FAs; FAME = fatty acid methyl ester; 300 SA = stearic acid; LA = linoleic acid; LNA = linolenic acid; ARA=arachidonic acid; EPA = eicosapen-301 taenoic acid; DPA = docosapentaenoic acid; DHA = docosahexaenoic acid; SFA = sum of the indi-302 vidual saturated fatty acids; UFA = sum of the individual unsaturated fatty acids; MUFA = sum of 303 the individual monounsaturated fatty acids; PUFA = sum of the individual polyunsaturated fatty 304 acids; OCFA = odd-chain fatty acids; BCFA = branched-chain fatty acids, sum of iso- and anteiso-305 FA; OBCFA = odd- and branched-chain fatty acids, sum of odd-, iso-, and anteiso-FA; SCFA, short-306 chain fatty acids (sum of individual fatty acids from C4:0 to C10:0); MCFA = medium-chain fatty 307 acids, sum of the individual fatty acids from C11:0 to C17:0; LCFA = long-chain fatty acids, sum of 308 the individual fatty acids from C18:0 to DHA; PUFA n-3 and PUFA n-6 = sum of individual n-3 and 309 n-6 fatty acids, respectively; CLA = sum of individual conjugated linoleic acids; TI = thrombogenic 310 index; AI = atherogenic index; h:H = hypocholesterolemic to hypercholesterolemic ratio; in the same 311 row indicate the significant differences (p < 0.05). 312

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#### 5. Conclusions

This survey permitted to characterize the colostrum composition of Sarda dairy ewes 314 in terms of macrocomposition (i.e., total proteins and fat), antioxidant properties (meas-315 ured in terms of FRAP and ABTS), seven trace elements (Cd, Cu, Mn, Ni, Pb, Se and Zn) 316 and IgG concentration. The high correlation between IgG and total protein evidenced that 317 the latter can be used to estimate immunoglobulin content of colostrum in sheep farm. 318 The colostrum antibodies are correlated positively with Se and Zn, both "well known" as 319 essential for stimulating the animal's immune system. The average amounts of toxic ele-320 ments were always below the established EU level. The fatty acid profile of colostrum 321 highlights a high variability in fatty acid groups between farms but evidenced meaningful 322 differences with the fatty acid profile observed in the mature milk. The amounts of trace 323 elements could be an important marker of nutritional and healthy quality of colostrum, 324 but also of environmental contamination of an area in which animals are breed. In con-325 clusion, this survey provides new data about the fatty acid profile of colostrum. Further 326 investigations are necessary to evaluate the factors of variation associated with the quality 327 of ewe colostrum, especially those associated with the variability of the IgG concentration. 328

Author Contributions: Conceptualization, A.N., G.B. and G.P.; methodology, A.N., G.B., G.P. M.D.329and G.S.; validation, A.N., E.T. and G.S; investigation, M.F.G., L.C., and I.L.; data curation, G.B, G.S.,330E.T. and A.N.; all authors were involved in the manuscript preparation and approved the final manuscript; supervision, A.N, G.S., M.D., and G.P. All authors have read and agreed to the published331version of the manuscript.333

Funding: This research was funded by the Autonomous Region of Sardinia, development and co-334hesion fund 2014-2020 SELOVIN project; grant number CUP: J86C17000190002"335

Institutional Review Board Statement: The study was approved by the Ethics Committee of the<br/>University of Sassari (n. 139652 03/11/2021 with the authorization of Ministero della Salute n°<br/>676/2021-PR based on art. 31 D.lgs. 26/2014).336<br/>337

Informed Consent Statement: Not applicable.

**Data Avail ability Statement:** The data presented in this study are available on request from the orresponding author. 340

Acknowledgments: All the activities in this investigation were carried out within the SEL-OVIN342project "quantitative and qualitative relationship of the selenium cycle in the dairy sheep industry343in Sardinia: determination of pedological, floristic and animal variability".344

The design of the investigation was planned in collaboration with Cargill srl and Department of 345 Nutritional Physiology and Feeding in the Faculty of Animal Sciences and Aquaculture of the Ag-346 ricultural University of Athens. The authors express their gratitude to all the farmers for their help 347 and willingness to carry out the work and to M.Sc. Antonio Piras and Giovanni Pinna (Cargill-Pu-348 rina) for technical assistance at farm level. Similarly, the authors thank M.sc. Achetti Ivonne from 349 the Istituto Zooprofilattico "Bruno Umbertini" of Lombardia and Emilia Romagna. Likewise, the 350 authors thank all technicians and colleagues that help us during the survey in sampling collection 351 and analysis. 352 Conflicts of Interest: The authors declare no conflict of interest.

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