
Acta Scientiarum. Animal Sciences
ISSN: 1806-2636
ISSN: 1807-8672
Editora da Universidade Estadual de Maringá - EDUEM

Oliveira, Carolina Oreques de; Tavares, Amauri Telles; Castro, Júlia Parada Nobre; Ávila, Sérgio Leandro Costa de; Gheller, Stela Mari Meneghello; Soares, Sara Lorandi; Gonçalves, Fernanda Medeiros; Bongalhardo, Denise Calisto

Reproductive parameters and weight gain of roosters fed with waste oil from olive culture

Acta Scientiarum. Animal Sciences, vol. 44, e53597, 2022
Editora da Universidade Estadual de Maringá - EDUEM

DOI: https://doi.org/10.4025/actascianimsci.v44i1.53597

Available in: https://www.redalyc.org/articulo.oa?id=303171455017

Acta Scientiarum
http://periodicos.uem.br/ojs
ISSN on-line: 1807-8672
Doi: 10.4025/actascianimsci.v44i1.53597



# Reproductive parameters and weight gain of roosters fed with waste oil from olive culture

**Carolina Oreques de Oliveira[1*], Amauri Telles Tavares[1], Júlia Parada Nobre Castro[1], Sérgio Leandro Costa de Ávila[1], Stela Mari Meneghello Gheller[2], Sara Lorandi Soares[2], Fernanda Medeiros Gonçalves[3] and Denise Calisto Bongalhardo[4]**

[1]Programa de Pós-Graduação em Zootecnia, Universidade Federal de Pelotas, Campus Capão do Leão, Av. Eliseu Maciel, s/nº, Cx. Postal 354, 96160-000, Capão do Leão, Rio Grande do Sul, Brazil. [2]Programa de Pós-Graduação em Veterinária, Universidade Federal de Pelotas, Capão do Leão, Rio Grande do Sul, Brazil. [3]Departamento de Gestão Ambiental, Universidade Federal de Pelotas, Pelotas, Rio Grande do Sul, Brazil. [4]Departamento de Farmacologia e Fisiologia, Instituto de Biologia, Universidade Federal de Pelotas, Capão do Leão, Rio Grande do Sul, Brazil. *Author for correspondence. E-mail: carolina_oliveira2004@hotmail.com

**ABSTRACT.** Waste oil from olive oil extraction industry was used, instead of soybean oil, in heavy roosters' diet in order to evaluate birds' reproductive parameters. A total of forty roosters were housed individually in boxes with 1.2 $m^2$. Two experimental diets were used: control diet, based on corn, soybean meal, and soybean oil; and test diet, where soybean oil was totally replaced by waste oil. In order to verify weight gain and feed intake, animals were individually weighed weekly. Seven semen collections were performed with fifteen-day interval. Reproductive variables analyzed sperm volume, motility, concentration, and morphology. No statistical difference ($p > 0.05$) was observed between treatments at the different collection periods for the variables sperm volume, motility, and concentration. There was a statistically significant difference between treatments for body weight in periods three ($p = 0.04$), and seven ($p = 0.04$). Statistical differences ($p = 0.01$) were also observed between treatments for abnormal sperm morphology. Among collection periods, statistical difference was observed for motility ($p = 0.00$), and sperm concentration ($p = 0.01$). Total replacement of soybean oil by waste oil from olive oil extraction in young heavy roosters' diets does not affect sperm volume, motility, and concentration; reduces defects in sperm tail, and promotes better weight gain control.

**Keywords:** broiler breeders; industrial waste; males; olive trees; semen.

Received on May 8, 2020.
Accepted on August 4, 2020.

## Introduction

Olive trees cultivation and olive oil extraction are practices exploited for thousands of years. It is estimated that approximately 800 million olive trees cover the Mediterranean region, producing about 2 million tons of olive oil and almost 3 million tons of solid waste annually (Khdair, Abu-rumman, & Khdair, 2019; Simonato, Trevisan, Tolve, Favati, & Pasini, 2019). The growth of waste production, and the concern about how and where to reuse these materials, has gone together in companies and organizations worldwide, so that the environment is preserved.

Animal nutrition accounts for between 50-70% of total production costs, so industry and producers should be aware of strategies that maximize the efficiency of their business, in order to obtain low-cost animal protein (Alqaisi, Ndambi, & Williams, 2017).

Residues generated by the olive industry have a high nutritional value, and can be reused for other purposes, such as animal nutrition. The chemical composition of olive residues can contain up to 8% of lipids, 7% of proteins, 4% of minerals, and 1% of phenols (Dermeche, Nadour, Larroche, Moulti-mati, & Michaud, 2013). In the absence or scarcity of the major grains, such as corn and soybeans, using alternative foods from industrial processes in animal nutrition acts not only on the reduction of costs, but also on the sustainable destination of these materials (Nunes, Zanine, Machado, & Carvalho, 2007).

Breeders performance in poultry farming is attributed not only to breeding programs, but also to the nutritional quality of diets. Currently in the industry, poultry diets are formulated to meet nutritional requirements of the female matrix, providing the same diet to males. Physiologically, females and males have different nutrient needs due to the different products that each animal produces: eggs (females) and sperm

(males). Thus, intervention in roosters' diets quality, providing adequate nutritional levels, would allow better fertility rates in commercial flocks (Borges et al., 2006).

Males are responsible for fifty percent of the genetic material of a farm, however their importance is increased by the fact that one single male is used for every 10 females. Bongalhardo, Dionello, Cardellino, and Braccini Neto (1994) found a positive correlation between breeding fertility and egg fertility, enhancing the economic importance of reproductive factors in poultry farming.

Lipids are one of the main chemical components of sperm and sperm membranes (Silva & Guerra, 2011). High concentration of polyunsaturated fatty acids in sperm membrane increases the risk of peroxidation, being necessary to protect them with antioxidant substances.

The olive oil extraction industry may have an excellent alternative to waste management, since the waste oil (WO) from olive oil extraction may be used for animal nutrition. This waste has potential to meet bird's nutritional requirements, and may protect sperm cell from reactive oxygen species, since it contains important antioxidant substances like phenols, tocopherols, selenium, and vitamin E (Cioffi et al., 2010; D'amato et al., 2014).

Thus, the objective of this study was to evaluate the inclusion of waste oil from olive oil extraction in heavy roosters' diets on their reproductive parameters and weight gain between 25 and 39 weeks of age.

## Material and methods

Waste oil was collected in February 2017, from an olive oil extraction industry located in southern Brazil; and the experiment was performed from February to June, 2017. This experiment was approved by the Ethics Committee of the Federal University of Pelotas under the number 9088.

Forty roosters of a heavy commercial strain, with an initial age of 25 and final 39 weeks, were used. Birds were housed in an area of 100 $m^2$, with all environmental variables (temperature, humidity, and luminosity) controlled according to physiological needs of males breed and age. The animals were distributed in a completely randomized design, with twenty males per treatment. Each male was individually housed in Polyvinyl chloride (PVC) boxes, with dimensions of 120 cm of length, 100 cm of width, and 70 cm of height. Boxes contained a semiautomatic tubular feeder and two nipple drinkers. Bed covering the floor was composed of rice husk, with 10 cm of height. Each box, containing a male, was considered an experimental unit.

The light program provided 14 hours of artificial light from the birds' 22 weeks of age, with light intensity of 40 to 60 lux, and 10 hours of darkness. Photoperiod was controlled by an analogic timer (TMAØBC, Exatron, Brazil) turning the lamps on at 7:00a.m., and turning them off at 9:00p.m. The light program remained the same throughout the whole experimental period. Room temperature and humidity were controlled by a thermal sensor connected to an exhaust system, with thermal temperature limit of 20°C for hoods activation.

Two diet formulations for heavy-males were used, corresponding to nutritional requirements of pre-breeding (18 to 27 weeks) and breeding phase (28 weeks to the end), according to lineage recommendations.

The experiment had two treatments, the first one (control) consisted of a diet based on corn, soybean meal, and soybean oil; and the second, the test diet (WO), had the soybean oil totally replaced by the waste oil of olive oil extraction. Diets composition for both treatments at different reproductive stages can be observed in Table 1 and the nutritional levels in Table 2. In addition, Table 3 shows the fatty acid composition of the waste oil. Super Crac program (TD Software Ltda®, Brazil) was used to calculate diets, which were isoenergetic and isoproteic for both treatments and life stages.

To monitor weight gain, males were individually weighed weekly, on a commercial digital scale (Elgin® DP15, Brazil) with a capacity of 15 kg. After weighing, treatment mean weight was calculated, and feed intake was adjusted for each animal according to its individual weight. Every fifteen days, ration increments of 0.1 to 0.4 g were performed to stimulate reproductive performance and to prevent weight loss. Feed was supplied once a day, always at the same time (first hour in the morning), and water was ad libitum.

Experimental diets supply, and semen collection training, started fifteen days before trials period. After that, weekly semen collections, using dorsal-abdominal massage method proposed by Burrows and Quinn (1937) were made, however, only biweekly data were considered for males' reproductive performance evaluation. Thus, in total, thirteen semen collections were performed during the experimental period, but for analysis, only seven were used.

**Table 1.** Experimental diets for heavy roosters in different phases of life.

| Ingredients (%) | Pre-Breeding | | Breeding | |
|---|---|---|---|---|
| | Control | WO | Control | WO |
| Corn grain | 61.50 | 61.00 | 66.00 | 65.50 |
| Soybean meal | 20.00 | 20.00 | 13.50 | 13.50 |
| Wheat bran | 4.00 | 4.00 | 5.00 | 5.00 |
| Dicalcium phosphate | 2.00 | 2.00 | 2.00 | 2.00 |
| Limestone | 1.00 | 1.00 | 1.00 | 1.00 |
| Soy oil | 2.25 | 0.00 | 2.25 | 0.00 |
| Waste oil | 0.00 | 2.25 | 0.00 | 2.25 |
| Premix[1] | 0.70 | 0.70 | 0.70 | 0.70 |
| Sodium bicarbonate | 0.30 | 0.30 | 0.30 | 0.30 |
| Common salt | 0.25 | 0.25 | 0.25 | 0.25 |
| DL - methionine | 0.02 | 0.02 | 0.02 | 0.02 |
| Inert | 7.98 | 8.48 | 8.98 | 9.48 |
| Total (%) | 100.00 | 100.00 | 100.00 | 100.00 |

[1]Guarantee levels per kilogram of product: Biotin 0.04 g, Copper 2.4 g, Iron 8.0 g, Iodine 0.3 g, Total Lysine 8.7%, Manganese 15.0 g, Total Methionine 13.8%, Calcium Pantothenate 2.6 g, Selenium 0.072 g, K3 Vitamin 0.4 g, A Vitamin 2,000,000 IU, Zinc 20 g, Choline 77 g, Halquinol 6,000 mg, B1 Vitamin 0.4 g, B12 Vitamin 0.004 g, B2 Vitamin 1.60 IU, B6 Vitamin 0.6 g, D3 Vitamin 600,000 IU, E Vitamin 10,000 IU, Folic Acid 0.48 g. WO = waste oil.

**Table 2.** Nutritional levels of the experimental diets for heavy roosters in different phases of life.

| | Nutritional levels | | | |
|---|---|---|---|---|
| | Pre-Breeding | | Breeding | |
| | Control | WO | Control | WO |
| ME (kcal $kg^{-1}$) | 2760 | 2760 | 2758 | 2758 |
| CP (%) | 15.54 | 15.54 | 12.93 | 12.93 |
| Ca (%) | 1.00 | 1.00 | 0.95 | 0.95 |
| P avail. (%) | 0.47 | 0.47 | 0.46 | 0.46 |
| Total Met. (%) | 0.36 | 0.36 | 0.33 | 0.33 |
| Met. + total cystine (%) | 0.54 | 0.54 | 0.47 | 0.47 |
| Total Lysine (%) | 0.82 | 0.82 | 0.65 | 0.65 |
| Na (%) | 0.20 | 0.20 | 0.20 | 0.20 |

ME = metabolizable energy; CP = crude protein; Ca = calcium; P avail. = available phosphorus; Met. = Methionine; Na = total sodium; WO = waste oil.

**Table 3.** Chemical composition of waste oil (WO) from the olive oil extraction process.

| Fatty Acids | Composition (%) |
|---|---|
| Tetradecanoic C14 | 0.01 |
| Pentadecanoic C15 | 0.01 |
| Hexadecanoic C16 | 14.13 |
| Eicosanoic C20 | 1.06 |
| Tricosanoic C23 | 0.22 |
| Tetracosanoic C24 | 0.33 |
| Hexadecenoic C16:1 | 1.09 |
| Oleic C18:1 | 71.72 |
| Linoleic C18:2 | 11.07 |
| Eicosenoic C20:1 | 0.19 |
| Eicosadienoic C20:2 | 0.01 |
| Eicosatrienoic C20:3 | 0.10 |
| Eicosatetranoic C20:4 | 0.04 |
| Docosenoic C22:1 | 0.01 |
| Docosahexanoic C22:6 | 0.01 |

After collection, semen was stored in Falcon tubes with 0.1 mL graduation, and transported in a thermal box, to protect from ambient temperature variations. Reproductive variables analyzed were sperm volume, motility, concentration, and morphology.

Semen volume was checked directly in the collection tube after ejaculate sedimentation to tubes bottom. Immediately afterwards, semen was diluted 1:1 (vol/vol) with Lakes diluent (Bootwalla & Miles, 1992) at room temperature.

For sperm motility evaluation, 10 µL of diluted and homogenized semen was placed on a glass slide, and cells were visualized in an objective microscope (BX 41 Olympus America, Inc., São Paulo, São Paulo State, Brazil) at 400× magnification (Daramola et al., 2016). Sperm motility evaluation was subjective, always

performed by the same technician, and expressed in percentage (%), assigning values of 0 - 100%, where 0% represented all cells immotile and 100% all motile. Evaluation was always made in duplicate and the final value is the arithmetic mean of two observations.

Sperm concentration was analyzed using spectrophotometer at a wavelength of 450 nm (Micronal B542®, Brazil), adding 6 µL of semen diluted in Lakes in 3 mL of 2.9% sodium citrate with 0.4% glutaraldehyde (final dilution of 1:1000). Observed values were converted to billions of sperm per milliliter of ejaculation

To evaluate sperm morphology, eosin-nigrosin staining was used, and damaged portions of cells were visualized under optical microscope (BX 41 Olympus America, Inc., São Paulo, São Paulo State, Brazil) (Daramola et al., 2016). Quantification of sperm defects was performed by counting 100 cells, which were classified as normal or with head or tail defects. Morphology values are presented in percentages.

Repeated measures experimental design was used, with twenty animals per treatment, and each rooster representing an experimental unit. Statistical analysis was performed in Statistix 8.0 program (Analytical Software®, USA). Data normality of each variable was verified through Shapiro-Wilk test. When data presented normal distribution, they were submitted to repeated measures analysis of variance, and means were compared by Tukey test using five per cent of significance. For non-parametric data, transformation by square root arc sine [Arcsin (sqrt)] or logarithmic [Log (x)] was made before analysis. Data that did not present normal distribution, even after transformation, were submitted to Kruskal-Wallis non-parametric test. For better interpretation purposes all data are presented in their original scale.

## Results and discussion

Means and standard errors of sperm volume are demonstrated in Table 4 and there was no statistical difference between treatments at the different collection periods for this variable ($p > 0.05$). In period 2, 4, and 6 it was necessary to transform the seminal volume data for [Arcsin (sqrt)] in both treatments.

**Table 4.** Seminal volume (mean ± standard error) of roosters fed diets control or containing waste oil (WO) from the extraction of olive oil during different collection periods.

| Collected | Seminal volume (mL) | | |
|---|---|---|---|
| | Control | WO | P-Value |
| 1 | 0.48 ± 0.10 | 0.32 ± 0.07 | 0.164$^{ns}$ |
| 2 | 0.57 ± 0.1 | 0.50 ± 0.10 | 0.763$^{ns}$ |
| 3 | 0.48 ± 0.09 | 0.67 ± 0.08 | 0.097$^{ns}$ |
| 4 | 0.56 ± 0.06 | 0.59 ± 0.10 | 0.817$^{ns}$ |
| 5 | 0.53 ± 0.09 | 0.49 ± 0.08 | 0.950$^{ns}$ |
| 6 | 0.57 ± 0.06 | 0.56 ± 0.06 | 0.870$^{ns}$ |
| 7 | 0.33 ± 0.06 | 0.43 ± 0.06 | 0.245$^{ns}$ |

$^{ns}$There was no statistical difference by the Tukey test ($p > 0.05$).

Factors such as temperature, diet, sanity, lineage, and semen collection method can influence on seminal volume (Bongalhardo, 2013). Results observed in the present work for this variable are above values found for roosters local breed aged 54 weeks by Kacel and Iguer-Ouada (2018), who obtained a mean seminal volume of 0.34 mL for control animals and 0.39 mL for males who received 0.2 mL daily of olive oil.

Regarding diets, lipid source may influence on volume ejaculated, since oils are sources of cholesterol, which is a precursor of steroid hormones such as testosterone (Hall, Irby, & Kretser, 1969). Testosterone, in turn, promotes better development of tests, whose size and weight have a positive correlation with reproductive parameters (Wilson, Krista, Mcdaniel, & Sutton, 1988; Rosa, Stefanello, & Ferrufino, 2012; Feng et al., 2015). Kacel and Iguer-Ouada (2018) used only 0.002% of olive oil "*on top*" when feeding males. Thus, it is possible that the higher seminal volume observed in our study is related to the higher level of oil (2.25%) in the diet.

Evaluating seminal quality of rabbits submitted to diets with different sources of oil, including olive oil, Lancellotti et al. (2013) did not find differences for seminal volume. However, in a second experimental period, males previously submitted to an hypercholesterolemic diet (6.5% saturated fat and 0.05% cholesterol), and later supplemented with a diet containing olive oil, had better reproductive indexes, demonstrating that fatty acid profile present in olive oil promoted animals reproductive efficiency recovery.

As in the seminal volume, there was no statistical difference between treatments for a sperm motility and concentration variables. Means and standard errors can be seen in Table 5.

**Table 5.** Sperm motility and concentration (mean ± standard error) of roosters fed diets control or containing waste oil (WO) from the extraction of olive oil during different collection periods.

| Collected | Sperm Motility (%) | | | Sperm Concentration ($10^9$ mL$^{-1}$) | | |
|---|---|---|---|---|---|---|
| | Control | WO | P-Value | Control | WO | P-Value |
| 1 | 99.00 ± 1.00 | 93.57 ± 2.83 | 0.153$^{ns}$ | 2.90 ± 0.52 | 4.00 ± 0.44 | 0.135$^{ns}$ |
| 2 | 80.83 ± 8.97 | 82.00 ± 8.07 | 1.000$^{ns}$ | 1.99 ± 0.34 | 2.47 ± 0.31 | 0.314$^{ns}$ |
| 3 | 94.64 ± 2.94 | 94.00 ± 1.77 | 0.295$^{ns}$ | 2.29 ± 0.30 | 2.87 ± 0.29 | 0.167$^{ns}$ |
| 4 | 91.36 ± 4.16 | 97.27 ± 1.24 | 0.712$^{ns}$ | 2.89 ± 0.34 | 2.46 ± 0.39 | 0.420$^{ns}$ |
| 5 | 93.07 ± 3.02 | 95.29 ± 1.51 | 0.893$^{ns}$ | 2.81 ± 0.32 | 2.78 ± 0.28 | 0.946$^{ns}$ |
| 6 | 82.63 ± 5.27 | 89.00 ± 3.71 | 0.362$^{ns}$ | 3.34 ± 0.30 | 3.54 ± 0.29 | 0.630$^{ns}$ |
| 7 | 74.17 ± 0.11 | 73.18 ± 0.11 | 0.903$^{ns}$ | 2.04 ± 0.24 | 1.91 ± 0.30 | 0.669$^{ns}$ |

$^{ns}$There was no statistical difference by the Tukey test ($p > 0.05$).

Sperm motility has a positive correlation with sperm membrane total lipid composition, and a negative correlation with saturated fatty acids composition. Changes in fatty acid profile through different sources of fat offered to animals could result in differences in motility (An-im, Kirkwood, Techakumphu, & Tantasuparuk, 2011). However, this was not observed in our study, where sperm motility was not distinct between treatments.

Cardoso et al. (2014) also did not observe any significant difference in rodents that received, through gavage, different substances, among them olive oil. The same was verified by Lancellotti et al. (2013) and El-Kholy et al. (2015), who did not observe changes in motility by including olive oil in diets of rabbits and rats, respectively. Mangiagalli, Martino, Smajlovic, Cavalchini, and Marelli (2010) did not observe a significant effect on sperm motility of Ross-308 roosters of control group (70,24%) and animals of group supplemented with lycopene (76,20%), an important pigment present in fruits like tomatoes and which has antioxidant activity, promoting protection of cell membranes against oxidative stress. (Garrido et al., 2012).

In our study, a low motility value was observed in period seven, probably due to the low temperature (13.8°C) and high humidity (91%) recorded on the day of collection, which may have caused cold thermal stress to birds. According to Lara and Rostagno (2013), stress represents an organism reaction to stimuli that unbalance body homeostasis. Literature reports that temperatures between 18 and 25°C are within the thermal comfort zone, or thermoneutrality, of adult birds. Therefore, values lower than 18°C may result in cold stress and impact on animals performance (Tinôco, 2001; Zhang et al., 2016).

Malik et al. (2013) evaluated semen of domestic roosters and observed a mean concentration of 2.73 billion cells mL$^{-1}$, which is similar to our results. However, several studies evaluating reproductive performance of domesticated roosters have found higher values.

Mangiagalli et al. (2010), for heavy roosters aged 25 - 42 weeks, observed a mean concentration of $3.37\times10^9$ mL$^{-1}$ for control animals and $4.24\times10^9$ mL$^{-1}$ for animals receiving lycopene in the diets, with no statistical difference between treatments. Triques et al. (2016) worked with 50-week old heavy roosters and obtained a mean concentration of $3.39\times10^9$ mL$^{-1}$ for control and $4.69\times10^9$ mL$^{-1}$ for animals that received antioxidants in the diet. Moyle, Yoho, Whipple, Donoghue, and Bramwell (2012) observed an even higher average of 6.41 billion/mL of sperm cells in the ejaculate of roosters subjected to light stimulation at 21 weeks of age.

The difference of values of sperm concentration found in this study among studies by Moyle et al. (2012), Mangiagalli et al. (2010), and Triques et al. (2016) can be justified due to the low seminal volume found by these authors.

According to Bongalhardo (2013), sperm concentration is influenced by seminal plasma volume and can range from one to five billion sperms per milliliter. Therefore, there is a tendency for lower sperm concentrations when there is greater seminal plasma production.

Evaluating reproductive parameters of rabbits, Lancellotti et al. (2013) did not observe a statistical difference between diets (control and with olive oil) in sperm concentration. However, they observed that inclusion of olive oil, containing saturated fatty acids and 0.05% of cholesterol, promoted a reestablishment of sperm concentration. Similarly, El-Kholy et al. (2015) found that the addition of 30% of olive oil in the diet of albino rats promoted significant improvements in sperm cell concentration.

Although there was no statistical difference between treatments for motility and sperm concentration when all values are evaluated regardless of the treatment applied there was a difference between the collection periods for both variables (Table 6).

**Table 6.** Sperm motility and concentration (mean ± standard error) of heavy roosters during different collection periods.

| Collect | N | Sperm motility (%) | Sperm concentration ($10^9$ $mL^{-1}$) |
|---|---|---|---|
| 1 | 12 | 96.29 ± 2.73$^{a}$ | 3.55 ± 0.36$^{ab}$ |
| 2 | 27 | 81.42 ± 1.82$^{ab}$ | 2.26 ± 0.23$^{b}$ |
| 3 | 29 | 94.32 ± 1.76$^{a}$ | 2.59 ± 0.21$^{ab}$ |
| 4 | 22 | 94.32 ± 2.02$^{a}$ | 2.68 ± 0.26$^{ab}$ |
| 5 | 30 | 94.19 ± 1.73$^{a}$ | 2.80 ± 0.21$^{ab}$ |
| 6 | 39 | 85.82 ± 1.52$^{ab}$ | 3.44 ± 0.20$^{a}$ |
| 7 | 23 | 73.67 ± 1.97$^{b}$ | 1.98 ± 0.19$^{b}$ |
| P-Value | | 0.004 | 0.018 |

$^{ab}$Means with different letters in the same column differ from each other by the Tukey test ($p < 0.05$).

In period seven, motility was lower (73.67%) than in periods one, three, four, and five (96.29, 94.32, 94.32, and 94.19%, respectively). For sperm concentration, periods two and seven presented the lowest values of sperm cells per mL of ejaculate (2.26 and 1.98, respectively), differing from period six, which recorded the highest mean (3.44 billion cells $mL^{-1}$).

In the collection period seven, as previously discussedin motility, the lowest temperature and highest humidity were recorded, which may have been a determinant factor also in sperm concentration. In the second period, the reverse occurred: the highest temperature (26.8°C) and the maximum relative humidity (86%) were recorded. Birds, in particular, are species that suffer more from thermal stress than other animals, because they have higher metabolic rates and thus produce greater body heat. In the present study, effect of heat stress on sperm motility was not statistically significant. Karaca, Parker, and Macdaniel (2002), in a study with 57 week old heavy roosters submitted to elevation of body temperature concluded that thermal stress results in damage to sperm physiology and depression in male fertility.

Table 7 presents means and standard errors of sperm morphology. It was necessary to transform the tail morphology data to [LOG (x)] in both treatments. There was no statistical difference ($p > 0.05$) between treatments regarding normal sperm cells and cells with head abnormalities. However, a significant difference ($p = 0.01$) was observed between treatments for cells with tail defects, where birds fed with residual oil in the diet had a lower incidence of defects.

**Table 7.** Sperm morphology of heavy roosters fed control diets or containing waste oil (WO) from the extraction of olive oil.

| Diets | N | Normal (%) | Head (%) | Tail (%) |
|---|---|---|---|---|
| Control | 89 | 84.80 ± 1.32$^{ns}$ | 4.70 ± 1.12$^{ns}$ | 10.50 ± 0.21$^{a}$ |
| WO | 87 | 87.83 ± 0.82$^{ns}$ | 4.33 ± 0.48$^{ns}$ | 7.84 ± 0.22$^{b}$ |
| P-Value | | 0.134 | 0.200 | 0.013 |

$^{ab}$Means with different letters in the same column differ from each other by the Tukey test ($p < 0.05$). $^{ns}$There was no statistical difference by the Tukey test ($p > 0.05$).

Defect in spermatozoa tail can be considered a decisive factor in fertility, since this structure is fundamental for sperm movement throughout the oviduct till the ovum. Abnormalities in this portion may cause decline in sperm motility and prevent fertilization (Al-Ani, 2013).

Lancellotti et al. (2013) observed that animals whose treatments contained olive oil in the diet presented the lowest percentage of abnormal spermatozoa (on average 20% of lesioned cells). Al-Ani (2013) tested the action of olive oil on the toxicity of cadmium inclusion in the diet of rats and concluded that olive oil promoted protection of the testicles of the animals against free radicals, which resulted in better reproductive indices, among them the percentage of normal sperm cells (68%), which was significantly better than the other treatments that did not contain olive oil.

Oil from olive trees has important chemical properties that act in sperm cell preservation and quality (Banihani, 2017). Rich in oleic acid, antioxidants, and phenolic compounds, olive oil prevents the formation of free radicals that promote lipid peroxidation. Thus, there is a decrease in the occurrence of plasma membrane damage, and spermatozoa functionality and morphology.

Figure 1 shows weight gain curves of animals from both treatments, as well as the expected lineage curve. There was a statistically significant difference between treatments during period three ($p = 0.04$) and seven ($p = 0.04$). In both periods, animals whose diet had soybean oil totally replaced by waste oil had lower body weight than control animals.

Oil from olives has high concentration of oleic acid, about 70%, and low concentrations of saturated fatty acids, mainly myristic and lauric (Mendoza et al., 2013; Wedyan, Abu Hanieh, Al Harasheh, & Altawaha,

2017). In soybean oil, concentrations are very different from olive oil: it presents only 14.5% of oleic acid, while myristic and lauric acids have high concentrations, 41% and 14.4%, respectively (Sadamade, Oyedepo, & Bolaji, 2013). According to Zambiazi, Przybylski, Zambiazi, and Mendonça (2007), ingestion of sources rich in saturated fatty acids are related to higher rates of plasma cholesterol and obesity. However, when increasing consumption of polyunsaturated fatty acids and monounsaturated ones present in olive oil, for example, the saturated profile is improved.

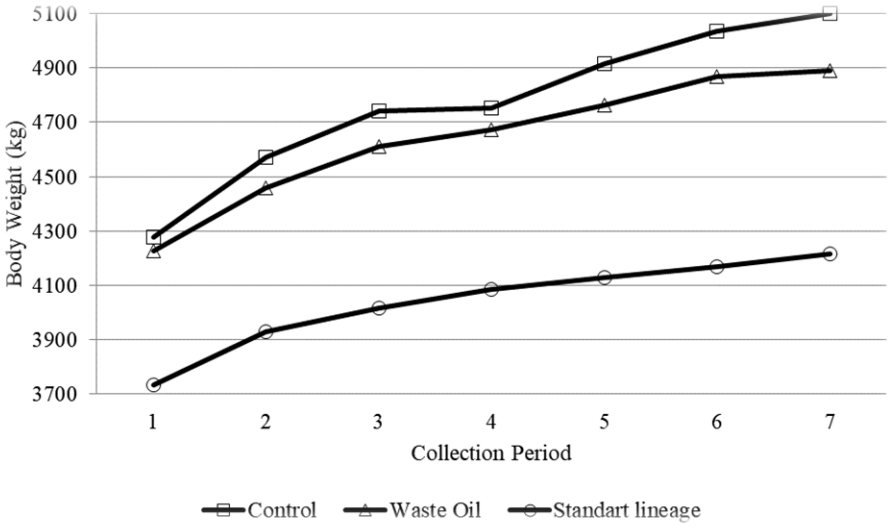


**Figure 1.** Weight gain curve of heavy roosters fed diets control or containing waste oil from the extraction of olive oil during different periods of collection.

It is expected that heavy matrices will gain weight as the age advances, and this was observed in our work. Lighter males enhance flocks' fertility and prolong their longevity. In addition, there is a decrease in males foot and joint injuries occurrence as well as incidence of females lesions caused by natural mating (Lara, 2015). Hocking and Bernard (2000) emphasize the importance of understanding that excessive weight gain in male breeding animals causes a decrease in batch fertility, not only due to structural problems in animals anatomy that prevent natural mating, but also due to dominance of heavier males over the lighter ones.

In broiler breeders, weight gain and reproductive traits have negative correlation (Djermanovic, Mitrovic & Djekic, 2013). Silveira et al. (2014) found better fertility and egg hatchability in batches whose males received diets specific to their nutritional requirements and, therefore, presented better control of weight gain compared to control animals, resulting in higher productivity.

In a study with broilers fed diets containing bovine fat (control) or olive oil, Zhang, Zhou and Kim (2013) observed that weight gain of animals that received olive oil in the diet, in 0-35 days old period, was lower than control animals, demonstrating the influence of lipid source on body weight control. Therefore, use of residual oil in heavy roosters diet may help to control the animals' weight gain.

## Conclusion

Total replacement of soybean oil by waste oil from olive oil extraction in young heavy roosters' diet does not affect reproductive characteristics such as sperm volume, motility, and concentration. It reduces morphological injuries in spermatozoa tail and promotes better control of male weight gain.

## Acknowledgements

The company Azeites Batalha® for supplying the waste to carry out the work and Polinutri® Alimentos S.A. for the supply of vitamins, minerals, and amino acids to the roosters' diets.

## References

Al-ani, N. K. H. (2013). Protective influence of olive oil on reproductive parameters in male rat treated with cadmium. *Global Journal of Bio-Science and Biotechnology*, *2*(4), 500-505.

Alqaisi, O., Ndambi, O.A. & Williams, R.B. (2017). Time series livestock diet optimization: cost-effective broiler feed substitution using the commodity price spread approach. *Agricultural and and Food Economics*, *5(25)*. DOI: https://doi.org/10.1186/s40100-017-0094-9

An-im, N., Kirkwood, M., Techakumphu, M., & Tantasuparuk, W. (2011). Lipid profiles of sperm and seminal plasma from boars having normal or low sperm motility. *Theriogenology*, *75*(5), 897-903. DOI: https://doi.org/10.1016/j.theriogenology.2010.10.032

Banihani, S. A. (2017). Semen quality as affected by olive oil. *International Journal of Food Properties*, *20*(2), 1901-1906. DOI: https://doi.org/10.1080/10942912.2017.1357044

Bongalhardo, D., Dionello, N. J., Cardellino, R. A., & Braccini Neto, J. (1994). Repetibilidade e correlações fenotípicas do caráter volume de sêmen de galos Withe Leghorn. *Revista da Sociedade Brasileira de Zootecnia*, *23*(6), 1002-1007. DOI: https://doi.org/10.1590/S1516-35982000000500009

Bongalhardo, D. C. (2013). Production and preservation of rooster semen. *Revista Brasileira de Reprodução Animal*, *37*(2), 131-135.

Bootwalla, S. M., & Miles, R. D. (1992). Development of diluents for domestic fowl semen. *World's Poultry Science Journal*, *48*(2), 121-128. DOI: https://doi.org/10.1079/WPS19920012

Borges, C. A. Q., Rostagno, H. S., Silva, J. H. V., Albino, L. F. T., Torres, C. A. A., Jordão Filho, J., & Ribeiro, M. L. G. (2006). Protein requirements and carcass composition of male broiler breeders from 27 to 61 weeks. *Revista da Sociedade Brasileira de Zootecnia*, *35*(5), 1971-1977. DOI: https://doi.org/10.1590/S1516-35982006000700013

Burrows, W. H., & Quinn, J. P. (1937). The collection of spermatozoa from the domestic fowl and turkey. *Poultry Science*, *16*(1), 19-24. DOI: https://doi.org/10.3382/ps.0160019

Cardoso, T. F., Varela Junior, A. S., Silva, E. F., Vilela, J., Hartmann, A., Jardim, R. D., ... Corcini, C. (2014). Influence of mineral, olive or sunflower oils on male reproductive parameters in vitro – the wild rodent *Calomys laucha*. *Andrologia*, *46*(7), 722-725. DOI: https://doi.org/10.1111/and.12138

Cioffi, G., Pesca, M. S., Caprariis, P., Braca, A., Severino, L., & Tommasi, N. (2010). Phenolic compounds in olive oil and olive pomace from Cilento (Campania, Italy) and their antioxidant activity. *Food Chemistry*, *121*(1), 105-111. DOI: https://doi.org/10.1016/j.foodchem.2009.12.013

D'amato, R., Proietti, P., Nasini, L., Del Buono, D., Tedeschini, E., & Businelli, D. (2014). Increase in the selenium content of extra virgin olive oil: quantitative and qualitative implications. *Grasas & Aceites*, *65*(2), 1-9. DOI: https://doi.org/10.3989/gya.097313

Daramola, J. O., Adekunle, E. O., Onagbesan, O. M., Oke, O. E., Ladokun, A. O., Abiona, J. A., ... Adeleke, M. A. (2016). Protective effects of fruit-juices on sperm viability of West African Dwarf goat bucks during cryopreservation. *Animal Reproduction*, *13*(1), 7-13. DOI: https://doi.org/10.4322/1984-3143-AR726

Dermeche, S., Nadour, M., Larroche, C., Moulti-mati, F., & Michaud, P. (2013). Olive mill wastes: Biochemical characterizations and valorization strategies. *Process Biochemistry*, *48*(10), 1532-1552. DOI: https://doi.org/10.1016/j.procbio.2013.07.010

Djermanovic, V., Mitrovic, S., & Djekic, V. (2013). Rooster body weight influence on the reproductive performance of the broilerparents. *Biotechnology in Animal Husbandry, 29*(1), 83-91. DOI: https://doi.org/10.2298/BAH1301083D

El-kholy, T. A. F., Al-abbadi, H. A., Qahwaji, D., Al-ghamdi, A. K., Shelat, V. G., Sobhy, H. M., & Hilal, M. A. (2015). Ameliorating effect of olive oil on fertility of male rats fed on genetically modified soya bean. *Food & Nutrition Research*, *59*(1), 1-6. DOI: https://doi.org/10.3402/fnr.v59.27758

Feng, Y., Ding, Y., Liu, J., Tian, Y., Yang, Y., Guan, S., & Zhang, C. (2015). Effects of dietary omega-3/omega-6 fatty acid ratios on reproduction in the young breeder rooster. *BMC Veterinary Research*, *11*(73), 1-7. DOI: https://doi.org/10.1186/s12917-015-0394-9

Garrido, M., Gonzáles-flores, D., Marchena, A. M., Prior, E., García-Parra, J., Barriga, C., & Moratinos, A. B. R. (2012). A lycopene-enriched virgin olive oil enhances antioxidant status in humans. *Journal of the Science of Food and Agriculture*, *93*(8), 1820-1826. DOI: https://doi.org/10.1002/jsfa.5972

Hall, P. F., Irby, D. C., & Kretser, D. M. (1969). Conversion of cholesterol to androgens by rat tests: Comparison of interstitial cells and seminiferous tubules. *Endocrinology*, *84*(3), 488-496. DOI: https://doi.org/10.1210/endo-84-3-488

Hocking, P. P., & Bernard, R. (2000). Effects of the age of male and female broiler breeders on sexual behaviour, fertility and hatchability of eggs. *British Poultry Science*, *41*(3), 370-388. DOI: https://doi.org/10.1080/713654925

Kacel, A., & Iguer-ouada, M. (2018). Effects of olive oil dietary supplementation on sperm quality and seminal biochemical parameters in rooster. *Journal of Animal Physiology and Animal Nutrition*, *102*(6), 1608-1614. DOI: https://doi.org/10.1111/jpn.12983

Karaca, A. G., Parker, H. M., & Mcdaniel, C. D. (2002). Elevated body temperature directly contributes to heat stress infertility of broiler breeder males. *Poultry Science*, *81*(12), 1892-1897. DOI: https://doi.org/10.1093/ps/81.12.1892

Khdair, A. I., Abu-rumman, G., & Khdair, S. I. (2019). Pollution estimation from olive mills wastewater in Jordan. *Heliyon*, *5*(8), 1-6. DOI: https://doi.org/10.1016/j.heliyon.2019.e02386

Lancellotti, T. E. S., Boarelli, P. V., Romero, A. A., Funes, A. K., Cid-Barria, M., Cabrillana, M. E., ... Fornés, M. W. (2013). Semen quality and sperm function loss by hypercholesterolemic diet was recovered by addition of olive oil to diet in rabbit. *Plos One*, *8*(1), 1-8. DOI: https://doi.org/10.1371/journal.pone.0052386

Lara, L. J. C. (2015). Reprodução nas aves: desafios do manejo e da nutrição. *Revista Brasileira de Reprodução Animal*, *39*(1), 85-90.

Lara, L. J., & Rostagno, M. A. (2013). Impact of heat stress on poultry production. *Animals*, *3*(2), 356-369. DOI: https://doi.org/10.3390/ani3020356

Malik, A., Haron, A. W., Yusoff, R., Nesa, M., Bukar, M., & Kasin, A. (2013). Evaluation of the ejaculate quality of the red jungle fowl, domestic chicken and bantam chicken in Malaysia. *Turkish Journal of Veterinary and Animal Science*, *37*(5), 564-568. DOI: https://doi.org/10.3906/vet-1107-26

Mangiagalli, M. G., Martino, P. A., Smajlovic, T., Cavalchini, L. G., & Marelli, S. P. (2010). Effect of lycopene on semen quality, fertility and native immunity of broiler breeder. *British Poultry Science*, *51*(1), 152-157. DOI: https://doi.org/10.1080/00071660903401540

Mendoza, M. F., Gordillo, C. M., Expósito, J. M., Casas, J. S., Cano, M. M., Vertedor, D. M., & Baltasar, M. N. F. (2013). Chemical composition of virgin olive oils according to the ripening in olives. *Food Chemistry*, *141*(3), 2575-2581. DOI: http://dx.doi.org/10.1016/j.foodchem.2013.05.074

Moyle, J. R., Yoho, D. E., Whipple, S. M., Donoghue, A. M., & Bramwell, R. K. (2012). Sperm production and testicular development of broiler breeder males reared on shortened growth cycles. *Journal of Applied Poultry Research*, *21*(1), 88-94. DOI: https://doi.org/10.3382/japr.2011-00363

Nunes, H., Zanine, A. M., Machado, T. M. M., & Carvalho, F. C. (2007). Foods Alternative in diet of sheep. *Archivos Latinoamericanos de Producción Animal*, *15*(4), 141-151.

Rosa, A. P., Stefanello, C., & Ferrufino, R. (2012). Influence of total or partial withdrawal of the crest on the reproductive status of broiler males. *Revista Portuguesa de Ciências Veterinárias*, *107*(581-582), 63-68.

Sadamade, A., Oyedepo, T. A., & Bolaji, O. S. (2013). Fatty acids composition of three different vegetable oils (soybean oil, groundnut oil and coconut oil) by high performance liquid chromatography. *Chemistry and Materials Research*, *3*(7), 26-29.

Silva, S. V., & Guerra, M. M. P. (2011). Cryopreservation effects on sperm cells and alternatives for crioinjury reduction. *Revista Brasileira de Reprodução Animal*, *35*(4), 370-384.

Silveira, M. M., Freitas, A. G., Moraes, C. A., Gomes, S. F., Litz, S. H., Martins, J. M. S., ... Fernandes, E. A. (2014). Feeding management strategy for male broiler breeders and its effects on body weight, hatchability and fertility. *Brazilian Journal of Poultry Science*, *16*(4), 397-402. DOI: http://dx.doi.org/10.1590/1516-635x1604397-402

Simonato, B., Trevisan, S., Tolve, R., Favati, F., & Pasini, G. (2019). Pasta fortification with olive pomace: Effects on the technological characteristics and nutritional properties. *LWT - Food Science and Technology*, *114*(8), 1-6. DOI: https://doi.org/10.1016/j.lwt.2019.108368

Tinôco, I. F. F. (2001). Industrial aviculture: New concepts of materials, conceptions and constructive techniques available for Brazilian poultry houses. *Revista Brasileira de Ciências Avícolas*, *3*(1), 1-26. DOI: https://doi.org/10.1590/S1516-635X2001000100001

Triques, G. E., Schimidt, J. M., Oro, C. S., Bordignon, H. F., Donin, D. G., & Fernandes, J. I. M. (2016). Effect of dietary antioxidant supplementation on reproductive characteristics of male broiler breeders during the post-peak production phase. *Semina: Ciências Agrárias*, *37*(4), 2557-2566. DOI: https://doi.org/10.5433/1679-0359.2016v37n4Supl1p2557

Wedyan, M., Abu Hanieh, B., Al Harasheh, A., & Altawaha, A. R. (2017). Chemical characterization of olive pomace in the northern region of Jordan. *Bulgarian Journal of Agricultural Science*, *23*(5), 866-872. DOI: https://doi.org/10.20944/preprints201703.0150.v1

Wilson, J. L., Krista, L. M., Mcdaniel, G. R., & Sutton, C. D. (1988). Correlation of broiler breeder male semen production and tests morphology. *Poultry Science*, *67*(4), 660-668. DOI: https://doi.org/10.3382/ps.0670660

Zambiazi, R. C., Przybylski, R., Zambiazi, M. W., & Mendonça, C. B. (2007). Fatty acid composition of vegetable oils and fat. *B. CEPPA Digital Library of Journals*, *25*(1), 111-120. DOI: http://dx.doi.org/10.5380/cep.v25i1.8399

Zhang, Z. F., Zhou, T. X., & Kim, I. H. (2013). Effects of dietary olive oil on growth performance, carcass parameters, serum characteristics, and fatty acid composition of breast and drumstick meat in broilers. *Asian-Australasian Journal of Animal Sciences*, *26*(3), 416-422. DOI: https://doi.org/10.5713/ajas.2012.12486

Zhang, S., Su, H., Zhou, Y., Li, X., Feng, J., & Zhang, M. (2016). Effects of sustained cold and heat stress on energy intake, growth and mitochondrial function of broiler chickens. *Journal of Integrative Agriculture*, *15*(10), 2336-2342. DOI: https://doi.org/10.1016/S2095-3119(15)61314-4