Effects Of Feeding Graded Levels Of Crude Oil-Containing Diets On Hematological Parameters Of Growing Pigs

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Abstract: Effects of feeding graded levels of crude oil-containing diets were studied on haematological parameters in growing pigs. 24 pigs of average body weight (BW) of 9 ± 1.4 (Mean ± SD) kg were used in the study. Animals were randomly assigned to their individual experimental pens. There were 4 graded crude oil dietary treatments: 0g (control group), 10g, 15g and 20g of crude oil/kg of diet. There were 4 replications per dietary treatment. Animals were fed at 5% of their BW for 4 weeks. Blood samples were collected from all animals and snap frozen. Red blood cell (RBC), haemoglobin (Hb), packed cell volume (PCV), white blood cell (WBC) and differentials: neutrophils (NEU) and lymphocytes (LYM) were analysed. Eosinophil (EOS), basophils (BAS) and monocytes (MON) were absent. There were significant ($P < 0.05$) differences in the RBC, Hb and PCV contents. The RBC counts of treatments 1 and 2 were similar ($P > 0.05$) and were significantly ($P < 0.05$) higher than those of treatments 3 and 4 with treatment 4 having the lowest RBC counts. Similar trend was also observed for Hb and PVC concentrations. WBC counts of treatments 1 and 2 were similar ($P > 0.05$) and significantly ($P < 0.05$) higher than those of treatments 3 and 4; with treatment 4 showing the lowest count. NEU of treatment 1 was significantly higher ($P < 0.05$) than those of treatments 2, 3 and 4 with treatment 4 having the lowest. Conversely, treatment 4 significantly ($P < 0.05$) showed the highest LYM concentration compared with treatments 1 to 3 with treatment 1 showing the lowest concentration. It was concluded that growing pig's threshold for crude oil ingestion lies between 10g and 15g of crude oil/kg of diet as beyond the 10g blood constituents were negatively impacted and the ratio of NEU to LYM was altered.

Key words: Contamination, Crude Oil, Hematological Parameters and Pig

1 Introduction

The assays of blood composition as it relates to blood constituents are always used to ascertain the health status of the animal in respect to its environment, particularly its nutrition. To this end, blood parameter has been shown to be one of the major indicators of the physiological, pathological and nutritional state of the animal. [5] postulated that changes that are observed in blood constituents are a good guide to the animal producer about the metabolic state of the animal, its feeding and well-being. In expanding this feeding concept on the well-being of the animal, [9] further demonstrated that haematological parameters can be used to better understand the physiological relationship between the animal and its nutrition regarding the health of the animal. From the fore stated, it has been shown that some ingested constituents of diets can cause leukemia, reduced erythrocytes, lymphocytes, neutrophils and in this way predispose the animal to pathogenic attacks thereby significantly reduce the animal genetic potential for production [19]. Accordingly, a later study [18] reported decreased erythrocytes, platelets and packed cell volume in rabbits exposed to crude oil. [10] again showed that crude oil hydrocarbons reduced erythrocytes and other blood constituents in rats that ingested them. Other workers [14] reported similar observations in goats, [2] in broiler chickens and [3] in broiler finishers. To this point, there is a need for data on the pig although we have previously demonstrated that growing pigs can ingest up to 10g of dietary crude oil/kg of diet without any negative effect on the blood constituents [16]. Therefore, the objectives of this study are to investigate the influence of higher (above 10g crude oil/kg of diet) graded levels of dietary crude oil on the haematological parameters of the growing pig.

2 Materials and Methods

Animals and their Handling

24 landrace growing pigs weighing on average 9 ± 1.4 (mean ± SD) kg body weight (BW) were acquired from Cape Farms, Irete, Imo State transported under humane conditions to the Rivers State University Teaching and Research Farm. On arrival the animals were randomly assigned to their individual pens. Before their arrival pens were washed and disinfected to ensure a 'free pathogen' environment for the animals. The animals were given 14-d to proper adjust to their new environment. They were also injected with amoxyciline intramuscularly for further protection against disease-causing organisms. They were fed similar grower diet during this 14-d adaptation period. At the end of this period, the animals were offered their experimental diets at 5% of their BW (as-fed basis) according to [11] twice daily at 09:00h (half of the daily meal) and 16:00h, respectively. Water was given ad libitum through low water pressure nipples. Pens were insured to be cleaned throughout the

experimental period. There were four replications per dietary treatment group.

Procedure for Crude Oil Addition to Diets

In this study, Bonny Light crude oil obtained from the Nigerian Agip Oil Company Limited specifically was used. The crude oil was initially exposed to sunlight for 24 h in shallow pans according to the method of [18] as to achieve a stable product that simulate natural form during pollution as the light volatile portions escaped during exposure to sunlight. Six corn-soybean meal-based diets formulated to be isocaloric and isonitrogenous to meet or exceed the [15] recommended nutrient requirements of growing pigs of 10 – 20 kg BW were used in the study. The experimental diets contained dietary crude oil at 0g (control diet), 10g, 15g and 20g of crude oil/kg of diet, respectively resulting in four dietary treatment groups. The experiment lasted for 28-d.

Experimental Procedure, Data Collections, Design and Analyses

At the end of study period, blood samples were humanely collected into EDTA treated tubes and immediately snap frozen for analyses of blood parameters, namely: RBC, Hb, PCV; WBC and its differentials: NEU, LYM, MON, EOS and BAS. Blood were analyzed by hematology auto-analyzer machine (BC-2300). The experimental data were analyzed as a CRD. Data were subjected to analysis of variance (ANOVA) using PROC GLM of SAS (SAS Inst. Inc., Cary, NC) according to the experimental model: $Yij = \mu + D_i + Eij$; where Yij is the observation, μ = overall mean common to all treatments, D_i = the effect of the i^{th} diet and E_{ij} = the error term. Means were compared using Tukey's test and α-level of 0.05 was used for all statistical comparisons to represent significance.

3 Results and Discussion

Animals in treatments 1 and 2 readily consumed their respective diets except animals in treatment groups 3 and 4 that orts were seen from early third week of study and more so with treatment 4. This might be an indication of probably some discomfort the animals in these groups might be experiencing with their diets postprandial [15]. The results of the RBC counts, Hb and PCV concentrations are shown in Table 1.

Table 1. RBC, Hb and PCV contents of Pigs fed varied crude oil-contained diets

	DIETS					
Item	Diet 1 n = 4	Diet 2 n = 4	Diet 3 n = 4	Diet 4 n = 4	SEM	P-value
RBC (x 10^9/l)	5.00^a	4.79^a	3.00^b	2.25^c	0.125	0.001
Hb (g/dl)	13.00^a	12.81^a	9.00^b	6.75^c	0.177	0.002
PCV (%)	31.00^a	30.60^a	25.32^b	20.43^c	0.770	0.001

Means with different superscripts with the same row are significantly (P < 0.05) different

Table 1 shows the RBC, Hb and PCV contents of the animals on the four crude oil dietary treatments of this study. The RBC contents of animals on diets 1 and 2 were not significantly different (P > 0.05) and significantly (P < 0.05) higher than those of diets 3 and 4. The RBC content of diet 3 nevertheless was significantly (P < 0.05) higher than those of 4 that had the lowest RBC content. The Hb contents of diet 1 animals were similar (P > 0.05) with those of diet 2 that were significantly higher (P < 0.05) than those of diets 3 and 4. However, the Hb contents of diet 3 animals were significantly higher (P < 0.05) than those of 4. The PCV contents of the animals on diets 1 and 2 were similar (P > 0.05) but generally also mimicked or mirrored those of RBC and Hb as PCV significantly (P < 0.05) linearly reduced as dietary crude oil content increased. The results of the WBC and their differentials are shown in Table 2.

Table 2. WBC, NEU and LYM contents of Pigs fed varied crude oil-contained diets

	DIETS					
Item	Diet 1 n = 4	Diet 2 n = 4	Diet 3 n = 4	Diet 4 n = 4	SEM	P-value
WBC (x10^9/l)	6.00^a	5.99^a	4.00^b	3.00^c	0.02	0.005
NEU (%)	58.00^a	41.25^b	35.5^c	29.25^d	1.94	0.004
LYM (%)	42.00^a	58.75^b	64.50^b	70.75^c	2.01	0.006

Means with different superscripts within the same row are significantly (P < 0.05) different

The WBC contents of animals on diets 1 and 2 were not significantly different (P > 0.05) but were significantly (P < 0.05) higher than those of diets 3 and 4 groups. In any case, the animals on diet 3 had a significantly (P < 0.05) higher WBC contents than those of 4 group. However, for the neutrophils animals on diet 1 group had a significantly (P < 0.05) higher content compared with the animals on diet 2 group that was also significantly (P < 0.05) higher than those of diets 3 and 4 groups. However, animals on diet 3 group had a significantly (P < 0.05) higher neutrophil content compared with those of diet 4 group. For the lymphocytes, diet 1 animals demonstrated a significantly (P < 0.05) lower value compared with those of diet 2 group that showed a significantly (P < 0.05) lower value than those of diets 3 and 4 groups. It is also imperative to note that animals on diet 4 group had a significantly (P < 0.05) higher value of lymphocytes compared with those of diet 3 group. Crude oil had long been shown to be a toxic material for domesticated animals [6]. It has been demonstrated that haematological parameters are affected by factors like nutrition, environment, health condition and physiological status of the animal [15]. Furthermore, [2] indicated that hematological factors are one of the best measures in assessing the health status of the animal. The views of [12] were corroborated by the findings of [1]. In this study, there was no significant difference between treatments 1 and 2 in Hb, RBC and PCV contents. However, beyond these treatments as dietary crude oil content increased to 15g and 20g/kg of diet, these parameters were compromised. These were indications that the health status of animals in these treatment groups were compromised as the serum levels of these parameters were significantly reduced. These findings agree with the observations of [1], [3], [17]. These findings are also indicators of the fact that the animals on dietary treatments 3 and 4, respectively suffered from anemia as judged by their significant reduced levels. Furthermore, the animals in these groups would also be generally weak primarily due to low circulating Hb and RBC further triggered by low packed cell volume of the circulating blood. The welfare of the animals on diets 3 and 4 were further compromised as evident by the significant reduced WBC counts as the dietary crude oil level increased to 15g and 20g in the diets, respectively. Reduction

in the WBC contents is also an indication of the fact that the animals in these dietary treatment groups suffered from leukemia suggesting that the health status of the animals have been serious compromised as also found in the studies of [12]. This is a major fact that can easily predispose the animals to diseases and some other environmental stressors [8], 13]. From these findings therefore, it can equally be speculated that if the current study had continued beyond the 4 weeks' duration, the animals on diet 2 could have probably experienced chronic effects of the ingested crude oil as the effects of the ingested crude oil were very acute within the study duration for diets 3 and 4 groups. Thus, it can equally be deduced that if the study period was extended beyond the 4 weeks the animals in groups 3 and 4 could have experienced sudden death usually referred to as 'sudden death syndrome' in swine [15]. These observations also readily come to the fore as the health of the animals particularly those on diets 3 and 4 had already started deteriorating as evidenced in their reduced hematological properties. This assertion is further supported by the fact that apart from the control treatment diet 1 the ratio of LYM to NEU generally increased in the high crude oil-contaminated diets which could have been a physiological response of the animals via stimulation of the immune system as the ingested crude oil would have been perceived as xenophobes [15]. These findings also justify the current agitation by the Ogoni ethnic nationality struggle in the Niger Delta Region of Nigeria for the clean-up of their polluted environment by crude oil exploration [4], [12], [20]. This assertion is further justified by the fact that the pig model has been recognized globally as the animal model for biomedical research [7].

4 Conclusion

It was concluded that pigs can consume up to 10g crude oil/kg of diet without compromise on its blood constituents. However, crude oil consumption beyond 10g/kg diet results in acute negative effects on hematological parameters of the growing pig and increased the ratio of LYM to NEU. Therefore, the growing pig's threshold for crude oil ingestion lies between 10g and 15g of crude oil/kg of diet.

5 References

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