Research Article

Effects of Feeding Exhausted Olive Cake on Rabbit Performances, Visceral Development and Carcass Characteristics

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Abstract | Utilizing by-products from the olive oil sector in animal feed can become economically feasible for producers by offering alternative sources of dietary fiber, thereby expanding the potential applications of these by-products. This study aimed to assess the effects of incorporating Exhausted Olive Cake (EOC) into commercial rabbit diets on zootechnical performance, carcass characteristics, and visceral development. To achieve this, a total of 60 hybrid rabbits (New Zealand x Californian) were employed. Three distinct diets were formulated, progressively substituting alfalfa with EOC at levels of 0%, 5%, and 10%. Zootechnical performance was monitored by recording weekly feed consumption and growth performance. Visceral development and carcass traits were evaluated in 10 rabbits from each treatment group at 64 days of age. No significant differences were observed among the treatment groups, except for caecum length, which was greater in the control diet compared to the EOC diets, and cecal content dry matter, which was lower in the control diet relative to the EOC10 diet. Consequently, this study validates the potential utility of exhausted olive cake as a valuable component in rabbit fattening diets, serving as a substitute for dietary fiber in traditional formulations and contributing to reduced feed expenses.

Keywords | Exhausted olive cake, Rabbit, Carcass characteristics, Growth performance, Visceral development

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INTRODUCTION

The global consumption of olive oil has exhibited a consistent annual increase, likely attributed to its sensory qualities and beneficial health attributes (Dermeche *et al*., 2013; Gullón *et al*., 2020). Over the past six decades, olive oil production has witnessed a threefold growth, resulting in an annual yield of 3,000,000 tonnes (IOC, 2022). This escalation in production has also led to a proportional rise in by-products. The olive oil extraction process contributes to 20% of olive oil, with the remaining 80% constituting waste, encompassing 30% crude oil cakes (COC) and 50% olive mill effluent (Nasopoulou *et al*., 2011; Dermeche *et al*., 2013; Hansen, 2022). Following initial oil extraction through pressing and centrifugation, the COC can be repurposed for animal feed, compost, or undergo solvent-based extraction to capture residual olive oil, giving rise to refined olive oil and exhausted olive cake (EOC) (Nunes *et al*., 2016; Gullón *et al*., 2020).

In alignment with the objective of sustainable production and by-product valorization, diverse applications for EOC have been explored, predominantly in the energy sector (Christoforou and Fokaides, 2016), as constituents of thermoplastic composites (Siracusa *et al*., 2002), within concrete aggregates (Alkheder *et al*., 2016), and in soil enrichment (Khdair and Abu-Rumman, 2020). However, the integration of this by-product into animal feed remains an underexplored area. EOC holds potential as a valuable dietary fibre source for livestock animals, particularly monogastric species. While research predominantly centres on ruminants, such as cattle (Tufarelli *et al*., 2013; Tzamaloukas *et al*., 2021; Bionda *et al*., 2022; Marcos *et al*., 2022), limited studies exist with a focus on pigs, investigating enhancements in skin quality and processed products (Joven *et al*., 2014; Leite *et al*., 2022).

In the context of rabbit production, numerous investigations have examined the inclusion of olive cake in rabbit diets, primarily utilising COC. These trials have demonstrated the viability of incorporating olive cake into fattening diets without compromising growth performance. The consensus suggests that such inclusion improves the economic efficiency of rabbit production, given that feed constitutes a substantial proportion of overall costs (Kadi *et al*., 2004; Chaabane *et al*., 1997; Dorbane *et al*., 2016; Salama *et al*., 2016; Bakr *et al*., 2019; Dorbane *et al*., 2019; Rupić *et al*., 2019; Wahed *et al*., 2019; Azazi *et al*., 2020). Conversely, the use of EOC in rabbit production remains relatively unexplored.

Therefore, this study seeks to evaluate the effects of incorporating EOC into growing rabbit diets, with the dual objectives of reducing production costs and substituting other fiber sources.

MATERIALS AND METHODS

The experimental trial was conducted at UTAD research facilities. The study adhered to the guidelines outlined in Decree-Law n°113/2013 of 7th August, which enacts the Directive n°2010/63/EU concerning the protection of animals used for scientific purposes in Portuguese law. Additionally, the study followed the approved protocol by UTAD´s Animal Welfare Body (ORBEA), reference number 1058-e-DZ-2019.

Exhausted olive cake and experimental diets

In this study, extracted olive cake sourced from an industrial facility situated in the northeast region of Portugal was employed. The olive cake underwent dehydration in a forced ventilation dryer at 40°C for a duration of four days using a (Memmert UL 80) dryer. Subsequently, the Exhausted Olive Cake (EOC) was ground to an approximate particle size of 3 mm.

For the formulation of the feed, raw materials were procured and a basic fattening diet was formulated. Three dietary treatments were established, including a control diet (EOC0), and two experimental diets featuring 5% EOC (EOC5) and 10% EOC (EOC10). The incorporation of EOC was achieved by substituting lucerne with EOC.

All raw materials were finely ground, appropriately mixed, and subsequently processed through a granulator to create pelleted feed (refer to Tables 1 and 2 for details).

Table 1: Ingredients of the different experimental diets.

	Diets		
	EOC0	**EOC5**	**EOC10**
Ingredients (g/kg DM)			
EOC	00	50	100
Dehydrated lucerne	310	260	210
Sunflower pomace	158.4	158.4	158.4
Wheat	95.4	95.4	95.4
Beet pulp	94.1	94.1	94.1
Barley	45.1	45.1	45.1
Sugarcane molasse	53.7	53.7	53.7
Citrus pulp	54.2	54.2	54.2
Corn distillates	45.0	45.0	45.0
Wheat bran	44.1	44.1	44.1
Grape pomace	33.3	33.3	33.3
Sunflower husks	18.0	18.0	18.0
Vegetable oil	11.7	11.7	11.7
L-lysine	1.44	1.44	1.44
DL-Methionine	0.45	0.45	0.45
Salt	2.70	2.70	2.70
Monocalcium phosphate	9.90	9.90	9.90
Binder	18.0	18.0	18.0
Vitamin and mineral mix*	4.50	4.50	4.50
	1000.0	1000.0	1000.0

EOC0, EOC5 and EOC10 - diets with 0, 5 and 10% of exhausted olive cake, respectively. *vitamin A, 10,000 IU; vitamin D3, 1080 U; vitamin E, 36 mg; vitamin K, 1 mg; vitamin B1, 2 mg; vitamin B2, 6 mg; vitamin B6, 2 mg; vitamin B12, 10 mg; niacinamide, 50 mg; Ca- pantothenate, 20 mg; folic acid, 5 mg; Fe, 78 mg; Cu, 14 mg; Co, 0.5 mg; Mn, 20 mg; Zn, 60 mg; Se, 0.05 mg; I, 1.1 mg; choline chloride, 260 mg. In addition, Calcium carbonate, Luctarom 1408-Z, L-Threonine, Toxmystat, Luctanox, Sepiolite, NL-510-R, salt, Biolys 70 and Sodium bicarbonate.

Throughout the experimental duration, rabbits had *ad libitum* access to drinking water via nipple drinkers, as well as pelleted feed. Notably, no antibiotics were administered through the feed or drinking water during the course of this study.

Animals and experimental design

For this study, a total of 69 rabbits of both sexes, comprising

New Zealand x Californian rabbits (NZ x C), were selected. These rabbits were initially aged 35 days (weaning age) and possessed an average body weight of 1086± 54.8g. The study period extended to 64 days, corresponding to the slaughter age.

Table 2: Chemical composition of the diets and exhausted olive cake and lucerne.

Chemical composition (%)	Diets			EOC	Lucerne
	EOC0	EOC5	EOC10		
Organic matter	86.2	86.5	87.1	94.1	90.4
Neutral detergent fibre[1]	43.0	44.4	42.9	56.4	66.1
Acid detergent fibre	26.6	26.3	26.2	44.3	44.0
Acid detergent lignin	9.40	9.60	11.0	21.7	10.4
Crude protein	15.2	14.9	14.8	7.0	11.4
Crude fat	2.8	2.8	3.0	2.0	1.2
Gross energy (MJ/kg DM)	17.0	17.1	17.4	20.4	17.9
Digestible energy[2] (MJ/kg DM)	10.2	10.3	10.3	6.0	6.1

[1] NDForm; [2] Values obtained through the method by Fernandez-Carmona *et al*. (2005).

Of these 69 rabbits, 60 were randomly allocated across three distinct treatment groups and were individually housed in cages measuring 25x40x40 cm. It's noteworthy that littermates were evenly distributed among the various treatment groups, resulting in 20 replicates per treatment. An additional nine animals were equally divided into three collective cages, with each group comprising three rabbits. These collective cages were included to account for any mortalities that might occur within the respective treatment during the initial week of the study. All rabbits were provided *ad libitum* access to feed and had constant access to water.

The study was conducted within a controlled, enclosed facility, maintaining an ambient temperature range of 17°C-24°C. The facility featured a forced ventilation system and operated on a 12-hour light regimen (7:00 am to 7:00 pm).

Growth performance and slaughtering

During the 29-day trial period (from 35 to 64 days of age), growth performance parameters including animal weight and feed consumption were monitored on a weekly basis, while mortality rates were assessed daily. Any remaining feed in each cage was collected weekly, weighed, and factored into the calculation of feed consumption and the feed conversion ratio. As such, key metrics such as live weight (LW), average daily feed intake (ADFI), average daily gain (ADG), and feed conversion ratio (FCR) were determined.

The obtained results are presented for three distinct periods: the first period (35-49 days of age), the second period (49-64 days of age), and the overall trial period (35-64 days of age) encompassing the fattening phase.

At 64 days of age, a random selection of 10 rabbits per treatment, inclusive of both sexes, underwent slaughter following a fasting period of two hours. The slaughter process was performed via sudden cervical dislocation, adhering to the guidelines set forth by the overseeing body for animal welfare (ORBEA), as stipulated by legislation governing animal welfare in experimental research (Decree-Law No. 1/2019).

Gastrointestinal parameters

Following the slaughter and skinning of the rabbits, various organs including the stomach, small intestine, cecum, colon, heart, liver, spleen, and lungs were excised and their respective weights recorded. Additionally, the hot carcass weight was determined. Measurements were also taken for the lengths of the small intestine, cecum, and colon.

Immediately post-slaughter, the pH of the cecal contents was measured using a digital potentiometer (pH91, WTW, Germany).

In each animal, the cecum was initially weighed while containing its contents, then it was cleaned and reweighed when empty. The quantity of cecal content was calculated by taking the difference between these two weights. Prior to cleaning the cecum, two samples of cecal content were extracted and subjected to drying in an oven at 60°C for 24 hours to ascertain dry matter content.

Carcass and meat characteristics

The refrigerated carcass was obtained within a well-ventilated area and subsequently cooled to 3°C for a duration of 24 hours. The carcass was then sectioned, employing the technical cutting procedure outlined by Blasco *et al*. (1993). Subsequent to the carcass division, individual weights were recorded for the head, loin, ribs, legs, and shoulder of each animal.

Incisions were made in the leg sections to measure the ultimate pH (pHu) within the meat using a potentiometer (pH91, WTW, Germany). Furthermore, a colorimeter (Minolta CR-300 Chroma Meter, Osaka, Japan) was utilised to assess the external meat colour in the lumbar region, as well as the internal colour within the longissimus dorsi muscle.

Chemical analysis

Samples of both feed and Exhausted Olive Cake (EOC) were subjected to drying at 50ºC within an air-forced oven (Venticell, MMM Group, Munich, Germany). Post-drying, these samples were ground through a 1 mm mesh screen (Tecator Cyclotec 1093 Sample Mill, Foss SA, Sweden) and subsequently prepared for chemical analysis.

The determination of various nutritional parameters followed the procedures outlined by AOAC (2016). Specifically, the analysis included: Dry matter (DM, method no. 934.01), Organic matter (OM), Ash (method no. 942.05), Ether extract (EE, method no. 920.39), Total Nitrogen content, measured as N-Kjeldahl (method no. 954.01). Crude Protein (CP) content was computed based on the nitrogen content as per the CP method (method no. 954.01). Total Dietary Fiber, comprising Soluble and Insoluble Fiber (method no. 991.43), Additionally, Neutral Detergent Fiber without the use of sodium sulfite and α-amylase, as described by Van Soest *et al.* (1991) and Van Soest and Robertson (1980), was determined.

Statistical analysis

Statistical analysis was conducted using the JMP software (JMP® Pro 16.2.0). The data underwent one-way analysis of variance (ANOVA), with the dietary composition serving as the primary factor, employing the General Linear Model procedure. For the comparison of multiple means, a Tukey Honestly Significant Difference (HSD) test was executed. The predetermined threshold for statistical significance was set at 5%.

RESULTS AND DISCUSSION

Gender distribution was balanced for performance and slaughter tests. The effect of gender is not shown, as it did not influence any of the evaluated parameters, which was to be expected, since up to this age (about 70 days of age) the animals have not yet reached puberty. In current commercial strains, there seems to be little or no effect of gender on the parameters that were evaluated throughout the experiment, as referred by other authors (Iyeghe-Erakpotobor and Adeyegun, 2012; Martínez-Bas *et al.*, 2018).

Diets

The substitution of lucerne with exhausted olive cake (Table 1) at the assessed proportions in this study (5% and 10%) did not result in any significant alterations to the feed characteristics. The experimental diets exhibited comparability in their chemical composition (Table 2) across key parameters.

Growth performance

When analyzing the effects of EOC on the growth performance of the rabbits, no significant differences were found ($p > 0.05$), as shown in Table 3. During the trial, only two animals died in the first week (1 from the control and one from the EOC5 treatment) which were immediately replaced by rabbits that were fed the same diet. Due to the small number of dead rabbits, the effect of treatment on mortality was not analyzed.

Table 3: Effect of increasing levels of EOC supplementation on growth performances (n=20 per treatment).

	Diets			SEM	p value
	EOC0	EOC5	EOC10		
n	20	20	20		
Live weight (g)					
35 d	1093	1090	1123	23.15	0.824
49 d	1768	1759	1735	28.18	0.927
64 d	2502	2443	2424	33.6	0.621
Average daily gain (g/d)					
35-49 d	47.9	45.4	43.1	1.05	0.235
49-64 d	49.6	47.5	45.7	0.90	0.130
35-64 d	48.9	46.6	44.8	0.69	0.089
Average daily intake (g/d)					
35-49 d	134.2	137.9	129.6	3.82	0.701
49-64 d	162.0	149.2	152.4	3.49	0.348
35-64 d	147.9	143.6	140.5	2.70	0.546
Feed conversion ratio					
35-49 d	2.82	2.87	2.94	0.040	0.497
49-64 d	3.26	3.28	3.34	0.046	0.806
35-64 d	3.04	3.08	3.13	0.034	0.616

Visceral development

Visceral weight, either in absolute terms or relative to carcass weight, remains unaffected by dietary factors (Table 4). Dry matter content in the cecum exhibits differential outcomes across treatments, with animals subjected to the EOC10 treatment demonstrating elevated values compared to the control group.

In relation to the dimensions of the digestive organs, a significant effect ($p \leq 0.05$) was observed in the length of the cecum (Table 5). Animals on the control diet displayed the greatest lengths, which differed significantly from those that received EOC as part of their dietary regimen. Notably, there was a disparity of 5.7 cm for the EOC5 treatment and 5.6 cm for the EOC10 treatment in comparison to the EOC0 treatment.

Carcass characteristics

No significant differences were observed when examining the impact of various dietary regimens on slaughter weight, carcass yield, and drip loss. Likewise, no disparities were identified following the segmentation of the carcass into distinct sections (Table 6).

Table 4: Effect of increasing levels of EOC supplementation on viscera weight (n=10 per treatment).

	Diets			SEM	p-value
	EOC0	EOC5	EOC10		
Weight (g)					
Liver	99.4	106.5	105.1	2.80	0.563
Kidney	23.1	23.4	23.2	0.44	0.958
Heart	8.66	8.21	8.19	0.24	0.688
Lungs and thymus	23.2	21.6	22.6	0.70	0.644
Stomach	95.2	107.4	94.9	4.54	0.468
Small intestine	137.3	137.2	145.2	3.77	0.621
Colon	84.8	88.9	93.6	2.53	0.374
Full cecum	201.5	184.4	188.8	4.49	0.284
Empty cecum	34.8	36.1	35.8	0.65	0.735
Fresh cecal content	166.6	148.4	153.0	0.45	0.242
Dry cecal content	35.3	32.1	35.1	0.92	0.305
Weight (g/kg carcass)					
Liver	7.18	7.34	7.41	0.15	0.845
Kidney	1.67	1.62	1.64	0.03	0.846
Heart	0.63	0.57	0.58	0.01	0.380
Lungs and thymus	1.68	1.50	1.60	0.05	0.401
Stomach	3.95	3.97	4.38	0.178	0.564
Small intestine	5.69	5.52	6.06	0.155	0.367
Colon	3.52	3.59	3.87	0.538	0.751
Full cecum	8.35	7.47	7.83	0.161	0.081
Empty cecum	1.44	1.47	1.49	0.030	0.762
Cecum pH	6.11	6.04	5.90	0.043	0.136
Dry matter of cecal content (%)	21.2^{b}	21.7^{ab}	23.1^{a}	0.310	0.029

Table 5: Impact of increasing levels of EOC supplementation on viscera length (n=10 per treatment).

	Diets			SEM	p value
	EOC0	EOC5	EOC10		
Length (cm)					
Small intestine	341.4	352.9	329.5	6.65	0.358
Colon	121.7	125.3	125.9	2.68	0.800
Cecum	41.7^{a}	37.6^{b}	37.4^{b}	0.78	0.001
Length (cm/kg carcass)					
Small intestine	14.2	14.4	13.7	0.34	0.751
Colon	5.06	5.11	5.25	0.123	0.836
Cecum	2.39^{a}	2.12^{b}	2.17^{b}	0.041	0.014

Regarding carcass color and longissimus dorsi pHu, no statistically significant variations were detected among the treatment groups (Table 7).

The principal aim of this study was to investigate the feasibility of incorporating Exhausted Olive Cake (EOC) as a partial substitute for traditional dietary fiber in rabbit diets, and to ascertain its potential effects on performance and carcass characteristics within our experimental conditions. The utilization of agricultural by-products in animal nutrition has seen a notable surge in recent years, with many materials previously destined for energy generation now being redirected for the production of animal protein (Mutwedu *et al*., 2022). Exhausted olive cake is one such by-product that can serve as a valuable source of dietary fiber, enriched with lignin, in rabbit diets (Dorbane *et al*., 2016).

Table 6: Impact of increasing levels of EOC supplementation on carcass characteristics (n=10 per treatment).

	Diets			SEM	p value
	EOC0	EOC5	EOC10		
Weight (g)					
Final live weight	2370	2467	2401	33.7	0.507
Hot carcass	1430	1498	1474	24.3	0.527
Chilled carcass	1383	1451	1421	23.5	0.512
Carcass yield (%)					
Hot	60.2	60.69	60.43	0.352	0.395
Chilled	58.2	58.7	59.2	0.32	0.480
Drip loss (%)	3.32	3.15	3.62	0.132	0.358
Carcass segments					
Hot weight (g)					
Head	143.5	150.1	148.5	2.49	0.541
Legs	398.4	418.9	398.9	6.07	0.300
Loin	344.3	362.5	343.5	7.31	0.488
Ribs	142.9	155.2	139.6	3.88	0.231
Blades	155.8	170.6	162.5	2.87	0.108
Weight (g/kg chilled carcass)					
Head	10.4	10.4	10.5	0.14	0.949
Legs	28.9	28.9	28.1	0.29	0.428
Loin	24.9	25.0	24.1	0.27	0.423
Ribs	10.3	10.3	9.84	0.207	0.254
Blades	11.3	11.8	11.4	0.17	0.201
Dissected fat	2.67	2.99	3.12	0.125	0.318

Table 7: Impact of increasing levels of EOC supplementation on meat characteristics. (n=10 per treatment).

	Diets			SEM	p-value
	EOC0	EOC5	EOC10		
pHu (after 24h)	5.86	5.95	5.86	0.02	0.053
Color of the Lumbar region					
L	49.6	47.3	49.2	0.68	0.357
a	13.0	12.8	13.2	0.88	0.989
b	12.3	12.0	12.5	0.41	0.927
Internal color of *Longissimus dorsi*					
L	54.2	51.2	52.5	0.53	0.065
a	6.29	7.56	6.79	0.27	0.153
b	12.4	11.9	12.5	0.33	0.719

In this study, the partial replacement of lucerne with EOC did not exert any discernible impact on the chemical composition of the diets. Furthermore, no disparities in growth performance were observed among the animals receiving the different dietary formulations, implying that EOC represents a potential feedstock for rabbits without compromising their productive performance. While the literature on this specific topic in rabbits remains limited, our findings align with several studies employing varying levels of crude olive cake inclusion, ranging from 5% to 25%, which similarly reported no adverse effects on animal growth performance (Dorbane *et al*., 2016, 2019; Bakr *et al*., 2019; Wahed *et al*., 2019).

Regarding visceral development, differences between treatments were only observed in the dry matter content of cecal contents ($p < 0.05$) and cecal length ($p < 0.001$). The cecal dry matter content increased in the EOC10 treatment, indicating a direct influence of EOC on the percentage of dry matter within the cecum. This outcome aligns with the expectation that the percentage of dry matter in the cecum would increase with higher levels of indigestible fiber in the diet, as reported by Caîsin *et al*. (2020). In terms of cecal length, the control group exhibited the expected higher values, consistent with the normal range for cecal length in this breed, as indicated by Nath *et al*. (2016), which falls between 38.32-43.96 cm. However, for the treatments incorporating EOC, a reduction in cecal length was observed. This is in line with findings from Krieg *et al*. (2012) and Nicodemus *et al*. (1999), who reported a similar trend in their studies, where increased dietary lignin concentration led to a reduction in cecal development.

Regarding carcass characteristics, no statistically significant differences were detected in carcass yield, the weight of different cuts, pH, or color. Ben Rayana *et al*. (1994) also reported no differences in organ weights and carcass components when 11% olive pomace was included in the diet. Similarly, Bakr *et al*. (2019) did not observe differences in the development of certain organs (head, kidney, heart) and carcass components (empty carcass weight, edible part), even with olive pomace inclusions of up to 25%.

CONCLUSIONS AND RECOMMENDATION

In conclusion, the utilization of EOC did not yield significant gains, nor did it result in losses. EOC stands as a feasible raw material for incorporation into rabbit diets, offering economic advantages due to its status as a by-product of the olive oil industry, which is cost-effective compared to certain other sources of raw fiber. Moreover, it contributes to a more sustainable resource utilization, aligning with the principles of a circular economy.

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AUTHOR'S CONTRIBUTION

VP and PN: Conceptualization.
PN, JT, VP, JM and DM: Methodology.
PN: Software.
VP, JM, JT and DM: Validation.
JT, VP, DM: Formal analysis.
PN and VP: Investigation.
VP, JM and DM: Resources.
VP and PN: Data processing.
PN: Writing original draft preparation.
PN, VP, JT, JM and DM: Writing review and editing.
VP, JM and DM: Funding acquisition.
All authors have read and agreed to the published version of the manuscript.

Abbreviations

EOC, exhausted olive cake; COC, crude olive cake; LW, live weight; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio.

Conflicts of interest

The authors have declared no conflict of interest.

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