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THE EFFECT OF ROSEMARY (*SALVIA ROSMARINUS*) SUPPLEMENTED DIET ON REPRODUCTIVE AND PRODUCTIVE TRAITS OF LIBYAN LOCAL PIGEON

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ABSTRACT

The objective of this study was to investigate the effect of rosemary leaves powder supplement on reproductive and productive performance of local pigeon. Thirty pairs of Libyan local pigeons were randomly allocated into three groups, each with 10 replicates. Rosemary leaves powder (RLP) was added to isonitrogenous and isoenergetic ration at 0 % (control, C), 0.5 % (T1) or 1 % (T2). The experiment lasted for a complete breeding cycle. RLP did not affect egg weight, egg number or incubation period. Egg volume (13.82, 10.73 and 11.42 cm^3), egg hatchability (100, 55.56 and 50.00 %) and hatching weight (16.63, 14.91 and 14.89 g) were higher in C group in comparison with T1 and T2, respectively ($p < 0.05$). Egg surface area (30.25, 26.30 and 27.74 cm^2) and egg width (2.67, 2.44 and 2.53 cm) were higher in C than in T1 ($p < 0.05$) and tended to be higher than T2 ($p > 0.05$). Egg length, egg shape index, fertility rate and pre-weaning survival rate tended to decrease with the increased RLP inclusion rate; however, these differences were not significant ($p > 0.05$). Squab weight at weaning was lower in C than in T2 group, (310.35, 312.08, 337.98 g for C, T1, and T2, respectively) ($p < 0.05$). Peroxide value of fresh meat tended to decrease in T2 compared to T1 or C groups (0.6, 0.49, 0.37 meq/kg for C, T1 and T2, respectively) ($p > 0.05$). Malondialdehyde (MDA) concentration in fresh meat (0.16, 0.16, 0.09 mg MDA/kg) and blood (1.66, 0.83, 0.61 mg MDA/L) for C, T1 and T2, respectively, was lower in T2 compared to C group ($p < 0.05$). In conclusion, adding RLP to feed negatively affected the reproductive performance despite expected improvement of the productive performance and oxidative status in pigeons.

Key words: pigeon; rosemary; reproduction; production; oxidative stress

INTRODUCTION

North Africa is one of the native homes for rock pigeon (*Columba livia*) (Shapiro and Domyan, 2013). Domestic pigeons descend from rock pigeons (Gilbert and Shapiro, 2013). Nowadays, domestic pigeons could be divided into three categories: flying, fancy and utility pigeons (Harlin, 1994). The pigeon is a monogamous bird that breeds all year round, with peak fertility during spring and summer (Johnston and Janiga, 1995). Pigeons are altricial birds in which squabs depend on both parents until weaning (Dumont, 1965). In comparison with other poultry species, information about the nutritional requirements of the domestic pigeon is limited. This may be attributed to the nutritional dependency of squabs on their parents until weaning (Sales and Janssens, 2003). Abou Khashaba *et al*. (2009) reported that protein levels of 14–16 % and energy of about 2800 $Kcal.kg^{-1}$ of diet could meet the productive and reproductive requirements of pigeons in different seasons. Aromatic plants and their derivatives have

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been used as feed additives in poultry due to their potential antimicrobial properties, improving nutrient digestion, inhance immune system and antioxidant capacity (Jin *et al*., 2020). Rosemary is one of widely investigated aromatic plants in poultry nutrition and it was reported to increase weight gain in broilers (Ghazalah and Ali, 2008) and quails (Sarmad *et al*., 2020), improve the physical characteristics of eggs (Abo Ghanima *et al*., 2020) and improve egg hatchability and fertility (Radwan *et al*., 2008; Borghei-Rad *et al*., 2017). However, studies of rosemary feed supplement effect on pigeon's reproductive and productive performance are still limited (Dal Bosco *et al*., 2005). The variation of egg volume and shape within the same species could be a result of food quality and weather conditions, and this variation in volume and shape could affect squab mortality (Janiga, 1996). Measurements of egg weight, shape, volume and surface area are considered important indicators in the poultry industry and biological studies, and these measurements correlate with hatchability and hatch weight (Narushin, 2005). Reproductive and productive performance of Libyan pigeons are not well documented in comparison with other poultry species, especially broiler and layer chickens.

The objective of this study is to evaluate the effect of the inclusion of rosemary leaves as a feed additive on the productive and reproductive performance of Libyan pigeons. In addition, the reproductive and productive traits, determined in this study, could be considered as a reference to Libyan pigeon as long as these traits concerned.

MATERIAL AND METHODS

This study was conducted at the animal house of Faculty of agriculture, Omar Al-Mukhtar University, Libya. The room was 4 x 6 m and 3 m in height. Cages were 30 cm long, 23 cm wide and 20 cm in height and equipped with feeders and waterers. Before an arrival of the birds, the room was cleaned with disinfectants, the ceiling and walls were painted with lime and sprayed with insecticide, then closed for 24h. Thirty pairs of Libyan pigeons 12 – 24 months of age were purchased from local farmers. Birds were adaptated to the experimental room for 30 days, during this period the birds received the control diet. Proper heater was used to maintain room temperature around 20 °C. Iso-nitrogenous and iso-caloric diets were prepared in pelleted form in a private feed factory. Rosemary plant was obtained from Al-Bayda suburbs. The plant was dried in a shade with a well-ventilated place without applying any heat treatment. After dryness, the leaves were separated from branches and ground to the fine powder. Rosemary leave powder was mixed with a ration during feed manufacturing.

Diet ingredients and chemical composition are presented in Table 1. Water was provided *ad libitum* and feed quantity was adapted to feed intake. The experiment began in November 2019 and ended in January 2020.

Pigeon pairs were randomly allocated to three groups: control (C) and two treatments groups (T1 and T2), with 10 replicates per each group. Pairs (male and female) of each group were randomly distributed to cages. The control group received the standard diet, and the other two treatment groups T1 and T2 received rosemary powdered leaves either at 0.5 % or 1 % of the ration, respectively.

Table 1. Ingredients and chemical composition of experimental diet

Ingredients	%
Corn grain	66
Wheat grain	10.5
Soybean meal	18.6
Dicalcium phosphate	1.2
Calcium carbonate	2.4
Salt	0.3
Vitamin-mineral mix*	1.0
Chemical composition	
Dry matter	88
Crude protein	15.5
Crude fiber	2.71
Ether extract	3.88
Ash	1.00
Soluble carbohydrates	76.91
ME** (MJ.kg^{-1} diet)	13.36

*Vitamin-mineral mix compose of (kg): Vit A. 1000000 IU, D_3 300000 IU, E 5000 mg, B_1 500 mg, B_2 400 mg, B_6 400 mg, B_{12} 2 mg, K_3 12.5 mg, C 2500 mg, Nicotinic acid 1500 mg, Calcium pantothenate 2000 mg, Choline chloride 25000 mg, Methionine 3500 mg, Lysine 10000 mg, NaCl 9800 mg, Mg 3000 mg, Mn 3150 mg, Zn 5100 mg, Fe 3100 mg, Cu 250 mg, Co 247 mg, I 137.6 mg, Dical. Phosphate 68300 mg.
** Estimated from the sum of ingredients ME.

Measurements

Egg weight, hatching and weekly weight until weaning on 28 days were recorded using a digital precise balance (XB320 M Precisa Instruments, Dietikon, Switzerland). In addition, clutch size and incubation period were recorded. Egg length and width were recorded by calibre Vernier and the following parameters were obtained:

- Egg volume (cm^3) = 0.51 . egg length . (egg width)^2 (Hoyt, 1979)
- Egg shape index (%) = egg width/egg length . 100 (Narushin and Romanov, 2002)
- Egg surface area (cm^2) = (3.155 − 0.0136 . egg length + 0.0115 . egg width) . egg length . egg width (Narushin, 2005)
- Fertility: The eggs that were not hatched or abandoned by the parents were examined and fertility was calculated as follows:
  Fertility (%) = N. of fertilized eggs/N. of total eggs . 100
- Hatchability was assessed as follows:
  Hatchability (%) = N. of squab hatched/N. of total eggs . 100

Chemical analysis

Proximate analysis of diet was done according to AOAC protocol (AOAC 2000).

Total Phenols in the ground rosemary leave sample were determined according to Singleton and Rossi (1965). The results were expressed as milligrams of Gallic acid equivalent (GAE) per gram of sample dry matter.

Total Flavonoids in the ground rosemary leave sample were determined according to the approach proposed by Yoo *et al.* (2008) and expressed as milligrams of Catechin equivalent (CE) per gram of sample dry matter.

Finally, Oxidative stress marker analysis was done as follows: At the end of the experiment, 5 squabs were randomly chosen from each treatment and killed. Samples of blood and meat were taken for determination of Malondialdehyde (MDA) by TBA assay according to the methods of Placer *et al*. (1966) and Vyncke (1970), respectively.

Data were subjected to a statistical analysis using the SYSTAT 13 software © (Copyright 2009, Systat Software, Inc.). The data were analysed based on a one-way analysis of variance. Treatment means were then compared by LSD (Least Significant Differences) test at $p < 0.05$ significance level. Hatchability, fertility and mortality rates were subjected to a Chi-square test (χ^2) at $p < 0.05$ significance level.

RESULTS AND DISCUSSION

Total phenols and flavonoids in rosemary leaves

The content of phenols and flavonoids in rosemary leaves is shown in Table 2. Rosemary leaves used in this study contained 76.97 mg GAE.g^{-1} of total phenols and 49.90 mg CE.g^{-1} of flavonoids. Antioxidant capacity of rosemary is related mainly to its content of phenols and flavonoids (Yeddes *et al*., 2019). In comparison with our results, studies in Algeria and Tunisia (two neighboured countries) recorded lower concentrations of total phenols and flavonoids in rosemary leaves when we consider a change of their results from mg GAE.g^{-1} extract to mg GAE.g^{-1} of the sample (Hendel *et al*., 2016; Yeddes *et al*., 2019). This variation may be due to intrinsic factors of the plant itself or to geographical location, plant maturity stage, pre-extraction treatment and extraction conditions (Pérez *et al*., 2007; Yeddes *et al*., 2019; Aljabri, 2020).

Effect of rosemary supplement on the productive performance of Libyan pigeons

Rosemary supplement decreased squab hatching weight (Table 3.). Increasing rosemary supplement from 0.5 to 1 % in the diet did not affect hatching weight ($p > 0.05$). After hatching, the effect

Table 2. Total content of phenols and flavonoids in rosemary leaves

	Total phenols*	Total flavonoids **
Rosemary leaves	76.97	49.90

*mg GAE/g of sample, ** mg CE/g of sample

Table 3. The effect of rosemary supplementation on squab weight from hatching to 4 weeks of age ($\bar{X}$ "g" ± SE)

Rosemary		
C	T1	T2
16.63[a] ± 1.37	14.91[b] ± 1.55	14.89[b] ± 1.12
115.43 ± 17.24	126.91 ± 10.37	115.67 ± 25.74
208.50[b] ± 13.75	257.04[a] ± 19.61	250.34[a] ± 23.11
294.08[b] ± 11.66	315.92[a] ± 25.67	326.74[a] ± 24.50
310.35[b] ± 14.20	312.08[ab] ± 30.00	337.98[a] ± 39.20

[a,b] Means with different superscripts in the same row differ significantly ($p < 0.05$).

of rosemary supplement changed. The body weight of birds in C group was lower than the body weight of the birds in T1 and T2 groups from two-week age until weaning ($p < 0.05$). Hatching weight in broilers is highly correlated with egg weight, however, the growth in the stage that follows hatching, especially from the fifth day onward, was not affected by egg weight, and was highly influenced by nutrition (Pinchasov, 1991).

Studies have shown that rosemary supplements may enhance weight gain in broilers (Al-Kassie, 2008; Ghazalah and Ali, 2008) and quails (Sarmad *et al*., 2020). However, Sarmad *et al*. (2020) stated that the inclusion of rosemary into the diet at 0.5 and 1 % was better than that of 2.5 %. Intrestingly, the same results were reported by Elnaggar *et al*. (2016). Several reports, on the other hand, mentioned that rosemary supplements may not improve weight gain in layers (Alagawany and Abd El-Hack, 2015) and broilers (Ghozlan *et al*., 2017; Abd El-Latif *et al*., 2013). Thus, Ghozlan *et al*. (2017) demonstrated that the inclusion of rosemary into the diet at more than 0.5 % had negative effect on the productive performance of broilers. Contrarily, our results showed that rosemary supplement improved the growth performance of squabs.

Sarmad *et al*. (2020) concluded that the effect of rosemary on the improvement of growth in quails could be attributed to the natural antioxidants in rosemary (Etter, 2005; Nieto *et al*., 2018), and this may explain the improvement in growth rate we observed in our experiment. It is noteworthy that oxidative stress in domestic birds leads to many diseases that affect their growth and production performance (Estévez, 2015).

Effect of rosemary supplement on the reproductive performance of Libyan pigeons

The effect of rosemary supplements on the reproductive performance and external physical characteristics of eggs are presented in Table 4. Egg weight and incubation period was not significantly affected by the rosemary supplement. However, rosemary supplements at the amounts mentioned in this study lead to deterioration of some reproductive traits of local pigeons.

Egg volume and egg hatchability in treatment groups were lower than in C group ($p < 0.05$). Egg width and egg surface area tended to be decreased with the rosemary supplement and this decline was significant between T1 and C groups ($p < 0.05$). Fertility rate, egg length and egg shape index tended to be lower in rosemary supplement groups in comparison with C group, but the difference was not significant ($p > 0.05$).

Pre-weaning mortality rates tended to be higher in treatment groups when compared to C group, but the difference was not significant ($p > 0.05$). In contrary to our findings, Șimșek *et al*. (2015) reported that adding rosemary oil to the ration of quails affected neither the physical characteristics of eggs nor the reproductive performance of birds. In addition, rosemary supplements in the poultry diet improved the physical characteristics of eggs (Abo Ghanima *et al*., 2020), egg hatchability and fertility (Radwan *et al*., 2008) or egg fertility without affecting hatchability (Borghei-Rad *et al*., 2017).

To our knowledge, no study has mentioned a negative effect of rosemary supplement on reproductive performance of birds. In other species, negative effects of rosemary on reproductive perfor-

Table 4. The effect of rosemary supplementation on the reproductive traits, physical egg characteristics and pre-weaning mortality rate of squabs ($\bar{X}$ ± SE)

	Rosemary		
	C	T1	T2
Egg weight (g)	17.95 ± 0.58	16.21 ± 1.03	17.61 ± 0.57
Egg number/nest	1.80 ± 0.20	1.8 ± 0.13	1.6 ± 0.16
Egg shape index (%)	74.60 ± 1.42	71.80 ± 1.10	72.20 ± 0.73
Egg volume (cm^3)	13.82^a ± 0.74	10.73^b ± 0.73	11.42^b ± 0.30
Egg length (cm)	3.59 ± 0.08	3.40 ± 0.10	3.50 ± 0.04
Egg width (cm)	2.67^a ± 0.05	2.44^b ± 0.07	2.53^{ab} ± 0.03
Egg surface area (cm^2)	30.25^a ± 1.13	26.30^b ± 1.27	27.74^{ab} ± 0.48
Fertility rate (%)	100	77.78	87.50
Hatchability (%)	100^a	55.56^b	50.00^b
Incubation period (days)	17.94 ± 0.15	17.79 ± 0.15	18.40 ± 0.40
Mortality rate (%)	11.11	20	25

^{a,b} Means with different superscripts in the same row differ significantly ($p < 0.05$).

mance of rats (Nusier *et al*., 2007) or insects (Douiri *et al*. 2014) have been reported. In the case of rats, a decline in androgen production was supposed to be a result of rosemary ingestion (Nusier *et al*., 2007).

The effect of rosemary supplement on the oxidative status

The results of the effect of rosemary supplements on oxidative stress of squab blood and meat are presented in Table 5.

Hydroperoxides are the major product of oxidation and it can be characterized by a peroxide value. Peroxide value has been long time considered as a good indicator of the degree of meat oxidation which characterizes the primary stage of the oxidation process (Domínguez *et al*., 2019).

Gheisari (2011) stated that higher peroxide values indicate clearly to rancidity of fat, but moderate or lower peroxide values are not an indicator of the absence of rancidity, therefore, it is difficult to correlate between standard peroxide values and degree of rancidity. In our study, meat samples of squabs were fresh and the results showed that peroxide value tended to decline with rosemary supplements, however the differences between treatments and C group were not significant ($p > 0.05$). The oxidative status of treated birds has been improved by the rosemary supplementation at 1 % of the diet, as presented in Table 5. The quantity of MDA was significantly lower in the meat and blood of the T2 squab group in comparison to C group ($p < 0.05$). The quantity of MDA tended to

Table 5. The effect of rosemary supplementation on the oxidative stress parameters in meat and blood of squabs

		C	T1	T2
Meat	Peroxide value (meq.kg^{-1})	0.60 ± 0.25	0.49 ± 0.10	0.37 ± 0.01
	TBARS (mg MDA.kg^{-1})	0.16^a ± 0.01	0.16^a ± 0.01	0.09^b ± 0.01
Blood	TBARS (mg MDA.l^{-1})	1.66^a ± 0.02	0.83^{ab} ± 0.03	0.6^b ± 0.01

^{a,b} Means with different superscripts in the same row differ significantly ($p < 0.05$).

be lower in the blood of T1 squab group compared to control, but the difference was not significant ($p > 0.05$). MDA is one of the final products of lipid oxidation in the cells and its quantity is used as a parameter of oxidative stress (Tsikas, 2017).

In contrary to our results, Dal Bosco *et al*. (2005) did not find a significant difference in fresh meat MDA concentration between squabs consumed rosemary supplemented diet and the control group.

However, the rosemary supplement decreased MDA concentration in meat after 90 days of freezing. MDA concentration has been decreased in broiler meat (Yesilbag *et al*., 2011; Ghozlan *et al*., 2017) or in quail blood and meat (Cetin *et al*., 2017) after receiving a rosemary-supplemented diet.

In addition, Botsoglou *et al*. (2007) reported that the oxidative status in turkey meat has been improved by the consumption of a rosemary-supplemented diet. Lower levels of MDA revealed slowness or inhibition of lipid oxidation as a result of improvement of oxidation status in treated birds. Rosemary is rich in phenolic compounds, which can inhibit the free radical formation and hence lipid oxidation (Yesilbag *et al*., 2011).

CONCLUSION

The addition of rosemary leave powder to the pigeon's diet caused two different responses:

1) Improvement of productive performance and oxidative status in pigeons,
2) deterioration in some of the reproductive traits. Investigating the effect of rosemary supplement for more than one breeding cycle is recommended before making a final conclusion.

AUTHOR CONTRIBUTIONS

Conceptualization: Akraim, F.

Methodology: Akraim, F., Bellail, A. A.

Investigation: Akraim, F., Alfakhri, M. Y. M., Bellail, A. A.

Data curation: Alfakhri, M. Y. M., Bellail, A. A.

Writing-original draft preparation: Akraim, F.

Writing-review and editing: Akraim, F., Alfakhri, M. Y. M.

Project administration: Akraim, F.

All authors have read and agreed to the published version of the manuscript.

INFORMED CONSENT STATEMENT

Not applicable.

DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the corresponding author.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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