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RESEARCH ARTICLE

Elemental Distribution and Fatty Acid Profile of Raw Goat Milk: Effect of Dietary Olive Pellets on Milk Nutritional Value

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Abstract: ***Background*:** Milk is one of the important drinks for human and serves many nutrients including trace elements required for growth. In terms of prediction of element availability in food, fractionation analysis is helpful for understanding element partitioning.

***Objective*:** The current study was conducted to examine the effect of dietary olive pellets on goat milk. The milk samples of goats which were fed with basal diet and olive pellet added basal diet were investigated.

***Method*:** Lipid, protein and serum fractions of milk were separated and elements including Fe, Cu, Zn, Mn, Mg, Ca, Cr, Ni, Mo, Al, Ba, Pb, Sn, V, Co and Cd were detected using inductively coupled plasma optic emission spectrometry.

***Results*:** The distribution tendency of the elements was variable. The relatively highest bioavailable metal species were found in the milk of olive pellet fed goats, and the percentages were between 5.4 and 83.6%. Sum of the element contents of lipid, protein and serum fractions was compared with whole sample analysis and recoveries were between 93.3 and 110.7%. Fatty Acid (FA) profile of the milk samples was determined using gas chromatography. Classification of milk samples was carried out based on FA contents by principal component analysis. The milk obtained from goats fed with olive pellets have been shown to be similar in terms of polyunsaturated, monounsaturated and long chain FA contents.

***Conclusion*:** The results of the present study indicated that feeding the goats with olive pellets improves the quality of milk in terms of FA content. On the other hand, distribution of the elements between investigated fractions was not remarkable.

Keywords: Element fractionation, fatty acid composition, goat milk, olive pellet.

1. INTRODUCTION

Milk has a unique role in human diet and is a significant provider of protein, fat, vitamin and mineral. Furthermore, milk and dairy products contribute to rural economy in many countries. More than any other livestock, the goat is the main supplier of dairy and meat products for rural people. Recently, goat milk has gained more importance than cow and sheep milk in human diet especially for infants and mothers who are breastfeeding. Compared to cow milk, digestion of goat milk is easier due to smaller fat globules. It was recommended as a valuable nutrition considering its less allergenicity and better digestibility. On the other hand, goat milk includes higher percentage of short and medium chain (C_6 - C_{14}) fatty acids and it has been concluded that it reduces Low Density Lipoprotein (LDL) level in the blood. In general, fatty acids such as butyric, caproic, caprylic, capric, lauric, myristic, palmitic and linoloic acid are significantly higher in goat milk. It was concluded that, lactose content of goat milk is less than cow milk and goat milk has therapeutic effects [1-3].

It has been reported that different feeding supplementations for ruminants affect and modify the main constituents, element content and fatty acid composition of milk [4-10]. Toral *et al*. [11] concluded that adding fish oil and plant oils to starch diets increases the fatty acid levels in goat milk. Similarly, addition of sugar and sunflower oil to diet of Saanen goats increased the milk yield [10]. It was observed that feeding systems supplemented with plant oils, oilseeds or agro-industrial by-products allow to produce milk and dairy products rich in mono- and poly- unsaturated fatty acid [7, 9, 10, 12].

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In the literature, element composition of goat milk has been extensively investigated using various detection techniques [13-15]. The researches on this field mostly are based on the total amount of elements. However, it is known that biological behavior of the element strongly depends on its chemical form. Therefore, fractionation studies have been more informative than total element determination to elucidate bioavailability and/or toxicity of the measured elements in food samples [16]. Fractionation has been defined as classification of analyte(s) from a certain sample according to physical and/or chemical properties. It has been concluded that fractionation analysis is of great importance to predict the availability of nutrients [17].

The olive tree cultivation is an important agricultural industry in Turkey. According to the International Olive Council (IOC) report, 390 thousand tons of table olive and 160 thousand tons of olive oil were produced in Turkey in 2014/2015 [18]. This industry is an important part of Turkish economy and exportation. On the other hand, the sector generates large amounts of liquid and solid wastes during the process as well as all over the world. The wastes contain high amount of organic compounds that are not easily degradable. It is known that, there are various studies to regard olive wastes as soil conditioner, fertilizer, biomass fuel and forage [19, 20]. In Turkey, olive pellet feed was produced and used as a by-product of olive-olive oil industry, recently.

In our previous work [21] goat and breast milk showed similarity in terms of the distribution of elements and total concentrations. Considering the similarity of the distribution of the elements between the fractions, our data was found valuable for the possible usage of goat milk as a baby nutrient. Starting from this point of view, milk samples which were obtained from goats fed with and without olive pellet were investigated for elemental distribution and fatty acid composition. The lipid, protein and serum fractions of milk were separated and the elements such as Fe, Cu, Zn, Mn, Mg, Ca, Cr, Ni, Mo, Al, Ba, Pb, Sn, V, Co and Cd in mentioned fractions were detected using inductively coupled plasma optic emission spectrometry (ICP-OES). Additionally, fatty acid profile of the milk samples was determined using Gas Chromatography (GC). Due to limited number of studies on element fractionation analysis in goat milk samples, the current study presents valuable data about nutritional quality.

2. MATERIALS AND METHODS

2.1. Instrumentation

Element determination was performed using a Perkin Elmer 3100 XL (Waltham, MA, USA) inductively coupled plasma optic emission spectrometry (ICP-OES). The instrumentation was equipped with an echelle-based polychromator, a standard axially viewed glass torch and a cross flow nebulizer coupled to glass cyclonic spray chamber. Transport of the solutions to the nebulizer was achieved using a peristaltic pump at 1.5 mL min^{-1}. Plasma, auxiliary and nebulisation gas flows were 15.0, 0.5 and 0.5 L min^{-1}, respectively. The wavelengths (nm) used for the elements were; 238.204 for Fe; 327.393 for Cu; 206.200 for Zn; 257.610 for Mn; 285.213 for Mg; 317.933 for Ca; 267.716 for Cr; 231.604 for Ni; 202.031 for Mo; 308.215 for Al; 233.527 for Ba; 220.349 for Pb; 189.927 for Sn; 290.880 for V; 228.616 for Co and 228.802 for Cd.

Fatty acid methyl ester (FAME) profile was determined by an Agilent 7890A Gas Chromatograph (GC) equipped with Agilent HP-88 capillary column (100 m x 0.25 mm x 0.2 μm) and flame ionization detector (FID). The profile of FAME in a 1 μL sample at a split ratio of 1:100 was determined using a temperature gradient program. GC conditions were as follows: initial oven temperature 140°C maintained for 5.0 min, increased to 220°C at 4°C min^{-1} and held for 5.0 min, then increased to 240°C at 2°C min^{-1} and maintained for 5.0 min. Detector and injector temperatures were 260°C and nitrogen was used as the carrier gas.

A TE214S model Sartorius analytical balance (Goettingen, Germany) with 0.0001 g accuracy, MR 3001K model Heidolph (Schwabach, Germany) heating magnetic stirrer, Thermo Scientific (Waltham, MA USA) Orion 5 Star model pH meter, an Elektro-mag (İstanbul, Turkey) M815P and a Sigma Laborzentrifugen (Harz, Germany) centrifuges were used during the fractionation studies.

2.2. Chemicals

All reagents used in the assay procedures were of analytical or chromatography grade. All aqueous solutions were prepared in water, supplied by a reverse osmosis system. n-Hexane, trichloroacetic acid (TCA) (50 %, w/w) and acetone (Merck, Darmstadt, Germany) were utilized in fractionation analysis. Digestion of the whole samples and the fractions were performed with concentrated HNO_3 (65 %) and H_2O_2 (30 %) (Merck, Darmstadt, Germany). 1,000 mg L^{-1} standard solutions of Fe, Cu, Zn, Mn, Mg, Ca, Cr, Ni, Mo, Al, Ba, Pb, Sn, V, Co and Cd (all Merck) were used to prepare a multi element calibration standard. Supelco® 37 Component FAME mix (Sigma Aldrich) was used as fatty acids standard.

2.3. Sample Collection

This study was carried out with milk samples of Saanen goats from 3 different farms of Aydın. The diet of the goats was based on alfalfa hay, corn silage and grain. Dietary treatments of the goats were designated as (A) basal diet without olive pellets, (B) olive pellet added basal diet for 1 year and (C) olive pellet added basal diet for more than 1 year.

The goats were milked by hand twice a day, morning and evening. Milk samples were collected in the farms, kept at 4°C in polyethylene containers and transferred to the laboratory. The milk samples were unpreserved and extraction of FA and fractionation analysis were achieved in fresh samples. Additional aliquots of unpreserved milk samples were stored at -20°C.

2.4. Fractionation Process

The three main fractions lipid protein and serum were isolated in real matrix of raw milk samples. A previously suggested sequential fractionation scheme (Fig. **1**) [21, 22] was used for separation of the main fractions.

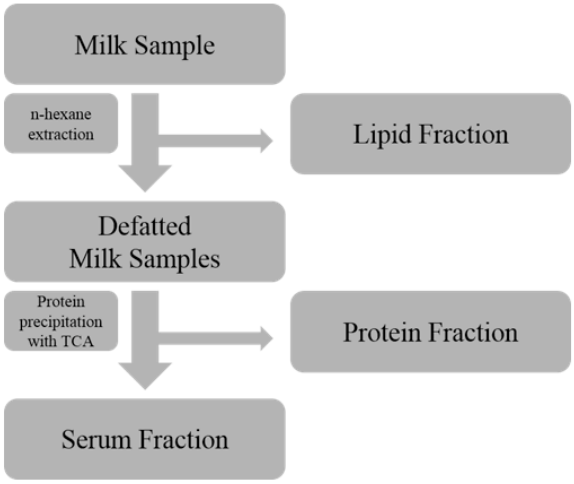

Fig. (1). Element Fractionation scheme [20, 21].

2.5. Isolation of Fractions

2.5.1. Lipid Fraction

A 5.0 mL portion of fresh milk sample was transferred in a 50 mL falcon tube and 10.0 mL of n-hexane was added to remove lipids. The mixture was shaken for 5 min and centrifuged at 4,000 rpm for 15 min for the separation of immiscible phases. Then, the n-hexane phase was pipetted, evaporated from separated phase and the fraction was called *lipid fraction*. The residue was used for subsequent extraction steps.

2.5.2. Protein Fraction

The obtained defatted residue was diluted with 15 mL of purified water. The pH of the sample was adjusted to 4.8 and 1.0 mL of 50 % (w/v) TCA was added for precipitation of proteins. To accelerate coagulation, the sample was heated up to 60°C and then incubated at 4°C for cooling. Prior to separation of precipitate, the sample was centrifuged as similar in lipid fractionation. The obtained precipitate was washed with cold acetone and called *protein fraction*.

2.5.3. Serum Fraction

The final solution after removing lipid and protein fractions was called *serum fraction*. Water soluble constituents of milk such as low molecular compounds and ions were included in the serum fraction.

2.6. Digestion of Whole Milk Samples and Fractions

The whole milk matrix and the isolated fractions were acid digested prior to ICP-OES analysis. The digestion for whole matrix was carried out as follows: 10.0 mL of concentrated HNO_3 was added on 5.0 mL of sample and treated for 1 h under reflux. Then, a 5.0 mL portion of H_2O_2 (30%, v/v) was added on the mixture and continued to digestion under reflux for another 1 h. After digestion, the sample was allowed to cool and clear solution was diluted to 25.0 mL. Digestions of the isolated fractions was achieved in open vessels on hot plate. Accordingly, the fractions were taken into a 100 mL glass beaker, 5.0 mL of concentrated HNO_3 was added and covered with watch glass. The mixture was simmered until completion of sample decomposition resulting in a clear solution without scattering. After cooling, the digests were transferred to a volumetric flask and diluted to 10.0 mL. Blank solutions were also prepared by the procedure above without sample [20, 21]. All the analyses were performed in triplicate run and the results were reported as mean ± standard deviation.

2.7. Calibration Procedure

External calibration procedure was employed for the quantitative analysis of elements. Standard solutions were prepared in 3% HNO_3 by diluting a multi element standard solution that was also prepared using single element standards. The calibration curves for all the analytes were built on five different concentrations between 10.0-750.0 µg L^{-1}. The correlation coefficients for all the calibration curves were at least 0.9999. The instrumental drift was checked by the analysis of standards on regular basis. Limit of detection (LOD) values of the instrument were given in Table **1**.

2.8. Fatty Acid Analysis

For analysis of the fatty acid composition, a 100 mL portion of fresh milk was extracted by 5 mL of n-heptane for 1 hour. Briefly, milk fat samples were dissolved in 4 mL of isooctane and esterification was completed by using 0.4 mL of 1 M KOH in methanol. The mixtures were vortexed for 30 s and then centrifuged for 3 min at 3000 rpm. The clear layers containing the FAMEs were transferred to autosampler vials and analyzed by gas chromatograph. Individual fatty acid methyl ester (FAME) peaks were identified by comparison of their retention times with FAME mix standard and fatty acid contents were expressed in percentage of total amount of the fatty acids identified.

Table 1. Detection limits of the instrument.

Limit of Detection (LOD) Values (µg L^{-1})							
Fe	**Cu**	**Zn**	**Mn**	**Mg**	**Ca**	**Cr**	**Ni**
8.7	4.0	6.7	3.0	18.6	28.9	2.9	4.5
Mo	Al	Ba	Pb	Sn	V	Co	Cd
2.6	10.3	2.4	1.8	5.9	3.7	5.2	4.8

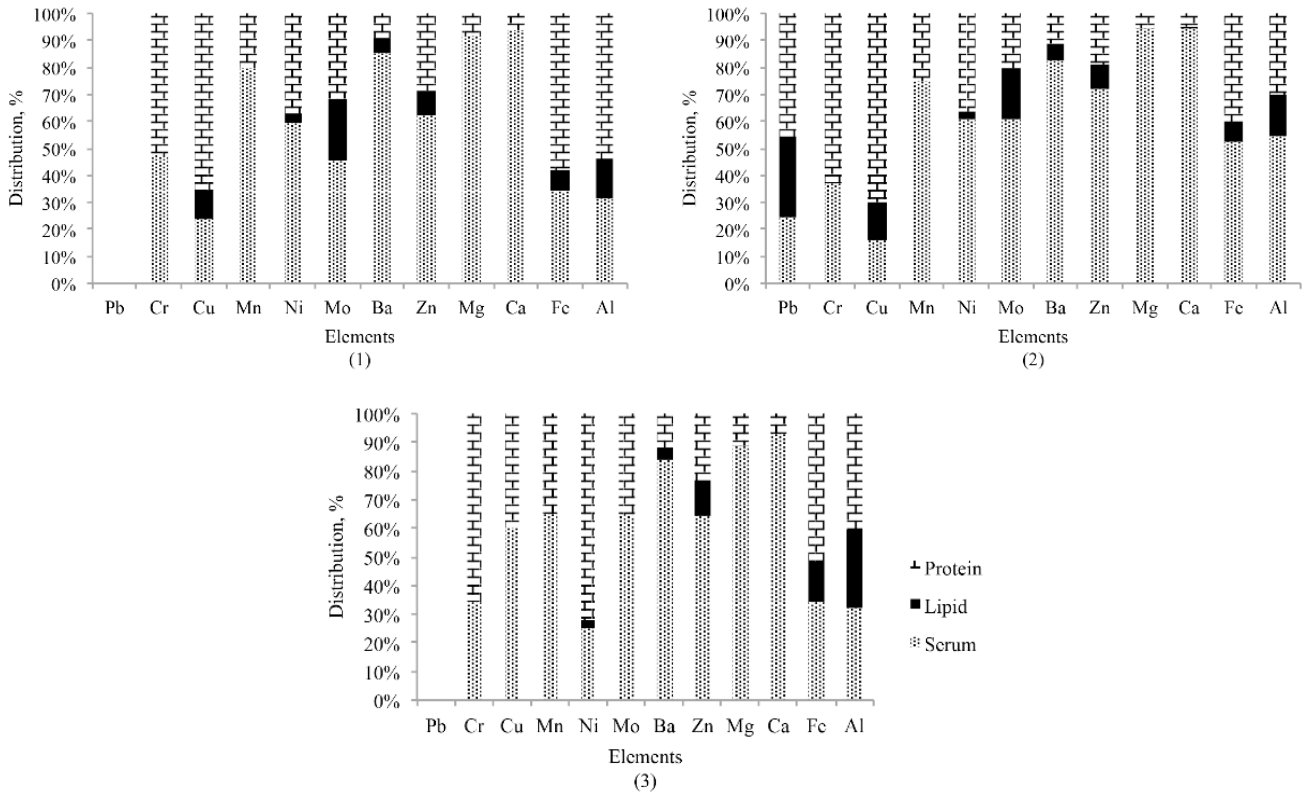

Fig. (2). Distribution of elements within fractions: milk obtained from (1) basal diet fed goats, (2) olive pellet fed goats (for 1 year), (3) olive pellet fed goats (for more than 1 year).

2.9. Statistical Analysis

The FA results were statistically compared by one-way ANOVA procedure of the SPSS software package for Windows (version 15.0, SPSS Inc., Chicago, IL, USA). Differences among the mean values were performed using the Duncan's multiple range test and the significance levels of the results ranged from 0.1 to 0.001. Milk samples were considered as factor and the replicated results were considered as dependent values. Additionally, principal component analysis (PCA) has been used to explore correlations. Data processing was performed by the software XLSTAT.

3. RESULTS AND DISCUSSION

3.1. Distribution Pattern of the Elements

The concentrations of Pb, Cr, Cu, Mn, Ni Mo, Ba, Zn, Mg, Ca, Fe and Al measured in isolated fractions are presented in Table **2**. Additionally, distribution percentages of the elements between fractions were visualized in Fig. (**2**). Sn, V, Co and Cd content of the fractions were below detection limits. Fractionation patterns of Ca and Mg ions were similar in all milk samples. The ions which were predominantly present in cationic form were found in serum fraction. Ca and Mg contents of serum fractions varied between 93.1-94.8% and 88.7-94.6%, respectively. These findings were in harmony with the literature outcomes [23-25]. In the case of Mn, Ba, Mo and Zn, the distribution of this species also showed similarity with Ca and Mg and was mainly found in serum fraction. The percentages of element contents in serum fractions were between 64.8-79.4% for Mn, 82.8-85.2% for Ba, 45.4-64.7 % for Mo and 61.5-72.3% for Zn. It has been previously reported that, Ca, Zn and Mg found in serum fraction binds with low molecular weight (LMW) ligands such as citrate [26]. Therefore, high binding constant of these ligands may allow less amount of elements to be bound to proteins and/or lipids. Correspondingly, it was seen that lipid bound forms of these elements were remarkably lower or below LOD.

On the other hand, in the case of fractionation of Ni, Fe, Al and Cu, noticeable variations were ascertained within the milk samples. Ni was mainly found in serum fractions of A and B milk samples with 59.8% and 61.1 %, respectively. On the contrary, it was mainly found in protein fraction of C sample (over 70 %). Additionally, Fe concentration of A and C milk samples were relatively higher in protein fraction and found as 1.4±0.2 mg L^{-1} (58.3 %) and 1.5±0.1 mg L^{-1} (51.9 %), respectively. Lipid bounded iron content were varied between 7.3 - 13.5 % within the milk samples. It was reported that [26] Fe links to amino acids of polypeptide chains of casein. Correspondingly, Fe was mainly bounded to high molecular weight compounds (lipid and protein bounded). In contrast to the literature [27], copper followed same distribution pattern with iron and considerably higher proportion was found in protein and lipid fractions. Al was mainly distributed in protein fraction except B milk sample. The percentages of protein bounded Al were 53.9 % for A, 30.1 % for B and 40.7 % for C milk samples. Similarly, in our previous work [21], the protein fraction had been reported to have included the most of the Al concentration in UHT cow milk and UHT semi-skimmed goat milk. Chro-

Table 2. The distribution of elements.

Sample	Fractions	Elements (μg L^{-1}; *mg L^{-1})											
		Pb	**Cr**	**Cu**	**Mn**	**Ni**	**Mo**	**Ba**	**Zn***	**Mg***	**Ca***	**Fe***	**Al**
A	Serum	nd	37.0±2.5	45.6±4.4	56.8±1.2	1329.1±7.2	44.5±2.1	175.2±5.1	2.4±0.1	134.8±3.4	1716.3±40.1	0.82±0.03	146.7±4.0
	Lipid	nd	nd	20.5±0.2	nd	70.7±4.3	22.1±2.6	12.3±0.9	0.36±0.01	0.120±0.001	1.2±0.2	0.193±0.006	67.1±2.4
	Protein	nd	43.7±0.6	126.0±2.9	14.7±0.7	822.4±29.1	31.5±0.3	18.0±1.5	1.10±0.03	11.9±0.1	119.5±1.9	1.4±0.2	249.7±13.7
B	Serum	22.2±0.7	37.4±4.9	22.5±3.2	48.8±2.0	1030.6±34.9	36.8±2.7	91.1±9.6	3.4±0.3	127.4±1.9	1164.4±35.5	1.24±0.02	343.7±11.7
	Lipid	27.4±3.0	nd	18.8±4.1	nd	41.1±0.9	11.7±2.8	6.6±0.2	0.4±0.03	0.13±0.02	3.2±0.1	0.173±0.006	95.8±8.5
	Protein	41.9±2.2	63.2±2.5	96.1±1.3	16.3±2.9	614.3±15.9	12.0±1.8	12.3±1.3	0.9±0.1	7.1±0.2	61.1±0.2	0.96±0.04	189.4±27.2
C	Serum	nd	35.9±5.9	68.0±4.4	29.8±2.4	270.8±0.6	49.8±3.1	136.6±9.2	1.62±0.08	136.4±6.0	1880.3±39.2	1.0±0.1	201.9±4.5
	Lipid	nd	nd	nd	nd	33.2±1.5	nd	6.5±0.8	0.30±0.01	0.094±0.002	3.2±0.1	0.39±0.04	169.1±17.9
	Protein	nd	68.8±6.0	45.6±4.0	16.2±2.4	783.0±89.1	27.2±2.3	19.5±2.0	0.59±0.01	17.2±0.4	136.1±1.7	1.5±0.1	255.1±2.1

Table 3. Comparison of theoretical and experimental total element content.

Samples		Elements (μg L^{-1}; *mg L^{-1})											
		Pb	**Cr**	**Cu**	**Mn**	**Ni**	**Mo**	**Ba**	**Zn***	**Mg***	**Ca***	**Fe***	**Al**
A	Sum of Fractions	nd	80.7±2.6	192.1±5.3	71.5±1.4	2222.2±30.3	98.1±3.4	205.5±5.4	3.9±0.1	146.82±3.40	1837.0±40.1	2.4±0.2	463.5±14.5
	Experimental Total	nd	77.8±7.8	188.6±10.7	71.9±5.6	2233.7±262.0	99.3±14.1	216.3±15.6	3.8±0.2	151.8±3.5	1842.4±25.3	2.2±0.2	460.2±47.5
	Rec. %	-	103.7	101.8	99.4	99.5	98.8	95.0	102.6	96.7	99.7	109.1	100.7
B	Sum of Fraction	91.5±3.8	97.8±5.5	137.4±5.4	65.1±3.5	1686.0±38.4	60.5±4.3	110.0±9.7	4.7±0.3	134.63±1.9	1228.7±35.5	2.37±0.04	628.9±30.8
	Experimental Total	94.7±10.1	103.4±5.1	128.8±6.1	68.2±7.0	1565.7±8.8	58.4±5.1	103.4±7.7	4.5±0.4	139.8±5.7	1187.7±80.9	2.3±0.1	647.2±52.0
	Rec. %	96.6	94.6	106.7	95.4	107.7	103.6	106.4	104.4	96.3	103.4	103.0	97.2
C	Sum of Fractions	nd	104.7±8.4	113.6±5.9	46.0±3.4	1087.0±89.1	77.0±3.9	162.6±9.4	2.5±0.1	153.7±6.0	2019.6±39.2	2.89±0.15	626.1±18.6
	Experimental Total	nd	104.9±3.7	112.1±13.1	42.9±4.3	1165.2±6.0	73.6±7.8	163.6±16.3	2.6±0.2	161.6±6.9	2008.8±52.0	2.65±0.06	565.7±29.4
	Rec. %	-	99.8	101.3	107.2	93.3	104.6	99.4	96.1	95.1	100.5	109.0	110.7

Rec. %=(Sum of fractions/Experimental Total)x100

mium contents were distributed between serum and protein fractions. Lipid bound Cr species were below detection limit. Pb was only found in B milk sample fractions. Apparently, the concentrations of Pb were 22.7±0.7 μg L^{-1} in serum fraction, 27.4±3.0 μg L^{-1} in lipid fraction and 41.9±2.2 μg L^{-1} in protein fraction of B sample. This difference between Pb contents within the milk samples could be presumably attributed to the basal diet, natural variation or environmental factors.

The relatively highest lipid bound metal species were found in B and C milk. The lipid fraction of A milk appeared to be the less element containing fraction. This meant that bioavailable fraction in these milk samples was higher. Such occurrence could be attributed to the olive pellet diet of the goats.

3.2. Direct Determination of Total Elements in Milk Samples

The results concerning the total amounts of elements (Table **3**) were in good correspondence with those in the literature for goat milk analyzed after complete digestion [28, 29]. The contents of Ca and Mg were found in the range of 1187.7 - 2008.8 mg L^{-1} and 139.8 - 161.6 mg L^{-1}, respectively. Milk obtained from one year fed olive pellet goats contained markedly higher concentrations of Ca and Mg. Additionally, Ca and Mg concentrations found in present study were higher than those reported previously by Khan *et al*. [15] and Bağdat Yaşar *et al*. [21]. Similarly, total iron concentration was higher in C sample (2.65±0.06 mg L^{-1}). In a research in Turkey [30], Ca, Mg and Fe concentrations have been reported as 1342±49.87, 510±25.73 and 3.88±0.16,

Table 4. Fatty acid composition of milk samples.

Fatty Acids		Milk Samples			SEM	*P*-value
		A	B	C		
		% by wt of Total FAs				
C4:0	Butyric acid	1.31[b]	1.53[ab]	1.71[a]	0.080	†
C6:0	Caproic acid	1.51	1.68	1.45	0.028	ns
C10:0	Capric acid	7.39[a]	6.74[b]	6.66[b]	0.321	†
C11:0	Undecylic acid	0.25[b]	0.34[b]	0.67[a]	0.098	*
C12:0	Lauric acid	4.33[a]	2.88[b]	3.25[b]	1.135	**
C14:0	Myristic acid	10.74[a]	8.80[c]	9.82[b]	1.883	**
C14:1	Myristoleic Acid	0.71[ab]	0.61[b]	0.89[a]	0.040	†
C15:0	Pentadecylic acid	1.05[ab]	0.88[b]	1.19[a]	0.040	†
C15:1	Ginkgolic acid	0.35[a]	nd	nd	0.082	**
C16:0	Palmitic acid	26.66	27.56	27.66	0.607	ns
C16:1	Palmitoleic acid	2.83[b]	3.29[a]	1.44[c]	1.855	**
C17:0	Margaric acid	0.80[a]	0.40[b]	nd	0.320	**
C17:1	cis-10-Heptadecenoic acid	0.31[a]	0.35[a]	nd	0.073	**
C18:0	Stearic acid	10.33[a]	7.63[c]	8.74[b]	3.683	**
C18:1n9t	Elaidic acid	0.58[b]	1.32[a]	nd	0.875	***
C18:1n9c	Oleic acid	24.20[a]	25.28[a]	28.31[b]	9.080	**
C18:2n6c	Linoleic acid	5.11	nd	nd	17.408	***
C18:2n6t	Linolelaidic acid	nd	5.86[b]	8.22[a]	35.826	***
C20:0	Arachidic acid	0.64[b]	3.19[a]	nd	5.696	***
C18:3n6	γ-Linolenic acid	0.49[a]	0.57[a]	nd	0.190	**
C20:1	Eicosenoic acid	0.41[b]	1.06[a]	nd	0.571	***
SFA[1]		65.01	61.63	61.15	8.851	ns
MUFA[2]		29.39[b]	31.91[a]	30.64[ab]	3.175	†
PUFA[3]		5.60[c]	6.43[b]	8.22[a]	3.571	**
Short Chain[4]		10.46	10.29	10.49	0.023	ns
Medium Chain[5]		47.78[a]	44.77[ab]	44.25[b]	7.264	†
Long Chain[6]		41.76[b]	44.91[a]	45.27[a]	7.457	†

[1] SFA= Saturated FA (C4:0 +C6:0+C10:0+C12:0+C14:0+C15:0+C16:0+C17:0+C18:0+C20:0)
[2] MUFA= Monounsaturated FA (C14:1+C15:1+C16:1+C17:1+C18:1+C20:1) (*c*- and *t*- isomers)
[3] PUFA= Polyunsaturated FA (C18:2+ C18:3) (all isomers)
[4] Short Chain (C4:0 to C11:0)
[5] Medium Chain (C12:0 to C17:1)
[6] Long Chain (C18:0 to C20:1)
[a, b, c] Means in a raw with different letters are significantly different: $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$ and $^{\dagger}P<0.1$
ns: non-significant ($P>0.1$)
nd: not detected
SEM: standard error of means

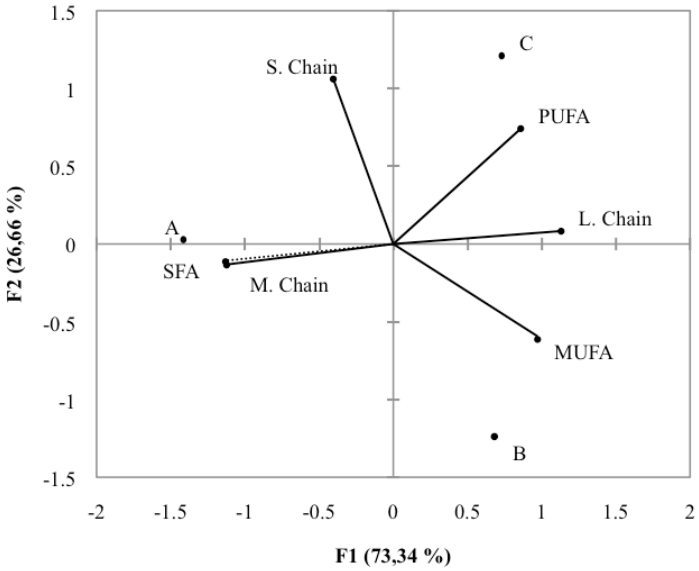

Fig. (3). PCA model in correlation of milk samples and FA contents.

respectively in goat milk. The difference between the results may be attributed to the season, region and breed of goats. Concerning Cu, Cr, Mn, and Zn, the outcomes were in good agreement with the literature [14, 21, 29]. Pb content was below LOD for A and C milk samples. On the other hand, Pb concentration was 94.7±10.1 µg L^{-1} in B milk. This difference could be attributed to possible contamination from basal diet. Additionally, Ni and Al contents were within the range of 1.16-2.23 and 0.46-0.65 mg L^{-1}, respectively. With regard to Mo and Ba, the obtained results were similar with those in previous studies [13, 21].

Certainly, for all elements, sum of the fractions separated was well compared to the total concentrations determined. The harmony between the results is shown in Table **3**. Moreover, this concordance consolidated discriminably the isolation of fraction in milk matrix. As it can be apparently seen, the recovery percentages were found to change from 95.0 - 109.1 % for A, 94.6 - 106.7 for B and 93.3 - 110.7 for C sample.

3.3. Fatty Acid Composition of Milk Samples

Mean values of the fatty acid composition identified by GC in the milk samples are given in Table **4**. The results were in line with other reports [9, 10, 12]. Previously reported studies concluded a decrease in saturated fatty acid (SFA) and an increase in mono unsaturated fatty acid (MUFA) and poly unsaturated fatty acid (PUFA) content of milk obtained from olive pellet fed goats. In this study, MUFA and PUFA contents were significantly higher (P<0.1 and P<0.01, respectively) in the milk of olive pellet supplemented goats. Additionally, differences between two olive pellet groups were smaller. It could be concluded that, olive pellet affected the fatty acid composition in a short period. According to the results, the largest increase in unsaturated FAs caused by oleic (C18:1n9c) and linolelaidic (18:2n6t) acid. Particularly, the high content of oleic acid can be attributed to olive pellet diet. The increase in oleic acid has been found to be valuable according to its beneficial effects on human health. Lopez-Huertas [31] reported that oleic acid rich milk reduced LDL cholesterol in blood serum. Furthermore, oleic acid has significant effect on immune system of cancer patients and reducing effect on tumor volume [32]. Despite the reduction of SFA amount in milk samples of olive pellet fed goats, there was no significant statistical difference in SFA contents. Indeed, the discrepancy could be attributed to stage of lactation and breeds of goats, basal diets and season which were not investigated in this study. Additionally, milk from the normally fed goats contained the highest concentration of SFA such as capric (P<0.1), lauric (P<0.01), myristic (P<0.01), margaric (P<0.01) and stearic (P<0.01) acids. However, no substantial differences were observed in the concentration of caproic acid (C6:0) and palmitic acid (C16:0) between the milk samples (P>0.1).

Additionally, for discriminating the milk samples by SFA, MUFA, PUFA, short chain FA (S. Chain), medium chain FA (M. Chain) and long chain FA (L. Chain) contents, the two principal components (PC1 and PC2) were plotted in Fig. (**3**). Plots of scores of PC1 versus PC2 depicted the best visualization in the main trends for the milk samples. The samples were divided into groups along PC1: with SFA and medium chain FA contents, A sample was located at the negative end of PC1. On the other hand, B and C samples were gathered at the positive side with PUFA, MUFA and long chain FA contents. These results also visually proved the diet effect on differentiation of milk FA composition. However, it should be noted that a clear classification of milk samples could be achieved with analysis of large number of samples.

In conclusion, an application of the previously developed element fractionation procedure was conducted on raw milk that was obtained from goats fed with olive pellets. Additionally, FA composition was investigated. The results of the present study demonstrated that feeding the goats with olive pellets increased the MUFA and PUFA contents in milk. Correspondingly, principal component analysis showed that, milk samples of basal diet fed and olive pellet fed goats were separated into two groups. Accordingly, milk of basal diet fed goats was rich in SFA and medium chain FA and milk of

olive pellet fed goats were rich in PUFA, MUFA and long chain FA.

The distribution of the elements between serum, protein and lipid fractions was not remarkable for milk samples. Mainly, Ca, Mg, Mn, Ba, Mo and Zn were found in serum fraction. On the other hand, Fe and Cu were predominantly bound to protein fraction. Unfortunately, the outcomes of the fractionation analysis for elements in raw goat milk could not be compared well with the literature because of limited data. So, the results obtained in this study provide useful information on nutritional value of goat milk. Considering genetic factors and seasonal changings, a detailed research with large number of milk samples would be more informative for nutritional assessment.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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