



Impact of Royal Jelly Supplement on Ewe's Milk Fat Content and Fatty Acid Profile During the Early Stage of Lactation

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Abstract: The aim was to study the possible effects of royal jelly (RJ) supplementation on milk fat content and fatty acids (FA) profile of ewes during the early stage of lactation. Randomly, thirty-six Ossimi ewes were divided into two groups (18 animals each). The first group was offered the basal diet which was considered as a control group, whereas the second group was fed the basal diet, in addition to a supplement of a single bolus of RJ (1000 mg/head) every two days as a treated group. Regardless the effect of time, the RJ-supplemented group recorded a non-significant increase in daily milk yield (1.22 kg) when compared with the control (1.08 kg) group ($P < 0.063$). While, the RJ-supplemented ewes showed significantly increase in milk fat percentage ($P < 0.008$) as compared to control group. Lactating ewes in the control group produced significantly higher contents of milk C14:0 ($P < 0.036$) and C18:0 ($P < 0.027$) saturated FA than that in the RJ group. However, the contents of milk C6:0 and C17:0 in the RJ group were significantly greater than that estimated in the control ($P < 0.050$ and 0.041 , respectively). Furthermore, Lactating ewes supplemented with RJ produced significantly higher contents of milk C16:1 ($P < 0.001$), C18:1 ($P < 0.001$) and C18:2 ($P < 0.046$) unsaturated FA than the control group. It can be concluded that supplementation of ewes during the early stage of lactation with RJ can improve the nutritive value of milk fat, and appears to be an opportunity to modify the concentrations of certain milk fatty acids.

Keywords: Royal Jelly, Lactating Ewes, Milk Yield, Fatty Acids

1. Introduction

In the few recent decades, natural additives have been extensively used in animal feeding due to their positive effects [1]. While, Beehive products are commonly used, as they contain some phenolic compounds which have several antimicrobial properties. To a certain degree, such compounds may modify the fermentation process in ruminants, based on their origin and supplementation level in the diets [2]. Accordingly, research has been practiced using beehive

products; including royal jelly (RJ), bee pollen and Propolis; for their biological functions and pharmaceutical benefits [3].

RJ is a secretion produced in the cephalic glands of nurse bees and serves as the most important part of honeybee larvae diet, while playing a major role in caste differentiation [4]. RJ is widely used in both folk and official medicine and is a controversial effectual and beneficial dietary supplement. It has a complex composition (water, proteins, lipids, carbohydrates, amino acids, mineral salts, vitamins, enzymes, hormones, oligo-elements and natural antibiotics) comprising 67% water, 12.5% crude protein (including small amounts of

many different amino acids), 11% simple sugars (monosaccharides) and 5% fatty acids. It also contains many trace minerals, some enzymes, antibacterial, antibiotic components and trace amounts of vitamin C [5, 6]. RJ has been associated with antioxidant, antibiotic, anti-inflammatory, immunomodulatory, anti-allergic and general tonic pharmacological activities [7]. Meanwhile, information on the mechanism of action of chemical and bioactive compounds of RJ is not fully explored [8].

It has been known that milk fat contents and fatty acid (FA) profiles can be enhanced by make changes in composition of the diet for lactating animals, increasing the ability to respond to market preferences and human health requirements [9]. Additionally, flavonoids originating from beehive products have been found to alter the fermentation process in the rumen and simulate the ruminal digestion under laboratory conditions [10]. Therefore, the data about the effect of RJ on the milk fat contents and FA profile in lactating ewes are limited and controversial. Thus, the objective of the present study was to evaluate the possible effects of RJ supplements on milk fat contents and FA profiles in Ossimi ewes during the early stage of lactation.

2. Materials and Methods

This study was carried out at the experimental farms of the Nuclear Research Center, the Egyptian Atomic Energy Authority, which was conducted in the desert region of Inshas, Egypt.

2.1. Animals, Experimental Design and Management

Thirty-six multiparous ewes (Ossimi; 38 ± 1.5 kg BW) were randomly selected. Phenotypically, all ewes are healthy and have symmetrical udders. After lambing, animals were monitored and accommodated to one of the experimental groups. At the 3rd day post-lambing, the selected ewes were assigned randomly into two equal groups (18 animals each). The first group was offered the basal diet which was considered as a control group, whereas the second group was fed the basal diet, in addition to a supplement of a single bolus of RJ (soft gelatin capsules; 1000 mg/animal) every two days as a treated group. The current study continued for 4 weeks and completed under a temperate environmental conditions during the winter season ($23.2 \pm 1.2^\circ\text{C}$).

The crude RJ was collected and prepared by the Pharco Company (Royal Jelly 1000 mg, Pharco pharmaceuticals, Egypt). This commercial bolus form was prepared from a raw RJ that collected during the April month. At this time, the chemical composition of RJ is more stable and homogeneous. Using the gas chromatography–mass spectrometry (Shimadzu QP 2010 Plus, Japan), the chemical composition of RJ was calibrated according to [11]. The column of gas chromatography was a Zebron™ (ZB-1; ZB-5MSplus Capillary GC) methyl poly siloxane ($30 \text{ m} \times 0.25 \text{ mm i.d.} \times 0.25 \mu\text{m}$ film thickness). The chemical composition of RJ is described in Table 1. The main Flavonoids components are: Chrysin, Pinocembrin, Tectochrysin and Pinostrobinchalcone.

Table 1. The chemical composition (major flavonoids and fatty acids) of Royal Jelly.

Components	¹ TIC %	² RT (min)
10-Hydroxydecanoic acid	19.609	19.372
Octanoic acid, 8-hydroxy	3.138	14.791
Pinocembrin	1.844	33.72
3-Hydroxydecanoic acid	1.459	16.799
Chrysin	0.843	35.97
Pinostrobinchalcone	0.732	32.34
Oleic acid amide	0.679	32.407
Furfuryl alcohol	0.648	2.27
Tectochrysin	0.383	35.13

¹TIC%: The ion current generated depends on the characteristics of the compound concerned; ²RT: Retention time.

Ewes were housed in a shaded free stall barn and the experimental groups were kept under the same environmental conditions. Feed allowances were offered once a day in the morning at 10 a.m. individually, ewes fed the recommended diet with a subsequent measuring of daily feed intake for each animal. The basal diet was given in a form of well-formulated, mixed ration, before and after lambing. The concentrate mixture consisted of yellow corn, soybean, cottonseed cake, wheat bran, sugar beet bulb, and vitamin premix. On a dry matter basis, the analyses to explore the chemical composite of the diet were performed as described in Table 2 [12]. The alfalfa hay, straw, and concentrate were in a 35:20:45 ratios. The mixed ration was given in well-constructed mangers (allowing feeder space=0.40 m per animal). Fresh drinking water was available to all animals at all times in clean basins full of fresh water.

Table 2. Ingredients and chemical composition of the concentrate mixture during the experimental period.

Item	¹ BD	Ingredients				
		² YC	³ CSM	⁴ WB	⁵ SBB	⁶ AH ⁷ S
Ingredients (%)						
Yellow corn	25					
Wheat bran	20					
Sugar beet bulb	30.4					
Soybean meal	7					
Undecorticated cotton seed meal	15					
Dicalcium phosphate	1					
Sodium chloride	1					
Mineral mixture*	0.5					
Vitamin mixture**	0.1					
Chemical composition (%)						

Item	¹ BD	Ingredients					
		² YC	³ CSM	⁴ WB	⁵ SBB	⁶ AH	⁷ S
⁸ CP	17.68	8.42	28.74	15.55	9.39	18.8	3.6
⁹ CF	15.5	2.23	17.38	42.8	20.92	32.2	31.1
Fat (ether extraction)	2.87	3.99	7.58	4.05	0.94	1.7	1.3
Nitrogen-free extract	47.28	72.08	29.4	38.51	56.72	34.4	42.3
Ash	6.01	1.29	5.15	4.96	11.63	7.7	16.4
Net energy (MJ/kg)	6.32	14.17	7.94	9.04	3.42	6.8	4.1

¹BD: basal diet (DM basis); ²YC: yellow corn; ³CSM: cotton seed meal; ⁴WB: wheat bran; ⁵SBB: Sugar beet bulb; ⁶AH: alfalfa hay; ⁷S: straw; ⁸CP: crude protein; ⁹CF: crude fiber.

* mineral mixture contains: 5g Cu, 30g Fe, 40g Mn, 45g Zn, 0.3g I, 0.1g Se and 881.6g Caco3/ kg mixture.

**vitamin mixture contains: 20 million (I.U) vit A, 2 million (I.U) vit D3 and 2g vit E / kg mixture.

2.2. Milk Yield, Fat Content and Fatty Acids Profile

Every day, all ewes tested for the presence of clinical signs of mastitis, such as high temperature, distressing response or glandular changes. In order to evaluate the daily milk yield, lambs were separated from their dams at 4 p.m, and being hand milked (0800 h) in the next morning [13]. Throughout the experiment (four consecutive weeks), this practice was done in each week. Hand milking had been practicing with two expert milkers have the same fitness. On a weekly basis and from each ewe, milk samples were collected (100 ml approximately). The samples of milk were kept save by the 2-bromo-2-nitropropane-1,3 diol product to detect the milk fat contents by a milk analyzer (MilkoScan 6000; Hillerød, Denmark).

On the 4th week of the experiment, one milk sample (100 ml approximately) was collected without the preservative from each animal and maintained frozen to determine milk FA composition. The concentrations of milk FA (g/100 g fat) were measured using the GC method [14]. The extraction of milk samples was prepared with Folch's solution, chloroform: methanol (2:1) then vortex for 2 min and centrifuged for 10 minutes at 4000 rpm. The trionanoin was used as internal standard. The esterification procedure was completed by mix-up the supernatant with 2ml of a mix of (95: 5 methanol: sulphuric acid), at that point set it in an oven for 2 h (at 80 °C), then extracted with 2 ml hexane. Finally, samples were prepared for injection in the GC set loop.

SupelcoSP2330 columns (30mm X 0.32mm X 0.2µm film thickness) were standardized and used as described by the standard protocols (Cat. No. 24073, Sigma-Aldrich, St. Louis, MO, USA). The standardized Gas chromatography was performed with an Agilent Technologies (7890A GC, Agilent Technologies, Inc, USA). The flow rates at the split vent and through the column were 50 and 2.5 ml/min, respectively. Initially, the gradient temperature started with 70°C and linearly increased to 170°C (20°C/min). Further, a delayed linear increase to 170°C (0.8°C/min) was practiced to separate the closely-eluting fatty acids, followed by an increase to reach 220°C (20°C/min), and a final 2.5 min hold. Considering the flame ionization detector (FID), the whole run time was 20.1 minutes. The major milk saturated and unsaturated fatty acids were considered in this study.

2.3. Statistical Analysis

The normality of the available data was confirmed by

Kolmogorov-Smirnov test ($P > 0.05$). The repeated measure data (daily milk yield and milk fat contents) were interpreted using the repeated measures analysis of variance as a MIXED procedure of SAS statistical system Package (SAS Institute, Cary, NC, USA). The model for repeated measures was defined as: $y_{ijk} = \mu + T_i + Ewe(T_i)j + Time_k + (Time \times T)_{ki} + e_{ijk}$, where, y_{ijk} =daily milk yield and milk fat contents, T_i =fixed effect of treatment, $Ewe(T_i)j$ =random effect of ewe nested in treatment, $Time_k$ =fixed effect of time, $(Time \times T)_{ki}$ =interaction of time and treatment, and e_{ijk} =random error term.

Regarding the milk FA profile, the model was fitted to include the fixed effects of treatment (two levels: CON and RJ, parity (two levels: primiparous and multiparous) and the random effects of the dairy animal nested within parity. The differences between the means of the control and treated group were determined by a Student's t-test adjusted with a Tukey correction.

3. Results

Regardless the effect of time, the RJ-supplemented group recorded a non-significant increase in daily milk yield (1.22 kg) when compared with the control (1.08 kg) group ($P < 0.063$). The influence of the RJ on milk fat contents is illustrated in Figure 1. Regardless the effect of time, the RJ-supplemented ewes produced significantly higher milk fat percentage than the control group ($P < 0.008$). However, the effect of time on the milk fat percentage was non non-significant ($P < 0.068$, as a repeated measure value).

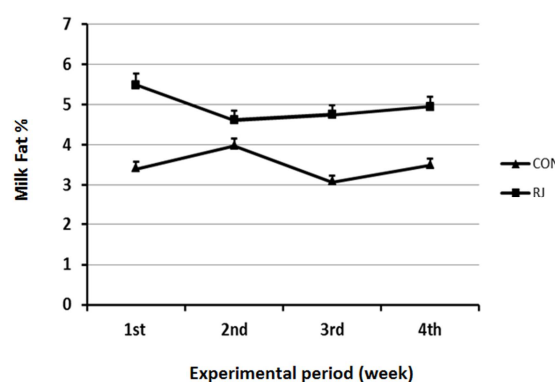


Figure 1. Effect of royal jelly (RJ) supplement on the percentage of milk fat in lactating ewes ($P < 0.008$). The effect of time on the milk fat percentage was non non-significant ($P < 0.068$, as a repeated measure value)).

Table 3. Milk Fatty acid profile (g/100 g of total lipids) of lactating ewes supplemented with Royal Jelly.

Fatty acids	Experimental groups		SEM ³	P-values
	CON ¹	RJ ²		
C4:0	3.41	4.39	0.21	0.163
C6:0	3.96	4.67	0.24	0.050
C8:0	2.57	3.64	0.09	0.098
C10:0	6.68	9.02	0.29	0.103
C12:0	3.68	5.08	0.28	0.083
C14:0	10.94	6.87	0.69	0.036
C15:0	1.70	1.71	0.10	0.603
C16:0	25.61	25.24	0.71	0.320
C17:0	2.34	3.09	0.16	0.041
C18:0	12.48	7.43	0.56	0.027
C14:1	0.113	0.144	0.02	0.383
C16:1	0.647	0.907	0.03	0.001
C18:1	0.506	0.692	0.05	0.001
C18:2	2.01	2.49	0.08	0.046
C20:1	0.111	0.146	0.004	0.356
C20:4	0.167	0.211	0.005	0.085
C20:5	0.018	0.023	0.001	0.436

¹CON: control group; ²RJ: Royal jelly-supplemented group; ³SEM: standard error mean.

The effects of RJ on the contents of milk FAs (g/100g fat) in dairy ewes are described in Table 3. Lactating ewes in the control group produced significantly higher contents of milk C14:0 ($P<0.036$) and C18:0 ($P<0.027$) saturated FAs than that in the RJ group. However, the contents of milk C6:0 and C17:0 in the RJ group were significantly greater than that recorded in the control group ($P<0.050$ and 0.041 , respectively).

Additionally, no significant variations were recorded in the contents of milk FAs, C4:0, C8:0, C10:0, C12:0, C15:0 and C16:0 in both of RJ-supplemented and control groups. Lactating ewes supplemented with RJ produced significantly higher contents of milk C16:1 ($P<0.001$), C18:1 ($P<0.001$) and C18:2 ($P<0.046$) unsaturated FAs than the control group. Contrarily, no considerable differences were reported in the contents of milk FAs, C14:1, C20:1, C20:4 and C20:5.

4. Discussion

Commercially, the dietary supplements of RJ were ready for use in a form of soft gelatin cap containing 1000 mg of natural product. Although the current study reported non-significant increases in milk yield in the RJ group, the percentage of milk fat was significantly improved. Similarly, the propolis increased milk yield and fat content ($P<0.05$) of the supplemented ewes compared with control [15]. Furthermore, both experimental groups in the present trial be given identical energy density in the diet (6.36 MJ/kg), with a possible mechanism that stabilize the energy metabolism in lactating RJ-supplemented animals.

Keeping in mind, that milk fat contents are synthesized in the epithelial tissue of the udder alveoli, the positive effect of RJ may attribute to its chemical component and especially to its major proteins, which play an essential role in the cell membrane benignity [16]. The rate of lipid peroxidation was elevated when the level of milk

polyunsaturated FAs is increased [17]. Hence, the preferable milk fat constituent in the RJ group support the viable protective mechanism of phenolic compounds of RJ on plasma lipids, maybe by catch the free radicals or binding with metal ions [18]. On contrast, the propolis supplement did not affect the milk fat contents in dairy cows [1].

The changes in milk FAs contents can be attributed to the altered fermentation process in the rumen, and consequently, to the lipid metabolism. However, common biological benefits of RJ are due to a natural mixture of its components (flavonoids and organic compounds). Regarding the fatty acid profile, Milk from RJ-supplemented ewes had significantly lower contents of saturated Tetradecanoic (C:14) and Octadecanoic (C:18) FAs. The variability of saturated FAs contents in the control and RJ-supplemented ewes suggest that de novo fatty acid synthesis was influenced by supplementation of RJ.

Further, other feed additives as flaxseed oil caused reduction in milk saturated FA contents [19]. What's more, lactating ewes supplemented with RJ produce significantly higher contents of milk Palmitoleic (C16:1), Oleic (C18:1) and Linoleic (C18:2) unsaturated FAs than that noticed in the control group. The increase in the components of milk Oleic and Linoleic unsaturated FAs in the RJ-supplemented ewes may be due to the inhibitory action on the initial step of the ruminal biohydrogenation process. The propolis supplement suppress the growth of some species of rumen bacteria, such as *Butyrivibrio fibrisolvens* and *Ruminococcus albus* [20].

The phenolic compounds from the beehive products may be acting on these bacterial species in the biohydrogenation process, thus altering the components of the Linoleic isomers in milk fat. It is believed that unsaturated FAs are unsteady and easily affected by a loss of electrons and further oxidative processes [19]. Taking into consideration, the physiological functions of RJ and its remarkable antioxidant effects, this may elucidate its beneficial and protective effects on milk unsaturated FAs contents.

5. Conclusion

The RJ supplement improved the nutritional merits of milk fat in lactating Ossimi ewes by increasing the contents of milk Palmitoleic (C16:1), Oleic (C18:1) and Linoleic (C18:2) unsaturated FAs, and the contents of milk Hexanoic (C6:0) and Heptadecanoic (C17:0) saturated FAs. In a recommendation to use RJ in the diets of the animals producing milk to increase the nutritional value of milk and its quality for consumer. In Taking into consideration that the present study is a preparatory trial, the action of RJ in lactating animals still need to extra investigation to determine the effective treatment full length, perfect dose, and mode of action.

Ethical Standards

The experimental procedures of the present research were

approved by the Nuclear Research Center, Atomic Energy Authority, Egypt.

Conflict of interest

The authors declare that they have no competing interests.

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