Effects Of Feeding Graded Levels Of Crude Oil-Containing Diets On Antioxidants And Oxidants Statuses In Pigs

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Abstract: Effects of graded levels of crude oil-containing diets were studied on sera levels of antioxidant enzymes: glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and oxidant malondialdehyde (MDA) in growing pigs. 24 growing 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 pens. 4 graded crude oil dietary treatments: 0g (control group), 10g, 15g and 20g of crude oil/kg of diet were used with 4 replications per dietary treatment. Animals were fed at 5% of their BW for 4 weeks. GSH sera contents of animals on diets 1 and 2 were similar ($P > 0.05$) as there were no differences between them but significantly ($P < 0.05$) higher than those of animals in treatments 3 and 4 with group 4 animals demonstrating the lowest content. SOD contents of treatments 1 and 2 were similar ($P > 0.05$) and significantly ($P < 0.05$) higher than those of treatments 3 and 4 with diet 4 showing the lowest level. CAT sera contents of animals on treatments 1 and 2 were similar ($P > 0.05$) and significantly ($P < 0.05$) higher than those of treatments 3 and 4 with animals on diet 4 showing the lowest levels. For GSH-Px, there were no differences amongst groups. MDA sera contents of the animals on treatments 1 and 2 were similar ($P > 0.05$) but significantly ($P < 0.05$) lower than those of treatments 3 and 4 with treatment 4 showing the highest level. It was concluded that ingestion of 10g crude oil/kg diet had no effect on antioxidant enzymes; however at 15g and above antioxidants were suppressed while MDA levels increased. Beyond the 10g/kg of diet the health of animal was compromised as judged by lowered levels of antioxidants and increased MDA levels.

Key words: Antioxidants, Contamination, Crude Oil, Oxidants and Pig

1 Introduction

Growing pigs are amongst the known fast growing species in the commercial setting for quick turnovers in profit making [9], [11]. This characteristic of the growing pig has also been implicated in the sudden death syndrome often observed in growing pigs in the commercial setting [11]. Overall, the attendant effect of this negatively affects the hog farmer as its profit margins always plummet. Most times some environmental stressors trigger these negative effects through dietary means [7]. Some of these contents' of animals' diets predispose animals to disease conditions leading to the stimulation of the immune system of the animals resulting in diverting energy and nutrients meant for growth/development to maintenance of the animal [4] – [5]. This would often result in stunting the growth of the animal [5]. From the fore stated, ingestion of crude oil in the Niger Delta Region of Nigeria by domesticated animals in the region is a common occurrence due to incessant oil pollution of their environments [12]. Animals have naturally been endowed with inherent ability to defend its self against oxidative stress especially through the glutathione (GSH) defense system when they come into contact with foreign bodies, such as crude oil. In order to combat oxidative danger the animal uses its complex anti-oxidation systems to protect against peroxidation. As previously stated, GSH defense system and its cohorts, such as CAT, SOD and GSH-Px are activated and work synergistically together [1]. SOD catalyzes the dismutation of superoxide radical into oxygen and hydrogen peroxide. CAT converts hydrogen peroxide to carbon dioxide and also breaks down potentially harmful toxin in the animal's body. GSH-Px is also an active quencher of ROS activities and converts oxidized GSH to its active reduced form. GSH catalyzes the reduction of hydrogen peroxide to water and oxygen. However, there are data in the literature pointing out that crude oil ingestion suppresses glutathione defense system [1], [10] and therefore predisposes animals to disease condition, including feed refusal due likely to anorexia resulting in stunted animal growth [4]. MDA is the major byproduct of the suppression of the GSH system. Antioxidants play special roles in the health of the animal [6]. Crude oil being a toxicant may therefore reduce antioxidants levels while increasing MDA levels due to oxidative stress induced by the ingested crude. Therefore, the objectives of this study are to evaluate the effects of ingestion of graded levels of crude oil-containing-diets on the statuses of GSH, SOD, CAT, GSH-Px and MDA in the growing pig.

2 Materials and Methods

Animal Treatments during Study

Twenty four landrace growing pigs weighing on average 9 ± 1.4 (mean ± SD) kg body weight (BW) were acquired from Cape Farms, 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 allowed 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 during this adaptation period. After the adaptation period, the animals were served their experimental diets at 5% of their BW (as-fed basis) according to the method of [6] 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 Additions to Diets

The crude oil used in this current study was Bonny Light crude oil obtained from the Nigerian Agip Oil Company Limited. Before the use of the crude oil to contaminate the diets it was exposed to sunlight for 24 h in shallow pans according to the method of [13] as to achieve a stable product that feigns natural form during pollution as the light volatile fractions escaped during exposure to sunlight. Six corn-soybean meal-based diets formulated to be isocaloric and isonitrogenous to meet or exceed the [9] 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.

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 GSH, SOD, CAT and MDA, respectively. GSH, GSH-Px and MDA were analysed for according to the methods of [3]. CAT was analysed for according to the method of [2] and SOD was analysed according to the method of [8]. 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 in each of the dietary treatment groups readily ate their allotted diets; nevertheless orts were observed with diets 3 and 4 and more so with diet 4 indicating that the 15 and 20g crude oil/kg diet is above the level of crude oil growing pigs can handle and tolerate. With this observation, it can be asserted that the orts seen with diets 3 and 4 signified feed refusal by the animals in the 2 treatment groups. This also justified the ending of the experiment in the fourth week since the orts observations began towards the end of the fourth week. Feed remnants also observed with pigs in treatments 3 and 4 would have also been related to the pigs' ability to link discomfort associated with an ingested feed postprandial [11]. Table 1 shows the effects of consuming graded levels of crude oil-containing diets.

Table 1. GSH, SOD, CAT, GSH-Px and MDA sera levels of growing pigs fed graded levels of crude oil-containing diets

Item	DIETS					
	Diet 1 n = 4	Diet 2 n = 4	Diet 3 n = 4	Diet 4 n = 4	SEM	P-value
GSH (mg/dl)	4.00^a	4.00^a	2.00^b	1.00^c	0.01	0.005
SOD (U/mg)	1.40^a	1.37^a	1.04^b	0.43^c	0.012	0.004
CAT (IU/mg)	15.86^a	15.55^a	12.21^b	7.42^c	0.07	0.001
GSH-Px (nmol/dl)	1.25	1.00	1.00	1.00	0.125	1.00
MDA (nmol/ml)	0.67^a	0.67^a	0.81^b	0.97^c	0.011	0.001

Means with same superscripts within the same row are not significantly (P > 0.05) different

As shown in Table 1, the GSH sera contents of animals on diets 1 and 2 were similar (P > 0.05) as there were no differences between them and were significantly (P < 0.05) higher than those of animals in treatments 3 and 4 with group 4 animals demonstrating the lowest serum content of GSH. The SOD sera contents of the animals mimicked or mirrored those of GSH as the SOD contents of treatments 1 and 2 were similar and significantly (P < 0.05) higher than those of treatments 3 and 4 with diet 4 animals showing the lowest level. CAT sera contents of animals on dietary treatments 1 and 2 were similar (P > 0.05) but significantly (P < 0.05) higher than those of treatments 3 and 4 with animals on diet 4 showing the lowest CAT levels. There were no significant (P < 0.05) differences in sera contents for all dietary treatment groups. On the other hand the MDA sera contents of the animals on dietary treatments 1 and 2 were similar (P > 0.05) as there were no significant difference between them but significantly (P < 0.05) lower than those of treatments 3 and 4 with treatment showing the highest level. From these results increased dietary intake of crude oil suppressed GSH sera levels as GSH levels reduced linearly as the crude oil level increased in the diets. Furthermore, as the crude oil increased in the diets SOD and CAT sera levels also linearly reduced. However, crude oil intake had no effect on GSH-Px. Conversely, the MDA sera levels increased as dietary crude increased. From the fore stated therefore, crude oil compromised the health of the animals as the level of intake increased beyond 10g/kg of diet. This suggests that animals on those diets experienced oxidative stress. These findings in this study agree with literature data that crude oil suppressed the GSH defense system of the animal and as such can predispose the animals to diseases or at least make them more susceptible to environmental stressors, including pathogenic organisms [1], [4] – [5]. Additionally, ingestion of crude oil beyond the bench mark of 10g/kg of diet resulted in increased levels of MDA supporting the idea that the animals in treatments 3 and 4 truly experienced or suffer from oxidative stress. This finding is also in agreement with literature data [10].

4 Conclusion

It was concluded that the benchmark of crude oil ingestion for growing pig is 10g/kg of diet. Beyond this level the GSH defense system of the animal is suppressed leading to oxidative stress and consequently the health of the animal is compromised as judged by increased MDA levels in treatments 3 and 4, respectively.

5 References

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