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Effect on quanti-quality milk and mozzarella cheese characteristics with further increasing the level of dried stoned olive pomace in diet for lactating buffalo

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Objective: Following a previous report, an experiment was conducted to determine the effect of increasing level of dried stoned olives pomaces (DSOP) in the diet of lactating buffaloes on milk and mozzarella cheese yield and characteristics.
Methods: Sixteen pluriparous buffaloes distributed into two groups were fed an isoenergetic (0.9 milk forage unit/kg) and isoprotein (149 g/kg dry matter [DM] of crude protein) diet, with or without DSOP. Each animal received 17 kg DM/d. Samples of forages and concentrates were weekly collected and used for duplicate chemical analyses. Individual milk samples from each control were analyzed for chemical and coagulating parameters and daily production of mozzarella cheese was estimated. At the end of the trial, bulk milk of each group was processed to produce mozzarella cheese and chemical (fat, protein, ash, pH) composition, fatty acids composition, carotenoids and tocopherols content were determined. A sensory test was also performed. The oxidative stability was measured on mozzarella cheese and on governing liquid.
Results: No significant differences were observed, neither for milk yield and body condition score, nor for milk characteristics. The fat was higher ($p<0.05$) in mozzarella of DSOP fed group but, saturated fatty acids were lower and unsaturated higher ($p<0.01$). Furthermore, lower atherogenic ($p<0.01$), and thrombogenic ($p<0.05$) indices were found in mozzarella cheese of DSOP fed group. In addition, the administration of DSOP did not affect the mozzarella cheese oxidative stability and no negative effect was found in the sensory properties.
Conclusion: No contraindications appeared for the inclusion of DSOP in the diet of lactating buffaloes. Besides, important effects on mozzarella cheese quality were obtained, such as a modification of fat content and attributes with an increment in the mono-unsaturated. Additionally, a lower saturated/unsaturated ratio and atherogenic and thrombogenic indices suggest an improvement of dietetic and nutritional characteristics of mozzarella cheese.

Keywords: Dried Stoned Olive Pomace; Dairy Water Buffalo; Milk Production and Quality; Mozzarella Cheese Quality

INTRODUCTION

Olive production is very widespread over the world. In 2008, olive orchards occupied 10,839 thousand ha in the world with an increase of 2,888 thousand ha from 1998; Mediterranean countries represent 95% of the world's surface area of olive orchards and 96% of the world's production [1]. The use of pomace as feed for animals has been considered even if pomace production is seasonal and adequate preservation and storage techniques are required. Ensiling alone or mixed with other feeds or urea or treated with alkali could be considered the cheapest way to preserve pomace together with its inclusion in the farm diet [2]. Another interesting way to guarantee good preservation of the byproduct could be the drying process applied to olive

pomaces [3] allowing, by choosing the best operative conditions, to preserve the phenolic composition and to reduce the oxidative processes of the lipid fraction. Virgin olive oil mechanical extraction from stoned olive pastes is a new approach to the processing generally applied to improve oil quality but, at the same time, can also be considered a method to reduce the lignin content in the stoned pomaces, thereby increasing thus their attractiveness as animal feed [3]. Stoned olive pomaces contain residual oil, with a fatty acid composition dominated by oleic acid and, at the same time, show high amounts of bio-active phenols such as secoiridoids and lignans. For those reasons, they have been considered for their potential application as important sources of monounsaturated fatty acids (MUFAs) and natural antioxidants in animal feeding [4].

The dried stoned olive pomaces, obtained as extravirgin olive oil mechanical extraction residue and processed as previously described [3], have been already used in the diet of dairy ewes [5] and for dairy water buffaloes [6]: in both experiments an improvement of the milk fatty acids profile and an improvement of the milk oxidative stability have been observed, furthermore a study [6] reported the occurrence of hydroxytyrosol in milk. The cultivation of olives in the Mediterranean basin often coincide with the breeding of sheep and buffaloes whose productions are for local people an economic resource [6,7]. In Italy the main product of buffalo is the milk which is almost entirely converted into mozzarella cheese. This is a soft cheese and it is very appreciated by consumers in Italy and other EU countries and in the USA.

The aim of this work on the basis of previous outcomes [6] is to verify the effect of further increasing the level of dried stoned olives pomaces (DSOP) in diet for lactating Mediterranean buffaloes, on the quantity and quality of milk produced and dietary-sensory characteristics of mozzarella cheese.

MATERIALS AND METHODS

Animals and diets

Animals: Sixteen pluriparous Mediterranean buffaloes (*Bubalus bubalis*, L.) were allotted into two groups, control (C) and treated (DSOP), with no significant differences in milk yield and lactation length in their previous lactation and no significant differences in days in milking, milk yield, live weight and body condition score (BCS) at beginning of the trial (Table 1). The experimental period lasted 40 days. The animals were weighed at the beginning and the end of the trial and the nutritional status was determined using the BCS, utilizing the scale modified [8] for buffalo; this method provides for the use of a score from 1 to 9. Buffalo's cows were milked twice daily at intervals of 12 h using a milking machine and milk yields were measured at the on-set of the trial and after 15, 30, and 40 d after the beginning of the trial.

Diets: The diets were characterized by 26% 2nd cut alfalfa hay, 30% corn silage, and 44% concentrate for both groups, on a dry matter (DM) basis, and fed as a total mixed ration once a day.

Table 1. Buffaloes characteristics of the two groups (eight animals for each group)[1)]

	C	DSOP	RMSE
Milk yield in the previous lactation (kg)	2,046	2,131	333.06
Lactation length in the previous lactation (d)	303	291	42.54
Days in milking (d)	70	74	14.76
Milk yield (kg/d)	9.80	9.77	1.51
Live weight (kg)	665	721	62.66
Body condition score (BCS) (1÷9)	6.42	6.58	0.41

C, control; DSOP, dried stoned olive pomaces; RMSE, root mean square of error.
[1)] Values are expressed as mean (n = 8).

The formulation of concentrates and the chemical composition of the feedstuffs and of the two diets utilized are reported in supplementary material. The experimental concentrate contained 20.00% as fed of DSOP (Supplementary Table S1), dried according to the procedure proposed by other authors [3]. The control and experimental concentrates had a net energy content of 1.06 and 1.04 milk forage unit (FU)/kg DM and crude protein (CP) content of 192.0 and 190.2 g/kg DM respectively. The control and experimental diets were isoenergetic and isoproteinc, the control diet had 0.86 milk FU/kg DM and 149.5 g/kg DM of CP, experimental diet had 0.87 milk FU/kg DM and 148.7 g/kg DM of CP (Supplementary Table S2); each animal of two groups received 17 kg DM/d.

Each animal in the experimental group received daily 1.694 kg of stoned pomace corresponding to 1.602 kg of DM which accounted for 9.42% of the total DM of the diet.

Animals were kept, maintained and treated in accordance with International Guiding Principles for Biomedical Research Involving Animals that are accepted standards for animal welfare.

Feed sampling and analysis

Samples of forages, concentrates and dried stoned olive pomace were weekly collected and submitted for the duplicate chemical analyses.

The DM, CP, crude fibre (CF), ether extract (EE), and ash [9] as well as neutral detergent fibre, acid detergent fibre, and acid detergent lignin were determined [10]. In addition, the non-structural carbohydrates were calculated. The net energy of feeds, expressed as milk FU/kg DM, was calculated by using the chemical composition and the digestibility of the organic matter. The above listed parameters for both diets were calculated proportionally to the percentage of the feedstuffs components.

Milk sampling and analyses

At each control, individual milk samples from the morning and evening milking were collected and allotted into two aliquots for analysis. The first aliquot was processed to assess fat, protein (N× 6.38), lactose, urea and pH [11]; the second aliquot was processed to assess the milk coagulating parameters: rennet clotting time (r), curd firming time (K_{20}), curd firmness (A_{30}), estimated by means of the thromboelastograph Formagraph as previously re-

ported [12] at 35°C using a liquid rennet (1:15,000 rennet unit), 90% chymosin diluted 1:100 and added to whole milk as 200 μL/100 mL.

The daily production of mozzarella cheese was estimated [13].

Mozzarella cheese and governing liquid sampling, analysis and oxidative stability evaluation

All the bulk milk of each group, the last day of the test, was processed according to the EU rules [14] to produce mozzarella cheese. The mozzarella cheese and governing liquid samples were collected according to the following procedure: mozzarella cheese was divided into two halves and was subsequently homogenized with an Omnimixer homogenizer at low speed without other solutions; the governing liquid was filtered with Whatman No. 1 filters. Mozzarella cheese samples were collected and allotted into three aliquots. The first aliquot was immediately processed for the following duplicate analytical determinations: fat, protein, ash and pH according to the National official methods [15]. The second aliquot was stored at –80°C until fatty acids composition which was determined by gas chromatographic as the methyl ester derivatives after trans-esterification with sulphuric acid following the procedure reported in a former study [6]; to evaluate the risk for coronary disease, atherogenic and thrombogenic indexes were calculated [16]. The third aliquot was stored at –80°C until analysis to assess carotenoids and tocopherols [17].

To assess the oxidative stability of mozzarella cheese and governing liquid, three mozzarella cheese samples and governing liquid were collected immediately and after a conservation period of 4 days (96 hours) at 4°C and darkness, and then stored at –80°C, until duplicate analytical determinations. The oxidative stability of the mozzarella lipid fraction and governing liquid were measured by the dosage of thiobarbituric acid-reactive substances (TBARs) [18] and expressed in μg of malonyldialdheyde (MDA) per g of fat and μg of MDA per mL, respectively. The evolution of α-tocopherols in mozzarella cheese was also assessed [17].

Mozzarella cheese sensory evaluation

At the end of the trial, a sensory test was carried out by seven trained judges on mozzarella cheese obtained from the bulk of the milk produced by the two groups during the last day of experimentation, using a descriptive questionnaire [19].

Statistical analysis

All parameters except the tocopherols and oxidative status of mozzarella cheese and governing liquid oxidative status were evaluated using the PROC general linear model (GLM) of SAS [20] according to the following linear model:

$$Y_{ij} = \mu+\alpha_i+\varepsilon_{ij}$$

Fixed effect: dietary treatment C and DSOP.

The tocopherols and oxidative status of mozzarella cheese and governing liquid oxidative status data were evaluated using the PROC GLM of SAS [20] according to the following linear model:

$$Y_{ijk} = \mu+\alpha_i+\beta_j+(\alpha\beta)_{ij}+\varepsilon_{ijk}$$

Fixed effects: dietary treatment (C and DSOP); storage time (0 and 96 hours).

Results collected from the sensory evaluation of mozzarella samples have been elaborated by the Fisher's exact test using the PROC FREQ of SAS [20].

RESULTS AND DISCUSSION

Characteristics of stoned olive pomace

The chemical composition and the net energy of the DSOP used are reported in supplementary material (Supplementary Table S3). The DM (945.4 g/kg) was similar to that present in the literature [6] (956.7 g/kg). The chemical composition in terms of CP, CF, and EE (80.8, 325.6, and 135.4 g/kg DM) was similar to the data in the literature [21] for a stoned virgin pomace (92.0, 317.0, and 137.0 g/kg DM). The net energy (0.56 milk FU/kg DM) was similar to that of a mature oat hay or of a bloom tall fescue hay (both around 0.58 milk FU/kg DM) while the protein level was intermediate between those two kinds of hay (48.0 and 96.5 g/kg DM). The phenolic profile of the DSOP showed the occurrence of verbascoside and 3,4-Dihydroxyphenylethanol-elenolic acid di-aldehyde, an aglycon derivative of the oleuropein (3,4-DHPEA-EDA) as the most abundant phenolic compounds, other than p-Hydroxyphenylethanol-elenolic acid di-aldehyde (p-HPEA-EDA), 3,4-DHPEA, p-HPEA, and (+)-1-acetoxypinoresinol. The total amount of phenolic compounds was 26.7 g/kg of DM.

Animal performance and milk characteristics

Animal performances: Table 2 shows the DM intake, milk production, its quality, the live weight, the average daily gain and BCS. The amount of DM ingested (17.00 kg/d), at this stage of

Table 2. Effects of dietary treatment on milk yield and quality (number of replication = 4), live weight, body condition score (number of replication = 2) from each group[1)]

	C	DSOP	RMSE
DMI (kg/d)	17.00	17.00	-
Milk yield (kg/d)	8.42	8.53	1.54
Fat (%)	8.57	8.95	0.94
Protein (%)	4.78	4.65	0.44
Lactose (%)	4.88	4.57	0.29
Urea (mg/100 mL)	39.10	40.52	4.50
LW (kg)	673	729	62.83
ADG (g/d)	406	393	122.04
BCS	6.50	6.69	0.32

C, control; DSOP, dried stoned olive pomaces; RMSE, root mean square of error; DMI, daily dry matter intake; LW, live weight; ADG, average daily gain; BCS, body condition score (1 = poor to 9 = obese).

[1)] Values are expressed as mean (n = 8).

lactation, is the maximum capacity of ingestion for the buffalo species [22]. The DSOP administered in the experimental group reached 1.602 kg DM/head/d, representing the 9.42% of the total DM of the experimental diet, higher than that found in a previous experimentation with a similar diet composition [7], which accounted 6.17% (1.050 kg DM/head/d). This result is in agreement with a preceding study [23], where no negative influences on DM intake in dairy cows receiving around the 15% of DM of ensiled olive cake, were observed. Dietary treatment did not affect milk yield and milk composition, in fact by comparing milk yield from the two groups (8.42 and 8.53 kg/d in C and DSOP group, respectively) no significant differences were observed. Furthermore no significant differences in the average daily gain (406 and 393 g/d) and in the BCS (6.50 and 6.69) have been observed.

Milk characteristics: Fat, protein, lactose and urea content were 8.57%, 4.78%, 4.88%, 39.10 mg/100 mL in milk from C group and 8.95%, 4.65%, 4.57%, and 40.52 mg/100 mL in milk from DSOP group, without statistical differences between the two experimental groups. These results are consistent with literature [22] and provide further confirmation of the absence of a negative effect of long chain unsaturated fatty acids (UFA) provided by the DSOP on the activity of rumen bacteria [4,6]. There were no statistically significant differences between milk from C group and the milk from DSOP group in terms of pH (6.73 and 6.72), rennet clotting time (18.34 and 17.87 min), curd firming time (2.02 and 2.34 min), curd firmness (50.73 and 48.63 mm) and estimated yield of mozzarella cheese (2.23 and 2.25 kg/d). The results of the coagulation parameters were similar to those found by another investigation [22], and better, for milk of DSOP group, than those previously reported [6] for the lower value of rennet clotting time (r = 17.87 vs 22.35 min) and curd firming time (K_{20} = 2.34 vs 2.66 min) and for the highest value of curd firmness (A_{30} = 48.63 vs 35.25 mm). It could be considered that the higher level of dietary DSOP (9.42%) compared to the quantity administered (6.17%) in the aforementioned trial [6] leads to a milk with better attitude for cheese making; then adding in the diet the stoned pomace did not determine negative changes both in quantity and quality of milk.

Mozzarella cheese chemical composition

In Table 3 is reported the effect of DSOP on pH and chemical composition of buffalo "mozzarella cheese". All the parameters for the samples from the two groups were similar with the exception of the fat which was significantly higher in the mozzarella produced using the experimental group milk (25.24% vs 26.06%, p<0.05). Other researchers [24] obtained an average value of 26.70% of fat, and others [25] observed a slightly higher value (28.46%), whilst the datum from the National food composition tables [26] is lower (24.40%). Protein and ashes in the control and experimental groups (14.18% and 14.04%, 1.39% and 1.31%) were within the range (11.90%÷16.57%, 1.06%÷1.51%) described in a previous paper [25], and the National tables [26] reported

Table 3. Effect of DSOP on pH and chemical composition of buffalo mozzarella cheese

	On fresh matter			On dry matter		
	C	DSOP	RMSE	C	DSOP	RMSE
pH	5.28	5.58	0.11	-	-	-
Dry matter (%)	43.09	43.49	0.76	-	-	-
Fat (%)	25.24[b]	26.06[a]	0.36	58.81[b]	59.93[a]	0.20
Protein (%)	14.18	14.04	0.37	32.90	32.29	0.82
Ash (%)	1.39	1.31	0.07	3.22	3.01	0.16

C, control; DSOP, dried stoned olive pomaces; RMSE, root mean square of error.
Different letters indicate statistically significant differences between groups; [a,b] $p < 0.05$.

a protein concentration of 16.70%. Furthermore, also the results expressed on the basis of the DM are significantly different for the fat content (58.81% vs 59.93%, p<0.05), thus the amount of DSOP in the concentrate promoted this modification in the "mozzarella cheese".

Mozzarella cheese fatty acid composition

Table 4 reports the fatty acid composition of the lipid fraction in the mozzarella obtained with milk from the control and the

Table 4. Effects of dietary treatment on mozzarella cheese fatty acid composition (% FAME)

	C	DSOP	RMSE
C6:0	3.89[A]	2.44[B]	0.38
C8:0	3.01[A]	2.24[B]	0.21
C10:0	4.12[a]	3.60[b]	0.23
C12:0	0.09	0.08	0.02
C14:0	18.66[a]	16.92[b]	1.00
C16:0	39.00	36.07	2.00
C18:0	6.82	8.05	0.92
C18:1n9	14.06[B]	20.90[A]	1.57
C18:2n6	1.52	1.69	0.32
C18:3n3	0.52	0.47	0.53
C20:4n6	0.07	0.08	0.01
C20:5n3	0.06	0.08	0.03
C20:6n3	0.0009	0.0005	0.00
SCFA	11.00[A]	8.39[B]	0.59
MCFA	60.97[a]	55.29[b]	2.31
LCFA	27.92[B]	36.31[A]	2.31
SFA	77.69[A]	71.63[B]	1.79
UFA	22.31[B]	28.37[A]	1.79
MUFA	18.77[B]	24.65[A]	1.61
PUFA	3.53	3.71	0.75
n6	3.33	2.76	0.30
n3	0.74	0.81	0.10
n6/n3	3.76[a]	2.73[b]	0.54
Atherogenic index	4.95[A]	3.68[B]	0.44
Thrombogenic index	3.68[a]	3.14[b]	0.30

FAME, fatty acid methyl ester; C, control; DSOP, dried stoned olive pomaces; RMSE, root mean square of error; SCFA, short chain fatty acids; MCFA, medium chain fatty acids; LCFA, long chain fatty acids; SFA, saturated fatty acids; UFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
Different letters indicate statistically significant differences between groups; [a,b] $p < 0.05$; [A,B] $p < 0.01$.

experimental group. The percentages of C6:0, C8:0, C10:0, and C14:0 were significantly higher in the "mozzarella cheese" from the control group milk. The C18:1n9 content, in the "mozzarella cheese" from the milk of treated animals was higher (14.06% vs 20.90%, p<0.01); the oleic acid content of milk and consequently of "mozzarella cheese" could depend directly on the feed source and also on the mammary gland desaturation activity [27]. Among saturated fatty acids (SFA), C12:0, C14:0, and C16:0 are known to be able to increase the level of low density lipoprotein (LDL) cholesterol in the blood and in the serum with an aggravation of the coronary heart disease (CHD) risk in humans. On the contrary the augmentation of the consumption of dietary sources of MUFA and polyunsaturated fatty acids (PUFA) reduces the risk of the occurrence of CHD by lowering the level of LDL cholesterol in serum. Moreover MUFA are less sensitive to peroxidation respect to PUFA, thus their higher relative occurrence leads to a longer shelf-life in food products, with a slowdown in the onset of rancidity. A lower level of SFA (77.69% vs 71.63%, p<0.01), a higher UFA and MUFA content (22.31% vs 28.37%, p<0.01; 18.77% vs 24.65%, p<0.01), a lower n6/n3 ratio (3.76 vs 2.73, p<0.05) were found in mozzarella from buffalo cows fed with stoned pomace. Consequently lower atherogenic (4.95 vs 3.68, p<0.01) and thrombogenic (3.68 vs 3.14, p<0.05) indexes values were found in mozzarella cheese from DSOP group. These results put in evidence a strong improvement of the healthy value of "mozzarella cheese" produced by milk from DSOP group. In fact, the utilization of diets characterized by low values of these indices is correlated with a reduction of the potential risk of CHD [28].

Mozzarella cheese tocopherols and oxidative status

The effects of diet and storage on "mozzarella cheese" tocopherol content and oxidative status of cheese and governing liquid are shown in Table 5. The total tocopherols' concentrations found in the mozzarella samples from the control group and experimental group was 161.27 and 149.90 μg/100 g respectively, similar to those found in literature [29] in commercial mozzarella cheeses produced from water buffalo milk. However no statistical differences due to dietary treatment were found in the concentration of tocopherols. The storage period from 0 to 96 hours resulted in a significant reduction (p<0.01) in the α-tocopherol content, which was slight higher in mozzarella from the control group (Figure 1). In dairy products, tocopherols are inhibitors of the free radical action that catalyze the beginning and propagation of chain reactions in lipid peroxidation [30]. No significant differences were observed between the TBARs according to the two groups (2.69 and 2.57 μg MDA/g fat) and Figure 2 put in evidence the consumption of antioxidants such as tocopherols during storage. Also in the governing liquid, the differences found for the two groups were not significant (0.38 and 0.26 μg MDA/mL). Nevertheless, the slightly lower level of MDA in modulus, in the governing liquid of mozzarella obtained from treated animals could be related to a partial transfer of hydrophilic polyphenols, with antioxidant activity, from cheese to liquid. Furthermore, also in the governing liquid during the storage period a consumption of antioxidants such as tocopherols was observed (Figure 3).

Table 5. Effects of diet and storage on mozzarella cheese tocopherol content and oxidative status also of governing liquid

	C	DSOP	TRT	t	TRTxt	RMSE
Mozzarella cheese						
α-tocopherol (g/100 g)	159.39	148.25	ns	**	ns	23.31
γ-tocopherol (g/100 g)	1.88	1.65	ns	**	ns	0.36
Total tocopherols (g/100 g)	161.27	149.90	ns	**	ns	23.26
α-tocopherol (g/g fat)	6.13	5.68	ns	**	ns	0.88
γ-tocopherol (g/g fat)	0.07	0.06	ns	**	ns	0.01
Total tocopherols (g/g fat)	6.20	5.75	ns	**	ns	0.88
TBARs (g MDA/g fat)	2.69	2.57	ns	**	ns	0.60
Governing liquid						
TBARs (g MDA/mL)	0.38	0.26	ns	ns	ns	0.07

C, control; DSOP, dried stoned olive pomaces; TRT, effect of dietary treatment; t, effect of storage time; RMSE, root mean square of error; TBARs, reactive substances with tiobarbituric acid; MDA, malondialdehyde.
** p < 0.01.

Sensory evaluation of mozzarella cheese

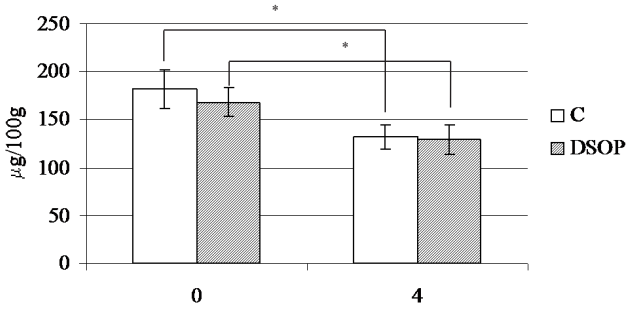

Figure 1. Effect of storage on α-tocopherol content in mozzarella cheese obtained with the milk from the two groups of buffaloes with different dietary treatments (C and DSOP). C, control; DSOP, dried stoned olive pomaces. * p<0.01.

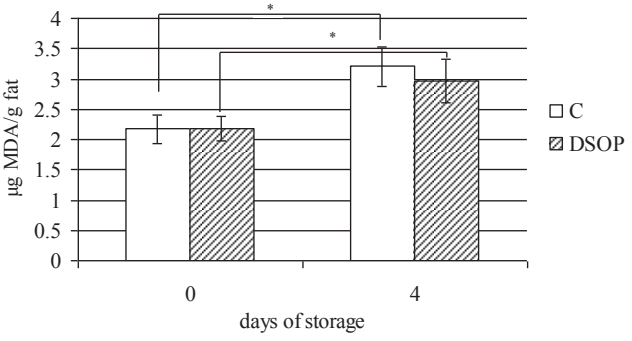

Figure 2. Effect of storage on the oxidative status (TBARs) of mozzarella cheese obtained with the milk from the two groups of buffaloes with different dietary treatments (C and DSOP). TBARs, thiobarbituric acid-reactive substances; C, control; DSOP, dried stoned olive pomaces. * p<0.01.

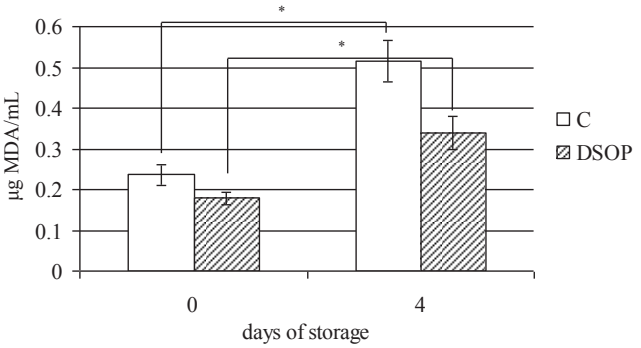

Figure 3. Effect of storage on the oxidative status (TBARs) of the governing liquid of mozzarella cheese obtained with the milk from the two groups of buffaloes with different dietary treatments (C and DSOP). TBARs, thiobarbituric acid-reactive substances; C, control; DSOP, dried stoned olive pomaces. * p<0.01.

In Table 6, the results of the sensory evaluation of the mozzarella cheese samples obtained from the bulk milk of the two groups are reported as number of attributions per descriptor. No statistical differences were observed between the two samples for all descriptors using the Fisher's exact test. Most of the judges have indicated for the surface a pearly white color and a smooth rind; the internal aspect has been described as pearly white and with good compactness. For both kind of mozzarella samples, the described olfactory sensations were mostly lactic and the prevalent taste sensation was sweet. Texture resulted mainly soft and juicy, in particular in mozzarella from the DSOP fed group. As a general consideration, the two mozzarella types received a similar and positive sensorial evaluation confirming the inclusion of the virgin stoned pomace in the experimental diet not to cause any negative effect not even in the sensory properties of the mozzarella cheese.

IMPLICATIONS

The results from this study have confirmed that the inclusion of a dried stoned olive pomace, at the tested concentration in dairy water buffalo feed does not provoke any damage or significant performance worsening without reduction in the quantity and quality of milk produced. On the contrary, important effects on mozzarella cheese quality were shown; the dried stoned olive pomace supply led to a modification of fat content and the fatty acid profile with an increment in the MUFAs. Further, the dietetic-nutritional characteristics of the mozzarella cheese are improved due to a better fatty acid composition determined by a decrease in the saturated/unsaturated ratio and of the atherogenic and thrombogenic indices.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Table 6. Sensory evaluation (n = 7) of mozzarella cheese samples produced with bulk milk from the two groups (number of attributions per descriptor)

	C	DSOP	Sign.
Surface appearence			
Colour			NS
White	3	3	
Pearly white	4	4	
Ivory white	-	-	
Rind			NS
Smooth	4	5	
Rough	2	2	
Toad skin	1	-	
Internal appearence			
Colour			NS
White	3	3	
Pearly white		4	4
Ivory white	-	-	
Body			NS
Compact	4	6	
Hollow	3	1	
Olfactory sensations			NS
Lactic	5	5	
Herbaceous	-	1	
Animal	2	1	
Tasty sensations			NS
Sweet	6	5	
Salty	-	1	
Acid	-	-	
Bitter	-	-	
Astringent	1	1	
Structure (*)			-
Soft	3	4	
Hard	1	-	
Strident	2	1	
Elastic	1	1	
Rubbery	1	1	
Juicy	3	4	
Dry	-	-	

C, control; DSOP, dried stoned olive pomaces; NS, not significant.
* Fisher's exact test was not used since each judge could give 2 ratings.

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REFERENCES

1. FAOSTAT. Food and Agriculture Organization of the United Nations. Statistics Division; 2010 [cited 2016 August 31]. Agricultural statistics. Available from http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor
2. Molina-Alcaide E, Morales García EY, Martín García AI. Effect of feeding multinutrient blocks on rumen fermentation, intake, digesti-

bility and milk yield and composition in dairy goats. In: Proceeding of the 11th Seminar of the Sub-Network FAO-CIHEAM on Sheep and Goat Nutrition 2005; 2005 Sept 8-10; Catania, Italy; FAO-CIHEAM; 2005. p. 77.
3. Servili M, Pauselli M, Esposto S, et al. "New approach to the use of stoned olive pomaces for animal feeding". In: Proceedings of the ISRIM International Conference: "New technologies for the treatment and valorisation of agro by-products"; 2007; 2007 Oct 3-5; Terni, Italy.
4. Chiofalo B, Liotta L, Zumbo A, Chiofalo V. Administration of olive cake for ewe feeding: effect on milk yield and composition. Small Rumin Res 2004;55:169-76.
5. Pauselli M, Servili M, Esposto S, et al. Effect of destoned olive cake as animal feed on ewe milk quality. In: Proceedings of the ISRIM International Conference of New Technologies for the Treatment and Valorization of Agro By-products 2007; 2007 Oct 3-5; Terni, Italy.
6. Terramoccia, S, Bartocci S, Taticchi A, et al. Use of dried stoned olive pomace in the feeding of lactating buffaloes: effect on the quantity and quality of the milk produced. Asian-Australas J Anim Sci 2013;26: 971-80.
7. Rancourt M, Fois N, Lavin MP, Tchakérian E, Valleran F. Mediterranean sheep and goats production: An uncertain future. Small Rumin Res 2006;62:167-79.
8. Campanile G, De Filippo C, Di Palo R, Taccone W, Zicarelli L. Influence of dietary protein on urea levels in blood and milk of buffalo cows. Livest Prod Sci 1998;55:135-43.
9. Cunniff P; Association of Official Analytical Chemists. Official Methods of Analysis of AOAC International 16th ed. Association of Official Analytical Chemists. Washington, DC: AOAC International; 1995.
10. Goering HK, Van Soest PJ. Forage fibre analysis (apparatus, reagents, procedures and some applications). Agriculture Hand Book 379. Washington, DC: ARS USDA; 1970.
11. Associazione Scientifica di Produzione Animale; ASPA. Methods of milk analysis of the main species of zootechnical interest. Perugia, Italy: University of Perugia; 1995.
12. Zannoni, M, Annibaldi S. Standardization of the renneting ability of milk by Formagraph. Sci Tec Latt-Casearia 1981;32:79-94.
13. Altiero V, Moio L, Addeo F. Estimate of mozzarella yield based on the fat and protein content of buffalo milk. Sci Tec Latt-Casearia 1989;40: 425-33.
14. Ministero delle Politiche Agricole, Alimentari e Forestali. Official methods of analysis for cheeses. Official Gazette of the Italian Republic; 229; 1986 Oct 2; 1986.
15. Commission of the European Communities. Commission Regulation (EC) No 103/2008 of 4 Feb 2008; L 31; 2008 Feb 5; 2008.
16. Ulbricht TLV, Southgate DAT. Coronary heart disease seven dietary factors. Lancet 1991;338:985-92.
17. Schüep W, Rettenmaier R. Analysis of vitamin E homologs in plasma and tissue: high-performance liquid chromatography. Method Enzymol 1994;234:294-302.
18. Fenaille, FP, Mottier R, Turesky SA, Guy PA. Comparison of analytical techniques to quantify malondialdehyde in milk powders. J Chromatogr 2001;921:237-45.
19. Risvik E, Popper R, Rogers R. Expert systems and their application in sensory evaluation. Food Qual Prefer 1989;1:183-4.
20. SAS. SAS/STAT 9.3 User's Guide. 19th ed. Cary, NC: SAS Institute Inc., 2011.
21. Malossini F, Verna M, Settineri D. Nutritional value of different feeds expressed in traditional UFs and according to new energy systems. In: Proceedings of the conference of Associazione Scientifica di Produzione Animale, La stima del valore nutritivo degli alimenti: metodi classici e concezioni moderne 1983; 1983 Sept 20-21; Rome, Italy. Associazione Scientifica di Produzione Animale; 1983. p. 111-28.
22. Bartocci S, Terramoccia S, Tripaldi C. The utilization of a high level energy/protein diet for lactating mediterranean buffaloes: intake capacity and effects on quanti-qualitative milk parameters. Livest Sci 2006;99:211-9.
23. Hadjipanayiotou M. Feeding ensiled crude olive cake to lactating Chios ewes, Damascus goats and Friesan cows. Livest Prod Sci 1999; 59:61-6.
24. Addeo F, Emaldi GC, Masi P. Tradition and innovation in the "Mozzarella di Bufala Campana cheese" production. In: Gigli S, Chupin D, Galal S et al., editors. Proceedings of the International Symposium on Buffalo Products 1996; 1996 Dec 1-4; Paestum, Italy. Rome, Italy: 1996. p. 23-39.
25. Claps S, Pizzillo M, Agoglia E, et al. Chemical and texture characteristics and sensory properties of "mozzarella" cheese from different feeding systems. Ital J Anim Sci 2007;6:1143-6.
26. CREA-NUT. Food composition table. Rome, IT: Research Center for Food and Nutrition; 2009 [cited 2016 August 31]. Available from http://nut.entecra.it/646/tabelle_di_composizione_degli_alimenti.html?idalimento=164810&quant=100
27. Chilliard Y, Ferlay A. Dietary lipids and forages interactions on cow and goat milk fatty acid composition and sensory properties. Reprod Nutr Dev 2004;45:467-92.
28. Fehily AM, Pickering JE, Yarnell JW, Elwood PC. Dietary indices of atherogenicity and thrombogenicity and ischaemic heart disease risk: the caerphilly prospective study. Br J Nutr 1994;71:249-57.
29. Balestrieri M, Spagnuolo MS, Cigliano L, et al. Evaluation of oxydative damage in mozzarella cheese produced from bovine or water buffalo milk. Food Chem 2002;71:293-9.
30. Madhavi DL, Deshpande SS, Salunkhe DK. Introduction. In: Madhavi DL, Deshpande SS, Salunkhe DK, editor. Food antioxidants: technological, toxicological, and health perspectives. New York: Marcel Dekker Inc.; 1996. p. 1-4.