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Fatty Acids Profile, and Δ^9 -Desaturase Index of Milk from Barki Ewes Fed Diets Supplemented with *Spirulina Platensis* or Fish Oil

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Abstract

The current study aimed to evaluate milk fat and fatty acids profile, nutrients digestibility and lactating ewes' performance as a response to supplementing diets with spirulina algae or fish oil in lactating barki ewes. Three experimental diets were used as follow: Control diet based on 60:10:30 concentrate feed mixture (CFM), clover hay, and bean straw (T1); control diet supplemented with 10 ml of fish oil/ kg DM (T2), control diet supplemented with 5 g *Spirulina Platensis* / kg DM (T3). The results showed that T2 significantly improved nutrients digestibility (DM, OM, NDF, ADF, EE) as compared with control (T1). No differences ($p>0.05$) were recorded between experimental groups in different blood serum parameters, milk yield, energy corrected milk (ECM), milk fat (%) and milk total solids yield. Supplementing diet with *Spirulina platensis* (T3) increased CLA, C18:3 *n6*, C18:3 *n3*. T2 and T3 increased milk poly-unsaturated fatty acid and declined Δ^9 desaturase index as compared with T1. The results of the current study suggested that supplementing diets with fish oil or *Spirulina platensis* had a potential positive impact in milk fatty acid profile which has a healthy benefit.

Keywords: fish oil; *Spirulina Platensis*; milk; fatty acid; conjugated linoleic acid; Δ^9 desaturase index

1. Introduction

Milk is one of the high biological value animal products. Globally there are increase interesting with the knowledge about the nutritional and health importance of milk. Milk considered an ideal natural food due to the presence of different nutrients and bioactive component and immunological characteristics[1]. One of the most important components of milk is fatty acid composition with crucial health benefits. Milk fats and fatty acid profile are affect by many factor such as stage of lactation, genetic factors, season, and diet which play a major role in modulating the fatty acid composition of ruminant milk[2]. Many recent studies have linked the whole lipid contents with chronic disorders, such as diabetes and cardiovascular diseases[3]. Results from different experiments revealed that some saturated fatty acids (lauric, myristic and palmitic, trans-fatty acids may be responsible for the increase in blood cholesterol concentration[3]. On the other hand, several studies showed potential benefits of conjugated linoleic acid (CLA) in lowering blood total cholesterol content, anti-carcinogenic, anti-diabetic and immunomodulation effects. Also, omega-3 *(n-3)* fatty acid prevent heart disease and improve immune response[4][5]. Enriching milk with highly functional components especially (CLA) for their activity and its benefits have been studied [6]. Conjugated linoleic acid refers to a mixture of positional and geometric isomers of linoleic acid (18 carbons) with two double bonds separated by one single bond[5][7]. Conjugated linoleic acids isomers naturally produced by rumen bacteria as intermediates in the biohydrogenation of dietary long chain un-saturated fatty acid mainly linoleic acid

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C18:2, with cis-9, trans-11 CLA being the predominant isomer found in ruminants products [8].

Many studies have been carried out to investigate the impact of addition of different sources of oils such as fish oil, sunflower oil, olive oil and soybean oil in lactating animals' diets in a try to increase Conjugated Linoleic Acid (CLA) concentration in milk[8–11]. Supplementing diet of lactating cows with combination of fish oil (FO) and a high-linoleic oil source was the most efficient dietary regimen to increase milk cis-9, trans-11 CLA [10,12]

The use of microalgae for animal feed started in the early 70s. Microalgal properties give them the ability to enhance feed nutritional content, improving their effect in animal health[13–16]. Spirulina is a photosynthetic, filamentous, spiral, non-heterocysts, multicellular blue green algae which grows in wide range fresh, marine and brackish water. To our knowledge, only few studies evaluated spirulina as a diet supplement for lactating ruminants. From few published experiments had noted that supplementing grazing cows with a low amount (15 and 30 g/d) of spirulina during the transition period did not affect body weight, milk yield, and composition in the subsequent lactation[17]. The current study hypothesized that supplementing ruminant diets with fish oil or spirulina algae could improve fatty acid profile of lactating barki Ewes without any negative impacts on milk production and feed efficiency.

1. Experimental

1.1. Study site

The current study was carried out at the experimental farm of Animal production department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt belonging to the Laboratory of Dairy Animal Production, National Research Centre (Egypt). The area has a climate with winter rains and an annual average rainfall of 22 mm and means annual temperature between 14 and 28 °C. Ewes were cared and handled in accordance with the Guide for the Care and Use of Agricultural Animals in Research and Teaching.

1.2. Animals, feeding and experimental design

Thirty lactating barki ewes weighing 40 ± 2.3 kg, were randomly assigned to three experimental groups in a complete randomized design form 7 days post parturition and extended for 60 days. Ewes were individually housed in pens (1.5 m^2/ewe), with a free access to water, and experimental diet (*ad libitum*), to meet their nutrients requirements according to NRC[18]. The control diet was based on 60:10:30 concentrate feed mixture (CFM), clover hay, and bean straw, respectively. The chemical composition of the ingredients and diet are shown in table (1). The experimental diets were as follow: control diet with no additives (T1), control diet plus 10 ml of blend of fish oil/ kg DM (T2), control diet plus 5 g *Spirulina Platensis* / kg DM (T3). Diets were offered twice daily at 07:00 and 17:00 h.

Table (1): chemical composition of ingredients and experimental diet (g/kg DM)

Item	CFM	Clover	Bean straw	Experimental diet
Dry matter (DM)	883.4	900.1	876.5	883.00
Organic matter (OM)	948.3	911.5	925.1	937.66
Crude protein (CP)	138.3	85.9	66.3	111.46
Ether extract (EE)	139.3	105.6	94.7	122.55
Neutral detergent fiber (NDF)	319.0	516.1	583.9	418.18
Acid detergent fiber (ADF)	134.4	325.3	434.9	243.64
Hemicellulose	184.6	190.8	149.0	174.54
Non-structural carbohydrate (NSC)	351.7	203.9	180.2	285.47
Ash	51.7	88.5	74.9	62.34

CFM: Concentrate Feed Mixture

1.3. Nutrients digestibility and chemical analysis

Nutrients digestibility were carried out using acid insoluble ash as an internal indigestible marker as described by Khattab et al. [19]. Fecal grap samples were collected from animals, twice daily during 30 and 60 days of the experiment at 07:00 and 18:00 h. samples were dried at 105 ^{o}C for 12 h in a forced oven[20]. Feed and fecal samples were ground to pass a 1-mm screen using a Wiley mill grinder. Feed and fecal samples were analyzed for ash as described by AOAC[20] (method ID 942.05), ether extract (EE) using diethyl ether in a Soxhlet extractor (method ID 920.39) according to AOAC [20] official methods. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by ANKOM 200 Fiber Analyzer according to Van Soest et al. [21], without the use of alpha amylase but with sodium sulfite.

Acid detergent fiber (ADF; method ID 973.18) was analyzed according to AOAC [20], after digestion with sulfuric acid and cetyl trimethylammonium bromide, and expressed exclusive of residual ash. Non-structural carbohydrate (1000 – [NDF + crude protein (CP) + EE + ash], hemicellulose [NDF – ADF], and organic matter (OM; [1000 – ash]) were calculated.

1.4. Sampling and analyses of blood serum

On the last day of the 4th, and 8th weeks of the experiment, blood samples (10 mL) were taken 4 h after feeding from the jugular veins of all ewes into plain clean dry tubes without anticoagulants.

Serum blood samples were centrifuged at 4000 × g for 20 min. Serum was separated into 2-mL clean dried eppendorf tubes and frozen at –20 ◦ C, until analysis. Serum glucose, total protein, albumin, triglycerides, aspartate aminotransferase (AST), alanine aminotransferase (ALT) were analyzed using specific kits (Stanbio Laboratory, Boerne, TX, USA) and following manufacturer instructions. Globulin concentration was calculated as the difference between total protein and albumin.

1.5. Milk Sampling and Analysis

The animals were hand milked twice daily at 6.00 am and 6.00 pm during the last three days of each experimental period. Milk samples were analyzed for total solids, solids not fat, total protein, fat, ash and lactose using infrared spectroscopy (Bentley 150, Infrared Milk Analyzer, Bentley Instruments, USA). Energy Corrected Milk (ECM) was calculated as described by khattab et al.[22] ECM (kg day−1) = milk (kg day−1) × [38.3 × fat (g kg−1) + 24.2 × protein (g kg−1) + 16.54 × lactose (g kg−1) + 20.7]/3140. Milk fatty acids methyl esters (FAME) were analyzed as described by Abo El-Nor & Khattab [10] by preparing base-catalyzed methanolysis of the glycerides according to international standard [23], FAME were separated using Cp-Sil 88 fused-silica capillary column (100X0.25 mm i.d. X0.2 µm film thickness, chrompack, Middelburg, Netherlands) on a Perkin-Elmer chromatograph equipped with a flame ionization detector. The column was held at 100°C for 1 min after injection, temperature-programmed at 7°C min^{-1} to 170°C, held there for 55 min, then temperature programmed at 10°C min^{-1} to 230°C and held there for 33 min. helium was the carrier gas with a column inlet pressure set at 30 psig and a split ratio of 1:20. The injection volume was 0.2 µL. total run time was of 105 min. The Δ^9 desaturase index were used as an indicator of the Δ^9 desaturase activity using fatty acids that are substrates and products for Δ^9 desaturase and calculated as described by Lock & Garnsworthy [7].

1.6. Statistical analysis

Nutrients digestibility, blood serum parameters, milk yield and milk constituent's data were statistically analyzed using PROC MIXED procedure of SAS software (Version 9.4). Significant differences between means of treatments were carried out by the Duncan's test and the significance threshold was set at $p<0.05$. Also, non-orthogonal contrasts were used to compare mean (T1 vs. others) (T2 vs. T3).

3. Results and discussion

3.1. Nutrients digestibility

Digestibility of DM, OM, CP, NDF, ADF, and EE were significantly greater ($p<0.05$) for T2 than for control (T1) as shown in Table (2). While, T3 significantly increased EE digestibility and decreased DM digestibility ($p<0.05$) than values for T1. The current results reflect no negative impacts for fish oil or *Spirulina platensis* in rumen fermentation and ruminal microbial activity, the specific composition of spirulina especially polysaccharide content of was reported to be approximately 13% of DM may also affect rumen microbes and thus ruminal fermentation of nutrients digestion [17][6].

Table (2): effect of different supplementation on nutrients digestibility (%)

Items	Treatments			±SEM	*p-value*	Contrasts	
	T1	T2	T3			T1 vs. other	T2 vs. T3
DM	76.23 b	79.405 a	75.26 c	0.792	< 0.0001	< 0.0001	0.0007
OM	79.015 b	82.095 a	78.025 b	0.786	0.0047	0.0022	0.0980
CP	78.945 a	79.025 a	74.67 b	1.003	0.0748	0.1514	0.0494
NDF	68.23 b	71.735 a	67.005 b	0.930	0.0190	0.0090	0.2167
ADF	64.26 c	70.59 a	66.54 b	1.183	0.0030	0.0016	0.0242
EE	85.41 b	87.845 a	87.27 a	0.478	0.0125	0.0158	0.0131

T1: Control diet based on 60:10:30 concentrate feed mixture (CFM), clover hay, and bean straw, T2: control diet supplemented with 10 ml of fish oil/ kg DM, T3: control diet supplemented with 5 g *Spirulina Platensis* / kg DM.Means in the same row with diferent superscripts difer, $P < 0.05$. *P-value* is the observed significance level of the *F-test* for treatment;

3.2. Blood serum measurements

Both Fish oil (T1) or *Spirulina platensis* (T2) supplementation did not affect (p>0.05) concentrations of different blood serum glucose, total protein, albumin, globulin, A/G ration, tri-glycerides, ALT, and AST (Table 3). All blood metabolites were within the reference ranges [24]. Feeding fish oil or *Spirulina platensis* did not a□ect serum total protein, albumin, globulin, and tri-glycerides suggesting unchanged nutritional status of ewes [6] . Values of liver enzymes, AST and ALT, were within normal physiological ranges revealing normal liver activity and function. The result suggests no liver pathological lesions[25].

Table (3): effect of different supplementation on some blood serum parameters

Items	Treatments			±SEM	*p-value*	Contrasts	
	T1	T2	T3			T1 vs. other	T2 vs. T3
Glucose (mg/dl)	81.35	84.85	85.11	0.932	0.350	0.400	0.0950
Total Protein (g/dl)	5.06	4.94	4.92	0.055	0.5673	0.6756	0.3236
Albumin (g/dl)	2.98	2.50	3.04	0.024	0.3128	0.2291	0.3660
Globulin (g/dl)	2.08	2.44	1.88	0.052	0.3496	0.9083	0.1511
A/G ratio	1.43	1.64	1.61	0.064	0.4889	0.7191	0.2516
Tri-Glycerides (mg/dl)	85.23	97.52	91.77	7.244	0.7896	0.5585	0.7216
ALT (unit/L)	50	48	53	0.777	0.120	0.11	0.115
AST (unit/L)	100	97	103	1.569	0.2873	0.1633	0.4971

T1: Control diet based on 60:10:30 concentrate feed mixture (CFM), clover hay, and bean straw, T2: control diet supplemented with 10 ml of fish oil/ kg DM, T3: control diet supplemented with 5 g *Spirulina Platensis* / kg DM.

3.3. Milk yield, composition, and fatty acid profile

Milk yield and energy corrected milk (ECM), total solids, fat, and lactose values were not differed (p>0.05) between the three experimental groups (T1, T2, and T3) (table 4). While, total protein, solid not fat (SNF) significantly decreased (p<0.05) in T2 than other groups (T1, and T3, respectively). Energy-corrected milk was similar among diets, in agreement with studies when supplemented diet with fish oil [26][27] [28] [29].

Table (4): effect of different supplementation on milk yield and composition

Items	Treatments			±SEM	*p-value*	Contrasts	
	T1	T2	T3			T1 vs. other	T2 vs. T3
Milk yield (g/d)	590.4	595.5	542.2	39.24	0.161	0.1779	0.1925
ECM (g/d)	730.7	733.03	714.97	59.70	0.219	0.1379	0.4017
Milk constitutes (%)							
Total solids	16.01	16.45	16.75	0.293	0.605	0.9071	0.3211
Fat	5.04	5.62	5.37	0.263	0.344	0.2902	0.2888
Total protein	4.31 a	4.16 b	4.33 a	0.030	0.050	0.0172	0.8333
Lactose	6.12	5.96	6.15	0.057	0.048	0.0165	0.7276
Ash	0.91 a	0.85 b	0.90 a	0.007	0.001	0.0005	0.5069
SNF	11.34 a	10.83 b	11.38 a	0.090	0.016	0.0047	0.8294
Milk constitutes yield (g)							
Total solids	94.52	97.96	90.82	7.07	0.231	0.1827	0.3123
Fat	29.76	33.47	29.12	3.02	0.212	0.0942	0.6645
Total protein	25.45	24.77	23.48	1.63	0.204	0.2771	0.1722
Lactose	36.13	33.35	33.35	2.34	0.224	0.2959	0.1842
Ash	5.37	5.06	4.88	0.34	0.238	0.3735	0.1626
SNF	66.95	64.49	61.70	4.33	0.229	0.3137	0.1800

T1: Control diet based on 60:10:30 concentrate feed mixture (CFM), clover hay, and bean straw, T2: control diet supplemented with 10 ml of fish oil/ kg DM, T3: control diet supplemented with 5 g *Spirulina Platensis* / kg DM. Means in the same row with di□erent superscripts di□er, $P < 0.05$. *P-value* is the observed significance level of the *F-test* for treatment;

Fatty acid profile shows (Table 5) a rise of short chain fatty acids (SCFA), medium chain fatty acid fatty acids (MCFA), CLA, and poly-unsaturated fatty acids (PUFA) in T2, and T3 compared with control (T1). Vaccenic acid (VA) was decreased in T2 and T3 as compared with T1, these results as associated with increasing CLA in both treatments than control, VA is a common intermediate in the biohydrogenation of both linoleic and linolenic acids. As well as rumenic acid, VA is then taken up in the gut and desaturated to rumenic acid by Δ9-desaturase in the mammary gland. This is regarded as the most important source of milk rumenic acid[2]

Table (5) : Milk fatty acids profile of different experimental treatments

Items	Experimental treatments			±SEM	*p-value*
	T1	T2	T3		
C4:0	1.13	1.34	1.35	0.050	0.1404
C6:0	1.53	1.84	1.49	0.073	0.0742
C8:0	1.53 b	2.45 a	1.94 b	0.145	0.0042
C10:0	5.86 b	8.87 a	7.48 ab	0.485	0.0078
C10:1	0.46 a	0.00 c	0.25 b	0.067	<.0001
C12:0	3.89	4.22	4.34	0.137	0.4364
C14:0	10.12	10.65	10.67	0.315	0.7743
C14:2	0.71 a	0.48 b	0.44 b	0.044	0.0020
C15:0	1.34 a	0.92 b	0.97 b	0.073	0.0062
C15:1	0.43 b	0.52 a	0.40 ab	0.021	0.0352
C16:0	22.63	26.16	28.48	1.132	0.0825
C16:1 *n9*	1.78	1.68	1.91	0.061	0.3570
C17:0	1.34 a	0.90 b	0.79 b	0.089	0.0013
C17:1	0.57 a	0.30 b	0.28 b	0.048	0.0002
C18:0	12.90 a	9.18 b	7.25 b	0.877	0.0013
C18:1	29.39	25.63	23.82	1.119	0.0993
C18:2 *cis*	2.59 b	3.20 b	5.70 a	0.489	0.0002
C18:3 *n3*	0.57 b	0.44 b	0.76 a	0.049	0.0018
C18:3 *n6*	0.82 b	0.80 b	1.20 a	0.070	0.0038
C20:0	0.41	0.41	0.48	0.017	0.1555
Short chain fatty acids	14.40 b	18.72 a	16.85 ab	0.790	0.0523
Medium chain fatty acids	37.00	40.42	42.88	1.439	0.2736
Long chain fatty acids	48.59	40.86	40.27	1.835	0.1017
Saturated fatty acids	62.67	66.95	65.25	1.975	0.7319
Mono-unsaturated fatty acids	32.63	28.13	26.66	1.233	0.1030
Poly-unsaturated fatty acids	4.69 b	4.92 b	8.10 a	0.577	0.0008
Total-unsaturated Fatty acids	37.33	33.05	34.75	1.188	0.3840
PUFA/SFA	0.075 b	0.073 b	0.124 a	0.008	0.0010
Δ^9 -desaturase index	0.07 a	0.05 ab	0.04 b	0.004	0.0044
VA/CLA ratio	11.36 a	7.99 b	4.18 c	1.064	0.0001

T1: Control diet based on 60:10:30 concentrate feed mixture (CFM), clover hay, and bean straw, T2: control diet supplemented with 10 ml of fish oil/ kg DM, T3: control diet supplemented with 5 g *Spirulina Platensis* / kg DM. Means in the same row with di□erent superscripts di□er, $P < 0.05$. *P-value* is the observed significance level of the *F-test* for treatment

Δ^9 desaturase index showed a slight change with supplementing diets with *Spirulina platensis* or fish oil. Δ^9 desaturase index compares the product to precursor fatty acid ratios and important for production of CLA and MUFA. VA/CLA ratio values show a reduction with T2, and T3 vs. T1, this reduction is a result of desaturase activity increment consequently led to increase in milk CLA. The values of PUFA/SFA showed that the good benefits of supplementing diet with *Spirulina platensis* as recording the highest value compared with other groups. PUFA /SFA above 0.45 is an indication for some negative health diseases such as coronary heart disease and cancer [1].

4. Conclusions

In conclusion, the fatty acid composition of milk varies with different supplementation either fish oil or *Spirulina platensis*. Poly-unsaturated fatty acids (PUFA) were higher with *Spirulina platensis* and fish oil than control. CLA concentration increased with supplementing diets with *Spirulina platensis*. Δ^9 desaturase index enhanced with both different supplements. These results reflect the healthy benefits of milk produced from ewes fed diet supplemented with fish oil or *Spirulina platensis* without any negative impacts on nutrients digestibility, blood biochemical indices and animal productive performance.

5. Conflicts of interest

The authors report no conflict of interest.

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