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Effects of Propolis, Royal Jelly, Bee Pollen and Ronozyme Supplementation in Diets of Japanese Quails (*Coturnix Coturnix Japonica*) on Yolk Lipid Peroxidation

Research Article

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Abstract

This study discovers the possible effect propolis, royal jelly, bee pollen and Ronozyme supplementation in diets that can be beneficial for Japanese quails (*Coturnix coturnix japonica*). Total one hundred and sixty Japanese quails at 43 days of age were used and divided randomly into 4 replicate groups each containing 32 animals. The experimental groups as follows: control group was feed a basal diet, royal jelly group was added to the water with 500 mg/kg diet, propolis group was feed orally on a basal diet supplemented with 4 g/kg diet, bee pollen group was feed orally on a basal diet supplemented with 1 g/kg diet, Ronozyme group was feed orally a basal diet supplemented with 1 g/kg diet for 74 days. Malondialdehyde (MDA) levels of the yolk were found highest in the control, royal jelly and Ronozyme groups as compared with bee pollen and propolis groups ($p<0.05$). MDA levels was significantly improved in the bee pollen and propolis groups as compared with royal jelly and Ronozyme groups ($p<0.05$). In conclusion, this experiment demonstrated that quails supplemented with propolis and bee pollen could produce egg rich. This study will help the researcher to uncover the critical areas of egg quality that many researchers were not able to explore. Thus a new theory on these compounds may be arrived at.

Keywords: Malondialdehyde; Yolk; Quail; Propolis; Royal Jelly; Bee Pollen; Ronozyme.

Introduction

Eggs have a high nutritional value and contain a variety of necessary components for the maintenance and the normal function of the human organism [1].

Lipid oxidation by free radicals is one of the primary mechanisms of quality deterioration in eggs. It is initiated in the highly unsaturated fatty acid fraction of membrane phospholipids, leading to the production of hydroperoxides, which are susceptible to further oxidation or decomposition to secondary reaction products such as short-chain aldehydes, ketones and other oxygenated compounds that may adversely affect lipids, pigments, proteins, carbohydrates, vitamins and the overall quality by causing loss of flavour, colour and nutritive value and limiting shelf-life [2]. Malondialdehyde (MDA) is one of the major lipid peroxidation products. This aldehyde is formed during lipid peroxidation of fatty acids with three or more double bonds [3].

Studies to improve oxidative stability of eggs and meat via dietary antioxidant enrichments [2, 4] reveal an inverse relationship between levels of dietary antioxidant and MDA contents in yolk, meat, a lipid peroxidation indicator in the poultry products. In the past, synthetic antioxidants were used with the intention of preventing lipid oxidation by scavenging chain-carrying peroxyl radicals or diminishing the formation of lipid radicals [5]. In the last decade, there has been a strong tendency towards using organic antioxidants from natural sources for the protection of animal health and their products against oxidation [6]. As a result, considerable interest has arisen in the use of natural supplements that could serve as alternatives to synthetic supplements on purpose to improve egg quality [7-9].

Royal jelly also contains vitamins, minerals, enzymes and antibiotic components. Royal jelly has been determined to exhibit a variety of pharmacological activities including antitumor,

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antimicrobial, antioxidant activity, vasodilative and hypotensive activities, as well as growth stimulating and infection preventing, antihypercholesterolemic and anti-inflammatory activities.

Propolis (bee glue) is a natural resinous hive product collected by bees from plants, particularly from flowers and leaf buds. Propolis generally contains 30% beeswax, 5% pollen, 50-55% resinous substances and 15% essential oils [10]. Propolis has shown diverse biological activities: antimicrobial activity against different bacteria and yeast, parasites; antioxidant, cytotoxic and potential antitumor activity, antipsoriatic, anti-inflammatory and analgesic activities, anti-obesity and hepatoprotective effects [11, 12].

Bee pollen is a powder-like material produced by flowering plants pollen, mixed with nectar and bee secretions and gathered by the honey bees. Pollen is called the only perfectly complete food and the main biological components of bee pollen are the phenolic acid derivatives and polyphenolic compounds, mostly flavonoid glycosides. The flavonoids are so called secondary plant compounds which have different important physiological and pharmacological activities. They possess diverse biological properties such as antioxidant, anti aging, anticarcinogenic, anti-inflammatory, antiatherosclerosis, cardioprotective and improve the endothelial function [13, 14].

Incorporating Ronozyme® ProAct in diets helps reduce feed costs without compromising performance. Protein is one of the most expensive components of any livestock diet, but is essential for growth. The challenge, therefore, is to use protein as efficiently as possible so as to reduce feed costs without compromising animal health. Proteases are enzymes that break down protein molecules into the amino acids and peptides needed by animals. Specifically developed for inclusion in animal diets, Ronozyme significantly increases the digestibility of protein. It complements naturally occurring proteases in feed, and considerably increases amino acid and peptide supply so as to enhance animal performance. It improves the digestibility of a wide range of protein sources and cereals, allowing savings in diet costs [15].

This experiment was conducted to investigate the effects of royal jelly, propolis, bee pollen and Ronozyme supplementation in diets of Japanese quails (*Coturnix coturnix japonica*) on contents of MDA in yolk.

Materials and Methods

Total one hundred and sixty Japanese quailsat aged of 43 days were used and divided into 4 replicate groups each containing 32 animals. All of birds were placed into cages with an internal dimension of 40×32 cm. The experimental groups as follows: Group 1 (control) was feed a basal diet. Group 2 was added to the water with 500 mg/kg dietroyaljelly [16]. Group 3 was feed a basal diet supplemented with 4 g propolis/kg diet. Group 4 was feed a basal diet supplemented with 1 g bee pollen/kg diet [17]. Group 5 was feed a basal diet supplemented with 1g Ronozyme/kg diet. The experiment was continued during 74 days.

The ingredients and chemical composition of the diets are presented in Table 1. Chemical composition of feed ingredients

Table 1. Ingredient Composition and Chemical Composition of the Experimental Diets (g/kg).

Ingredients	Starter	Finisher
Maize	539.8	621.3
Soybean meal	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-methionin	2.8	1.3
Vitamin–mineral premix[a]	3.5	3.5
Lysine	1.6	1.6
Calculated content		
ME, kcal/kg[c]	3300	3030
Dry matter, %[b]	89.4	90.50
Crude protein (CP), %[b]	23.62	19.25
Ether extract, %[b]	6.1	8.9
Ash, %[b]	5.1	4.3
Crude cellulose, %[b]	5.0	5.6
Calcium[c]	10	10.2
Total phosphorus[c]	5.5	5.6

[a] Vitamin–mineral premix provided per kilogram of diet: retinol, 4.5 mg; cholecalciferol 0.125 mg; Alpha-tocopherol, 100 mg; menadione, 4 mg; thiamine, 3 mg; riboflavin, 8 mg; niacin, 60 mg; pyridoxine, 5 mg; Ca-Dpantothenate, 18 mg; folic acid, 2 mg; D-biotin, 0.20 mg; Mn, 100 mg; Zn, 80 mg; Fe, 80 mg; Cu, 8 mg; Co, 8mg; Se, 0.3 mg; Iodine, 1 mg, Mo, 1 mg; choline chloride, 500 mg.
[b] Analysed AOAC(1995). [c] Based on NRC (1994) feed composition tables.

(dry matter, crude protein, aether extract and ash) as dried samples was analysed using AOAC [18] procedures. Corn and soybean meal-based feeds were formulated to be isonitrogenic and isoenergic according to the NRC [19] recommendation. All groups were kept under the same environmental conditions. Feed and water were given ad libitum.

Royal jelly and bee pollen were obtained from a commercial firm in Turkey. Royal jelly dissolved in distilled water, and kept frozen at -20°C until used. Chemical composition of royal jelly was assessed by gas chromatography-mass spectrometry (GC-MS, Agilent GC 6890 gas chromatograph) analyses (Table 2). Ronozyme was provided DSM Nutritional Products Inc. Pollen and Ronozyme were homogeneously mixed carefully to the basal diet. Propolis samples were collected from the Karabük Province (Middle Black Sea). Afterwards, propolis samples were kept desiccated in the dark until the processing. The samples were extracted for a week with 100 ml of 70% ethanol at room temperature to obtain the extract. After filtration, the extract was evaporated using a vacuum evaporator at 45°C and then used in the experiment. GC-MS was carried out to detect main components of propolis by the gas chromatograph coupled to the Agilent MSD 5973 mass detector under electron impact ionization. The main compounds of the propolis sample were identified and are listed in Table 3.

At the end of the experiment, 42 eggs per each group (2 eggs from each replicate) were randomly chosen to determine the MDA concentration of yolk. The yolk samples were mixed with 1.15% KCl at the 1:10 ratio (weight/volume) then homogenized onto fragmented ice. The homogenates were centrifuged at 18.000xg (+4°C) for 30 min to determine MDA concentrations. The MDA concentration as a marker of lipid peroxidation were determined according to the method of Placer et al., [20] based on the reaction with thiobarbituric acid. The concentrations of reagent (10%, w/v trichloroacetic acid and 0.168%, w/v TBA) were mixed in a glass tube. The solution was warmed for 20 min. The precipitate was removed by centrifugation at 4.000 g for 10 min. The formed MDA created a pink complex with thiobarbituric acid and the absorbance was read at 532 nm. The MDA content was expressed as nmol/ml.

Statistical Analysis

The results are expressed as mean ± standard error (S.E.). Statistical significance between the different groups was determined by using one way analysis of variance (ANOVA) in the SPSS 21 software package. Post hoc test was performed for between-group comparisons by using the Tukey multiple comparison test. All data are expressed as mean ± S.E. The level of significance was set at $p<0.05$.

Results

All eggs were collected on end of experiment and the yolk was evaluated MDA levels. MDA levels of the yolk were found highest

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.

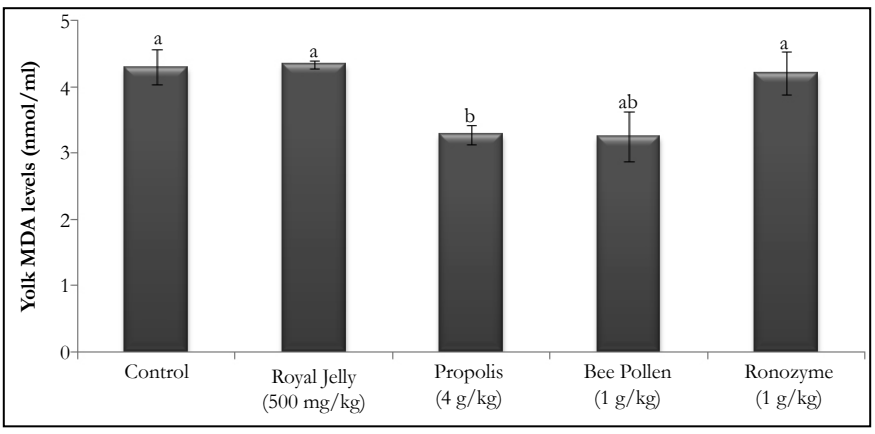
Table 3. Chemical Composition of Propolis as Assessed by GC-MS.

RT(m)	Contents	TIC(%)
Aromatic Alcohols		
4.787	Benzyl alcohol	0.279
11.334	2-Methoxy-4-vinylphenol	0.55
11.047	Cinnamic alcohol Aromatic Acids	0.455
8.309	Benzoic acid	1.123
24.097	İsoferulic acid	0.908
28.866	p-coumaric acid	0.404
14.418	Cinnamic acid	1.043
Esters		
24.433	Dimethyl caffeic acid	1.798
32.845	Cinnamyl cinnamate	1.863
Fatty acids		
26.121	Palmitic acid	0.418
29.268	Oleic acid	1.959
29.065	Linoleic acid	0.626
Flavanoids		
36.679	Chrysin	18.168
33.714	Pinosembrin	16.828
32.281	Pinostrobin chalcone	7.129
37.381	Galangin	7.887
35.214	Tektochrysin	9.503
40.607	Pectolinarigenin	0.316
37.555	Sakuranetin	1.055
Terpens		
34.117	Abietic acid	0.311
19.479	Alfa eudesmol	0.557
33.398	Dehydroabietic acid	0.678
19.345	Beta-selinenol	0.728
Others		
9.222	4- vinylphenol	0.438
33.039	5-Methyl-1,3-benzendicarbocsilic acid	2.478
37.840	İsomaturinin	0.833
38.316	3-phenyl pyrrolidine	0.611
39.099	3-ciyano-5,6-dimethoxy-2-methylthio-1-felnilindole	0.464
17.251	Methyl-4-phenyl-3-bütenoat	0.86
29.660	Pyridine,1-asetil-1,2,3,4-tetrahidro-5-(2-pyrrolidinil)	0.998
35.870	Rheochrysidin	0.573
39.817	Chrysophanic acid	1.14

RT: Retention time, minute

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

Figure 1. The Effect of royal jelly, propolis, bee pollen and ronazyme supplementation on yolk MDA level.



in the control, royal jelly and Ronozyme groups as compared with bee pollen and propolis groups ($p<0.05$). MDA levels of the yolk in bee pollen and propolis groups were significantly lower than those of other groups ($p<0.05$). MDA levels of yolk in bee pollen and propolis groups were found to be similar.

Discussion

The egg is the most complete food in nutritional point of view. Lipid oxidation is a process that has significant effect on the food industry because it can alter food quality (rancidity, flavor, odor, and color) and may lead to toxic end product accumulation. Lipid oxidation of foods is of great interest because it results in decreased nutritional value and sensory quality [21, 22]. The oxidative stability of shell eggs in storage has not generally been a major problem because they contain several naturally occurring compounds that protect the in-shell system from oxidation. Phosvitin, a yolk protein, and conalbumin, an albumen protein, have both been shown to exert antioxidant activity by inhibiting Fe^{2+} and Cu^{2+} catalysed reactions, whereas other yolk constituents including α-tocopherol, xanthophylls and lecithin, have all been shown to be very effective in preventing oxidation of yolk lipids [5]. Although shell eggs are relatively stable against oxidation, processed eggs have been shown to be subject to lipid oxidation. Recently, the susceptibility of processed eggs to lipid oxidation has been of increased concern due to the production of 'modified' eggs that are highly unsaturated and may, therefore, be more prone to oxidation during storage or processing, particularly, at low pH. As emerging egg technology produces more 'modified' eggs, there may be an increasing interest on the oxidative deterioration of marketed eggs [8, 23].

Several studies have reported the effects of dietary supplementation as a means of improving the oxidative stability of eggs. In a study, to investigate the effect of diet on lipid oxidation of shell eggs during refrigerated storage, 4 freshly collected eggs from each subgroup, totalling 16 eggs from each dietary treatment were used. Four eggs, each from a different subgroup of each dietary treatment, were directly analysed for yolk MDA content, while the others were stored at +4°C to be analysed in sets of 4 eggs at 30, 45 and 60 d of storage. The extent of lipid oxidation, as measured by MDA formation, differed between treatments but did not change with storage time. The concentrations of MDA found in yolks must have been due to consumption and subsequent deposition of MDA already formed in the diets or to *in vivo* production and deposition of MDA by the hens during digestion [8].

The extent of lipid oxidation is commonly determined by an assay based on reaction between 2-thiobarbituric acid (TBA) and MDA during heating at acidic pH. Experienced investigators have cautioned that this assay may give misleading results due to the contribution of other compounds in addition to the TBA-MDA complex formed and, hence, the term 'thiobarbituric acid reactive substances' (TBARS) is frequently used [24]. TBA reactivity can be influenced by several factors including the formation of MDA as an artefact in the analysis during the assay itself and the occurrence of MDA in various bound forms [25]. The spectrophotometric method used in the present study substantially improves the reliability of the measurements because the applied third-order derivative spectral analysis of the TBA-MDA complex eliminates potential interference from other reactive compounds, whereas the sample preparation procedure inhibits lipid oxidation occurring *in vitro* during the assay itself.

Dietary inclusion of royal jelly and Ronozyme did not affect the yolk MDA concentration. Similarly, Yalçın et al., [26] found that yeast autolysate supplementation did not affect the MDA concentration of eggs. However, Zhang et al., [27] reported that dietary supplementation with *S. cerevisiae* improved the oxidative stability of broiler meat and suggested that this was due to some antioxidant factors present in *S. cerevisiae* shifting the oxidative fat or fatty acid profile in the meat.

Living organisms are able to adapt to oxidative stress by inducing the synthesis of antioxidant enzymes and damage removal/repair enzymes [28]. It was found that the MDA levels in yolk were significantly higher in the control group compared to the bee pollen and propolis groups. Ahmad et al., [29] reported a decrease in circulating triglycerides in the birds due to increased unsaturated fatty acids intake, which can reduce the availability of lipids for the formation of yolk. They also reported that unsaturated fatty acids could affect the circulating estradiol and suggested that the dietary unsaturated fatty acids could alter the hormonal metabolism of the birds. Galal et al., [30] reported that eggshell quality was significantly affected by propolis supplementation, whereas the percentage and thickness of eggshell were significantly increased in the egg produced from hens fed diet containing 100 and 150 mg propolis. Tatli Seven [31] reported that under heat stress condition propolis supplementation significantly increased egg shell thickness and egg shell weight in laying hens. This may be due to improved calcium digestibility and absorption resulting from the acid derivatives such as benzoic, 4-hydroxy-benzoic which are found in propolis.

Babaei et al., [32] indicated that the addition of 5000 mg/kg propolis, 5000 mg/kg bee pollen could be beneficial in improving growth performance of quail chicks. This may be due to the antimicrobial and immunostimulant activity of honey bee products. It opens perspective uses of honey, bee pollen and propolis as a feed additive to improve poultry performance. A similar finding was reported that *Alternanthera brasiliana* and propolis extracts increased body weight gain from 14 to 21 days [33]. Increased dietary 5000 mg/kg propolis and 5000 mg/kg bee pollen supplementation and 2.20% aqueous honey tended to improve feed efficiency. In another study it was reported that addition of propolis powder at 0.5, 1.0 and 1.5 g/kg diet increased the growth parameters of quail chicks [34]. Supplementation of propolis in broiler diets at a level of 500 mg/kg increased body weight gain by 20.00%. This improvement of weight gain could be due to the high content of flavonoids in propolis diets and increased Feedintakes (FI) compared to control group [35]. Experimental work of some investigators showed that propolis supplementation to the ration of pullets improved FCR [36]. FI and FCR significantly decreased by increasing levels of bee pollen and propolis supplementation. When 400 mg/kg and 800 mg/kg bee pollen extract were added in feed mixtures for feeding broiler chickens, was increased in experimental groups compared to control group [37].

Bee pollen is widely used as a natural supplement, as it contains most of the essential elements needed for growth and developments in human beings and animals [38-40]. Bee pollen contains significant amounts of flavonoids, carotenoids and phytosterols. Flavonoids

are polyphenolic substances. Polyphenols have strong antioxidant capacity [41]. They are capable of scavenging free radical due to metal chelation properties [42, 43]. They analysed antioxidant properties of chesnut bee pollen before the treatment and determined that the chestnut bee pollen possessed many phenolic compounds, which are the factors of high antioxidant properties. It was reported that bee pollen supplementation decreases markers of oxidative stress, enhancing the antioxidant system of animals under stress [44]. Bee pollen might be suggested for useful properties in the prevention of disease in which free radicals ocur [45]. Besides, it has been reported that bee pollen has metal chelation properties that react with free radicals [42]. Their function is to hunt down free radicals and neutralize them. In so doing, they not only prevent free radicals from causing damage but also repair any damages [46]. They analysed antioxidant properties of chesnut bee pollen before the treatment and determined that the bee pollen possessed many phenolic compounds, which are the factors of high antioxidant properties. It was reported that bee pollen supplementation decreases markers of oxidative stress, enhancing the antioxidant system of animals under stress [44].

In the present study, increasing of yolk MDA level in the control group may be evidence of oxidative stress. The results of the current study showed that propolis and bee pollen supplementation to quails diet has improved much more than those of royal jelly and Ronozyme supplementation to lipid peroxidation. This may be due to more powerful antioxidant content of bee pollen and propolis than that of royal Jelly, Ronozyme and its antiimmune properties. Inclusion of bee propolis and/or pollen to diet decreased lipid oxidation and prolonged the shelf life of egg, in case of propolis and bee pollen having unsaturated fatty acid composition consumed by layers. This may be due to the antimicrobial and immunostimulant activity of honey bee products.

Conclusion

Propolis and bee pollen used as alternative to antioxidants had positive effects on lipid peroxidation. Therefore, propolis and bee pollen can be recommended as a egg promoter in quail production. They can be used as preventing lipid oxidation. It opens perspective uses of bee pollen and propolis as feed additive to improve poultry performance. In summary, the present study suggested that bee pollen and propolis supplementation to diet had potential protective activity on lipid peroxidation. Propolis and bee pollen can be used as an additive in the ration of poultry because of antioxidant capacity.

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