Alleviant Garlic Responses of Growing Rabbits Fed Crude Oil-Contaminated Diets

Ntinya C. Johnson[1], Victor M. Ogbamgba[1] and James T. Mbachiantim[2]

[1]Rivers State University, Port Harcourt, Department of Animal Science, Nigeria

[2]Federal University of Agriculture, Makurdi, Department of Nutrition and Dietetics, Nigeria

Abstract. Alleviant responses of growing rabbits due to garlic consumption following crude oil ingestions were investigated. 24 males New Zealand White rabbits weighing 870-925 grams were used in the study. The rabbits were randomly assigned to their experimental diets for 2 weeks following 2 weeks of pre-conditioning of the animals to their new environment. There were three dietary treatments as: T_0 (the negative control diet; received no crude oil and garlic), T_1 (received crude oil-contaminated diet at the concentration of 1.5g/kg of diet) and T_2 (received crude oil-contaminated diet as in T_1 but in addition received 20 g of garlic/kg of diet). At the end of study, blood samples were collected from all animals for blood parameters analyses. For all blood parameters studied: hemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC) and monocytes (MON) crude oil ingestion significantly depressed ($P < 0.05$) Hb, PCV, RBC, WBC and MON. Garlic consumption following the crude oil ingestions had no reversal effects induced by the crude oil on these hematological parameters. It was concluded that garlic had no alleviant effects or responses on the blood parameters of rabbits that ingested crude oil.

Key words: Crude oil, Garlic, Alleviant responses, Blood parameters and Rabbit

Introduction

Rabbit meat has been a popular source of animal protein in Nigeria, including the Niger Delta region where crude oil exploration is domiciled. Unlike beef, chevon, mutton and pork rabbit meat is less expensive as it represents low cost but high quality protein that employs only local forages and some food wastes of no direct value to humans (NRC, 1991). Furthermore, rabbit is cherished by consumers and it suffers no cultural and religious biases, such as beef and pork. To this extend therefore, in emergencies where other sources of proteins are lacking rabbit meat can rapidly and readily become the means of producing quality meat and a dependable protein source (NRC, 1991).

However, one of the major factors militating against successful rabbit production, especially in the Niger Delta region is the incidence of crude oil spills that are very common and frequent in the region (Udoudoh, 2011). Due to the cheap but quality protein from rabbit meat that is highly cherished, there is a need to decipher means of reducing the negative effects of the contamination or incessant crude oil pollutions of the animal environment. This comes to the fore because ingestion of crude oil via contaminated forages had been implicated in reduced PVC, red and white blood cells counts and hemoglobin leading to low quality of life and leukemia in animals that ingest crude oil (Berepubo, Johnson & Sese, 1994; Monsi, Kwuinji & Akpan, 1991).

The use of phytobiotic, such as garlic may be a means of reversing some of the negative effects of crude oil ingestion in rabbits, as it relates to hematological parameters of the animal; thereby serving as an alleviant means to the rabbits that ingest crude oil (Ankri & Mirelman, 1999; Cullen et al., 2005). This line of thinking is hinged on the data findings of Murray (1995). From clinical standpoint, garlic contains alliin and allicin which had been shown to improve quality of life even in human medicine (Agarwal, 1996; Ernst, 1997). We have also shown previously (Johnson & Iorliam, 2020) that garlic improved hematological parameters in the growing pig. Therefore, the objectives of this study are to investigate garlic alleviant responses of growing rabbits fed crude oil-contaminated diets.

Materials and Methods

Animals and Management

Prior to the introduction of the animals into their hutches, the hutches, feeding and the water troughs were thoroughly cleaned to ensure a 'pathogen-free' environment. After drying, twenty-four (24) male New Zealand White rabbits weighing 870-925 grams were acquired from one source in a single batch and weighed after which they were randomly assigned to their individual hutches to three dietary treatments with 8 rabbits per treatment. The animals were pre-conditioned or adapted to their new environment for two weeks. During the pre-conditioning period the animals were similarly fed and administered prophylactic coccidiosis as well as a broad spectrum antibiotics (terramycin) to properly stabilize the animals according to the method of Berepubo, Johnson and Sese (1994) prior to presenting the experimental diets.

Crude Oil Contamination

The crude oil used in this study is the Bonny Light acquired from Agip Oil Company Nigeria Limited. Prior to using the crude oil in contaminating the experimental diets, it was exposed for 24 hours in shallow pans according to the method of Ovuru and Ekweozor (2004) to enable its light fractions to evaporate leaving the stable product that mimics natural crude oil pollution form.

Experimental Diets

The rabbits were fed with grower mash supplemented with *Centrosema pubescens*, since rabbits are pseudo-ruminants. T_0 animals served as the negative control group while T_1 and T_2 were the positive control groups as: T_1 animals received 1.5g of crude oil (0.15%)/kg of diet and T_2 animals received similar amount of dietary crude oil as in T_1 but in addition garlic at the level of 20 g/kg of diet for 2 weeks, respectively according to the method of Johnson and Iorliam (2020). Therefore, the trial lasted for four weeks of 2 weeks of pre-conditioning period and 2 weeks of receiving experimental diets.

Blood Sample Collections

At the termination of trial, blood samples were collected from individual rabbits from each of the three treatment groups into ethylene diamine tetracetic acid (EDTA) treated tubes between 9 and 10 a.m. and immediately snap-frozen for later analyses by hematology auto-analyzer (BC-2300). Hb concentration, PCV, RBC count and total and differential WBC counts of each group were finally determined using standard laboratory procedures.

Statistical Analysis

Data obtained were subjected to analysis of variance (ANOVA) using the general linear model procedure of SAS. Treatment means were compared using Tukey's test. The experimental design was the CRD. Therefore, the model was $Y_{ij} = \mu + X_i + E_{ij}$; where: Y_{ij} = individual observation of any animal receiving a treatment, μ = population mean, X_i = effect of the ith diet (i = 1, 2, 3) and E_{ij} = the error term. An *α-level* of *0.05* was used for all statistical comparisons to detect significance.

Results

The results of the effects of crude oil and garlic ingestions on rabbits are shown in Table 1.

Table 1. Hb concentration, PCV and RBC counts of rabbits fed crude oil contaminated-diet and dietary garlic

Item	Diets			SEM	*P-value*
	T_0 n = 8	T_1 n = 8	T_2 n = 8		
Hb (g/dl)	13.33^a	5.49^b	6.66^b	0.77	0.001
PCV (%)	40.12^a	20.77^b	21.32^b	4.01	0.001
RBC (x 10^9/l)	7.06^a	4.11^b	4.86^b	0.58	0.02

Note: Means with different superscripts within the same row are significantly *(P < 0.05)* different. SEM = Standard error of the mean

Crude oil ingestion had very profound significant suppressive effects on Hb concentrations as its levels were significantly reduced *(P < 0.05)* in the animals' that received diets two and three compared with the negative control confirming that garlic had no effects on Hb concentrations as diets two and three showed no significant *(P > 0.05)* differences but had significantly lowered *(P < 0.05)* Hb concentrations compared with those of the negative control. Again, these trends found with the effects of crude oil and garlic were replicated in the PCV and RBC parameters. These also again confirmed that ingestion of crude oil significantly depressed *(P < 0.05)* the volumes of circulating blood in the animals of diets two and three as well as the levels of the RBC counts in animals that consumed diets two and three. These results again confirmed that crude oil causes or induces anemia and leukemia. Nevertheless, there were some forms of higher numerical values for these parameters with animals that consumed diet three but they were not significantly *(P > 0.05)* as earlier stated compared with diet two values. The results of the effects of crude oil and garlic consumptions on WBC counts and their differentials are shown in Table 2.

Table 2. Total and differential WBC counts in rabbits fed crude oil-contaminated diets and dietary garlic

Item	Diets			SEM	*P-value*
	T_0 n = 8	T_1 n = 8	T_2 n =8		
WBC (x10^9/l)	7.55^a	3.54^b	4.10^b	0.55	0.001
NEU (%)	51.00	49.00	48.50	3.36	0.22
LYM (%)	48.00	50.80	51.30	4.04	0.31
MON (%)	1.00^a	0.20^b	0.20^b	0.11	0.025
EOSI (%)	-	-	-	-	-
BASO (%)	-	-	-	-	-

Note: Means with different superscripts within the same row are significantly *(P < 0.05)* different. SEM = standard error of the mean.

The effects of crude oil consumption conspicuously reduced *(P < 0.05)* the WBC counts of animals of diets two and three, respectively compared with those of the negative control diet. Furthermore, dietary garlic had no effects on the WBC counts of rabbits as there were no significant differences *(P > 0.05)* in the WBC counts of animals of diets two and three. There were also no effects *(P > 0.05)* of dietary garlic on the WBC differentials ratio between NEU and LYM of rabbits except for MON where the animals on the negative control diet had a significantly higher *(P < 0.05)* value compared to those of the animals on diets two and three that had similar *(P > 0.05)* levels of MON.

Discussion

Garlic has long been known as a plant phytobiotic principally due to its bioactive compounds that can promote animal growth and health. This is built on the concept that garlic possesses antimicrobial, antiviral, antifungal and also active against some enteric parasites (Friedman, 2007). Furthermore, garlic has long again been used as complementary or alternative medicine in improving health and even cure some human and animal diseases (Bordia, 1981). To this extent, it is thought that garlic by its potential characteristics therefore would exert these potentials in the rabbit and thus serve as a remedy by reversing negative effects of crude oil ingestion particularly as it relates to the hematological parameters of the rabbit.

In this current study, however, garlic had no alleviant effects following the ingestion of crude oil. Additionally, the effects of crude oil were observed to be very acute in this study and thus agrees with the data of Berepubo, Johnson and Sese (1994). Crude oil is liquid in form and therefore, can easily find its way into the blood stream of the animal immediately after consumption and commenced its pathogenesis in the blood. Therefore, the severe effects of the ingested crude oil and the inability of garlic to demonstrate some alleviant influences might be related to the liquid nature of crude oil and the mode of action of garlic as a phytobiotic against the onset pathogenesis of crude oil ingestion (Murray, 1995; Agarwal, 1996).

Garlic potentials in the improvements of health are more related to its antimicrobial, antifungal, antiparasitic, antiviral as well as other health-related benefits, such as garlic ability to reduce hyperlipidemia and hypertension (Ankri & Mirelman, 1999). It has been found that the bioactive compound in garlic that earned it the potentials as a health-related phytobiotic is alliin (Murray, 1995). However, for alliin to exert its potentials it has to first of all be converted to allicin in the body and the rate of this conversion is approximately 40% effective (Murray, 1995). Therefore, crude oil being a liquid could easily find its way into the blood stream of the animals and commences its pathogenesis in the blood as earlier stated whereas that of alliin would first be converted to allicin (garlic bioactive compound) by which time crude oil would have ample of early opportunities in the blood such that by the time allicin is produced its potential to act as an alleviant to the negative effects of crude oil would have become belated to a large extent. Besides, the blood granulocytes consisting of neutrophils, eosinophils and basophils; neutrophils kill invading organisms by ingesting and digesting them. Eosinophils kill parasites that are involved in allergic reaction. Due to the special hydrocarbons in crude oil, such as benzene these blood components would not be able to engulf them as they are not necessarily pathogenic organisms. To this extent therefore, crude oil components though can be regarded as xenophiles may be difficult to isolate and identify by allicin. This may also explain in part the lack of effect of garlic to reverse the negative effects of crude oil consumption on the hematological parameters in the rabbit.

Conclusions

Crude oil ingestion suppressed all the hematological parameters investigated in this study, mainly Hb concentrations, PCV, red and white blood cells count. Furthermore, garlic consumption had no reversal impacts on the negative effects induced by the ingested crude oil-contaminated diet on above aforementioned blood components in the rabbit.

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