Revista de Ciências Agroveterinárias
Universidade do Estado de Santa Catarina (UDESC)

Research Article

Effect of dried oregano as a supplement in the diet of Extremadura Blue poultry on production and reproductive parameters

Efeito do orégano seco como suplemento na dieta de aves da raça Extremadura Blue sobre a produção e os parâmetros reprodutivos

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Submission: June 18th, 2024 | Acceptance: February 01st, 2025

ABSTRACT

The aim of this study was to investigate the effects of 2.5% dried oregano as a source of phytoestrogens in poultry diets on reproductive performance. A total of 120 hens of the Extremeña Blue breed and 10 cocks, 43 weeks old, housed individually without air conditioning in laying cages, were randomised into two groups and given water and feed *ad libitum*. After 90 days of feeding, an increase in the number of eggs laid was observed in the oregano supplemented group. However, none of the other parameters studied, such as fertility and hatchability, showed any significant difference. In the same way, the semen quality parameters did not differ between the two groups studied in terms of semen concentration, semen viability, semen motility and semen abnormalities (P>0.05).

KEYWORDS: Medicinal herbs. Autochthonous poultry breed. Sperm quality. Egg laying. Food supplement.

RESUMO

O objetivo deste estudo foi investigar os efeitos de 2,5% de orégano seco como fonte de fitoestrogênios em dietas de aves sobre o desempenho reprodutivo. Um total de 120 galinhas da raça Extremeña Blue e 10 galos, com 43 semanas de idade, alojados individualmente sem ar condicionado em gaiolas de postura, foram divididos aleatoriamente em dois grupos e receberam água e ração ad libitum. Após 90 dias de alimentação, foi observado um aumento no número de ovos postos no grupo suplementado com orégano. Entretanto, nenhum dos outros parâmetros estudados, como fertilidade e eclodibilidade, apresentou diferença significativa. Da mesma forma, os parâmetros de qualidade do sêmen não diferiram entre os dois grupos estudados em termos de concentração do sêmen, viabilidade do sêmen, motilidade do sêmen e anormalidades do sêmen (P>0,05).

PALAVRAS-CHAVE: Ervas medicinais. Raça de aves autóctone. Qualidade do esperma. Postura de ovos. Suplemento alimentar.

INTRODUCTION

Plant extracts, also known as phytobiotics, have been exploited in animal nutrition, particularly for their antimicrobial, anti-inflammatory, antioxidant, and antiparasitic activities. Oregano (*Origanum vulgare*) belongs to the Lamiaceae family. It has two important phenol compounds corresponding to 78–85% of the oil's composition, namely carvacrol (2-methyl-5-isopropyiphenol) and thymol (2-isopropyl-5-methylphenol) (MALAYOĞLU et al. 2010), which exhibits some biological activities

including anti-oxidant, anti-microbial, anti-inflammatory, and analgesic properties (CHENG et al. 2018, ZOU et al. 2016). Additionally, oregano is being evaluated for its positive effect on gastrointestinal health, improve nutrient digestion and also to improve egg productivity in laying hen farming (IVANOV & BOZAKOVA 2022).

Origanum vulgare is a plant of the mint family that also contains four phytoestrogens: apigenin, biochanin A, quercetin and luteolin (VAN MEEUWEN et al. 2007). Phytoestrogens are herbal compounds that imitate oestrogens and thereby elicit an oestrogenic response by reacting with oestrogen receptors. Phytoestrogens can have diverse antagonistic/agonistic effects depending on dose, type of tissue, oestrogen receptor (ER) subtype and the presence of endogenous hormone (DUSZA et al. 2006).

Dietary daidzein, a natural phytoestrogen, improved Shaoxing duck laying efficiency during the post-peak laying period (YASSEIN et al. 2015). DUSZA et al. (2006) showed that supplementation with daidzein improved egg development and eggshell thickness while lowering the broken eggs rate. Furthermore, SALEH et al. (2019), noted that improved production, egg quality, antioxidative status, hormonal profile, and steroidogenesis were observed with the dietary application of mixed phytoestrogen sources (flaxseeds and fenugreek seeds).

The aim of this trial is to evaluate the effects of supplementing the diet of Extremeña Blue breed laying hens and roosters with dried herb of *Origanum vulgare* on egg production and reproductive performance.

MATERIALS AND METHODS

Animal and diets

This experiment was conducted by the guidelines of the Department of Animal Production, Centre for Scientific and Technological Research in Extremadura (CICYTEX), Spain. All procedures used in this experiment were approved by the Animal Ethics Committee at the University of Extremadura. A total of 120 Extremeña Blue breed hens and 10 roosters at 48 weeks of age were randomly divided into 2 groups. The control group (CG) was fed a commercial production feed without any supplement (table 1), while the production feed of the experimental group (OG) was supplemented with 2.5% dried oregano (table 2), for a period of three months (March, April and May).

Oregano extracts

Oregano (*Origanum vulgare* subsp. *virens* (Hoffmanns & Link) Bonnier & Layens) crop was carried out under organic farming in the test plots of Finca la Orden (CICYTEX), in an area of approximately 100 m^2. The *Origanum vulgare* was used as dried herb, previous drying process under controlled conditions of temperature (20-25 ºC) and darkness, followed by threshing to remove the lignified stems. The dry matter obtained was added to the production feed for laying hens at a rate of 2,5% and pelletised.

Table 1: Ingredients and nutritional value of the commercial feed for laying hens (CG).

COMPOSITION: Maize (genetically modified), Soybean meal (from genetically modified soybeans), Wheat, Calcium carbonate, Palm oil (Palm oil from genetically modified soya), Wheat, Calcium carbonate, Palm oil, Dicalcium phosphate, Salt.
ANALYTICAL COMPONENTS: Crude Protein: 16.50%, Crude fat: 4.68%, Crude fiber: 2.75%, Cenizas: 12.71%, Calcium: 3.85%, Phosphorus: 0.54%, Sodium: 0.13%. Lysine: 0.88%, Methionine: 0.30%.
ADDITIVES (per kg of weight):
* VITAMINS AND PROVITAMINS
Vitamin A (E672) - 7,520 I.U.
Vitamin D3 (E671) - 1,500 I.U.
* TRACE ELEMENTS
Zinc (zinc oxide) (E6) - 40.04 mg
Manganese (manganous oxide) (E5) - 74.9 mg
Selenium (sodium selenite) (E8) - 0.2 mg
Copper (cupric sulphate pentahydrate) (E4) - 3.98 mg
Iron (ferrous sulphate monohydrate) (E1) - 30 mg
Yodo (yoduro potásico) (E2) - 1.8 mg
* ANTIOXIDANTS AND EMULSIFIERS
BHT (E321) - 0.13 mg
Propyl gallate (E310) - 0.13 mg
* ZOOTECHNICAL (ENZYMES)
6-phytase (3.1.3.26) - 300 FYT
* OTHERS
Canthaxanthin (2a161g) - 2.5 mg

Table 2. Ingredients and nutritional value of the commercial feed for laying hens supplemented with 2.5% dried oregano (OG).

COMPOSITION: Maize (genetically modified), Soya meal (from genetically modified soya), Wheat, Calcium carbonate, Palm oil, Dicalcium phosphate, Binder, Salt, Oregano (*Origanum vulgare*) (2,5%).
ANALYTICAL COMPONENTS: Crude Protein: 16.50%, Crude fat: 4.68%, Crude fiber: 2.75%, Cenizas: 12.71%, Calcium: 3.85%, Phosphorus: 0.54%, Sodium: 0.13%, Lysine: 0.88%, Methionine: 0.30%
ADDITIVES (per kg of weight):
* VITAMINS AND PROVITAMINS
Vitamin A (E672) - 10,000 I.U.
Vitamin D3 (E671) - 2,000 I.U.
Vitamin E (3a700) - 8 I.U.
* TRACE ELEMENTS
Manganese (manganous oxide) (E5) - 90 mg
Selenium (sodium selenite) (E8) - 0.22 mg
Copper (cupric sulphate pentahydrate) (E4) - 5 mg
Zinc (zinc sulphate monohydrate) (E6) - 65 mg
Iron (ferrous sulphate monohydrate) (E1) - 40 mg
Iron (ferric oxide) (E1) - 320 mg
Yodo (yoduro potásico) (E2) - 0.5 mg
* ANTIOXIDANTS AND EMULSIFIERS
Sepiolite (E562) - 1,000 mg

Semen collection

The cocks were managed intensively in a battery cage system and each cock was kept in an individual cage (52x45x38cm) and water and feed were offered *ad-libitum*. Feathers around the cloaca of each cockerel were clipped to minimise semen contamination during artificial insemination (AI). A single ejaculate of semen was collected from each cockerel one per week between 10:00 and 12:00 AM by the abdominal massage method (BURROWS & QUINN 1935). Macroscopic measurements were semen volume, semen colour, and semen pH. Microscopic measurements were semen concentration, semen viability, semen motility, and semen abnormalities.

Volume, colour and pH

Semen collection was make with the use of a graduated glass collection tube. The ejaculate volume was recorded directly from the semen collection tube and colour of semen was observed from the transparent tube and scored 1, 2, and 3 respectively by described (KABIR et al. 2007). Semen pH was determined with the aid of a highly sensitive p-Hydrion test paper (pH value ranges from 6.4 to 8.0) and compared with colours on a colour chart meter. Semen was then diluted 1:4 (semen: extender) using sodium chloride physiological solution (0,85% w/v). The four-fold diluted semen samples were brought to the laboratory for further analyses at the range of 22 to 25 ºC temperature during transport.

Sperm motility and concentration

For evaluation of motility, one drop of the diluted semen was placed on the slide and covered with glass cover slip. The sperm motility was estimated by microscopic observation at 400x magnification. Motility was expressed as the percentage of spermatozoa that are motile within the observed area. Several microscopic fields were examined for each sample.

The sperm concentration was measured using a direct cell count method using a haemocytometer chamber under 400X magnification of optical microscope after dilution with distilled water at 1:400 ratio and expressed as billion (10^9) per ml.

Eosin-nigrosin staining method

The live and dead sperm percent was calculated by differential staining technique using eosin–nigrosine (AKHLAGHI et al. 2014). A minimum of 200 sperms were counted in each slide for calculating live, dead and abnormal sperm per sample. Briefly, a 10µl drop of fresh semen was mixed with 200µl of eosin-nigrosin stain on a glass slide followed by making a thin smear of it. The spermatozoa which were appeared with pink colour (stained with eosin) were regarded as dead and spermatozoa which were appeared without any colour (no penetration of eosin stain) were regarded as live (Figure 1).

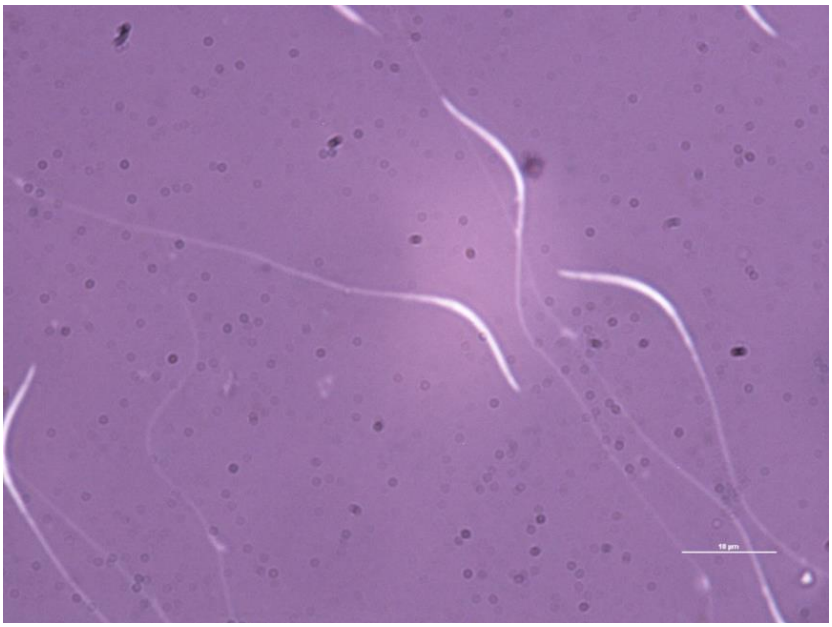

Figure 1. Microscopic image (X100) of spermatozoa stained with eosin-nigrosin stain. Spermatozoa stained light pink were considered dead and spermatozoa stained white without staining were considered alive.

Diff-Quik staining method

The Diff-Quik staining has the advantage of being very simple to perform, allowing for rapid analysis. By this method, normal spermatozoa and nuclei are lightly stained, while sperm with damaged or abnormal nuclei are very dark colour, making it easy to distinguish the degree of damage to the sperm (BENCHAIB et al. 2003, HENKEL et al. 2004). The stained samples were stored at room temperature and observed under a ×100 oil lens. Measurements were taken from at least four different locations to ensure a total of at least 200 spermatozoa per sample (Figure 2).

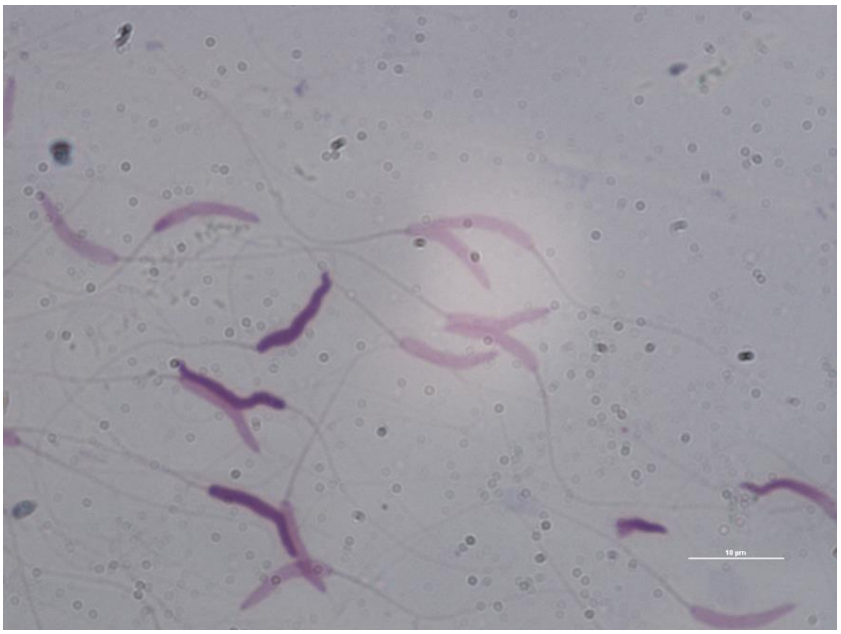

Figure 2. Microscopic image (X100) of spermatozoa stained with Diff-Quik stain. Spermatozoa stained light pink were considered to have normal chromatin and spermatozoa stained dark blue to purple were considered to have abnormal chromatin.

Artificial insemination

A total of 120 Extremeña Blue breed hens (48-week-old) were allocated in individual galvanized wire cages (50 x 45 x 35 cm) of an experimental poultry house without air conditioning and received water and feed *ad libitum*. Each experimental hen received 100 □l of its respective undiluted fresh semen one per week and was inseminated for four weeks to evaluate fertility and hatchability.

Fertility and hatchability evaluation

Throughout the experiment (3 months), the two groups were monitored for egg production. Eggs were collected each morning and the number of eggs was recorded separately for each group. The laying percentage was calculated as the number of eggs laid/day per hen × 100. Eggs were collected and stored at 15 °C until incubation two weeks later when they were also weighed to determine egg weight. Fertility was calculated as the proportion of fertile eggs among the incubated eggs, fertile eggs were determined by the conventional candling method after 18 days of incubation, infertile eggs and early dead embryos were removed, while hatchability was calculated as the proportion of hatched chicks among the total number of incubated eggs.

Statistical analysis

The data obtained were analysed using the SPSS statistical package (V23). After performing Levene's test for homogeneity of variances, the data were subjected to a one-way analysis of variance (ANOVA) with a 95% confidence interval. All variables were expressed as mean ± standard error (SE).

RESULTS AND DISCUSSION

Clearly, the fertility of poultry flocks is associated with the fertility of both males and females, but the low numbers of males used for natural or artificial insemination mean that their role is more important. While female breeders' fertility may be quantitatively measured by egg production, male's fertility is more difficult to assess

The characteristics and evaluation of the semen are important indicators of the reproductive potential of the breeding cocks. Semen characteristics, including volume; sperm count (total number); numbers of live, dead and abnormal sperm; and forward motility, are commonly tested to assess and predict male fertility in poultry (CHEN et al. 2017, DONOGHUE 1999, SUN et al. 2019).

The mean fresh semen volume collected in the oregano group was 0.6 ± 0.06 ml, while the mean fresh semen volume collected in the control group was 0.48 ± 0.33 ml. Nevertheless, the difference in ejaculate volume between oregano and control groups were not statistically significant. Similarly, the mean colour of fresh rooster semen was found to be creamy and its consistency thick in both the oregano and control groups. The values obtained for pH were within the normal range of 7.0 to 8.0 reported by DONOGHUE & WISHART (2000) and SIUDZIŃSKA & LUKASZEWICZ (2008). No effect of supplementation with oregano on semen pH was observed.

According to the result, as shown in Table 3, there was also no significant difference between the CG and the OG in terms of semen concentration, semen viability, semen motility and semen abnormalities ($P > 0.05$).

Table 3. Semen characteristics (mean ± SE) of the Extremeña Blue breed cock.

	CG	OG	*P*
Concentration	2.90*10^9 ± 0.18	3.17*10^9 ± 0.19	0.3101
Motility (%)	58.20 ± 0.17	62.60 ± 0.19	0.4223
Lives (%)	59 ± 0.07	61 ± 0.08	0.8712
Abnormalities (%)	16 ± 0.08	19 ± 0.075	0.9704

CG: control group; OG: oregano supplemented group; mean/error; P: p-value.

Three important semen characteristics necessary for the fertilization of an egg are sperm concentration, viability, and motility (PARKER et al. 2000). Even sperm motility are considered to be the most important characteristic associated with fertilizing capacity (VERSTEGEN et al. 2002). Values obtained in this study of semen characteristics of the Extremeña Blue breed cocks were within the acceptable range for artificial insemination in accord of SUN et al. (2019). The semen quality of local cocks did not differ significantly from that reported for other breeds of chicken, with semen volume and sperm production close to the upper end of the acceptable range (0.2-0.5 ml and 0.6-3.5 billion spermatozoa, respectively). It is important to select cocks with higher semen volume and total sperm count for breeding to achieve higher fertility. Accordingly, no significant difference in sperm fertility was found between the OG and the CG.

The characteristics and evaluation of the semen are important indicators of the reproductive potential of the breeding cocks. In this regard the present study provides the first sperm quality information of Extremeña Blue breed cock semen.

The egg production rate of the OG was significantly higher ($p<0.05$) than the CG, although egg weight at the end of three months was not affected by feeding. As indicated above, the diet significantly increased egg production. However, it did not affect reproductive performance in terms of fertility and hatchability (Table 4).

Table 4. Egg laying, fertility(%) and hatchability (%) (mean ± SE).

	CG	OG	*P*
Egg laying	59.10 ± 0.719	63.60 ± 0.90	0.0020
Fertility (%)	68.88 ± 5.83	67.46 ± 6.24	0.8680
Hatchability (%)	52.70 ± 5.09	56.48 ± 5.45	0.6140

CG: control group; OG: oregano supplemented group; mean/error; P: p-valour.

Improvement in egg production rate and egg weight is of critical economic value to the poultry industry. In the current study, our findings revealed that oregano dried herb supplemented at 2,5% in feeding, significantly improved egg production rates.

Similarly, CETINGUL et al. (2009) found that a dietary inclusion level of 20 g/kg Oregano Onites increased fertility ($P<0.02$), but above this level fertility was reduced in laying quails (*Coturnix coturnix japonica*). On the contrary, the study by CUFADAR (2018) showed that the addition of oregano essential oil to the diets had no effect on the performance and eggshell quality parameters of laying hens at 40 to 52 weeks of age.

There are only few *in vivo* studies that focus on herbs and their respective essential oils (EO) in laying hen diets. Conclusions of these studies tend to vary, although positive results dominate the observation. Nevertheless, the use of herbs, EO or their active plant compounds in diets for laying hens have been investigated only in a limited number of *in vivo* trials.

ZIARI et al. (2015) study suggests that *O. vulgare* dose-dependently affects ovarian oogenesis and gonadotroph cells. SHARBA et al. (2020) study revealed in female mice that aqueous hot extract of *O. Vulgare* act as improvement and development the uterus tissues and ovarian follicles with regulate progesterone and oestrogen levels; it has therapeutic properties in the reproductive system and uses possible as treated pathway of some problems fertility.

CONCLUSION

Dietary supplements containing phytoestrogens, such as *Origanum vulgare*, may have beneficial effects when used in laying hens. This study showed that adding 2.5% dried oregano to the diet of laying hens improved egg production performance. However, none of the other parameters studied, such as fertility, hatchability and sperm quality, showed a significant difference. Further studies are needed to determine the optimal dietary inclusion level and the exact mode of action of *O. vulgare* phytochemicals in improving production performance.

AUTHOR CONTRIBUTIONS

Conceptualization, methodology, and formal analysis**, Alfredo García, Francisco Vazquez and Carmen Barraso**; software and validation**, Carmen Barraso and Alfredo García;** investigation**, Carmen Barraso, Maria del Pilar Sánchez and Silvia Carretero**; writing-original draft preparation**, Alfredo García and Carmen Barraso**; writing-review and editing**, Francisco Vazquez**; visualization**, David García, Francisco Marquez, Maria del Pilar Sánchez and Silvia Carretero**; supervision, **Francisco Vazquez, Francisco Marquez and David García**; project administration, **Carmen Barraso and Alfredo García**; funding acquisition, **Francisco Vazquez and Alfredo García**. All authors have read and agreed to the published version of the manuscript.

FUNDING

This work was supported by The FEDER (European Regional Development Fund) funds in the 2014-2020 period.

INSTITUTIONAL REVIEW BOARD STATEMENT

Not applicable for studies not involving humans or animals.

INFORMED CONSENT STATEMENT

Not applicable because this study did not involve humans.

DATA AVAILABILITY STATEMENT

The data can be made available under request.

ACKNOWLEDGEMENTS

This work has been financed by COPLAGAN I+D+I project to be carried out in the framework of the operational program FEDER EXTREMADURA 2014-2020.

CONFLICTS OF INTEREST

Authors declare not conflicts of interest.

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