Effects Of Feeding Graded Levels Of Crude Oil-Containing Diets On Liver And Kidney Functions In The Growing Pig

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Abstract: Effects of feeding graded levels of crude oil-containing diets were studied on liver enzymes: alanine amino transferase (ALT), aspartic amino transferase (AST) and alkaline phosphatase (ALP) as well as on kidney biomarkers: blood urea nitrogen (BUN) and creatinine 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 animal in all the four dietary treatment groups at the end and snap frozen. ALT serum levels of diets 1 and 2 were similar ($P > 0.05$) but significantly ($P < 0.05$) lower than those of diets 3 and 4 with diet 4 having the highest ($P < 0.05$) significant serum value of ALT. The trends of ALT were mimicked by AST and ALP, respectively. BUN serum levels of diets 1 and 2 were similar ($P > 0.05$) but significantly ($P < 0.05$) lower than those of diets 3 and 4 again with diet 4 showing the highest ($P < 0.05$) significant value. Creatinine mirrored BUN fashion. It was concluded that ingestion of 10g of crude oil/kg diet had no effect on the liver and kidney; however at 15g and above of crude oil/kg of diet the crude oil negatively affected the health of the liver and kidney based on increased sera levels of their biomarkers as yardsticks.

Key words: Liver and Kidney Biomarkers, Crude Oil, Contamination and Pig.

1 INTRODUCTION

Both [9] and [14] had identified ALT as a hepatic enzyme as is mostly found in the liver. Therefore, when the liver is damaged or made to malfunction it leads to high levels of ALT in blood serum as a result of the leakage of the liver. To this point therefore, [2] showed that the major dependable biomarker of oxidative damage to the liver is ALT that can be done by evaluating serum level of ALT. This is true because [8] had shown that the activity of ALT in the liver is 3000 times its activity in the blood serum and any damage in the cells of the liver will result to the releasing of high amount of ALT in the blood of the organism. Again, another hepatic enzyme that is also used as a dependable biomarker to determine liver function is AST. Although AST is also found in the muscle, kidney and heart the liver is most associated with its production [9; 14]. Furthermore, [2] and [16] demonstrated that the production of reactive oxygen substances (ROS) produced by ingestion of foreign bodies, such as a toxicant is capable of damaging some tissues of the liver, thereby releasing AST into the blood, indicating liver malfunction also as a result of liver leakage. Another hepatic enzyme that has been demonstrated to be a good biomarker of liver function is ALP. It has also been shown that when ROS damages the liver, including the bile duct [16] it results in oxidative stress that also leads to ALP release into the blood stream due to leakages of the liver and the bile duct [9]. Another important organ of an organism is the kidney that is involved in the filtration of blood and release of waste products, such as blood urea nitrogen and creatinine. To this end, blood creatinine level is often used to determine the level of oxidative damage in kidney. The study of [3] has been used to lay credence to this. In that study, the blood creatinine level of the animal significantly increased when rabbits were treated with cisplatin due to its interference with kidney function suggesting that creatinine is a good biomarker of oxidative stress on the kidney. This also supports the idea that a high concentration of blood creatinine is indication of kidney damage or malfunction. Additionally, BUN level is another essential biomarker for determining kidney function. [3] and [9] demonstrated that high level of BUN indicates damage or malfunctioning of the kidney as BUN levels become abnormally increased. Therefore, conducting BUN test on an animal that ingested graded levels of a toxic substance, such as crude oil would be one of the best production practices to check the health status of the kidney. This in turn would help to determine the effect of the ingested toxicant on kidney fucntion of the animal. Therefore, the objectives of the Study are to measure serum levels of ALT, AST and ALP as well as serum levels of creatinine and BUN following the ingestion of dietary crude oil-containing diets as to be able to determine the health statuses of the liver and kidney, respectively of growing pigs that received the graded levels of crude oil-containing diets.

2 MATERIALS AND METHODS

Animals' Handling during Study

Twenty four landrace growing pigs that weighed on average 9 ± 1.4 (mean ± SD) kg body weight (BW) were bought from Cape Farms, Irete, Imo State and humanely transported to the Rivers State University Teaching and Research Farm. On arrival at the farm, they were randomly assigned to their individual pens. The animals were then given 14-d to adapt to their new environment. Amoxicillin antibiotic were given intramuscularly for protection to ensure their sound health and fed similar grower diet. After the adaptation period, the animals were presented with their experimental diets at 5% of their BW (as-fed basis) according to the method of [7] 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 cleaned throughout the experimental period. There were four replications per dietary treatment group.

Crude Oil Treatment and Addition to Diets

Bonny Light crude oil obtained from the Nigerian Agip Oil Company Limited was the crude oil type used in this study. Before the use of the crude oil proper it was exposed to sunlight for 24 h in shallow pans according to the method of [13] as to achieve a stable product that imitate 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 [12] 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 4 weeks.

Data Collections, Analyses and Experimental Design

At the end of study period, blood samples were humanely collected into EDTA treated tubes and immediately snaps frozen for sera analyses of ALT, AST, ALP, Creatinine and BUN. ALT and AST were analyzed according to the method of [17]. Alkaline phosphatase was done according to the method of [1]. BUN was determined according to the method of [10]. Creatinine was measured with alkaline picrate according to the method of [11]. The experimental data were analyzed as a completely randomized design (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

All animals consumed their diets, nevertheless some forms of feed refusals were observed in animals of treatments' groups 3 and 4 but more with treatment group 4 in the 4^{th} week of study. This also resulted in terminating the trial at the end of the 4^{th} week. The results of liver enzymes, namely: ALT, AST and ALP are shown in Table 1.

***Table 1**. ALT, AST and ALP serum levels of pigs fed graded levels of crude oil-containing diets*

	DIETS					
Item	Diet 1 n = 4	Diet 2 n = 4	Diet 3 n = 4	Diet 4 n = 4	SEM	P-value
ALT (µL)	21.25[a]	22.50[a]	53.00[b]	68.00[c]	0.71	0.001
AST (µL)	76.50[a]	76.25[a]	101.00[b]	126.25[c]	0.87	0.001
ALP (µL)	31.50[a]	31.00[a]	53.00[b]	79.25[c]	0.57	0.001

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

From the results as shown in Table 1, animals in the dietary crude oil treatments 1 and 2 had similar serum levels of ALT as they were not significantly (P > 0.05) different. However, as dietary crude oil increased beyond 10g/kg of diet ALT serum levels linearly increased significantly (P < 0.05) with the dietary 20g crude oil/kg of diet showing the highest serum levels for ALT, respectively. Furthermore, AST and ALP serum levels demonstrated similar patterns with ALT. The results of BUN and creatinine are shown in Table 2.

***Table 2**. BUN and creatinine serum levels of pigs fed graded levels of crude oil-containing diets*

	DIETS					
Item	Diet 1 n = 4	Diet 2 n = 4	Diet 3 n = 4	Diet 4 n = 4	SEM	P-value
BUN (mmol/l)	18.54[a]	19.10[a]	35.21[b]	43.23[c]	0.86	0.001
Creatinine (mmol/l)	2.15[a]	2.22[a]	8.43[b]	12.65[c]	0.91	0.001

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

BUN serum levels of animals on dietary treatment 1 and 2 groups were similar as they were not significantly (P > 0.05) different. However, as the dietary crude oil increased beyond 10g/kg of diet BUN serum levels linearly increased (P < 0.05) significantly compared with diets 1 and 2 resulting in the 4^{th} diet having the highest BUN serum levels. The trend in BUN serum levels were also mirrored by creatinine serum levels (Table 2). From biological standpoint, assays of the biomarkers of the liver and kidney are always used in modern science to assess the health statuses of these very important organs of the animal. Previous researchers had shown that crude oil ingestion can destruct the activities of hepatocytes leading to liver inflammation and necrosis. [6; 15] demonstrated a gross structural disintegration in the hepatocytes of rats that had contact with crude oil. [6] also demonstrated that the structural integrity of liver cells was affected at very low doses of undiluted Bonny Light crude oil. These authors also indicated that the effect of the crude oil also led to multiple bile duct proliferation with foci of hepatic necrosis.Additionally, [5] observed cellular infiltration in the liver of rabbits treated with 10ml crude oil/kg of feed widening of sinusoids, lymphatic infiltrates with interface destruction of hepatocytes and cytoplasmic vacuolation in those treated with 20ml crude oil/kg of feed while the liver of untreated rabbits showed no visible lesion, normal hexagonal architecture of the hepatocytes, hepatic veins and sinusoids. In this current study, our findings agreed with the literature data of [15] that showed deviations in the normal architecture of the liver as evidenced in our study via significant increased in all the serum biomarkers of the liver as also corroborated by the data of [5] and [6], respectively. Crude oil ingestion also interfered with the integrity of the kidney as its major two biomarkers increased exponentially as dietary crude oil increased in the diets beyond 10g of crude oil/kg of diet. These findings therefore implied that ingestion of crude oil beyond the 10g/kg of diet compromises the health statuses of the liver and kidney of the growing pig [4].

4 CONCLUSION

From the findings of this study, we concluded that pigs can tolerate crude oil in their diets up to 10g/kg of diet without interfering with the functions of the liver and kidney. However, beyond the 10g/kg of diet the functions of the liver and kidney were impeded as indicated by their biomarkers.

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