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The effects of royal jelly on performance and fatty acid profiles of different tissues in quail (*Coturnix coturnix japonica*) reared under high stocking density

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Abstract: To study the effects of royal jelly (RJ) on performance and fatty acid profiles, 168 unsexed 8-day-old quails (*Coturnix coturnix japonica*) were assigned to 4 treatment groups. Groups were arranged as follows: control (LSD; 160 cm^2/quail and no supplementation), high stocking density (HSD; 80 cm^2/quail and no supplementation), HSD-RJ 250 [80 cm^2/quail and 250 mg/kg body weight (BW) RJ given orally], and HSD-RJ 500 (80 cm^2/quail and 500 mg/kg BW RJ given orally). Body weight gain decreased significantly in the HSD group at day 42 ($P < 0.01$). Feed intake (FI) and feed conversion rate (FCR) were also significantly affected in the HSD group ($P < 0.01$). FI and FCR were improved by both doses of RJ. Both RJ doses increased total polyunsaturated fatty acid ratios in the breast, kidney ($P < 0.001$), leg, and liver ($P < 0.05$). Total saturated fatty acid ratios in leg ($P < 0.01$) and kidney ($P < 0.05$) tissues increased in the HSD group but decreased in the livers of HSD-RJ 250 group ($P < 0.05$). In conclusion, RJ supplementation improved performance parameters and unsaturated fatty acid ratios in the examined quail tissues.

Key words: Quail, stocking density, performance, fatty acids, royal jelly

1. Introduction

In recent years, there has been renewed interest in the investigation of bee products [honey, pollen, royal jelly (RJ), bee venom, and propolis] for their numerous functional, biological, and pharmaceutical beneficial effects (1–3).

RJ is a honey bee (*Apis mellifera* L.) secretion component for bee egg, larvae, and adult queen nourishment. This secretion is extremely rich in nutrients and has a yellowish-white, creamy liquid appearance and an acidic pH. It is secreted by the hypopharyngeal glands of nurse bees. The overall composition of RJ is 60%–70% water, 12%–16% crude protein, 10%–16% total sugar, and 3%–6% lipids, vitamins, and mineral salts. The composition varies according to the source of the exudates, climate, and some environmental conditions. RJ contains many bioactive ingredients. The main bioactive material is 10-hydroxy-2-decenoic (HDA, RJ acid), an unsaturated acid that is only found in RJ in nature. Numerous HDA effects, including antibacterial, antifungal, antiviral (4,5), and immunoactivating (6) effects, were reported. RJ contains major proteins with high levels of essential amino acids and peptides (7,8), possessing immunomodulating (9) and antioxidant properties (8). Phenols and polyphenols in major protein structures are responsible for significant antioxidant activity (4). RJ is rich in vitamins, especially water-soluble vitamins (vitamins B and C), and minerals such as potassium, calcium, magnesium, iron, zinc, sulfur, and copper (10).

The continuously increasing demand for protein for human consumption has prompted some producers and researchers to search for new approaches in animal production and husbandry practices in order to improve production efficiency. Quail is a good alternative animal protein due to its low housing, space, and feed requirements as well as diminished waste and higher productivity. Cage density is an important environmental factor affecting production levels and quality in quail meat (11). The goal of quail producers is to achieve a balance between production efficiency and bird welfare. Increasing the bird number per unit of space (density) reduces housing, equipment, and labor costs. It is, however, well documented that chickens housed at high density grow more slowly and have higher mortality and lower production quality (12,13). On the

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other hand, RJ has an antioxidant effect that counteracts the lipid peroxidation caused by free radicals under different stress conditions (4).

The purpose of this study was to examine the potential protective effects of RJ on performance and oxidative damage in different tissues caused by the stress of high stocking density. For this purpose, fatty acid profiles were analyzed in different muscle tissues and internal organs.

2. Materials and methods

2.1. Birds and experimental design

A total of 168 unsexed 8-day-old Japanese quails (*Coturnix coturnix japonica*) obtained from a commercial company were used for this study after obtaining local research ethics committee approval. Birds were kept in wire cages (40 × 32 cm^2) in a temperature-controlled room at 24 °C. Food and fresh water were provided ad libitum. The photoperiod (L/D) was 23 h/1 h. Birds were weighed and assigned to experimental groups balanced according to body weight (BW) and sex, with 3 replicates for each group. Quails in the control low stocking density (LSD; 160 cm^2/quail; 8 birds/pen) and higher stocking density (HSD; 80 cm^2/quail; 16 birds/pen) groups were reared for 8–42 days. The experimental groups were arranged as follows: no supplementation under optimum stocking density (control, LSD), no supplementation to basal diet under HSD, oral RJ (250 mg/kg BW) supplementation under HSD (HSD-RJ 250), and oral RJ (500 mg/kg BW) supplementation under HSD (HSD-RJ 500). RJ was obtained from a commercial firm in Turkey, dissolved in distilled water, and kept frozen at –20 °C until used. Ingredients and chemical composition of the basal diet are shown in Table 1.

Table 1. Ingredients and chemical composition of the basal diet.

Ingredients (g/kg)	Starter (day –21)	Grower (days 22–42)
Maize	539.8	621.3
Soybean meal (48%)	266.3	-
Full-fat soybean	124.8	276.5
Poultry meal	25	60
Soybean oil	9.4	14.3
Limestone	11.9	2.9
Bone meal	-	16.5
Dicalcium phosphate	11.9	-
Salt	3	2.1
DL-methionine	2.8	1.3
Vitamin-mineral premix*	3.5	3.5
Lysine	1.6	1.6
Chemical analysis		
ME, kcal/kg**	3300	3030
Dry matter, g/kg***	894.0	905.0
Crude protein, g/kg***	236.2	192.5
Ether extract, g/kg***	61	89
Ash, g/kg***	51	43
Crude fiber, g/kg***	56	50
Calcium, g/kg**	10	10.2
Available phosphorous, g/kg**	0.45	0.47
Total phosphorous, g/kg**	5.5	5.6

*: Vitamin and mineral premix provided per kilogram of diet: vitamin A, 15,000 IU; cholecalciferol, 5000 IU; vitamin E, 100 mg; vitamin K_3, 4 mg; vitamin B_1, 3 mg; vitamin B_2, 8 mg; vitamin B_3, 60 mg; vitamin B_6, 5 mg; Ca-D-pantothenate, 18 mg; folic acid, 2 mg; D-biotin, 0.20 mg; Mn, 100 mg; Zn, 80 mg; Fe, 80 mg; Cu, 8 mg; Co, 8 mg; Se, 0.3 mg; iodine, 1 mg; Mo, 1 mg; choline chloride, 500 mg.
**: Calculated from the tabulated value (National Research Council, 1994).
***: Analyzed according to AOAC (1995).

The diets used were formulated to be isonitrogenic and isoenergetic according to National Research Council (14) recommendations. Diets were formulated as starter (until day 21) and grower (between 22 and 42 days) diets. Chemical compositions of feed ingredients (dry matter, crude protein, ether extract, and ash) as dried samples were analyzed using AOAC (15) procedures, and crude fiber was determined by the Crampton and Maynard methods (16).

The quails were weighed every week and feed intake (FI) was measured weekly during the study. Weight gain and feed conversion ratio were calculated. Dead birds were recorded every day. At the end of the study (day 42), 10 quails from each group with body weights near the group average were slaughtered. *M. pectoralis profundus* of the breast, *M. gastrocnemius* of the leg, the whole liver, and the right kidney were collected. All samples were stored at –20 °C until analyzed. Subsequently they were thawed at 4 °C and homogenized for analysis.

Chemical composition of RJ was assessed by gas chromatography–mass spectrometry analyses (GC–MS) (Table 2). GC–MS analyses were carried out to detect the main components of RJ with an Agilent GC 6890 gas chromatograph coupled to an Agilent MSD 5973 mass detector in electron impact ionization mode. The gas chromatography column was a Zebron (ZB-1) methyl polysiloxane (30 m × 0.25 mm i.d. × 0.25 µm film thickness). Extraction of lipids from feed and tissue specimens was carried out with the Hara and Radin (17) method, in which a 3:2 (v/v) hexane/isopropanol mixture was used. For preparation of methyl esters, lipid extract in hexane/isopropanol phase was placed in 30-mL experiment tubes. Five milliliters of 2% methanolic sulfuric acid was added and the mixture was vortexed.

Table 2. Chemical composition of royal jelly assessed by GC–MS.

RT (min)	Contents	TIC (%)
Flavonoids		
36.337	Chrysin	0.847
33.502	Pinocembrin	1.842
35.114	Tectochrysin	0.382
32.176	Pinostrobin chalcone	0.735
Alcohol		
2.222	Furfuryl alcohol	0.279
Organic compounds		
8.871	Hydroxymethylfurfurole	0.656
Fatty acids		
16.924	3-Hydroxydecanoic acid	1.494
19.463	10-Hydroxydecanoic acid	19.815
32.398	Oleic acid amide	0.692
14.924	Octanoic acid, 8-hydroxy-(CAS)	3.226
Other		
2.896	2-Penten-4-olide	4.110
5.751	4,5-Diamino-2-hydroxypyrimidine	0.648
7.150	3,5-Dihydroxy-6-mehyl-2,3-dihydro-4H-pyran-4-one	1.409
3.891	Glutaconicanhydride	1.024
37.213	9-(4-Aminophenyl)acridine	0.489
42.011	Benzeneethanamine, N-[(4-nitrophenyl)methylene]	0.375
44.099	Ostreasterol	1.924
41.555	3-Hydroxydiphenylamine	0.336
22.868	3-(4-Nitrophenyl)propiolic acid trans-1,1-dichloro-2,3-diethylcyclopane	1.336

RT: Retention time. TIC: The ion current generated depends on the characteristics of the compound concerned and is not a true quantitation.

This mixture was left to methylate at 50 °C, incubated for 15 h, and cooled at room temperature, and then 5 mL of 5% sodium chloride was added and mixed. The produced fatty acid methyl esters were then extracted with 5 mL of hexane. The hexane phase was removed using a pipette and was treated with 5 mL of 2% $KHCO_3$. The solvent of the methyl ester-containing mixture was evaporated at 45 °C under nitrogen flow and was dissolved in 1 mL of hexane. Then it was placed in 2-mL closed autosampler vials and analyzed (18) using a Shimadzu GC 17 gas chromatographer. The main components of diets were determined by considering their areas as a percentage of the total ion current. Polyunsaturated fatty acid (PUFA), saturated fatty acid (SFA), and monounsaturated fatty acid (MUFA) ratios of the diets are shown in Table 3.

2.2. Statistical analysis

After the Shapiro–Wilk normality test, the data were subjected to analysis of variance, and significant differences were further subjected to Duncan's multiple range test (19). The results were considered significant at $P < 0.05$.

3. Results

As shown in Table 4, BW was similar among groups in the beginning of the study. This parameter was significantly lower in the HSD group at day 42 than in other groups ($P < 0.01$). RJ increased BW in experimental groups to levels comparable to the control group. FI and FCR were significantly deteriorated by HSD ($P < 0.01$). Groups supplemented with RJ demonstrated an increase in FI ($P < 0.01$). FCR improved in the HSD-RJ 250 and HSD-RJ 500 groups significantly ($P < 0.01$). There were no significant differences among the control, HSD-RJ 250, and HSD-RJ 500 groups in BW and FI values. Mortality rates were not substantially different among the groups ($P > 0.05$).

As shown in Table 5, the total PUFA ratio of breast muscle, especially n-6 PUFA, significantly decreased in the HSD group ($P < 0.001$). The total MUFA ratio in HSD was significantly higher ($P < 0.01$). RJ supplementation, also under HSD conditions, increased the PUFA ratio of the tissue, resulting in levels similar to the control. Total SFA and n-3 PUFA ratios were not significantly affected ($P > 0.05$) among any groups.

The total PUFA ratios of leg muscle were significantly higher in the control, HSD-RJ 250, and HSD-RJ 500 groups than in the HSD group ($P < 0.05$). The total SFA ratio of leg muscle was similar among the control, HSD, and HSD-RJ 500 groups. The total SFA ratios of leg muscle ($P < 0.01$) and liver ($P < 0.05$) tissues were lower in the HSD-RJ 250 group than in the control ($P < 0.01$). There were no statistically significant differences among the groups in total MUFA, n-3, and n-6 PUFA of the leg muscle.

In the liver and kidney tissues, the total PUFA ratio was significantly lower in the HSD group ($P < 0.05$), but similar between the other groups. The total MUFA ratios of liver tissue were higher in the control, HSD, and HSD-RJ 250 groups than in the HSD-RJ 500 group ($P < 0.01$). The difference in total MUFA ratios among the groups in kidney tissue, n-6 PUFA ratios of liver tissue, and n-3 PUFA ratios of liver and kidney tissues were not significant ($P > 0.05$). The total SFA ratio of liver tissue was the lowest in the HSD-RJ 250 groups ($P < 0.05$). The total SFA ratio of kidney tissue was the highest in the HSD group ($P < 0.05$) and similar among the other groups. The n-6 PUFA ratio was lowest in the HSD group in kidney tissue, whereas RJ supplementation under HSD conditions significantly increased the n-6 PUFA ratio in kidney tissue ($P < 0.001$).

Table 3. Fatty acid ratios of the experimental diets (starter and grower) (% of total).

Fatty acids	Starter	Grower
SFA	22.58	21.94
MUFA	30.54	21.93
PUFA	46.36	55.81
Total	99.48	99.68

4. Discussion

The effects of poultry rearing under HSD conditions have been studied for years. Similar to the performance results in the present study, HSD was shown to cause decreased FI, FCR, and BW gain, resulting in deteriorated poultry growth performance and welfare (11,12). When the number of birds per unit of space increases, microclimate conditions around the birds deteriorate. They move less and exhibit decreased walking ability, resulting in locomotion problems and difficulty accessing feeders and drinkers. The birds have to spend more time standing than resting, which results in social anarchy for resting birds (20). All of these problems cause physical and physiological stress to the birds (21). The use of different management practices, equipment, and several dietary alternatives has been recommended to alleviate such environmental stress (2,3). RJ has many flavonoids, organic compounds, fatty acids, and other active ingredients, as shown in Table 2. These chemical components can improve growth performance of quails under HSD stress, resulting in increased BW and FI and better FCR. The impact of these active ingredients, and especially flavonoids, on bird performance under stress conditions was reported previously (3,22).

Under stress conditions, adrenal glucocorticoids are released from the cortical region. These hormones initially mobilize lipids from adipose tissue to increase the required energy. Unsaturated fatty acids are mobilized first (21). Continued corticosterone administration under

Table 4. Effects of royal jelly on performance parameters of experimental groups.

	Days	Control (LSD)	High stocking density (HSD)	HSD-RJ 250	HSD-RJ 500	(P)
Body weight (g)	8	16.97 ± 0.61	17.05 ± 0.32	17.01 ± 0.38	16.97 ± 0.37	NS
	42	152.96 ± 4.36^{a}	136.14 ± 1.99^{b}	147.98 ± 2.70^{a}	146.88 ± 2.18^{a}	**
Body weight gain (g/days)	8–42	3.99 ± 0.13^{a}	3.50 ± 0.05^{b}	3.85 ± 0.07^{a}	3.82 ± 0.07^{a}	**
Feed intake (g/days)	8–42	15.01 ± 0.34^{a}	14.19 ± 0.04^{b}	14.81 ± 0.05^{a}	14.90 ± 0.02^{a}	* *
Feed conversion ratio (g feed intake/g body weight gain)	8–42	3.78 ± 0.12^{b}	4.10 ± 0.06^{a}	3.92 ± 0.09^{a}	3.96 ± 0.07^{a}	*
Mortality rates (%)	8–42	-	2.08	2.08	-	NS

NS: Nonsignificant, *: P < 0.05, **: P < 0.01.
$^{a, b}$: Mean values with different superscripts within a row differ significantly.

Table 5. Effects of royal jelly on fatty acid profiles of muscle and internal organ tissues under high stocking density conditions in Japanese quail (% of total).

	Control (LSD)	High stocking density (HSD)	HSD-RJ 250	HSD-RJ 500	P
Breast muscle (*M. pectoralis profundus*)					
PUFA	45.98 ± 0.66^{ab}	42.34 ± 0.80^{c}	49.68 ± 1.13^{a}	45.89 ± 1.69^{b}	***
MUFA	21.56 ± 1.55^{b}	25.90 ± 1.12^{a}	20.25 ± 1.72^{b}	23.77 ± 2.91^{ab}	**
SFA	31.48 ± 0.67	31.25 ± 0.49	30.02 ± 0.76	29.60 ± 1.37	NS
N3-PUFA	8.30 ± 0.78	8.74 ± 0.52	9.55 ± 0.56	7.05 ± 0.92	NS
N6-PUFA	38.11 ± 0.84^{b}	34.25 ± 0.62^{c}	40.13 ± 0.63^{a}	38.84 ± 1.17^{ab}	***
Leg muscle (*M. gastrocnemius*)					
PUFA	46.14 ± 1.68^{a}	43.56 ± 1.05^{b}	47.18 ± 2.53^{a}	46.43 ± 2.88^{a}	*
MUFA	25.20 ± 1.85	24.23 ± 1.28	26.34 ± 2.68	23.09 ± 3.55	NS
SFA	28.52 ± 1.38^{b}	31.24 ± 1.06^{a}	26.24 ± 1.58^{c}	29.76 ± 2.19^{ab}	**
N3-PUFA	6.65 ± 0.73	6.40 ± 0.56	7.55 ± 0.78	6.85 ± 0.62	NS
N6-PUFA	39.74 ± 2.48	37.62 ± 0.63	39.63 ± 2.26	39.39 ± 3.68	NS
Liver					
PUFA	40.50 ± 1.90^{ab}	35.08 ± 4.00^{b}	39.50 ± 3.88^{ab}	41.86 ± 0.85^{a}	*
MUFA	26.87 ± 2.10^{ab}	31.97 ± 3.67^{a}	34.78 ± 1.25^{a}	24.25 ± 2.61^{b}	**
SFA	30.98 ± 1.13^{a}	31.60 ± 1.26^{a}	26.30 ± 1.12^{b}	33.49 ± 0.59^{a}	*
N3-PUFA	4.73 ± 0.69	5.13 ± 0.77	4.97 ± 1.16	5.41 ± 0.21	NS
N6-PUFA	35.34 ± 2.20^{a}	30.12 ± 2.26^{b}	34.53 ± 2.73^{ab}	36.44 ± 0.72^{a}	**
Kidney					
PUFA	43.55 ± 0.79^{a}	35.10 ± 2.24^{b}	41.58 ± 0.98^{a}	42.59 ± 0.63^{a}	***
MUFA	19.05 ± 1.58	19.8 ± 1.94	19.55 ± 1.50	19.79 ± 1.29	NS
SFA	36.90 ± 1.44^{b}	44.15 ± 2.0^{a}	38.20 ± 1.53^{b}	36.78 ± 1.34^{b}	*
N3-PUFA	1.80 ± 0.33	1.44 ± 0.87	1.95 ± 0.54	1.70 ± 0.49	NS
N6-PUFA	41.38 ± 0.72^{a}	32.80 ± 1.82^{b}	39.63 ± 0.74^{a}	40.88 ± 0.92^{a}	***

NS: Nonsignificant, *: P < 0.05, **: P < 0.01, ***: P < 0.001.
$^{a–c:}$ Mean values with different superscripts within a row differ significantly.

chronic stress induces lipid peroxidation in the tissues (23). Morrissey et al. (24) reported that lipid peroxidation caused reduction of PUFA in the phospholipid fraction of the tissues during the adaptation period of the body to chronic stress (second stage of stress). Ongoing corticosterone secretion alters energy metabolism in line with fat synthesis, especially SFA synthesis, under stress conditions (25). Another factor affecting bird fattening is a decrease in physical activity under HSD conditions. Fattening results in increased SFA synthesis and accumulation in tissues (26). In accordance with these data, we found in the present study that the SFA ratio of leg muscle and kidney tissues was highest in the HSD group, which is probably related to chronic stress. In addition, PUFA (especially n-6 PUFA) ratios of all tissues were higher in RJ groups than in the HSD group. Moreover, the PUFA ratio of tissues in RJ groups had similar values to the LSD group, occasionally higher but not significantly. Bee products contain numerous phenolic compounds (4). Previous studies demonstrated that phenols and polyphenols had redox characteristics that acted as reducing agents, hydrogen donors, and singlet oxygen quenchers (27). They also possess metal chelator properties, reacting with free radicals and genotoxic substances or carcinogens (28). Guo et al. (8) reported that RJ proteins had strong antioxidative activity against the peroxidation of unsaturated fatty acids. They found 29 antioxidant peptides in RJ, and, among these, 12 small peptides had 2–4 amino acids that showed strong hydroxyl radical scavenging activity but neither metal-chelating activity nor superoxide-anion radical scavenging activity. Moreover, 3 dipeptides containing tyrosine residues at the C-terminal had strong hydroxyl-radical and hydrogen-peroxide scavenging activity. Zheng et al. (29) stressed that harvest time was important to the functional, biological, and pharmaceutical activities of RJ products. Freshly collected products showed stronger superoxide dismutase (an antioxidant enzyme) activity than stored products. Viuda-Martos et al. (4) mentioned that bee products had minor components such as carotenes, ascorbic acid, organic acids, and α-tocopherol showing antioxidant properties. Isidorov et al. (30) reported that the unique feature of RJ was a set of C8-, C10-, and C12-hydroxy fatty acids. Ten acid characteristics of this bee product were identified in different combinations, namely 7- and 8-hydroxyoctanoic, 3-hydroxydecanoic, 9-hydroxydecanoic, 9-hydroxy-2-decenoic, 10-hydroxydecanoic, 10-hydroxy-2-decenoic (10-HDA), 3,10-dihydroxydecanoic, 2-octene-1,8-dioic, and 2-decene-1,10-dioic acids. It also possesses some biological characteristics, especially 10-HDA acid. Likewise, results of fatty acid analysis such as the 10-HDA content of RJ used in this study are shown in Table 2. Most research emphasized the antimicrobial characteristics of the fatty acids (4,5); only a few mentioned their antioxidant properties (31). Nakajima et al. (32) noted the scavenging effects of caffeic acid from N-acetyl cysteine and vitamin C. All of these factors may have improved the fatty acid profiles of the muscles and inner organs by increasing PUFA ratios under HSD conditions. The effects of RJ were dose-dependent and varied between tissues. Increasing PUFA consumption could lead to an increase in the PUFA content of edible poultry tissues (33).

In conclusion, RJ is regarded as a valuable food supplement because of its functional, biological, and pharmaceutical properties. Our data suggest the potential protective activity of this bee product on performance and fatty acid composition of quail tissues under HSD stress. Further studies are needed to comprehensively assess biological activity, quality, and effects on allergy and toxicity.

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