Liver And Kidney Functions Of Growing Pigs Fed Graded Levels Of Crude Oil-Contaminated Diets

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Abstract: This study was designed to investigate the effects of ingesting graded levels of crude oil-contaminated diets on the liver and kidney functions of the growing pig using their biomarkers. 24 growing pigs weighing on average 8 ± 1.1 (mean ± SD) kg of body weight (BW) were acquired and used in the study. The pigs on arrival at the animal wing of the Rivers State University Teaching and Research Farm were randomly assigned to six dietary treatment groups of 4 pigs per treatment. Dietary treatment groups were as: 0g, 2g, 4g, 6g, 8g and 10g of crude oil/kg of diet, respectively. The experimental animals were fed at 5% of their BW and the experiment lasted for 28 d. Blood samples were collected from all treatment groups into EDTA treated tubes and immediately snaps frozen for later analyses of biomarkers for the liver: Alanine amino transferase (ALT), Aspartic amino transferase (AST) and Alkaline phosphatase (ALP) as well as for kidney biomarkers: blood urea nitrogen (BUN) and creatinine levels. There were no significant ($P > 0.05$) differences in all the liver biomarkers measured, ALT, AST and ALP. Similarly, there were no significant ($P > 0.05$) differences in the two biomarkers of the kidney measured, BUN and creatinine contents for all dietary treatment groups. It was therefore concluded that crude oil contaminations up to 10g/kg of diet did not affect the liver and kidney of growing pigs, since their functions were not impeded.

Key words: Crude Oil-Contaminated Diets, kidney biomarkers, Liver biomarkers and Pig.

1 Introduction

The liver and the kidney are very vital organs in living organisms, including the pig. In the presence of a toxicant, such as crude oil the functions of the liver and kidney can be compromised leading to health deteriorations of the affected animal due to leakage of the liver and possibly the bile duct [13] and for the kidney elevated levels of BUN and creatinine, respectively [2], [15]. The biomarkers of compromised or damaged liver functions are elevated levels of ALT, AST and ALP [13], [15]. During such times, the animal well-being is compromised, especially, in the absence of antioxidants to detoxify the products of reactive oxygen species (ROS) produced as a result of the oxidative stress [8], [12]. Furthermore, the efficiency of production is usually compromised as the animal is no more comfortable [4]. ROS and hydroxyl radicals so produced at these times lead to cells damages that can result in programmed cell death if the ROS are not removed from the cells of the animal [12]. ALT is mostly abundant in liver of animals [7], [16]. A high level of ALT in blood serum has been shown to be a dependable biomarker of oxidative damage in the cells of the liver of the animal [3]. [6] showed 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 amounts of ALT in the blood of the animal. AST is also an enzyme that is predominantly found in tissues of organs such as the muscle, liver, kidney and heart and particularly in the liver [7], [16]. [3], [18] demonstrated that ROS produced by some toxicants are capable of damaging some tissues of the liver, thereby releasing AST into the blood, resulting to its high levels in the blood. ALP has also been demonstrated to be a good biomarker of oxidative stress in animals [7]. ALP is released into the blood stream when there is an oxidative damage in the liver tissues, including the bile duct [18]. Blood creatinine is also used to determine the level of oxidative damage to kidney of animals. [19] demonstrated that blood creatinine increased when animals were treated with cisplatin suggesting that creatinine is a good biomarker of oxidative stress in studying the health status of the kidney. High concentrations of blood creatinine are therefore indications of kidney damage and malfunctioning. Different independent studies such as those of [7] and [9] had shown that high level of blood urea nitrogen is linked to damage or malfunctioning of the kidney suggesting that blood urea nitrogen test is one of the best practices in evaluating damage in the kidney. Therefore, the objectives of the Study are to determine the effects of ingesting graded levels of crude oil-contaminated diets on the health statuses of the liver and kidney using their biomarkers in growing pigs.

2 Materials and Methods

Animals and Management

Twenty four landrace pigs of average BW of 8 ± 1.1 (mean ± SD) kg were purchased from Cape Farms, Irete, Imo State, Nigeria and transported to the Rivers State University Teaching and Research Farm where the experiment was conducted. Animals were randomly assigned to their individual experimental pens. The pens were thoroughly washed and cleaned and allowed to dry before introducing the animals into them. The animals were given 14-days to acclimatize to their new environment. At this time, the animals were given ivermectin injection sub-cutaneous and amoxyciline antibiotic injection intramuscularly to ensure good health status before commencement of study and fed similar grower diet. After the adaptation period, the animals

were offered their crude oil-contaminated diets at 5% of their BW twice daily according to the method of [5] at 09:00h (half of the daily meal) and 16:00h, respectively. Water was provided ad libitum via low pressure nipples and pens were constantly kept cleaned throughout the experimental duration.

Crude Oil and Experimental Diets

The crude oil used in this study was Bonny Light from the Nigerian Agip Oil Company Limited. Before the commencement of study, the crude oil was exposed to sunlight for 24 h in a shallow pan to enable the escape of the light volatile fractions via evaporation thereby ensuring a stable product that feigns crude oil natural form during oil spillage and pollution [17]. Six corn-soybean meal-based diets that were isocaloric and isonitrogenous to meet or exceed the [13] recommended nutrient requirements of growing pigs of 10 – 20 kg BW were used in the study. Although the diets had similar nutrient levels they differed in their dietary crude oil contents as: diet1, the control diet (0g crude oil), diet 2, (2g crude oil), diet 3, (4g crude oil), diet 4, (6g crude oil), diet 5, (8g crude oil) and diet 6, (10g crude oil)/kg of diet, respectively. Animals received their respective experimental diets for 4 weeks.

Data Collections, Biomarkers Analyses and Experimental Design

At the end of the experiment blood samples were collected from all animals in each dietary treatment group into EDTA treated tubes and immediately snaps frozen for liver biomarkers, namely: ALT, AST and ALP as well as for kidney biomarkers (BUN and creatinine) analyses. ALT and AST were analysed according to the method of [14]. ALP was analysed according to the method of [1]. Urea creatinine concentration was determined according to the method of [10]. Creatinine concentration was measured according to the method of [11]. 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 all dietary treatment groups consumed their respective diets readily without any obvious signs of feed rejection indicating that the levels of crude oil in the diets were positively responded to. This was confirmed by the fact that there were no orts for all the dietary treatment groups. This suggests that growing pigs can tolerate crude oil up to 10g/kg of diet. This again suggests that all the dietary groups were palatable to the animals. Here, it is very imperative to note that pigs are the most sensitive animal species that can associate the postprandial experience of a diet. This simply means that a diet with negative postprandial effects is always rejected when such diets are present the next time [13]. This is also linked to the numerous test buds the species are known for [13]. The results of liver biomarkers are shown in Table 1 whereas the results of kidney biomarkers are shown in Table 2, respectively. As shown in Table 1, there were no significant ($P > 0.05$) differences in all the liver biomarkers studied. Similarly, as shown in Table 2, there were no significant ($P > 0.05$) differences in all the kidney biomarkers investigated. These results therefore, indicated that there were no leakages or damages to the liver and bile ducts of the pigs. In the same vein, there were no damages done to the kidneys and their functions at the levels of crude oil used in this trial in the presence of no feed refusal by the experimental animals, respectively.

Table 1. AST, ALT and ALP serum concentrations of growing pigs fed varied crude oil-contaminated diets.

	DIETS							
Item	Diet 1 n = 4	Diet 2 n = 4	Diet 3 n = 4	Diet 4 n = 4	Diet 5 n = 4	Diet 6 n = 4	SEM	P-value
AST (µ/L)	73.50	75.25	75.25	75.25	75.25	75.25	1.05	0.607
ALT (µ/L)	25.25	24.75	24.75	24.75	23.75	23.75	0.43	0.976
ALP (µ/L)	32.00	31.25	31.75	31.75	33.00	31.50	0.83	0.931

Table 2. BUN and creatinine serum concentrations of growing pigs fed varied crude oil- contaminated diets.

	DIETS							
Item	Diet 1 n = 4	Diet 2 n = 4	Diet 3 n = 4	Diet 4 n = 4	Diet 5 n = 4	Diet 6 n = 4	SEM	P-value
BUN (mmol/l)	19.10	19.11	18.97	19.00	18.99	19.00	1.56	0.876
Creatinine (mmol/l)	2.14	2.19	2.12	2.11	2.19	2.18	0.25	0.513

As shown in Tables 1 and 2, respectively the findings from this current study supported the fact that there were no differences in the levels of liver enzymes or biomarkers among the animals in all dietary treatments; thereby indicating that there were no leakages of the liver thus confirming the good health statuses of the liver of the animals in the study as well as that of the bile duct, including that of the kidney.

3 Conclusion

It was concluded that 10g of crude oil/kg of diet is below the threshold for growing pigs in respect of damaging the liver or the kidney. This is the first study to investigate the effects of crude oil ingestion in pigs. Further studies will therefore be conducted to be able to establish the threshold of crude oil for growing pigs as to be able to better characterize crude oil effects on liver and kidney functions in the growing pig.

4 References

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