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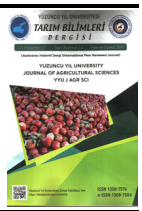

Research Article

Histopathological Effects of Different Levels of Palm Kernel Cake Fed to Dorper Lambs

Osama Anwer SAEED[1], Baker Tareq JABER[2], Umar Mohammed SANI[3], Awis Qurni SAZILI[4], Henny AKIT[5], Abdul Razak ALIMON[6], Anjas Asmara SAMSUDIN[*7]

[1,2]Department of Animal Production, College of Agriculture, University Of Anbar, Anbar, Ramadi, Iraq
[3] Department of Animal Science, Faculty of Agriculture, Taraba State University, P.M.P. 1167, Jalingo, Nigeria
[4,5,7]Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia
[6]Faculty of Animal Science, Universitas Gadjah Mada, Yogjakarta, Indonesia

[1]https://orcid.org/0000-0003-2706-1332 [2]https://orcid.org/0000-0002-5804-6779 [3]https://orcid.org/0000-0002-6170-9246
[4]https://orcid.org/0000-0002-2461-271X [5]https://orcid.org/0000-0003-1530-3496 [6]https://orcid.org/0000-0001-7235-1406
[7]https://orcid.org/0000-0002-9758-7973

* Corresponding author. anjas@upm.edu.my

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Abstract: Palm kernel cake (PKC) is a nutrient-rich by-product of oil mills that contained a high concentration of copper (Cu) among other minerals elements. The higher dietary concentration of Cu was reported to impair some physiological functions in some breeds of sheep due to Cu toxicity. Hence, this study was aimed to investigate the histopathological effects of PKC based diet on vital organs of Dorper lambs. Fifteen male Dorper lambs (8 month-old) were randomly assigned to three treatment groups. Lambs were fed with a diet supplemented with different levels of PKC based on dry matter (DM) (PKC 750 gm kg^{-1} DM, PKC 700 gm kg^{-1} DM, and PKC 650 gm kg^{-1} DM) for 120 days. At the end of the experiment, all the lambs were slaughtered, liver, kidney, and testis samples were examined for gross pathological lesions. Microscopic examination showed that the liver of PKC75 group animals was histologically normal. However, cell hepatocyte abnormality was observed in PKC70 and PKC65 groups. No clinical symptoms or severe histological lesions were noticed in the kidney and testis of all groups. Therefore, the histomorphological normal organs observed indicated the PKC contents provided detoxification and immune defense in male lambs.

Dorper Kuzularına Farklı Düzeylerde Hurma Çekirdekli Kek Yeminin Histopatolojik Etkileri

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Anahtar Kelimeler

Bakır Toksisitesi,
Palmiye çekirdekli kek,
Karaciğer,
Dorper kuzu,
Histopatoloji.

Öz: Hurma çekirdeği keki (PKC), diğer mineral elementler arasında yüksek konsantrasyonda bakır (Cu) içeren, yağ değirmenlerinin besin maddesi açısından zengin bir yan ürünüdür. Cu'nun daha yüksek diyet konsantrasyonunun, Cu toksisitesi nedeniyle bazı koyun ırklarında bazı fizyolojik fonksiyonları bozduğu bildirilmiştir. Bu nedenle, bu çalışma, PKC bazlı diyetin Dorper kuzularının hayati organları üzerindeki histopatolojik etkilerini araştırmak için yapılmıştır. On beş erkek Dorper kuzusu (8 aylık) rastgele üç muamele grubuna ayrılmıştır. Kuzular, 120 gün boyunca farklı seviyelerde PKC kuru maddeye dayalı (DM) (PKC 750 gm/kg DM, PKC 700 gm/kg DM ve PKC 650 gm/kg DM) ile beslenmiştir. Deneme sonunda tüm kuzular kesilerek karaciğer, böbrek ve testis örnekleri büyük patolojik lezyonlar açısından incelenmiştir. Mikroskobik incelemede, PKC75 grubu diyetle beslenen hayvanların karaciğerinin histolojik olarak normal olduğunu tespit edilmiştir. Ancak PKC70 ve PKC65 gruplarında hücre hepatosit anormalliği

gözlenmiştir. Tüm grupların böbrek ve testislerinde hiçbir klinik semptom veya ciddi histolojik lezyonuna rastlanmamıştır. Bu nedenle, histomorfolojik olarak organların normal gözlemlenmesi, erkek kuzularda detoksifikasyon ve bağışıklık savunması sağlayan PKC içeriğiden kaynaklandığı belirlenmiştir.

1. Introduction

Mineral elements are essential to animal wellbeing and survival. They aid physiological, structural, catalytic, and regulatory functions. Hence, the need to include ingredients that will supply the right amount of these minerals in the diet of animals is very essential (Bingöl and Bingöl, 2018; Das et al., 2018). However, if these minerals are ingested in excess due to the addition of mineral supplements and/or imbalance ration may trigger toxicity effects (Reis et al., 2010). Excess or deficiency mineral elements such as cadmium, lead, copper, chromium, iodine, manganese, molybdenum, selenium, and zinc exact some physiological changes in tissues and organs of animals (Asli et al., 2020). Palm kernel cake was reported to with 16-20% protein and served as a good alternative feed ingredient for feeding sheep (Ribeiro et al., 2011; Saeed et al., 2018). However, the higher dietary copper (Cu) concentration of up to 30 ppm in PKC is of concern in sheep ration. Clarkson et al., (2020) reported that excess dietary Cu is intolerable by sheep as it impaired the excretion of Cu in the bile which led to the excess aggregation of Cu in the liver and subsequent Cu toxicity. Similarly, excess dietary Cu concentration was reported to increase mortality, histopathological changes within the tissues of vital organs (Wu et al., 2020). Histopathological changes reported include mild liver toxicity, hepatocytes swollen, as well as vacuolated and necrotized localized at the medulla zone (Hair-Bejo et al., 1995). Copper concentrations were found to consistently increase in the kidney than liver of dead animals with signs of a hemolytic crisis; therefore, the kidney is considered the tissue of choice when the measurement of only one organ is requested (Rothuizen, 2020). Abnormal hepatic metabolism resulting from high dietary intake of Cu and altered biliary and urinary excretion of Cu or nutritional imbalances between Cu and other trace elements can lead to massive hepatic stores of Cu over time and subsequently lead to what has been referred to as chronic Cu toxicosis. Therefore, the present study was conducted to evaluate the histopathological effects of the palm kernel cake-based diet as a source of Cu on the liver, kidney, and testis of Dorper lambs.

2. Material and Methods

2.1. Experimental animals

The experiment was performed following the procedure of the Institutional Committee on Animal Use Ethics (Approval No. R064/2016). A total of 15 Dorper lambs with an initial live body weight 16 ± 0.38 kg and age 8 months were used in this study. Experimental was carried out at the research farm of University Putra Malaysia and lasted for a period of 120 days. Lambs were divided into three homogenous groups and randomly assigned to receive one of the three dietaries of varying inclusion levels of palm kernel cake as a source of Cu. The animals undergo an adaptation period of 3 weeks before starting the main experiment. Each pen was provided with a feeding trough and a water container, and feed was offered two times daily. Lambs were fed to different diets: a) supplemented with 750 gm PKC kg^{-1} DM per head (PKC75 group); b) supplemented with 700 gm PKC kg^{-1} DM per head (PKC70 group) and c) supplemented with 650 gm PKC kg^{-1} DM per head (PKC65 group), all diets were formulated to meet the body requirement according to (NRC, 2007). Analysis of diets minerals contents was done according to Kolmer et al., (1952). The concentration of minerals was presented in Table 1. The mineral premix was omitted in the diets in order to minimize the Cu level in the diets as PKC was reported to contain high minerals.

2.2. Histopathology of liver, kidney, and testis

After slaughter, the liver (right liver lobes), the right kidney (renal cortex), and right testis were removed and fixed in freshly prepared 10% formalin for at least 48 hrs. Samples were fixed in Bouin's solution for 16 hours. After fixation, the tissues were washed three times with 70% alcohol for an hour

per wash. The tissue processing was done automatically using a histokinette machine (Leica ASP 300 tissue processor, Germany). The machine with 11 containers that contained specific and time was adjusted for each container. The tissue samples were dehydrated by immersion with a graded series of low to high concentrations of alcohol until the tissue samples were saturated with 100% alcohol. Subsequently, the samples were infiltrated using 100% chloroform cleaning reagent. After being impregnated with paraffin wax, the samples were blocked in paraffin. The tissues were sectioned using a microtome (Leica, model RM 2155 rotary microtome, Germany) at a thickness of 5 µm and stained with Haematoxylin and Eosin (Bancroft and Gamble, 2008).

2.3. Statistical analysis

The general linear model procedure was used to examine data in a completely randomized design (CRD) in SAS 9.4 (SAS Inst. Inc., Cary, NC, USA). Duncan's multiple range test ($p < 0.05$) was used to calculate all multiple comparisons between means The proportion of lesions and healthy appearance of the liver were analyzed using Chi-Square analysis.

3. Results

This histopathological approach on Dorper lambs was conceived to provide biological insights on tissue changes induced by a diet with 750, 700, and 650 gm PKC/kg DM that showed the concentrations of the minerals in Table 1 accordingly.

Table 1. Experimental diet of minerals content (mg kg^{-1} dry matter (DM)

Item	Cu	Fe	Zn	Se
PKC75	4.84[a]	1073.63[a]	11.57	0.28
PKC70	4.52[b]	1002.05[b]	10.80	0.26
PKC65	4.19[c]	930.48[c]	10.03	0.24
SEM	0.101	21.131	0.476	1.085
p-value	0.003	0.001	0.153	0.429

PKC75; 750 gm PKC kg^{-1} DM. PKC70; 700 gm PKC/kg DM. PKC65; 650 gm PKC kg^{-1} DM.
[a,b,c] Means in the same column with different superscripts are significantly different.

The sections from the liver, kidney, and testis revealed organ-specific normal histological features. No remarkable histological of the eosinophilic hepatocytes, longestioc, and hepatic stellate with collagen fibrils were observed in the treatment group (Figure 1). However, the microscopic examination showed the liver of lambs fed on PKC70 and PKC65 presented with reversible histopathological changes ($p < 0.05$) as cell swelling hepatocytes while PKC75 unalterable histopathological alterations for the same character (hepatocytes) (Figure 1).

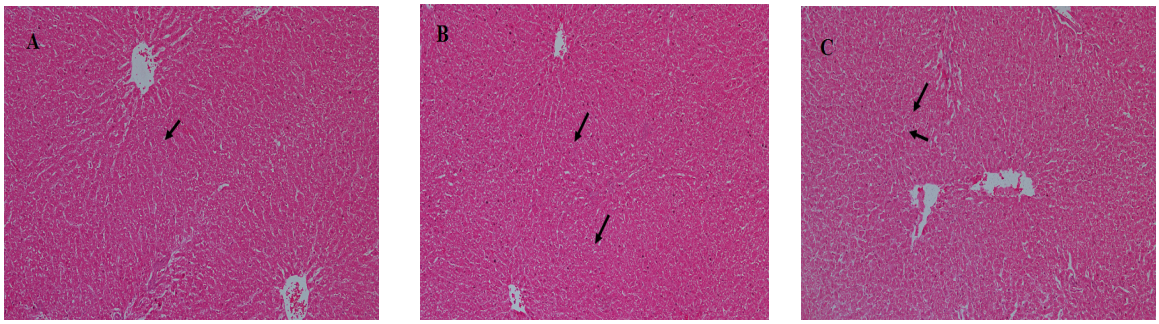

Figure 1. Histological Examination of the Liver in the Lambs Fed on Experimental Diets with H&E, 20x. (A) Liver in Lambs Fed 750 gm PKC kg^{-1} DM, Showing Normal Structure. (B) Liver in Lambs Fed 700 gm PKC kg^{-1} DM Shows Cell Swelling Hepatocytes. (C) Liver in Lambs Fed 650 gm PKC kg^{-1} DM Arrows Show Cell Swelling Hepatocytes.

The histological structure of kidney tissue of lambs in the PKC75, PKC70 and PKC65 are shown in figure 2. Histopathological findings of kidney in groups showed there were no signs of histopathological alteration of kidney tissues ($p > 0.05$) in multifocal interstitial nephritis and glomerular fibrosis in all groups and reminded normal.

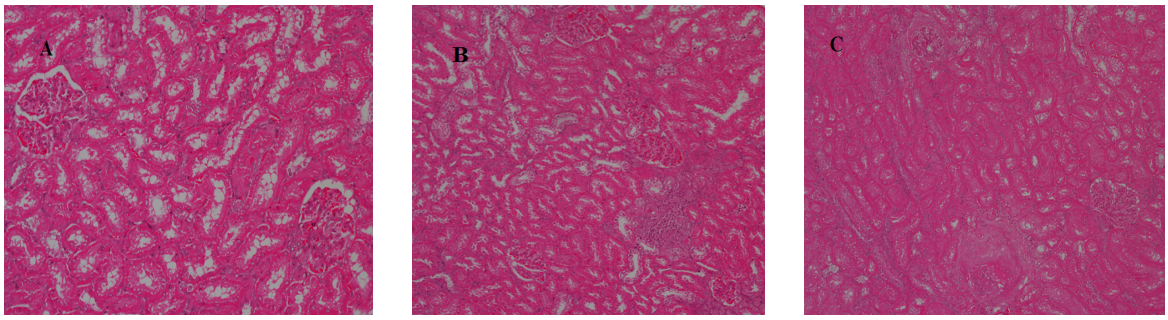

Figure 2. Histological Examination of the Kidney in the Lambs Fed on Experimental Diets with H&E, 20x. (A) The kidney of Lambs Fed 750 gm PKC kg^{-1} DM. There are no significant lesions. (B) The kidney of lambs fed 700 gm PKC kg^{-1} DM. There are no significant lesions.

The histopathology of the testis obtained by H&E staining is presented in figure 3. The testis in of all experimental groups no observable microscopic changes ($p > 0.05$) were seen in Sertoli cells and seminiferous tubules.

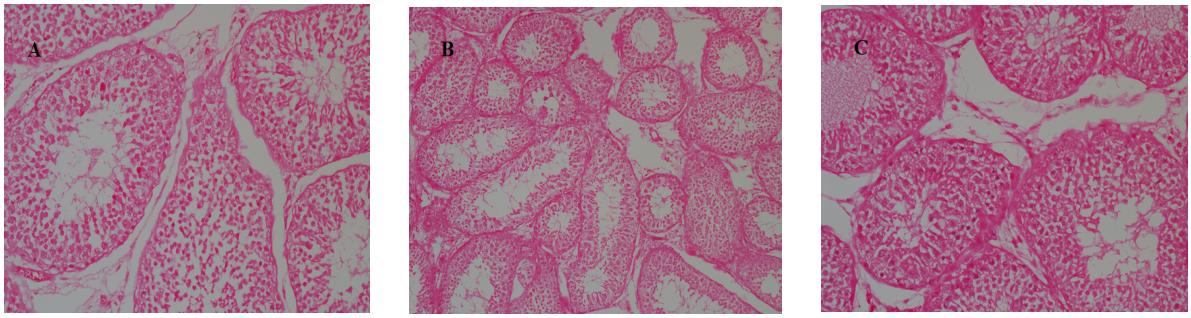

Figure 3. Histological examination of the testis in the Lambs Fed on Experimental Diets with H&E, 20x. (A) Testis of lambs fed 750 gm PKC kg^{-1} DM. There are no Significant Lesions. (B) Testis of Lambs Fed 700 gm PKC/kg DM. There are no Significant Lesions. (C) Testis of Lambs Fed 650 gm PKC kg^{-1} DM. There are no Significant Lesions.

There was no significant ($p > 0.05$) difference in the proportion of healthy and impaired liver between the different PKC inclusion levels Table 2. The highest inclusion level PKC750 showed 0% lesions as compared to 33.33% (1) lesions were observed in PKC650 and PKC700 respectively.

Table 2. Liver reaction to different PKC inclusion levels

Item	**Reaction**		
	Presence of Lesions	Normal Structure	Total
PKC75	0.00 (0)	33.33 (1)	33.33 (1)
PKC70	33.33 (1)	0.00 (0)	33.33 (1)
PKC65	33.33 (1)	0.00 (0)	33.33 (1)
Total	66.67 (2)	33.33 (1)	100 (3)
Chi Square test (Inclusion level vs Reaction) $\chi_{2,\,0.05}^{2} = 0.2231$			

PKC75; 750 gm PKC kg^{-1} DM. PKC70; 700 gm PKC kg^{-1} DM. PKC65; 650 gm PKC kg^{-1} DM.

4. Discussion and Conclusion

Feeding of PKC based diet to sheep has been reported to be a good source of dietary Cu. However, a higher concentration of dietary Cu in the sheep diet may probably cause mortality and histopathological changes within the tissues of vital organs due to toxicity. Findings from the present study are inconsistent with (Saeed et al., 2019a; Saeed et al., 2019b; Soren et al., 2017) on their studies on Cu poisoning in domesticated sheep. The histopathological findings confirm the mild liver toxicity observed in the present study agrees with (Hair-Bejo et al., 1995) who reported hepatocyte swollen, as well as vacuolated and necrotized, particularly at the medulla zone. However, no clinical signs of histological lesions were observed in the liver of all groups. Haywood et al., (2005) reported that Cu induced fibrosis in North Ronaldsay sheep results from hepatic stellate cell activation, simulating the pericellular fibrosis that characterizes infantile Cu toxicosis.

However, findings from this study have been equally conflicting because previous evidence indicated that the sheep died because of Cu toxicosis cannot be based solely on hepatic Cu concentrations but may also be due to hemolytic crisis caused by chronic Cu poisoning (Gupta, 2018). Although excess oral zinc administration could also cause liver damage in sheep (Najafzadeh et al., 2013).

No clinical signs were observed in the kidneys of all the treatment groups in the present study. Lesions of a chronic interstitial nonsuppurative nephritis in the kidney which often caused the death of animals are attributed to liver disease. Our finding agreed with Campos et al., (2019) who reported lambs fed agro by-product based diet during the feedlot period showed no renal failure. Similarly, the renal tubules in this study were also normal. Elimination of Cu in the urine may be greatly enhanced in the Cu-poisoned patient if the body storage sites are saturated (Roychoudhury et al., 2016).

The present study does not observe any effect to PKC based diets on the testicular features studied as reported by (Soren et al., 2017) no detrimental effect on the gross and histopathological composition of vital organs by replacing up to 50 % of soybean meal. However, the minor difference observed between treatment groups of study might be due to the age difference between samples. Kalwar et al., (2020), reported that seminiferous tubule diameter increases with an increased in age. The liver and kidneys, consistent with metallurgical relatively stable metabolism in the testis, are more susceptible to redox imbalance than the testis (Zhang et al., 2013).

There was no observable significant negative effect of feeding PKC based diet on vital organs (liver, kidney and testis). The non-injured sections of tissue could be because of the role of the Zn in PKC which led to induce detoxification in these organs. Further studies are required to unravel r the detoxification process.

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