

Haematological and lipid profile of rabbits fed ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*) additives to correct crude oil induced antioxidant challenges

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Abstract

Environmental pollution is a major global problem, posing a severe risk to humans and animals and limited data are available on the haematology of farm animals with heavy metal contamination due to exposure to crude oil pollution. This study was therefore conducted to assess the blood profile of rabbits fed ginger and turmeric additives to correct crude oil induced antioxidant challenges. The experiment was conducted using 40 mature rabbits of mixed breeds and sexes, randomly assigned to five treatments in a completely randomized design. Contaminated feed contained 0.035% of crude oil. Treatment 1 was a basal diet with no contamination and no test additive. Treatment 2 was offered contaminated feed with no test additives. Treatment 3 and 4 were contaminated feeds with 5g/kg body weight of ginger and turmeric, respectively. Treatment 5 had contaminated feed with a mixture of ginger and turmeric at 2.5g/kg body weight. At the end of the feeding trial which lasted for 10 weeks, blood samples were collected through the ear vein, and used for haematological and plasma lipid analysis. The results showed that additives improved the white blood cell count as both controls (Treatments 1 and 2) had depressed and significantly lower ($p > 0.05$) values from the treated groups. Lymphocyte percentage was significantly depressed in the positive control (19.03%), against the range of 73.85% - 86.80% obtained in the other treatments. Total cholesterol was highest in the negative control (45.00mg/dL) and least in Treatments 3 and 5 (28.00mg/dL). Triglyceride level was significantly higher ($p < 0.05$) in Treatment 4 (98.00mg/dL) and least (24.00mg/dL) in Treatment 3. In conclusion, ginger, turmeric, and their combination were beneficial in correcting the effects of crude oil contaminated feed on the blood profile of rabbits.

Keywords: : Ginger and turmeric, haematology, lipid profile, crude oil contamination

Profil hématologique et lipidique de lapins nourris avec des additifs de gingembre (*Zingiber officinale*) et de curcuma (*Curcuma longa*) pour corriger les défis antioxydants induits par le pétrole brut



Résumé

La pollution de l'environnement est un problème mondial majeur, posant un risque grave pour les humains et les animaux et des données limitées sont disponibles sur l'hématologie des animaux de ferme contaminés par des métaux lourds en raison de l'exposition à la pollution par le pétrole brut. Cette étude a donc été menée pour évaluer le profil sanguin des lapins nourris avec des additifs de gingembre et de curcuma pour corriger les défis antioxydants induits par le pétrole brut. L'expérience a été menée en utilisant 40 lapins matures de races et de sexes mélangés, assignés au hasard à cinq traitements dans une

conception complètement aléatoire. La charge contaminée contenait 0,035 % de pétrole brut. Le traitement 1 était un régime de base sans contamination et sans additif d'essai. Le traitement 2 s'est vu proposer des aliments contaminés sans additifs d'essai. Les traitements 3 et 4 étaient des aliments contaminés avec 5 g/kg de poids corporel de gingembre et de curcuma, respectivement. Le traitement 5 avait des aliments contaminés avec un mélange de gingembre et de curcuma à 2,5 g/kg de poids corporel. À la fin de l'essai d'alimentation qui a duré 10 semaines, des échantillons de sang ont été prélevés dans la veine de l'oreille et utilisés pour l'analyse hématologique et des lipides plasmatiques. Les résultats ont montré que les additifs amélioraient le nombre de globules blancs car les deux témoins (traitements 1 et 2) avaient des valeurs déprimées et significativement inférieures ($p > 0,05$) des groupes traités. Le pourcentage de lymphocytes était significativement diminué dans le contrôle positif (19,03%), contre la plage de 73,85% - 86,80% obtenue dans les autres traitements. Le cholestérol total était le plus élevé chez le témoin négatif (45,00 mg/dL) et le moins élevé dans les traitements 3 et 5 (28,00 mg/dL). Le niveau de triglycérides était significativement plus élevé ($p < 0,05$) dans le traitement 4 (98,00 mg/dL) et inférieur (24,00 mg/dL) dans le traitement 3. En conclusion, le gingembre, le curcuma et leur combinaison ont été bénéfiques pour corriger les effets du pétrole brut aliments contaminés sur le profil sanguin des lapins.

Mots clés : Gingembre et curcuma, hématologie, profil lipidique, contamination par le pétrole brut

Introduction

Environmental pollution is a major global problem, posing a severe risk to human and animals. The pollutants are spread through different channels, many of which finally enter the food chain of livestock and humans (Kaplan et al., 2010). There is an increasing concern about environmental pollutants getting into livestock production systems (Rajaganapathy, 2006). Some of these chemicals are present in crude oil, which seems to be the primary source of environmental pollution, especially in the oil-producing areas like the Niger Delta region of Nigeria. Research has shown that although the acute manifestations of exposures to crude oil and its heavy metal components are often mild and transient, severe exposures could result in acute renal failure (Otaigbe et al., 2012), hepatotoxicity (Eyong et al., 2004) and haemotoxicity (Sunmonnu et al., 2007). Rabbit farming has proven to be one of the most lucrative livestock farming as rabbits are characterized by small body size, short

gestation period, high productivity potential, rapid growth rate, genetic diversity, and their ability to utilize forages (Mailafia et al., 2010). Rabbit meat is of high quality, high in protein and low in fat content (Mailafia et al., 2010). Lane (1999) also stated that rabbit meat has less cholesterol, fewer calories, and a lower percentage of fat than beef, pork, chicken, lamb, and higher protein content, making it a perfect choice for human consumption. Despite the exceptional attributes and advantages of keeping rabbits, severe losses could be encountered if the animals are exposed to toxic elements such as crude oil contamination. This exposure can impair their physiological status leading to low productive capacity as well as pose a health threat to the humans that feed on it. Crude oil is reported to contain several poisonous compounds, which accumulate in the body and induces toxic symptoms that sometimes result in death (Heintz et al., 1999). The consumption of petroleum hydrocarbon contaminated diets has been

reported to cause liver enlargement, growth suppression, and haematological changes in animals leading to low productivity (Onwurah and Eze, 2000). Treating the animals with drugs and other antibiotics increases production costs and cannot be considered very safe. Hence, recent research has been carried out to determine the potential of natural medicinal plants and herbs to reduce the effect of heavy metal toxicity in animals which also indirectly affects the humans that consume the meat from such animals. Ginger (*Zingiber officinale*) is widely used as a spice and in the practice of traditional Chinese herbal medicine (Masuda *et al.*, 2004; Tapsell *et al.*, 2006). Ginger and its main compounds have shown various pharmacological effects including immune-modulatory, anti-tumorigenic, anti-inflammatory, anti-apoptotic, anti-hyperglycemic, anti-lipidemic, and antiemetic effects (Ali *et al.*, 2008). Turmeric is the root of a plant which is scientifically known as 'Curcuma longa'. Curcumin is a naturally occurring chemical compound found in the spice turmeric. Most of the protective effects attributed to the curcumin arise from its ability to trap free radicals (Tulani *et al.*, 2007). Hence dietary supplementation of turmeric could be considered as a natural method for detoxification of heavy metals. The study by Hewlings and Kalman (2017) highlights two critical biological properties of curcumin: antioxidant and anti-inflammatory effects. According to these researchers, curcumin exerts its antioxidant effect by trapping free radicals and by adjusting the activity of catalase, glutathione, and superoxide dismutase enzymes (Hewlings *et al.*, 2017). According to previous findings, regular consumption of herbal products may significantly reduce the absorption of heavy metals (Ovuru *et al.*, 1999). Plants such as ginger and turmeric are known to

possess antioxidative properties, which will effectively reduce free radicals produced by the ingestion of toxic chemicals, and the effect of these plants on animal health can be investigated through the blood (Lebda *et al.*, 2012). Although there are many studies on blood parameters of various animals and humans, data on haematology of farm animals with heavy metal contamination due to exposure to crude oil are scanty. Hence this study was carried out to examine the haematological response and lipid profile of rabbits fed ginger and turmeric to correct crude oil-induced antioxidant challenges.

Materials and methods

Site of experiment The experiment was carried out at the Rabbit Unit, Teaching and Research Farm, Department of Animal Science, University of Benin, Benin City, Nigeria. The farm is on a latitude of 6.20N and a longitude of 5.60E (FAAN, 2017) with a mean annual rainfall of about 2161mm and a mean relative humidity of 72.5% (Google Earth, 2017).

Experimental animals

The experiment was conducted using 40 mature rabbits of mixed breeds and both sexes. 8 rabbits were randomly allocated to each treatment. The rabbits were purchased from a supplier at Ogbomosho, Oyo state. The average weight of the rabbit was 1.8 kg. After purchase, the rabbits were treated against mange and other ecto- and endoparasites.

Experimental design

The experiment, which lasted for 12 weeks, was laid out as a completely randomized design with five groups replicated four times to give two rabbits per replicate. Each group consists of 8 rabbits making a total of 40 rabbits. Animals were randomly allocated into experimental groups and housed individually.

Preparation of experimental materials

Fresh ginger and turmeric were purchased from the Aduwawa market in Benin City, Edo State. The rhizomes and roots were cut into small pieces, air-dried and ground into powder form, and stored in an airtight container until use. The composition of the various treatments is presented in Table 1.

Experimental procedure

Animals were subjected to feed contaminated with 0.035% crude oil for

two weeks. Thereafter, the feed containing the experimental material was fed to the animals in the morning and evening while grass was fed to the animals in the afternoon to avoid feedlot bloat. The feeding trial lasted for 10 weeks. Water was given ad libitum throughout the experimental period.

Table 1: Experimental treatment

Treatment	Composition
T1 (negative control)	No contamination, no additives
T2 (positive control)	Contaminated with Crude oil, no additives
T3	Contaminated feed and treated with ginger at 5 g/kg body weight
T4	Contaminated feed and treated with Turmeric at 5 g/kg body weight
T5	Contaminated and treated with mixture of turmeric and ginger at 1:1

Table 2: Nutrient composition of basal diet

Nutrient	Composition (%)
Metabolizable Energy	2450.00 KCal/Kg
Crude Protein	18.00
Crude Fat	5.00
Crude Fiber	7.00
Calcium	1.60
Available Phosphorus	0.45
Lysine	0.75
Methionine	0.36
Salt	0.36

Housing and routine managements

The animals were housed individually in hutches made with wire mesh. The hutches had a dimension of about 60×60×80cm. Each rabbit compartment was provided with both feeding and drinking bowls which were molded round with cement to prevent turning over. Before the rabbits were brought in, the building and the hutches were cleared, swept, and washed thoroughly with detergents. The hutches and building were disinfected and left to dry for about a week before the animals were brought in. The feeders and drinkers were also adequately washed. Upon arrival, the rabbits were administered Vitalyte (an anti-stress/multi-vitamin) in cool, fresh

water and given feed ad libitum. Thereafter, all standard routine management procedures were carried out daily.

Data collection

At the end of the feeding period, four rabbits were randomly selected from each treatment group and starved of feed and water overnight. 5ml of blood samples was collected from the ear veins of the rabbits using a sterile syringe and put into bijou bottles containing EDTA (Ethylene diamine tetra-acetic acid) as an anti-coagulant and used for haematological analysis. Another 5ml was collected in heparin bottles and used for plasma lipid analysis. Haematological parameters were

determined using the automated multiparameter blood analyzer machine, "SYSMEX KX21", while the plasma lipid parameters were determined by using the diagnostic kit (Pars Azmoon Kit, IRI) on an automated analyzer (Abbot, model Alcyon 300, USA).

Statistical analysis

Data were subjected to statistical analysis. The analysis of variance (ANOVA) method was used to check for statistical differences between animals subjected to different treatments. Treatment means were separated using Duncan Multiple Range Test at $\alpha 0.05$.

Results

The haematological parameters presented in Table 3 showed existence of significant differences ($p < 0.05$) among the treated and controls groups in most of the parameters assessed. White blood cell counts were depressed in the negative and positive controls ($2.75 \times 10^6/\mu\text{L}$ and $2.6 \times 10^6/\mu\text{L}$, respectively), which were significantly different from the treated groups that recorded elevated levels, with treatment 5 having the highest value of $5.7 \times 10^6/\mu\text{L}$. The contaminated feed influenced the lymphocyte value as reflected in the positive control that recorded the least value of 19.03%, with a significant difference from the other treatments. On the other hand, the rabbits that received the additives and those on basal diet (negative control) had improved lymphocyte values in the range of 73.85% - 86.80%. The neutrophil was highest in the positive control (54.95%) and significantly higher ($p < 0.05$) than in the other treatments, with treatment 3 recording the least value (9.55%). Haemoglobin was significantly higher ($p < 0.05$) in the negative control (47.80g/dL) than was observed for other

treatments. Haemoglobin levels in the other treatments were within the range of 9.45g/dL in the positive control and 13.70g/dL in treatment 4. Platelet was highest and least in the positive and negative controls, respectively, with significant differences from the treated groups. Platelet distribution width was elevated and significantly different ($p < 0.05$) in both controls (8.60% and 10.65%, respectively) against the range of 3.50% - 5.15% obtained in the treated groups. Significantly higher ($p < 0.05$) mean cell volume were obtained in the treated groups (66.25fl - 69.55fl), while the negative and positive controls recorded 50.55fl and 63.05fl, respectively. Mean platelet volume was also elevated in both controls compared to the treated groups.

Total cholesterol increased significantly ($p < 0.05$) in the negative control (45.00mg/dL) as shown in Table 4, while the least value of 28.00mg/dL was recorded in both Treatments 3 and 5. No significant difference ($p > 0.05$) was observed between the positive control (39.50mg/dL) and Treatment 4 (39.00mg/dL). High-density lipoprotein was significantly higher ($p < 0.05$) in the negative control (32.00mg/dL) than in other treatments. The negative control with a 16.00mg/dL was similar (0.05) to Treatments 4 (16.00mg/dL) and 5 (13.50mg/dL). However, low-density lipoprotein was significantly higher ($p < 0.05$) in the positive control (15.50mg/dL) than in other treatments with lower values of 4.00 and 5.00mg/dL. Triglyceride level was significantly higher ($p < 0.05$) in Treatment 4 (98.00mg/dL) than in Treatment 5 (58.00mg/dL) and the negative control (47.00mg/dL). The positive control (29.00mg/dL) and Treatment 3 (24.00mg/dL) were similar.

Table 3: Haematological parameters of rabbits fed different plant additives to correct crude oil induced antioxidant challenges

Parameters	T1	T2	T3	T4	T5	SEM
White blood cells ($10^6/\mu\text{L}$)	2.75 ^b	2.6 ^b	4.5 ^a	3.15 ^b	5.7 ^a	0.32*
Basophils (%)	1.15	0.55	0.45	0.55	0.45	NS
Eosinophils (%)	2.60 ^a	1.70 ^b	1.10 ^c	2.50 ^a	1.95 ^b	0.07*
Lymphocytes (%)	74.10 ^b	19.03 ^a	86.80 ^b	73.85 ^b	75.30 ^b	12.2*
Monocytes (%)	1.05 ^a	1.45 ^{ab}	2.10 ^b	1.25 ^{ab}	1.45 ^{ab}	0.11*
Neutrophils (%)	21.10 ^b	54.95 ^c	9.55 ^a	21.85 ^b	20.85 ^b	3.17*
Red blood cell ($10^6 \mu\text{L}$)	15.37 ^b	4.59 ^a	5.83 ^a	5.90 ^a	5.46 ^a	4.46*
Haematocrit (%)	32.00 ^{bc}	28.85 ^c	38.5 ^{ab}	40.95 ^a	36.95 ^{ab}	2.31*
Haemoglobin (g/dL)	47.80 ^b	9.45 ^a	12.65 ^a	13.70 ^a	12.10 ^a	4.55*
Procalcitonin (%)	8.90 ^b	0.02 ^a	0.00 ^a	0.01 ^a	0.00 ^a	0.45*
Platelet larger cell ratio (%)	15.10 ^c	13.25 ^{bc}	9.85 ^b	8.00 ^b	3.50 ^a	1.97*
Platelets ($10^3/\mu\text{L}$)	12.52 ^c	37.00 ^a	24.50 ^b	28.00 ^b	24.00 ^b	0.65*
Platelet distribution width (%)	8.60 ^a	10.65 ^a	3.50 ^b	4.10 ^b	5.15 ^b	1.40*
Mean corpuscular haemoglobin (u/g)	19.90	20.65	21.70	23.25	22.10	NS
Mean corpuscular haemoglobin concentration (g/dm)	43.00 ^a	32.75 ^b	32.70 ^b	33.45 ^b	32.75 ^b	0.98*
Mean cell volume (fl)	50.55 ^c	63.05 ^b	66.25 ^a	69.55 ^a	67.65 ^a	0.77*
Mean platelet volume (μm^{-3})	11.35 ^c	7.00 ^b	3.80 ^a	3.85 ^a	3.00 ^a	2.10*

Different letters (a-c) within a row denote significant differences between treatments ($P < 0.05$).

Table 4: Serum biochemistry of rabbits fed different plant additives to correct crude oil induced antioxidant challenges

Parameters	T1	T2	T3	T4	T5	SEM
Total cholesterol (mg/dL)	45.00 ^c	39.50 ^b	28.00 ^a	39.00 ^b	28.00 ^a	1.78*
High Density Lipoprotein (mg/dL)	32.00 ^d	16.00 ^b	8.00 ^a	16.00 ^b	13.50 ^b	2.23*
Low Density Lipoprotein (mg/dL)	4.00 ^a	15.50 ^b	5.00 ^a	4.00 ^a	4.00 ^a	0.39*
Triglyceride (mg/dL)	47.00 ^b	29.00 ^a	24.00 ^a	98.00 ^d	58.00 ^c	4.45*

Different letters (a-c) within a row denote significant differences between treatments ($P < 0.05$).

Discussion

Blood parameters are valuable in monitoring feed toxicity, especially with feed constituents that affect the blood and the health status of farm animals (Oyawoye and Ogunkunle, 2004). Animals with low white blood cells are exposed to a high risk of disease infection, while those with high counts can generate antibodies in the process of phagocytosis and have a high degree of resistance to diseases (Soetan *et al.*, 2013) and enhanced adaptability to local environmental (Kabir *et al.*, 2011; Isaac *et al.*, 2013). In this study, the white blood cell decreased significantly in both the control treatments and those fed contaminated feed with turmeric additive (Treatment 4). observations here is in line

with the findings of Ngodigha *et al.* (1999), in which there was a reduction in total white cell count in goats fed crude oil contaminated diets, while supplementation with ginger and turmeric increased the white blood cell counts. The eosinophils obtained in this study were least in the ginger supplemented diet compared to both controls and other test groups. Eosinophils are primarily associated with parasitic infections, and a decrease in their number could indicate a healthy state of the animal, while an increase in their number may connote an unhealthy condition (Awodi, 2005). Most constituents of crude oil are highly toxic to biological membranes (Debra, 2003). Naphthalene, for instance, has been

reported to cause haemoglobin denaturation, which can be responsible for the development of haemolytic anaemia in oiled wildlife (Debra, 2003). This phenomenon may explain the variance in some haematological parameters recorded in the positive control in this study. Naphthalene, amongst other toxicants, suppresses the immune system, causes disruption or suspension of haematopoiesis (Hodgson and Smart, 2001); which corroborates the results in this study as observed for the rabbits on contaminated feed without the additives (positive control). The neutrophils were significantly highest in the crude oil contaminated group (positive control). The value was higher than the normal range of 20.00 – 45.55 reported by Mitruka and Rawnsley (1977). The contaminated feed with supplemental ginger had the least neutrophil value of 9.55, which was beyond the recommended range of Mitruka and Rawnsley (1977), while other treated groups and the negative control had values that were within the established range. The normal neutrophil levels suggests minimal or no inflammation, as neutrophils are the first responders to inflammation and cell damage. This result agrees with the report of Ovuru and Ekweozor (2004), where neutrophil values increased linearly with increasing concentration of crude oil contamination. The lymphocytes of the negative control and those that received contaminated feed with the various additives were within the documented range of 69 - 86% for rabbits (Mitruka and Rawnsley, 1977). Low Lymphocyte value was observed in the group that was offered crude oil contaminated feed with no additive (positive control) with a value of 19.03 % and a correspondingly low value of WBC ($2.60 \times 10^6 \mu\text{L}$) suggestive of poor health. Similar observations were made by Thompson and Lippman (1974) and Sudakov (1992) that ACTH and

glucocorticoids caused the regression of lymphoid tissue due to stress and feed contamination. According to Isaac *et al.* (2013), red blood cell is involved in transporting oxygen and carbon dioxide in the body. Thus, a reduced red blood cell count implies a reduction in the level of oxygen that would be carried to the tissues and the level of carbon dioxide returned to the lungs (Ugwuene, 2011; Soetan *et al.*, 2013). From this study, the negative control had significantly value, while the positive control and the treated groups were similar. Packed Cell Volume (PCV), also known as haematocrit or erythrocyte volume fraction, is the percentage (%) of red blood cells in the blood (Purves *et al.*, 2003). According to Isaac *et al.* (2013), increased PCV connotes better oxygen transportation capabilities, resulting in increased primary and secondary polycythemia. Furthermore, Chineke *et al.* (2006) posited that a high PCV indicates either increase in the number of RBC or a reduction in circulating plasma volume. The results from this study agree with the findings of Mitruka and Rawnsley (1977), on 30 – 45% PCV range for the rabbits. The inclusion of ginger, turmeric, and their combination, evidently improved the PCV value of the test rabbits as higher values were obtained in the treated groups than in negative and positive controls. According to Peters *et al.* (2011), PCV, haemoglobin, and mean corpuscular haemoglobin are major indices for evaluating circulatory erythrocytes and are significant in diagnosing anaemia). They also serve as valuable indices of the bone marrow capacity to produce red blood cells in mammals (Awodi *et al.*, 2005). Low levels of mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration indicates anaemia (Aster, 2004), while mean cell volumnae indicates variations in erythrocyte shape, size, and haemoglobin content. According to Berd *et al.* (2006), an increase in mean cell volume

could be attributed to the presence of a more significant number of reticulocytes in the circulating blood in comparison to mature red blood cells. Basavaraj *et al.* (2011) established that rabbit cholesterol level due to the dietary inclusion of turmeric powder at 0.00%, 0.15%, and 0.30% did not have effects on the cholesterol profile among the treatment groups. No significant difference in cholesterol level in rabbits fed 0.5% ginger supplement and the control was also reported by Abd El-Latif *et al.* (2019), contrary to results of this study. Rabbits fed diets incorporated with 0.5% ginger showed a significantly decreased triglyceride level (Abd El-Latif *et al.*, 2019), which agrees with the findings of this study, as triglyceride level in the ginger treatment was lower than both control treatments. According to Al-Sultan and Gameel (2004), the reduction of plasma triglyceride may be due to the enhancement of bile production by turmeric additive, which ultimately contributes to improving lipid digestion. Optimized fat digestion will indirectly increase the provision of substrates for β -oxidation or fat metabolism, which eventually enhances the production of succinyl-CoA through Krebs metabolic cycle (Cunningham and Klein, 2007). The result in this study agrees with the findings of Lebda *et al.* (2012), that male rabbits fed 2% ginger powder showed a significant decrease in triglyceride. Similarly, Hussein (2013), also demonstrated that triglyceride decreased with increasing turmeric levels in the diets. The results here conforms with the findings of Alagawany *et al.* (2016), that triglycerides, total cholesterol, and low-density lipoprotein concentrations were linearly and quadratically decreased with increasing dietary proportions of turmeric. Also, Hussein (2013) reported that triglycerides and total cholesterol were significantly decreased with increased

dietary turmeric levels to a 7 g/kg diet. The reduction of lipids profile (triglycerides, total cholesterol, and low-density lipoprotein concentrations) may be due to curcumin enhancing bile production and lipid digestion (Al-Sultan and Gameel, 2004). The lower the levels of LDL, the more the chances of preventing the onset of cardiovascular diseases, results on LDL in this study are in line with values reported by Malekizadeh *et al.* (2012).

Conclusion

It is concluded in this study that ginger, turmeric, and their combinations had beneficial effects on the haematology and lipid profile of rabbits and would play important roles as exogenous antioxidant in combating the challenges posed by ingestion of crude oil contaminated feeds.

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