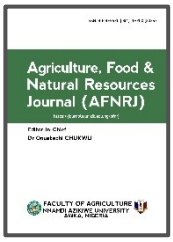
Agr. Foo. Nat. Res. J. Volume 4, Issue 2, Page 206 - 211

e-ISSN: 1597-7153 | p-ISSN: 3043-5420

Agriculture, Food and Natural Resources Journal

The Official Journal of the Faculty of Agriculture, Nnamdi Azikiwe University, Awka, Nigeria

Journal homepage: https://journals.unizik.edu.ng/afnrj



Original Article

Thermophysiology, haematology, reproductive hormones and antioxidative status of rabbit does fed graded levels of natural oils

OPEN ACCESS

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DOI: https://www.doi.org/10.5281/zenodo.17226807

Editor: *Dr Onyekachi Chukwu, Nnamdi Azikiwe University, NIGERIA*

Received: *February 28, 2025*
Accepted: *September 14, 2025*
Available online: *September 30, 2025*

Peer-review: *Externally peer-reviewed*

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Conflict of Interest: *The authors have no conflicts of interest to declare*

Financial Disclosure: *The authors declared that this study has received no financial support.*

ABSTRACT

The experiment investigated the effect of black seed oil (BSO), olive oil (OO), and shea butter oil (SBO) on selected physiological parameters in rabbit does to improve their productivity. The study lasted for 8 weeks. Twenty-eight rabbit does were randomly allotted to 7 treatments comprising control (0 ml), 0.5 ml, and 1.0 ml for each of BSO, OO, and SBO within a 2x3+1 factorial laid in a completely randomized design. Respiratory rate (RR) and rectal temperature (RT) of the does were determined twice a week throughout the study. Haematology, selected hormones, antioxidant enzymes, and capacity were evaluated at the end of the experiment. Data was subjected to analysis of variance, and significant means were separated using Duncan's Multiple Range Test ($\alpha<0.05$). Treatments had no significant effect ($p>0.05$) on RR. The interaction effect of the oils and their levels was significant on the RT of the does though only OO reduced RT as its level of inclusion increased. Oils and their levels had no effect on the blood profile of the does. The OO group had significantly higher ($p<0.05$) haemoglobin concentration, packed cell volume, and platelets compared to the control. Oestrogen was similar among treatments, while follicle-stimulating hormone and cortisol were significantly higher in does fed SBO and OO. Serum glucose, total cholesterol, total protein, antioxidant enzymes, and total antioxidant capacity were not different ($p>0.05$) among treatments. It was concluded that experimental oils did not impair the physiology, haematology and oxidative mechanism of studied animals. Hence, the oils could be utilized in improving rabbit does' production.

KEYWORDS: *Oestrogen, Oxidative mechanism, Physiology, Red blood cell, White blood cell*

INTRODUCTION

Rabbits are prolific and can efficiently convert fodder to food. Rabbits are raised for many different uses but the most common use is for meat, pelts, pet and laboratory use. However, rabbit production has recently gained attention as an effective means of alleviating poverty in developing countries (Siddiqui*et al*., 2023). Nevertheless, environmental and management factors such as ambient temperature as well as feed qualityimpede the production and reproduction of the rabbit (Ajao *et al*., 2022).

Farmers have sought many ways to diminish the constraints that militate against rabbit production in tropical regions including improved management practices, provision of quality nutrition and raising of hybrid rabbits among others (Siddiqui *et al*., 2023). However, the use of plant and plant extracts seems to be the most popular andnovel approach to achieve this objective (Daadeer *et al*., 2018). The bioactivity exhibited by these plants is due to phytochemicals which protect humans and animals

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against pathogens, stress conditions while enhancing reproductive efficiency (Ajao *et al*., 2024).

Respiratory rate and rectal temperature are among the foremost biophysical estimation of thermoregulation and can be used to assess stress or absence of it in mammals (Lenis *et al*., 2015).

Haematological evaluation can provide information on the effect of nutrition and environment on the productivity as well as welfare of farm animals (Olajide *et al*., 2024).

The antioxidant mechanism of an animal is ensured by antioxidant enzymes and non - enzymatic components. Antioxidant enzymes comprised superoxide dismutase, catalase and glutathione peroxidise while the non - enzymatic components are largely vitamins and other organic substances (Skowron *et al*., 2018). Hormones are the major biologic messengers that control body processes ranging from growth, reproduction and thermoregulation and their actions influence the robustness of the processes they mediate (Mattioli *et al*., 2021). Serum glucose, total cholesterol and total protein are important metabolic markers and are indicators of energy and protein metabolism as well as the health of important organs such as the liver and the kidney (Lozano *et al*., 2019).

Plants have complementary and overlapping mechanisms of action on the above-mentioned parameters. These include anti - stress effects, regulation of enzymes' actions, stimulation of the immune system and modulation of hormone secretion thereby enhancing animal's welfare and productivity (Farhan *et al*., 2021).Many studies have established the general efficacy of black seed (*Nigella sativa),* olive (*Olea europaea*) seed and shea (*Vitellaria paradoxa*) butter in improving the physiological responses of the rabbit in many part of the world (El-Ratel *et al*., 2021; Ahmed *et al*., 2019; Abd El-Monem *et al*., 2016).

However, there is dearth of information on the effects of black seed oil*,* olive oil *and* shea butter oil on the physiology of rabbit does in Nigeria. This information is needed in an age when plants and their extracts have become important in improving livestock reproduction, productivity and welfare especially with focus on the rabbit.

Hence, this study examined the effect of black seed oil*,* olive oil *and* shea butter oil on thermophysiology, haematology, reproductive hormones and antioxidative status of rabbit does.

MATERIAL AND METHODS

Experimental Site

The research study was conducted at a farm located in Oke - Odo Tanke, Ilorin, Kwara State. The research work lasted for 8weeks from the month of June 2023 to July 2023.

Experimental Animal and Management

The rabbits were sourced from reputable farmers in Kwara State. A total number of 28 growing female rabbit of average age of 5 months with an average weight of 1.5kg were used for the experiment. The rabbits were of mixed breed and were individually housed in cages made of wood and wire mesh. The rabbits were allowed to acclimatize for two weeks before starting the study. Feeders and water containers were thoroughly cleaned and washed on weekly basis throughout the experimental period. The animals were fed formulated concentrate (Table 1). The fed was divided into two and supplied to the animals in the morning (8:00am and 9am) and in the evening (5:00pm and 6:00pm) to avoid wastage. Feed and clean water were provided for the does *ad libitum*.

Table 1: Composition of Formulated Concentrate

Ingredients	Quantity (kg)
Maize	25.00
Wheat offal	45.00
Palm kernel cake	10.00
Soy bean cake	18.50
Salt	0.20
Vitamin premix	0.50
Lysine	0.50
Methionine	0.30
Total	100.00
Proximate Composition	
Crude protein (%)	18.10
Energy(kcal/kg)	2650

Experimental Design

The experimental animals were randomized to 7 treatment groups of black seed oil, olive oil and shea butter oil in a complete randomized design within a factorial outlay of 3x2+1. Each treatment group had 4animals. The experimental groups comprised of 0.0ml for the control and 0.5ml and 1.0ml for each of black seed oil, olive oil and shea butter respectively. The oils were fed to the animals at two days interval by means of a 2 ml syringe before feeding them in the morning.

Data Collection

Thermophysiology

Respiratory rate (RR) and rectal temperature (RT) of experimental rabbits were monitored twice in a week at 3 days interval. RR was determined by carefully observing and counting the flank movement of a still animal per minute after which RT was determined by using a digital clinical thermometer. The thermometer was inserted into the rectum of the rabbit and once the thermometer made a beeping sound, the digital figure displayed on the LCD of the thermometer was recorded.

Blood collection and haematological Analysis

At the end (week 8) of the study blood samples were obtained from the rabbits in each treatment group for the analysis of haematological parameters. The blood samples were collected from the rabbit's ear vein using a disposable 21-gauge needle. For haematological analysis, the blood samples were collected in bottles containing dipotassium ethylene diamine-tetra acetic acid (EDTA) as an anticoagulant. Haematological determined were red blood cells, white blood cells, haemoglobin, packed cell volume and platelets. An automatic haematological analyser was used for the analysis.

Hormonal, Biochemical and Antioxidant Assay

At the time of collecting blood samples for haematology, blood samples were also collected for hormonal, serum metabolites and antioxidant enzymes assay. Serum was harvested by centrifugation at 3500 revolution per minute for 15 minutes from the blood samples collected. Hormones assayed were oestrogen, follicle stimulating hormone and cortisol. Glucose, total cholesterol and protein were the serum metabolites assayed. Catalase, glutathione peroxidase, sodium dismutase and total antioxidant capacity of the studied animals were also investigated to determine their oxidative status. These parameters were assayed as described by El-Kholy *et al*. (2021) at the Bio Bridge Laboratory, Flower Garden, Ilorin, Kwara State.

Data Analysis

Data was analysed using the General Linear Method (GLM). Treatment means were separated using Duncan's Multiple Range Test with $\alpha < 0.05$. Statistical Package for Social Sciences version 25 was used for the statistical analysis.

RESULTS AND DISCUSSION

Respiratory rate (RR) (163.33FM/m - 166.35FM/m) (Table 2) was not significantly different ($p>0.05$) among experimental treatment regardless of the treatment levels. These values were lower than the findings (216.25c/m - 270.58c/m) of Ajao and Ola (2024) in heat stressed rabbits. In addition, interaction effect of treatments and their levels was not significant on RR implying that oil levels did not affect the impact of the oils. The observed non - significant effect of experimental oils on rabbit RR is similar to the reports of Abd El-Monem *et al*. (2016) implying that none of the administered oils affected the inflow or outflow of thermal energy of the animals. Rectal temperature (RT) (37.66°C - 40.30°C) was not significantly different ($p>0.05$) among treatments. In addition, RT was similar between oil levels with the exception of the olive oil (OO) group (as shown in the interaction effect of treatments and treatment levels) where RT value decreased (40.30°C to 37. 90°C) as level of oil increased. RT observed in the study with the exception of black seed oil (BSO) and OO at 2ml and 1ml respectively was lower than 39.24°C - 39.52°C reported by Al-Zafry and Medan (2012) in rabbits injected with vitamin E and selenium implying that the oils did not elicit the buildup of heat in the body. This can be due to secondary metabolites present in plants and their extracts can influence thermal homeostasis (Beale *et al*., 2018; Rahman *et al*., 2018).

The haematological profile of experimental rabbit does is shown in Table 3. Interaction effect of experimental oils and oil levels was not significant on the studied haematological parameters indicating that supplementation levels did not influence the impact of the oils. This result was different from the report of Umar *et al*. (2018) who fed black seed oil to male rabbits.

Table 2: Respiratory rate and rectal temperature of rabbit fed black seed oil, olive oil and shea butter oil

Factor	Respiratory rate (FM/m)	Rectal temperature (°C)
Treatment(T)		
Control	166.35	37.66
Blackseed Oil (BSO)	165.42	38.69
Olive Oil (OO)	163.38	39.11
SheabutterOil(SBO)	163.33	37.80
PSEM	1.12	0.60
Treatment Level (TL)		
0	166.35	37.66^{b}
BSO1	166.21	37.83^{b}
BSO2	164.63	39.55^{ab}
OO1	162.33	40.30^{a}
OO2	164.42	37.90^{b}
SBO1	162.63	37.72^{b}
SBO2	164.03	37.87^{b}
PSEM	1.49	0.80
P-value		
T	0.27	0.23
TL	0.59	0.77
T*TL	0.41	0.03

*^{ab}Means with different superscript within the column are significantly different ($p<0.05$). PSEM = Pooled standard error of mean; FM/m = Flank movement/minute; * = interaction*

Red blood cell (RBC) (4.54 - 5.37 x $10^{12}L^{-1}$) and white blood cell (4.96 - 5.09 x $10^{9}L^{-1}$) were not significantly different ($p>0.05$) among treatments and treatments levels. This implies that experimental oil did not disrupt RBC and WBC production. Haemoglobin concentration (54.33g/L - 109.17g/L), packed cell volume (22.77 % - 27.27 %) and platelets (22.33x $10^{9}L^{1}$ - 92.17x $10^{9}L^{1}$) were higher in rabbits fed olive oil compared to the control though animals fed black seed oil and shea butter oil had values similar to both. This report indicates that the administered natural oils improved the haematopoiesis process of the animals, maintain the blood oxygen carrying capacity and preserve their overall body defense. In addition, the result of this study is comparable to the conclusions of previous studies that fed natural additives including oils to rabbits which attributed the effect of the oils to their phytochemical content (El- Gindy *et al*, 2019, Alagbe and Oluwafemi, 2019, Idahor *et al.*, 2018, Ansah *et al.*, 2011).

Table 3: Haematological profile of rabbit does fed graded level of natural oils

Factor	Parameter				
	RBC ($x 10^{12}L^1$)	HGB (g/L)	PCV (%)	WBC ($x 10^9L^1$)	PLT ($x 10^9L^1$)
Treatment Level (TL)					
Control	4.54	54.33[a]	22.77[a]	5.00	22.33[a]
Black seed oil (BSO)	4.89	87.33[ab]	25.67[ab]	5.04	38.00[ab]
Olive oil (OO)	5.37	109.17[b]	27.27[b]	5.09	92.17[b]
Shea butter oil (SBO)	4.88	85.50[ab]	25.53[ab]	4.96	59.83[ab]
PSEM	0.22	10.75	0.89	0.06	14.22
Treatment Level (TL)					
0	4.54	54.33	22.77	5.00	22.33[a]
BSO1	4.68	81.00	25.27	5.00	51.67[ab]
BSO2	5.10	93.67	26.07	5.08	24.33[a]
OO1	5.38	114.00	27.73	5.10	80.33[ab]
OO1	5.36	104.33	26.80	5.08	104.00[b]
SBO1	4.52	59.33	23.37	4.81	38.67[ab]
SBO2	5.24	111.67	27.70	5.10	81.00[ab]
PSEM	0.36	17.81	1.47	0.10	23.57
Probability					
T	0.32	0.36	0.44	0.45	0.10
TL	0.22	0.23	0.26	0.19	0.51
T*TL	0.60	0.25	0.22	0.36	0.34

[ab]Mean values with different superscripts within the column differ significantly (p<0.05); PSEM = Pooled standard error of mean; 0 =Zero oil; 1 = 1ml; 2 = 2ml; RBC = Red blood cell; HGB = Haemoglobin concentration; PCV = Packed cell volume; WBC = White blood cell; PLT = Platelet number

Reproductive hormones comprising of follicle stimulating hormone (FSH) and oestrogen (E2) and stress hormone (cortisol) of the experimental rabbits are shown in Table 4. E2 level (322.70 pg/ml - 363. 43 pg/ml) is not significantly different (p>0.05) among treatments contrary to FSH (0.38 mIU/ml - 2.97 mIU/ml) which is significantly higher in olive oil and shea butter oil supplemented groups. This implies that olive oil and shea butter oil might improve the reproductive efficiency and fertility of the rabbit does similar to what is reported in rabbit bucks fed olive oil (El-Ratel *et al*., 2021; Ahmed *et al*., 2019). Cortisol levels among experimental treatments ranged between 7.94 ng/ml and 15.16 ng/ml with the least value in black seed supplemented group. The cortisol values observed in this study is within the normal range (Bozzo *et al*., 2022). In addition, the high cortisol levels recorded in OO and BSO did not hamper the secretions of the reproductive hormones in the treated animals suggesting that it would not affect their fertility.

Serum glucose (GLU) (6.57 mmol/l - 7.13 mmol/l) of the experimental animals did not differ within treatments with the exception of those allotted BSO with decreasing values as the supplementation level increased. Nonetheless, GLU was within the normal range for rabbits (Harcourt-Brown and Harcourt-Brown, 2012). Total cholesterol (1.57 mmol/l - 2.85 mmol/l) recorded in this study was below the observation of Ansah *et al*. (2011). Total protein (4.87g/dl - 7.81g/dl) of rabbit does fed shea butter oil at 1.00 ml was significantly higher (p<0.05) compared to those fed black seed oil at 0.50 ml while all other experimental treatments. Nonetheless, TP recorded in this study was within the values reported by Ajao *et al*. (2024). In general, observed values of GLU, TC and TP are suggestive of efficient energy and protein metabolism with preserved hepatic and kidney functions (Lozano *et al*., 2019).

Catalase (261.63u/ml - 432.80u/ml), glutathione peroxidase (118.16U/L - 213.16U/L), sodium dismutase (1.06u/ml - 1.42u/ml) and total antioxidant capacity (7.24mMTE - 9.36mMTE) of experimental rabbit does were presented in Table 5. Selected oils regardless of oil levels had no significant effect (p>0.05) on these parameters. This report is different from the conclusions of El-Ratel *et al*. (2021) and implies that the test oils did not disrupt the antioxidative defence of rabbit does.

Table 4: Selected hormone and serum metabolite of rabbit does fed black seed oil, olive oil and black seed oil

Factor	Parameter					
	E_2 (pg/ml)	FSH (mIU/ml)	CORT (ng/ml)	GLU (mmol/l)	TC (mmol/l)	TP (g/dl)
Treatment (T)						
C	322.70	0.38[a]	8.69[ab]	5.55	2.08	7.02
Blackseed Oil	353.82	0.45[a]	7.94[a]	5.83	2.42	7.64
Olive Oil	344.39	2.97[b]	10.24[ab]	5.80	2.66	7.49
Shea butter Oil	363.43	2.81[b]	15.16[b]	6.33	2.61	7.69
PSEM	86.98	0.71	2.14	0.34	0.20	0.53
Treatment Level (TL)						
0	322.70	0.38	8.69	5.55	2.08	7.02
1	348.96	2.12	11.46	6.03	2.58	7.42
2	358.79	2.03	10.77	5.95	2.54	7.79
PSEM	79.88	0.65	1.97	0.31	0.18	0.49
P -value						
T	0.99	0.02	0.04	0.42	0.60	0.95
TL	0.92	0.90	0.76	0.84	0.85	0.51
T*TL	0.80	0.84	0.11	0.94	0.66	0.45

[ab]*Mean values with different superscripts across the row differ significantly ($p<0.05$); FSH = Follicle stimulating hormone; E_2 = Oestrogen; CORT = Cortisol; GLU = Glucose; TC = Total cholesterol; TP = Total protein*

Table 5: Antioxidant enzymes and total antioxidant capacity of rabbit does fed black seed oil, olive oil and black seed oil

Factor	Parameter			
	CAT(u/ml)	GPX(U/L)	SOD(u/ml)	TAC(mMTE)
Treatment (T)				
Control	356.96	153.23	1.42	8.27
Blackseed Oil	432.80	118.16	1.06	7.24
Olive Oil	303.61	158.49	1.37	8.29
Shea butter Oil	261.63	213.16	1.33	9.36
PSEM	65.92	38.10	0.21	1.09
Treatment Level (TL)				
0	432.80	118.16	1.06	7.24
1	326.81	206.69	1.39	10.09
2	287.99	143.23	1.35	7.19
PSEM	60.37	34.89	0.20	1.00
P-Value				
T	0.56	0.46	0.95	0.70
TL	0.59	0.14	0.88	0.06
T*TL	0.87	0.87	0.26	0.46

CAT = *Catalase; GPX = Glutathione peroxidase; SOD =Sodium dismutase; TAC= Total antioxidant capacity*

CONCLUSION AND RECOMMENDATIONS

The study concluded that none of the experimental oils impaired any of the physiological parameters monitored during the study. Rabbit does fed olive oil regardless of the level had higher hemoglobin concentration, packed cell volume, platelets number and follicle stimulating hormone. It is recommended that the studied oils especially olive oil at 1ml and 2 ml could be utilized in improving the welfare and productivity of rabbit does.

Acknowledgement

Authors acknowledges farmers in Kwara State whom rabbits were sourced from.

Authors' Contributions

BHA designed and supervised the experiment. He also analysed the data and reviewed the manuscript. QAM, OSA, ROI and UBI managed experimental animals and collected data. TOB and MOA curate the data and drafted the manuscript.

Ethical Statement

The study was approved by the Research Committee of the Department of Animal Production, University Ilorin, Ilorin, Nigeria.

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