



Impact of feeding ration supplemented with silymarin-rich extract on milk quality of goat and utilization of milk in producing functional soft-cheese

Sameh M. Shedeed¹, Eman H. ElSabaawy², Mamdouh I. Mohamed², Hoda B. Mabrok^{3*} and Walaa M. Abd El-Wahab⁴



¹Dairy Technology Department, Animal Production Research Institute, Ministry of Agriculture, Dokki, Cairo, Egypt

²Animal Production Department, Agricultural and Biological Research Institute, National Research Centre, Dokki, Cairo, Egypt

³Nutrition and Food Science Department, Food Industries and Nutrition Research Institute, National Research Centre, Dokki, Cairo, Egypt

⁴Animal Nutrition Department, Animal Production Research Institute, Ministry of Agriculture, Dokki, Cairo, Egypt

Abstract

Silymarin is a flavonoid complex extracted from the milk thistle plant and acts as a strong antioxidant. Using natural compounds as additives in livestock nutrition could be a new goal in livestock production. The objective of this study was to investigate the effect of a ration containing different levels of silymarin-rich extract (10, 20, or 30 g) on fatty acids profile, production and quality of goat milk; and producing functional cheese from milk. Egyptian Nubian pregnant goats (n=16) were divided into four groups. Group 1 fed on a control ration. Groups 2, 3, and 4 were fed on a control ration and orally administrated with different levels of silymarin-rich extract at 10, 20, or 30 g/day, respectively. The feeding experiment lasted 4 months. The results showed that silymarin-rich extracts improved milk composition and quality by increasing protein and fat concentrations. Milk yield was significantly increased by silymarin-rich extracts supplementation. Feeding goats on rations supplemented with silymarin-rich extracts altered nutritional value of milk by increasing unsaturated fatty acids and decreasing the saturated fatty acids levels. Cheese produced from goat milk showed a high content of fat and protein. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis of cheese-protein showed that adding the silymarin-rich extract to the goat feed increases the protein level in particularly β -casein, α s1-casein and α s2-casein. It could be concluded that using silymarin-rich extracts in rations of goats improved milk production, milk quality, and milk fatty acids. Silymarin-rich extracts improved the quality of goat cheese.

Keywords: Silymarin; Goat Milk; Milk yield; Milk Fatty acids; Cheese; SDS-PAGE.

1. Introduction

Goat milk and its products, mainly cheese, have a great intention worldwide [1]. Goat milk has a higher digestibility and lower allergenic properties than the milk of cows [2]. Twenty percent of fatty acids in goat milk are short and medium chain fatty acids, making it more digestible and contributing to the specific aroma of goat milk [3]. It contains more unsaturated fatty acids and less content of n6/n3 ratio

(polyunsaturated fatty acids) than cow milk [4-5]. Goat milk have high protein content with present of essential amino acids [6]. The major proteins in goat milk are α s2-casein (α s2-CN), β -casein (β -CN), k-casein, β -lactoglobulin and lactalbumin. The hypo-allergenicity properties of goat milk is related to low content of α s1-casein (α s1-CN) [7]. Protein of goat milk is differs from cow milk in its genetic polymorphisms [8]. Moreover, milk of goat have

*Corresponding author e-mail: hoda.mabrok@gmail.com; (Hoda B. Mabrok).

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vitamins (riboflavin and niacin) and minerals (calcium, magnesium, potassium and phosphorus), also stimulates probiotic bacteria growth [9-11].

Milk thistle (*Silybum marianum*) seeds have several phyto-constituents which help in management of different disease. Milk thistle seeds contain flavonoids such as taxifolin, chrysoeriol, quercetin and eriodictyol [12]. Silymarin (flavonolignins) is the main bioactive component in milk thistle [13]. Milk thistle extract rich in silymarin promotes liver health through reducing lipid production and act as antioxidant agent [14]. Silymarin has shown potential anti-cancer, anti-microbial, neuro-protective, cardiovascular-protective and anti-diabetic effects [15]. Silymarin is recommended as dietary supplement for animals which suffer from liver disease [16]. Supplemented poultry rations with milk thistle seeds positively impacted the meat flavor characterization of the muscles [17]. Using alternative feeds containing bioactive compounds or phyto-additives in animal nutrition is a promising approach [18].

Numerous studies showed that changing in ruminant rations affect the milk quality [19, 20]. Milk thistle endosperm supplementation increased monounsaturated fatty acids in milk of cow, which promotes human health benefits [21]. Silymarin supplementation increased milk yield of dairy cows and speeded up the process of the metabolic adaptation at the beginning of lactation and beneficially affected lactation in the transition phase [22-24]. Feeding Greek mountain ewes with milk thistle oil increased milk yield with no change in fat content [25]. Chaji et al. [26] and Arviv et al. [27] reported that feeding on milk thistle has no negative effect on adult goat. Tedesco et al. [28] reported that silymarin supplementation (10 g/d) improved milk yield, however, milk quality parameters (fat, protein, urea, lactose and somatic cell count) were not differ between control and treated goats. There are not enough studies on the effect of silymarin water extract supplementation on goat milk production. Also information about its effect on fatty acids profile in milk goat is still lacking. Therefore, the present study was conducted to investigate the effect of silymarin-rich extract supplementation on milk composition, fatty acids profile and produce functional cheese from goat milk fed on rations supplemented with silymarin-rich extract.

2. Materials and methods

2.1. Plant material and extraction preparation:

Milk thistle seeds were obtained from local market and extracted with hot water (double distilled water) for 1 hour using soxhlet apparatus. The extract was filtered then concentrated under control reduced pressure at 40°C to obtain crude extract. Dried crude extract was stored at -20°C until further use.

2.2. Experimental ration composition and analysis:

Ingredients (%) of ration were consisted of corn (40%), soybean meal (15%), sunflower meal (23%), wheat bran (17%), molass (2%), limestone (2%) and sodium chloride (1%). Feed samples were subjected to proximate chemical analyses (crude protein, crude fat, crude fiber and ash) according to AOAC [29] method, while nitrogen free extract was calculated by difference.

2.3. The experimental animals and design:

The experiment was carried out in the Sheep and Goat Research Unit, Nubaria Agricultural Experimental Station, National Research Centre. Egyptian Nubian multiparous pregnant goats (3 months pregnant, n=16, 23±2kg) were selected and divided into four identical groups (four animals each). Animals were fed on either the control ration or the control rations with an orally administered with different levels of silymarin-rich extract. Group 1 fed on control ration only and serve as control. Group 2 fed on control ration and orally administered with 10 g/animal/day silymarin-rich extract. Group 3 fed on control ration and orally administered with 20 g/animal/day silymarin-rich extract. Group 4 fed on control ration and orally administered with 30 g/animal/day. Goats were housed in soil-surfaced pens, rations were offered twice a day at 7 am and 4 pm in quantities sufficient to meet their energy and nutrient requirements according to NRC recommendations [30]; animals have free access to clean fresh water. The experiment lasted 4 month.

2.4. Milk sampling, composition and fatty acids analysis:

Goats were hand milked twice a day. Daily milk yield was recorded. Milk sample on the last two days of the experiment were collected and stored at -20°C for analyses of protein, fat, ash, total solids, lactose,

galactose, and fatty acids profile according to standard procedures. Protein, fat, and ash content were measured using AOAC method [29]. Milk total solid was estimated according to method of Cipolat-Gotet et al. [31]. Lactose and galactose were determined according to the method of Ohlsson et al. [32].

Fatty acids were extracted from milk according to method of Smith and Jack [33]. Fatty acids profile analyzed by gas chromatography (GC). Fatty acids methyl esters were prepared by base-catalysed methylation according to the method of Luna et al. [34]. Diethyl ether (1 ml) were added to extracted fatty acids. Then, methyl acetate (50 μ l) and 1 M sodium methoxide in methanol (100 μ l) were added. The reaction was stopped by adding an oxalic acid (50 μ l) in diethyl ether saturate solution after 5 min at room temperature. After centrifugation (1500 \times g, 5 min), 200 μ l of upper layer of solution was used directly for GC analysis. Fatty acids analysis were carried out on HP 6890 series gas chromatograph system. Nitrogen was the carrier gas, and initial and final temperatures were set at 50 and 230°C, respectively, with detector and injector temperatures set 300 and 280°C, respectively. Individual fatty acids methyl esters were identified by comparing sample peak retention times with standard mixtures and pure standard methyl esters from sigma- aldrich and expressed as percentage of total fatty acids (FA).

2.5. Soft cheese preparation:

Cheeses were prepared from milk collected from goats of the different groups. Calcium chloride (0.01 g/5 L of milk) was added to warm goat milk (42° C) 30 min prior to the addition of the starter culture. Cooled milk was inoculated with starter culture. Diluted liquid chymosin was added after inoculation of culture depending on accomplishment of pH value. The milk was allowed to coagulate for two hours after the addition of chymosin. The coagulated curd was cut and let to stand for 10 min and then was poured into a plastic mold lined with a cheese cloth. There after the whey was drained off from the curd. The cheese samples were collected in sterile containers and weighed immediately prior to storage in the refrigerator at 4 \pm 1°C [35]. The weight of cheese sample was recorded, and the yield of the cheese was calculated as follow: cheese yield% = (weight of cheese)/ (weight of milk) \times 100.

2.6. Sodium dodecil sulphate polyacrylamide gel electrophoresis (SDS-PAGE):

SDS-PAGE was carried out according to the method of Laemmli [36] and modified according to the method of Basch et al. [37]. For SDS-electrophoresis, the cheese proteins were extracted according to the method described by Kalit et al. [38] with some modifications. Cheese samples were homogenized with lysis buffer composed of 55 mM Tris (pH 6.8), 2% SDS, 7% glycerol, and 5% β -mercaptoethanol for 30 min at 40 °C. The homogenized samples were centrifuged (15,000 \times g for 15 min at 4 °C), then the supernatant was collected and protein concentration was measured. Protein extract for each samples (50 μ l) were added to the same volume of loading buffer. Samples were boiled for 10 minutes in water bath, then 10 μ l bromophenol blue was added to each tube before sample loading (50 μ l). SDS-polyacrylamide gel electrophoresis was performed using stacking gel (4%) and separating gel (15%). The protein bands were visualized by commassie staining and were analyzed using gel analyser 19 software.

2.7. Statistical analysis

The results were expresses as mean \pm SD and analysed using ANOVA followed by Tukey's comparison test using the SPSS statistical program. Differences were considered significant at $p \leq 0.05$ [39].

3. Results

3.1. Milk yield and composition:

The chemical composition of the control ration were as follows: crude protein (15%), crude fat (3%), ash (6%), crude fiber (9%) and nitrogen free extract (67%). Data summarizing significant results for the effect of silymarin-rich extracts on milk production and milk composition are shown in table 1. There was a significant difference in milk production of groups fed on rations supplemented with silymarin-rich extracts (20g/day, $p \leq 0.05$ and 30 g/ day, $p \leq 0.01$). Milk protein content (%) was significantly increased by silymarin-rich extract (20 g/day, $p \leq 0.01$ and 30g /day, $p \leq 0.001$). Milk fat content (%) was significantly increased by silymarin-rich extract of 20 g/day and 30g /day ($p \leq 0.05$ and $p \leq 0.01$, respectively). However, 10 g silymarin-rich extract/animal/day did not affect milk protein and fat levels compared to the control. Total solids, ash, lactose, galactose percentages were comparable to the control.

Table 1: Impact of silymarin-rich extracts on goat milk composition and milk production

	Control	Silymarin-rich extract		
		(10g/day)	(20g/day)	(30g/day)
Milk yield (kg/day)	3.625a±0.479	4.25a±0.645	4.57b±0.289	5.00b±0.408
Fat (%)	3.427a±0.022	3.497ab±0.009	3.557bc±0.027	3.604c±0.068
Protein (%)	3.736a±0.026	3.782a±0.048	3.836b±0.049	3.863b±0.035
Total solids (%)	12.168a±0.075	12.313a±0.223	12.308a±0.090	12.553a±0.343
Ash (%)	0.770a±0.012	0.818a±0.051	0.853a±0.048	0.883a±0.082
Lactose (%)	43.114a±0.074	43.150a±0.049	43.200a±0.062	43.365a±0.225
Galactose (%)	0.044a±0.004	0.048a±0.009	0.051a±0.006	0.056a±0.004

Values were mean±SD, the same letter in same row means non-significant difference; different letter mean significant difference at 0.05 probabilities.

3.2. Influence of silymarin-rich extract on milk fatty acids composition:

Fatty acids profile in milk of goats among different experimental groups are presented in table 2. Short and medium-chain fatty acids (C4, C6, C8, C10,C12) were increased by silymarin-rich extract levels (10, 20, and 30 g/day) with values being 17.57%, 18.04% and 17.97%, respectively, compared to the control (17.36%). Palmitic, oleic, stearic and myristic acids were the abdominal fatty acids in milk of goats. Silymarin-rich extracts decreased the levels of palmitic and stearic acids. Total saturated fatty acids concentration decreased by supplementation of silymarin-rich extracts compared to the control. Total unsaturated fatty acids (UFA) and mono-unsaturated fatty acids (MUFAs), levels increased with silymarin-rich extracts supplementation (10, 20, and 30 g/day). The highest value of conjugated linoleic acids was observed with group 3, which fed 20 g silymarin-rich extracts /day. Silymarin-rich extract (20 and 30 g/day) increased the level of poly-unsaturated fatty acids (PUFAs). Feeding goat with ration supplemented with silymarin-rich extracts decreased the n6/n3 ratio.

3.3. Cheese yield and its chemical properties:

Cheese yield and its chemical properties results are presented in figure 1 and table 3. Cheese-products yield were significantly increased (25.67, 25.85, and 25.99 %) by silymarin-rich extracts 10, 20, and 30 g/day supplementation, respectively, compared to

control (25.51 %). The highest value of cheese yield was obtained from goat milk fed on silymarin-rich extract 30 g/day (figure 1). Cheese produced from milk collected from goat feeding on ration supplemented with silymarin-rich extracts (10, 20 or 30 g/day, respectively) have higher fat and protein % compared to the control (table 3). Moisture levels ranged from 55.59 to 55.71%. Storage for 7 days and 14 days increased fat and protein % compare to the control, while moisture levels decreased.

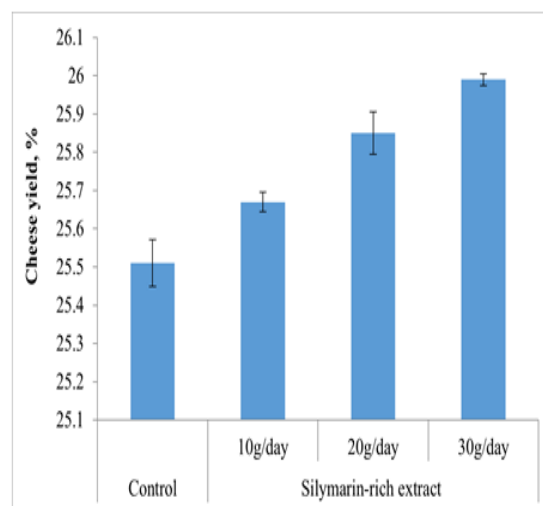


Fig 1: Yield of soft-cheese produced from goat milk fed on silymarin-rich extracts

Table 2: Fatty acids profile in milk of goats among different experimental groups feeding on rations supplemented with silymarin-rich extracts

Fatty acids (g/ 100 g of total FA)	Control	Silymarin-rich extract		
		(10g/day)	(20g/day)	(30g/day)
Butyric acid, C4:0	4.1	4.23	4.3	4.25
Caproic acid, C6:0	1.44	1.44	1.5	1.52
Caprylic acid, C8:0	2.52	2.54	2.6	2.53
Capric acid, C10:0	5.75	5.77	5.7	5.76
Undecylic acid, C11:0	1.02	0.99	0.9	0.93
Lauric acid, C12:0	2.53	2.6	3	2.98
Myristic acid, C14:0	9.5	9.23	9.6	9.88
Myristoleic acid, C14:1	0.28	0.32	0.3	0.35
Pentadecylic acid, C15:0	1.2	0.94	0.9	0.93
Palmitic acid, C16:0	26.94	26.2	25	24.8
Palmitoleic acid, C16:1	1.22	1.31	1.4	1.38
Margaric acid, C17:0	1.2	0.99	0.8	0.82
Stearic acid, C18:0	12.81	12.69	12	12.2
Elaidic acid, C18:1n9trans	2.95	2.95	3.3	3.29
Oleic acid, C18:1n9cis	24.68	25.9	26	26.6
conjugated linoleic acid (CLA), C18:2 cis-9,trans-11	0.15	0.15	0.2	0.15
conjugated linoleic acid (CLA), C18:2 trans-10,cis-12	0.45	0.45	0.6	0.5
α -Linolenic acid, C18:3n-3	0.13	0.14	0.2	0.16
γ -Linolenic acid, C18:3n-6	0.32	0.32	0.4	0.36
Arachidic acid, C20:0	0.81	0.84	0.7	0.74
Total saturated fatty acids (SFA)	69.82	68.46	68	67.3
Total unsaturated fatty acids (UFA)	30.18	31.54	32	32.7
Monounsaturated fatty acids (MUFA)	29.13	30.48	31	31.6
Polyunsaturated fatty acids (PUFA)	1.05	1.06	1.2	1.17
n6/n3 ratio	2.46	2.29	2.33	2.25

3.4. SDS-PAGE analysis of cheese protein:

Through sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis, the degradation of caseins extracted from fresh cheeses was assessed (figure 2). Cheese proteins were separated successfully to a clear bands and identified as α 2-casein (α 2-CN), α 1-casein (α 1-CN), β -casein (β -CN). The electrophoretic profile from SDS-PAGE analysis showed a major β -CN band, while less intensive bands for α -casein isoforms (α 2-CN

and α 1-CN) in control and other cheese samples. Silymarin-rich extracts used to feed goats appears to influence the protein profile of all cheese samples. As shown in figure 2, silymarin-rich extract (20 g/day and 30 g/day) increased the level of α 1-CN. The highest concentration of silymarin-rich extract (30g/d) promotes the formation of high-density bands for the insoluble high molecular weight co-aggregations compared to other products.

Table 3: Chemical properties of soft-cheese products produced from goat milk.

Cheese products		Fat	Protein	Moisture
		%	%	%
Fresh				
Control		23.68a ±0.10	16.62a±0.14	55.59a±0.12
Silymarin-rich extract	(10g/day)	23.75a ±0.08	16.69a±0.01	55.64a±0.06
	(20g/day)	23.92a ±0.07	16.85a±0.14	55.67a±0.10
	(30g/day)	23.99b ±0.14	16.95b±0.04	55.71a±0.08
Storage,7 days				
Control		24.65a ±0.05	17.62a±0.04	52.51a±0.08
Silymarin-rich extract	(10g/day)	24.71a±0.06	17.99b±0.12	52.56a±0.43
	(20g/day)	24.99b±0.11	18.05b±0.21	52.70a±0.35
	(30g/day)	25.0b3±0.16	18.12b±0.12	52.15a±0.05
Storage,14 days				
Control		25.85a±0.15	18.52a±0.03	50.10a±0.05
Silymarin-rich extract	(10g/day)	25.91a±0.07	18.57a±0.05	50.17a±0.06
	(20g/day)	25.97a±0.07	18.72b±0.12	50.01a±0.11
	(30g/day)	26.5a±0.10	18.75b±0.05	49.9a±0.11

Values are presented as Mean±SD; the same letter in same row means non-significant difference; different letter mean significant difference at 0.05 probabilities

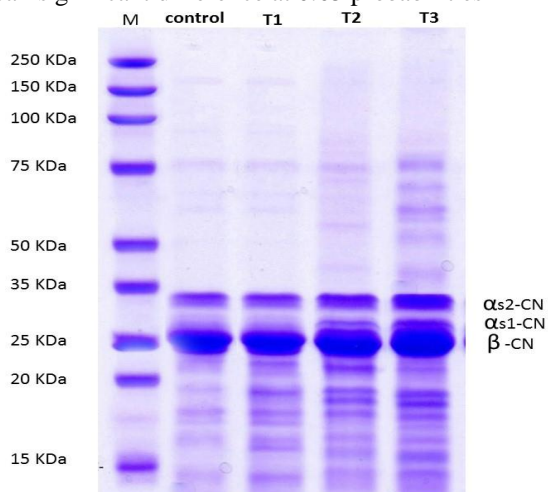


Fig 2: Sodium dodecil sulphate polyacrylamide gel electrophoresis (SDS-Page) of goat soft cheese protein.

M: maker; T1: cheese product-1 (silymarin-rich extract 10g/day); T2: cheese product-2 (silymarin-rich extract 20g/day); T3: cheese product-3 (silymarin-rich extract 30g/day); α s2-CN: α s2-casein; α s1-CN: α s1-casein; β -CN: β -casein.

4. Discussion

Goats are important livestock species in many countries all over the world. The first farm animals to be domesticated were goats. Goats have great adaptability and productivity [40]. Goat milk production and consumption have been continuously increasing [41]. Goat milk composition (fat, protein and ash) were higher than cow and human milk with lower lactose therefore, it recommended for lactose intolerant patients [42-43]. It contains higher short chain fatty acids and medium-short chain fatty acids than cow milk [43]. Milk composition is affected by several factors such as breed, nutrition, environment, age, lactation stage and feed [44]. Thus, using natural compounds as feed additives such as silymarin may affect milk production. The present study results proved that milk yield was improved after the administration of silymarin-rich extract, especially at levels 20 and 30 g/day. This may be due to presence of bioactive compounds such as flavonoids which have positive effect on milk production. Milk thistle extract contain 39.32 μ g/g flavonoids and 1669.47-

1609.23 mg/g silymarin [45-46]. Li et al. [47] reported that milk yield increased after supplementation with flavonoids-rich extract in buffalo. In goats, dietary supplementation with silymarin during peripartum period improved milk peak yield [28].

Protein, fat, and lactose contents are important technological criteria in goat milk quality [48]. Fat is one of the most important components of goat milk regarding its value, as lipids are involved in cheese yield, firmness, color and flavor of goat dairy product [49]. Furthermore, milk have different type of fatty acids (short-chain fatty acids, saturated fatty acids, unsaturated fatty acids, mono- and poly-unsaturated fatty acids) which have effect on human health [50]. Dietary change or feed additives are affect milk fatty acids profile [51]. Milk fatty acids profile was altered after feeding on unsaturated fatty acids-rich additive to lactating goats [52-53]. Cumin seeds supplementation (10g/day) increased milk fat level, total unsaturated fatty acids (by 9.7%) and conjugated fatty acids (by 23.1%), and decreased milk saturated fatty acids (by 3.9%) in damascus goat [54]. Feeding multiparous cows on endosperm of milk thistle increased fat content and unsaturated fatty acids (c18:0, 6-cisc18:1, 9-12-cisc18:2) in cow milk [21]. The present study showed that milk fat level and fatty acids profile were affected by silymarin-rich extract supplementation suggesting that supplementation with silymarin can be applied to modified fat formation in dairy goat.

About 40% of fatty acids in milk are synthesized in mammary gland and about 60% originated from plasma uptake [55]. In ruminants, short and medium chain fatty acids (c4:0 to c12:0) are synthesized de novo by the mammary glands, while long chain fatty acids are transport to the mammary gland from blood [56]. In the current study, goats fed silymarin-rich extract have higher milk total short and medium fatty acids content compared to the control, indicating trigger of de novo fatty acids synthesis by mammary gland in those groups. Stearic acid (18:0) content in milk change according to different factors such as $\Delta 9$ -desaturase activity in mammary gland and processes of biohydrogenation in rumen [56]. Stearic acid (18:0) is converted to oleic acid (18:1n9cis) in mammary gland to regulate milk fluidity [57]. Lock and Garnsworthy [58] reported that stearic acid content in milk reduced due to the elevation of $\Delta 9$ -desaturase activity in mammary gland or the reduction of biohydrogenation efficiency. Stearic acid

was decreased and oleic acid was increased in milk of goat after silymarin-rich extract supplementation compared to control in the present study. Poly-unsaturated fatty acids and the ratio PUFAs n-6 and PUFAs n-3 play important role in improving human health; PUFA contribute in cardiovascular diseases, diabetes and atherosclerosis treatments [59]. Also, the ratio PUFAs n-6 and PUFAs n-3 is an indicator for the nutritional value of fat [60]. Several studies reported the positive effect of dietary supplementation on PUFAs content in milk [61-63]. Fish oil, oilseeds, vegetable-seeds and algae administration in dairy sheep decreased the saturated fatty acids and increased PUFAs content with increasing the proportion of PUFA-n3 in milk [64-67]. Several studies reported that feeding rations contain high level of polyphenols elevated milk PUFA and reduced n6/n3 ratio [68-70]. Silymarin-rich extract increased the PUFA content in milk of goats and lowered the ratio of n6/n3 in the present study.

Goat milk is used to produce several dairy products such as cheese, yogurt and infant formula due to its high digestibility, nutritional value, and hypoallergenic effect [71-73]. Goat cheese is the most valued product as functional food product, it contains bioactive peptide, macro- and micro-nutrients which have a health benefit as antioxidant and antimicrobial effects [74-75]. The most important content in cheese production to form curd is milk casein for coagulating [76]. The concentration of $\alpha 1$ -CN in milk is important for cheese yield [77]. The total protein in goat milk is positively correlated to $\alpha 1$ -CN concentration and that related to elevates gel firmness and thereby yield of cheese [78-80], therefore, important for the economy of cheese producer. In the present study, the SDS-PAGE profile was mainly characterized by β -casein, $\alpha 2$ -casein and $\alpha 1$ -casein. Silymarin supplementation produce significant different for β -CN, $\alpha 2$ -CN and $\alpha 1$ -CN. The electrophoretic patterns showed that increase in silymarin-rich extract added to the goat's feed lead to increase in casein proteins in particular $\alpha 1$ -cn, this leads to an increase in the total protein and cheese yield.

5. Conclusion

Milk and dairy products are important in human food, so improving the nutritional quality of milk and its product is recommended. The use of silymarin-rich extract as dietary additive in goat rations increased

milk production and milk quality. Goat milk was high in its content of total protein, total fat, polyunsaturated fatty acids and low in saturated fatty acids. Also, silymarin affect cheese yield and cheese composition subsequent improved the quality of cheese product.

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7. Conflict of interest

All authors declare that they have no conflict of interest.

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