Uluslararası Tarım Araştırmalarında Yenilikçi Yaklaşımlar Dergisi
International Journal of Innovative Approaches in Agricultural Research 2022, Vol. 6 (4), 417-425
https://doi.org/10.29329/ijiaar.2022.506.12

Original article

Effect of the Dietary Royal Jelly Supplementation in Ewes of Bulgarian Dairy Synthetic Population on the Body Weight of the Lambs and the Milk Composition

Tanya Ivanova [a], Teodora Popova [a, *] & Ralitsa Balkanska [a]

[a] Agricultural Academy, Institute of Animal Science- Kostinbrod, Kostinbrod, Bulgaria

Abstract

The aim of the experiment was to assess the effect of the royal jelly supplementation in the diet of ewes from Bulgarian Dairy Synthetic Population on the body weight of the lambs and the milk composition. The trial was carried out with 20 ewes and their lambs, reared in the experimental sheep farm of the Institute of Animal Science- Kostinbrod, Bulgaria and lasted 30 days during the suckling period. The animals had the same date of lambing and lambs' body weight, and were divided into two groups, each containing 10 ewes. The feeding was according to the standards for this category of ruminants with ad libitum access to water, as each ewe from the supplemented group received twice a week 300 mg freeze dried royal jelly. The body weight of the lambs was controlled at the beginning and at the end of the trial period, and the weight gain was calculated. The chemical and the fatty acid composition of the milk was measured in individual samples. The fatty acid composition was used to calculate the atherogenic (AI) and thrombogenic (TI) indices. The live weight (LW) and the average daily gain (ADG) differed between the groups of lambs according to the dietary royal jelly supplementation. The lambs of the ewes that had received the supplement displayed higher LW (P=0.0138) and ADG (P=0.0062). The physicochemical composition of the milk showed lower fat content (P=0.0142) and higher density (P=0.0200) in the ewes receiving royal jelly. Significant increase of C18:0 and decrease of C17:1 proportion was observed. Additionally, the contents of the polyunsaturated C20:4n-6, C22:5n-3 and C22:6n-3 was considerably diminished in the milk of the group that received royal jelly. The results so far demonstrated the potential of the royal jelly to manipulate the fatty acid profile of the ewes' milk.

Keywords: Royal Jelly, Dairy Sheep, Sucking Period, Body Weight, Milk Composition.

Received: 23 August 2022 * **Accepted:** 23 September 2022 * **DOI:** https://doi.org/10.29329/ijiaar.2022.506.12

* **Corresponding author:**

Popova Teodora is a full professor in the Department of Fundamental research in animals in the Institute of Animal Science-Kostinbrod. Her research interests include quality of meat and meat products, food quality, fatty acid composition of foods.
Email: tlpopova@yahoo.com

INTRODUCTION

Animal nutrition is of great importance for the products derived, moreover the consumers' demands towards high quality and healthy animal products has been constantly increasing. The interest in the supplements for the diet of animals in recent years is towards natural products (Aguiar et al., 2014). In this regard, some plant extracts as well as the bee products have been widely used. The honey, propolis, bee pollen and royal jelly are rich in protein, carbohydrates, lipids, vitamins and minerals. In the last decade the interest towards their inclusion in the diet of farm animals (Seven et al., 2012; Aguiar et al., 2014; Madras-Majewska et al., 2015), however, the information about their effects on the animals remains scarce.

In a study with sheep, Husein et al. (1999) reported favourable effect of royal jelly on the reproductive performance. The mode of action of royal jelly on the reproductive system of the animals is still not completely clear. It is suggested that some compounds in it may change the hormonal secretion in the supplemented animals (Kridli and Al-Khetib, 2006). Royal jelly together with the Gonadotropin-releasing hormone are used for estrus synchronization in sheep (Kridli et al., 2003). The authors compared the royal jelly and equine chorion gonadotropin (eCG) for the control of the estrus in sheep. Further, Moradi et al. (2013) proved that royal jelly improved the sperm parameters in rams during storage and is a good source of antioxidants.

Royal jelly has antioxidant, hepatoprotective, antibacterial, antitumor, antibiotic, anti-inflammatory and immunomodulatory effects (Pavel et al., 2011). The studies for its application the sheep feeding are scarce. In their recent study on dietary supplementation of lactating ewes with fresh royal jelly, El-Tarabany et al. (2019) found effect on the lipids, proteins and dry matter in the milk. In the next experiment, El-Tarabany et al. (2020) investigated the effect of soft gelatin capsules of royal jelly added in the diet of sheep during the early stage of lactation on the fatty acid composition of the milk.

Some of the studies on the royal jelly as a nutrient on the health status of people and experimental animals have been widely reviewed by Strant et al. (2019). However, the works on the effect of the royal jelly in the diet of various farm animals and the products derived are rather insufficient. Hence, the aim of our study was to assess the effect of the dietary royal jelly supplementation in ewes from Bulgarian Dairy Synthetic Population on the weight of lambs and the composition of milk.

MATERIALS and METHODS

Animals and Diets

The study was carried out with Bulgarian Dairy Synthetic Population ewes (n=20) and their lambs, reared in the experimental sheep farm of the Institute of Animal Science - Kostinbrod. The trial was 30 days during the sucking period. The animals had the same date of lambing and similar body weights of the lambs and were divided in two groups – control (C) and supplemented (S) each containing 10 animals. The ewes were fed according to the standards for this category of animals, with *ad libitum* access to water and salt. The diet and the concentrate composition are presented in Table 1 and 2. Additionally the chemical composition of the diet is presented in Table 3. The animals from S group received individually (by hand) 300 mg of freeze-dried royal jelly twice a week. The body weight of the lambs was recorded at the beginning and at the end of the trial in the morning before feeding, after 12 h of fasting. The average daily gain was calculated.

Table 1. Daily diet of the ewes

Ingredients	kg
Meadow hay	1.320
Corn silage	1.870
Concentrate	0.740

Table 2. Concentrate composition

Component	%
Corn	32.1
Barley	21.2
Sunflower meal	22.0
Wheat bran	22.0
Salt	0.7
Dicalcium Phosphate	0.7
Calcium carbonate	1.2
Premix	0.1

Milk physicochemical composition

The milk physicochemical composition was measured in milk samples obtained from each ewe (20 ml) at the end of the trail period. The analyses of fat, protein, solids non fat and density were performed in duplicates on automatic milk analyser Ekomilk (of Bulteh 2000, Stara Zagora, Bulgaria).

Table 3. Chemical composition of feeds

Items	Forages		
	Meadow hay	Corn silage	Concentrate
Dry matter (DM), %	112.2	56.1	84.39
% of the DM			
Crude protein	9.8	2.2	17.19
Crude fibers	27.2	6.8	8.37
Ether extract	1.9	0.7	2.20
Nitrogen free extracts	41.6	14.8	52.2
Ash	6.4	2.1	3.5

Fatty acid profile

To analyse the fatty acid composition, milk lipids were extracted according to the method of Hara and Radin (1978). The samples were then methylated (Domínguez et al., 2022) and analysed through gas-liquid chromatography using C Si 200 chromatograph, equipped with capillary column (DM-2330:30 m×0.25 mm×0.20 µm) and hydrogen as a carrier gas. The temperature program was as follows: the initial temperature of the oven was set to 160°C for 0.2 min, the temperature was then increased to 220°C at a rate of 5°C/min and was held for 5 min. The temperature of the injector and detector was set to 230°C. The fatty acids were expressed as percentage of the total methyl esters identified (Christie 1973). The content of each fatty acid was used to calculate the atherogenic (AI) and thrombogenic (TI) indices according to the equations proposed by Ulbricht and Southgate (1991): $AI=(C12{:}0+4\times C14{:}0+C16{:}0)/[MUFA+\Sigma(n\text{-}6)+\Sigma(n\text{-}3)]$ (1).

$TI=(C14{:}0+C16{:}0+C18{:}0)/[0.5\times MUFA+0.5\times(n\text{-}6)+3\times(n\text{-}3)+(n\text{-}3)/(n\text{-}6)]$ (2).

Statistical evaluation

The results were evaluated using the statistical package Data Analysis, Excel, 2021 of Microsoft. The significance of the difference between the groups regarding the studied parameters was evaluated through t-test.

RESULTS and DISCUSSION

The weight development of the lambs during the trial is presented in Table 4. It was assured that the lambs from both groups had similar live weight at the beginning of the experiment. At the end of the trial, the lambs of the supplemented ewes had higher final live weight when compared to the lambs from the control group of ewes (15.98 kg *vs.* 11.81 kg, P=0.0138).

Table 4. Live weight and average daily gain in lambs (Mean ± SEM)

Item	Group C	Group S	P
LW (initial), kg	8.53 ±0.15	8.29±0.15	0.2980
LW (final), kg	11.81±1.11	15.98±0.84	0.0138
ADG, g/d	109.11±0.08	256.27±0.06	0.0062

The average daily gain was also higher (P=0.0062) in the lambs whose mothers received the royal jelly supplement. Still in the available literature, the nutritional effects of the royal jelly have been reported mainly in regard to the reproductive function and health status in animals. The results, concerning the performance traits are scarce, contradictory and depend on the animal species. El - Tarabany et al. (2019) when studying the effect of royal jelly in lactating ewes failed to observe effect of the dietary treatment on the weight changes and feed intake in the animals. Experiments with poultry, however, showed favourable effect of bee products on the performance. In Japanese quails, Seven et al.

(2016) showed that dietary supplementation of royal jelly increased significantly the final body weight in the female birds, in males, however the increase was lower and not significant. Babaei et al. (2016) reported that the dietary supplementation with royal jelly was associated with increased weight gain in Japanese quails. It is known that the royal jelly is rich in bioactive compounds. It could be suggested that the higher weight gain and body weight in the lambs suckling from the supplemented ewes is due to the flavonoids coming from the royal jelly. Dietary flavonoids have been reported to positively affect these traits in animals and poultry (Muqier et al., 2017; Park et al., 2020; Prihambodo et al., 2021).

Table 5 shows the data concerning the effect of the royal jelly supplementation on the physocochemical composition of milk.

Table 5. Physicochemical composition of the milk according to the royal jelly supplementation (Mean ± SEM)

Items	Group C	Group S	Sig.
Fat, %	3.91±0.308	2.59±0.28	0.0142
Protein, %	6.05±0.64	5.89±0.43	0.8419
Solid non fat, %	11.52±0.77	11.60±0.43	0.9266
Density, °G 20/4 °C	37.46±0.23	39.76±0.75	0.0200

The results show that the ewes consuming royal jelly produced milk with lower fat content (2.59%) when compared to the control group (3.91%). The protein and solid non fat content remained unaffected, nevertheless their values are within the range that is previously observed for the breed (Ivanova et al., 2011). Our results contradict to those of El-Tarabany et al. (2019), who observed considerably higher fat and protein content of the milk in ewes supplemented with royal jelly. The density of the milk was higher in the supplemented group.

A total of 22 fatty acids have been identified in the milk samples. (Table 6).

Table 6. Fatty acid composition (Mean ± SEM)

Fatty acid, %	Group C	Group S	Sig.
C8:0	1.76±0.16	1.96±0.12	0.3491
C10:0	6.26±0.79	6.64±0.28	0.6568
C11:0	0.76±0.04	0.89±0.04	0.0808
C12:0	4.30±0.54	3.97±0.14	0.5720
C14:0	11.26±0.84	10.56±0.26	0.4528
C14:1	0.60±0.02	0.63±0.04	0.6429
C15:0	0.99±0.03	1.06±0.07	0.3559
C16:0	26.93±0.91	25.52±0.36	0.1769
C16:1n-7	1.71±0.15	1.49±0.10	0.2460
C17:0	0.68±0.04	0.69±0.03	0.8619
C17:1	0.62±0.02	0.52±0.03	0.0398
C18:0	9.86±0.83	13.21±0.64	0.0080
C18:1n-9	29.14±2.25	27.83±0.55	0.5828
C18:2n-6	2.63±0.18	2.56±0.10	0.7559
C18:3n-3	0.84±0.07	0.90±0.10	0.6360
CLA	0.72±0.06	0.87±0.09	0.2181
C20:2n-6	0.17±0.01	0.13±0.01	0.0700
C20:3n-6	0.04±0.005	0.05±0.002	0.9213
C20:4n-6	0.40±0.02	0.28±0.02	0.0104
C20:5n-3	0.08±0.01	0.06±0.004	0.2572
C22:5n-3	0.18±0.01	0.13±0.01	0.0494
C22:6n-3	0.07±0.01	0.05±0.06	0.0450

Differences between the control and the supplemented group were observed in regard to the percentage of some individual fatty acids. As a whole, their content is low and the differences could not affect the total amounts of the different classes of fatty acids. The milk derived from the group receiving royal jelly showed higher percentage of C18:0 (P=0.008), compared to the control group, and also there was a tendency towards higher content of C11:0 (P=0.0808). The percentage of C17:1 decreased in the milk of the supplemented ewes (0.62% vs. 0.52%, P=0.03). Similar decrease was observed in regard to the polyunsaturated C20:4n-6 (P=0.0104), C22:5n-3(P=0.0494) and C22:6n-3 (P=0.0450). The results in Table 7 displayed that SFA had the highest content (62.80%, 64.50%, respectively for the control and the treated group), followed by MUFA (32.07%, 30.47%) and PUFA (5.13%, 5.03%). There was no visible effect of the royal jelly supplementation on the total amounts of the different fatty acid classes.

Table 7. Total amounts of fatty acids (%) and nutritional indices of the milk (Mean ±SEM)

Item	Group C	Group S	Sig.
SFA[1]	62.80 ±2.51	64.50±0.84	0.5297
MUFA[2]	32.07±2.30	30.47±0.61	0.5116
PUFA[3]	5.13±0.31	5.03±0.27	0.8028
n-6	3.24±0.20	3.02±0.13	0.3809
n-3	1.17±0.11	1.14±0.12	0.8369
AI	2.09±0.24	2.07±0.05	0.6520
TI	2.23±0.20	2.40±0.12	0.6408
n-6/n-3	2.77±0.17	2.65±0.39	0.8806

[1]SFA- saturated fatty acids, [2]MUFA-monounsaturated fatty acids, [3]PUFA- polyunsaturated fatty acids

The studies on the effect of the royal jelly on the fatty acid composition of milk are rather scarce. According to El-Tarabany et al. (2020), royal jelly when administered in the diet of ewes can be successfully used for manipulation of the fatty acid composition of milk. As the authors showed, the dose of 1000 mg per animal 4 times a week for a period of 4 weeks led to significant augmentation of some mono- (C16:1n-7 and C18:1n-9) and polyunsaturated fatty acids (C18:2n-6 and C20:4n-6), as well as reduction of C14:0 and C18:0. This is not in agreement with our results. The lack of changes in the fatty acid profile in regard to MUFA showed negligible effect of the royal jelly on the microbial population in the rumen. This could be due to the lower amount of supplement in the diet of the ewes in this experiment. Seven et al. (2014), observed considerable influence of two doses of royal jelly on the fatty acid composition of meat and organs in quails. The effect was however dependent on the dose and differed among the tissues as in terms of PUFA it revealed positive correlation with feed intake. The atherogenic and thrombogenic indices (Table 7) for the milk samples were comparable with the results of other authors (Gerchev et al., 2015; Sinanoglu et al., 2015; Voblikova et al., 2020). The n-6/n-3 ratio in both groups of ewes is <4, showing good dietetic and healthy qualities of milk.

Conclusion

The results of the study showed that the dietary supplementation of freeze-dried royal jelly in ewes in amount 600 mg/week affected the live weight and average daily gain of the lambs, leading to higher values of these traits. In addition, the physicochemical composition of the milk and its fatty acid profile differed considerably between the two groups. The milk of the supplemented ewes had lower fat but higher density. Furthermore, in regard to the fatty acid profile, the differences pointed towards significant increase in the percentage of C18:0 while the contents of C17:1, C20:4n-6, C22:5n-3 and C22:6n-3 were lower in the milk of the ewes receiving royal jelly. The results demonstrated the potential of the royal jelly to modify the chemical and fatty acid composition of the ewes' milk, however, further experiments are necessary to determine the optimal doses and duration of administration of the supplement for maximum positive effect on the studied parameters.

REFERENCES

Aguiar, S.C., Cottica, S.M., Boeing, J.S., Samensari, R.B., Santos, G.T., Visentainer, J.V., Zeoula, L.M., 2014. Effect of feeding phenolic compounds from propolis extracts to dairy cows on milk production, milk fatty acid composition, and the antioxidant capacity of milk. Animal Feed Science and Technology, 193: 148 – 154, https://doi.org/10.1016/j.anifeedsci.2014.04.006.

Babaei, S., Rahimi, S., Karimi Torshizi, M.A., Tahmasebi, G., Khaleghi Miran, S.N., 2016. Effects of propolis, royal jelly, honey and bee pollen on growth performance and immune system of Japanese quails. Veterinary Research Forum, 7(1):13-20.

Christie, W.W. 1973. Lipid analysis. Pergamon Press. Oxford. 352 p.

Domínguez, R., Crecente, S., Borrajo, P., Agregan, R., Lorenzo, J.M., 2015. Effect of slaughter age on foal carcass traits and meat quality, Animal, 9: 1713-1720, https://doi.org/10.1017/S175173111500067.

El-Tarabany, M.S., El-Tarabany, A.A., Atta, M.A., Ahmed-Farid, O.A., Mostafa, M.M., 2019. Effect of royal jelly on milk composition and blood biochemical parameters in lactating ewes. The Journal of Agricultural Science, 157 (2):1–6, https://doi.org/10.1017/S0021859619000352.

El-Tarabany, A.A.S, Ahmed-Farid, O.A., Emara, S.S., El-Tarabany., M.S., 2020. Impact of royal jelly supplement on ewe's milk fat content and fatty acid profile during the early stage of lactation. Advances in Applied Physiology, 5(2): 37-41, https://doi.org/10.11648/J.AAP.20200502.15.

Gerchev, G., Naydenova, N., Slavkova, S., Mihaylova, G., 2015. Fatty acid composition of milk fat in milk of Tzigay sheep and their F2 cross-breeds with Chios. Biotechnology in Animal Husbandry, 31(1), 45-53, https://doi.org/10.2298/BAH1501045G.

Hara, A., Radin, N.S., 1978. Lipid extraction of tissues with a low-toxicity solvent. Analytical Biochemistry, 90: 420-426, https://doi.org/10.1016/0003-2697(78)90046-5.

Husein, M.Q., Kridli, R.T., Humphrey, W.D., 1999. Effect of royal jelly on estrus synchronization and pregnancy rate of ewes using flourogestone acetate sponges. Journal of Animal Science, 77, 431-438.

Ivanova, T., Raicheva, E., Pacinovski, N., 2011. Yield of cheese and milk composition in sheep from Synthetic Population Bulgarian Milk and East-Friesian breed. Biotechnology in Animal Husbandry, 27(3): 1105-1111, https://doi.org/10.2298/BAH1103105I.

Kridli, R.T., Husein, M.Q., Humphrey, W. D., 2003. Effect of royal jelly and GnRH on the estrus synchronization and pregnancy rate in ewes using intravaginal sponges. Small Ruminant Research, 49(1): 25–30, https://doi.org/10.1016/S0921-4488(03)00057-9.

Kridli, R.T., Al-Khetib, S.S., 2006. Reproductive responses in ewes treated with eCG or increasing doses of royal jelly. Animal Reproduction Science, 92(1–2): 75-85, https://doi.org/10.1016/j.anireprosci.2005.05.021.

Madras-Majewska, B., Ochnio, L., Ochnio, M., 2015. Use of bee products in livestock nutrition and therapy. Medycyna Weterynaryjna, 71 (2): 94-99.

Moradi, A. R., Malekinejad, H., Farrokhi-Ardabili, F., Bernousi, I., 2013. Royal Jelly improves the sperm parameters of ram semen during liquid storage and serves as an antioxidant source. Small Ruminant Research, 113(2–3): 346-352, https://doi.org/10.1016/j.smallrumres.2013.03.003.

Muqier, Qi, S.,Wang, T., Chen, R., Wang, C., Ao, C., 2017. Effects of flavonoids from Allium mongolicum Regel on growth performance and growth-related hormones in meat sheep. Animal Nutrition, 3 (1): 33-38, https://doi.org/10.1016/j.aninu.2017.01.003.

Park, J.-H., Shanmugam, S., Ho, K. I., 2020. Influences of dietary flavonoid (quercetin) supplementation on growth performance and immune response of growing pigs challenged with Escherichia coli lipopolysaccharide. Journal of Animal Science and Technology, 62(5):605-613, https://doi.org/10.5187/jast.2020.62.5.605.

Pavel, C. I., L. Al. Marghitas, O. Bobis, D. S. Dezmirean, A. Sapcaliu, I. Radoi, M. N. Madas. 2011. Biological activities of royal jelly – review. Scientific Papers: Animal Science and Biotechnologies, 44(2): 108 – 118.

Prihambodo, T.R., M.M. Sholikin, N. Qomariyah, A. Jayanegara, I. Batubara, D.B. Utomo, N. Nahrowi, 2021. Effects of dietary flavonoids on performance, blood constituents, carcass composition and small intestinal morphology of broilers: a meta-analysis. Animal Bioscience, 34(3):434-442, https://doi.org/10.5713/ajas.20.0379.

Seven, I., Aksu, T., Tatli Seven, P., 2012. The effects of propolis and vitamin C supplemented feed on performance, nutrient utilization and carcass characteristics in broilers exposed to lead. Livestock Science, 148(1 – 2): 10 – 15, https://doi.org/10.1016/j.livsci.2012.05.001.

Seven, I., Şimşek, Ü. G., Gökçe, Z., Seven, P. T., Arslan, A., Yilmaz, Ö., 2014. The effects of royal jelly on performance and fatty acid profiles of different tissues in quail (*Coturnix coturnix japonica*) reared under high stocking density. Turkish Journal of Veterinary and Animal Sciences, 38(3): 271 – 277, https://doi.org/10.3906/vet-1303-62.

Seven, P.T., A. Sur Arslan, M. Özçelik, Ü. Gülcihan Şimşek, İ. Seven, 2016. Effects of propolis and royal jelly dietary supplementation on performance, egg characteristics, lipid peroxidation, antioxidant enzyme activity and mineral levels in Japanese quail. European Poultry Science, 80, https://doi.org/10.1399/eps.2016.138.

Sinanoglou, V. J., P. Koutsouli, C. Fotakis, G. Sotiropoulou, D. Cavouras, Iosif Bizelis. 2015. Assessment of lactation stage and breed effect on sheep milk fatty acid profile and lipid quality indices. Dairy Science & Technology, 95(4): 509-531, https://doi.org/10.1007/s13594-015-0234-5.

Strant, M., Yücel, B., Topal E., Puscasu A.M., Margaoan, R., Varadi, A., 2019. Use of royal jelly as functional food in human and animal health. J. Anim. Prod., 60(2), 131-144, https://doi.org/10.29185/hayuretim.513449.

Ulbricht, T.L. Southgate, D.A.T., 1991. Coronary heart disease: seven dietary factors. Lancet, 338: 985–992, https://doi.org/10.1016/0140-6736(91)91846-m.

Voblikova, T., Permyakov, A., Rostova, A., Masyutina, G., Eliseeva, A. 2020. Study of fatty-acid composition of goat and sheep milk and its transformation in the production of yogurt. International Applied Research Conference “Biological Resources Development and Environmental Management”, KnE Life Sciences, 742-751, https://doi.org/10.18502/kls.v5i1.6161.