Poultry Science

Heat detoxification of *Jatropha cucas* meal and its effect on productive and reproductive performance of quail

Youssef A. Attia,[*] Mayada R. Farag,[†] Mohammed A. Al-Harthi,[*] Fulvia Bovera,[‡] Adel D. Alqurashi,[*] Alessandro Di Cerbo,[§] and Mahmoud Alagawany[#,1]

[*]*Department of Agriculture, Faculty of Environmental Sciences, King Abdulaziz University, Jeddah 21589, Saudi Arabia;* [†]*Forensic Medicine and Toxicology Department, Veterinary Medicine Faculty, Zagazig University, Zagazig 44111, Egypt;* [‡]*Department of Veterinary Medicine and Animal Production, University of Napoli Federico II, via F. Delpino,1, 80137, Napoli, Italy;* [§]*School of Biosciences and Veterinary Medicine, University of Camerino, Matelica 62024, Italy; and* [#]*Poultry Department, Agriculture Faculty, Zagazig University, Zagazig 44511, Egypt*

ABSTRACT Jatropha is a large, multipurpose, drought-tolerant plant with many traits and great potential as a biofuel crop. It originates from Central America but is now distributed throughout the tropics, including Africa and Asia. The study determines whether the dietary inclusion of raw *Jatropha cucas* meal (**RJM**, 3.5%) had negative impacts on the reproductive and productive performances of male Japanese quail as well as whether these impacts could be mitigated by heating the jatropha meal at 100°C for 24 or 48 h (JH_{24} or JH_{48} respectively). One hundred twenty healthy mature male quails at the age of 12 wk were assigned randomly to 4 treatments. Every treatment had 6 replicates, with 5 birds per replicate. The RJM caused a considerable decline in fertility and a high mortality rate in quail, whereas heat-treated jatropha meal (JH_{24} or JH_{48}) decreased these unwanted effects. The RJM significantly increased triglycerides, aspartate aminotransferase (**AST**), and alanine aminotransferase (**ALT**), while reducing total protein and albumin. These values returned to normal in the JH_{24} and JH_{48} groups. The RJM significantly reduced the testosterone and increased estradiol and hepatic content of vitellogenin (**Vtg**) and estrogen receptor alpha (**ER*α***) while they were normal in JH_{48} group. Superoxide dismutase (**SOD**) and catalase (**CAT**) activities, and the reduced glutathione (**GSH**) content in testicular tissues were significantly reduced in the **RJM** group when compared to control. Protein carbonyl (**PC**), malondialdehyde (**MDA**), and 8-hydroxy 2 deoxyguanosine (8-**OHdG**) levels were significantly increased in the **RJM** group when compared to control. Heating of JM for 48 h reduced the 8-OHdG and MDA levels toward the control level better than JH24 and restored PC to normal. Based on the obtained results, The toxic components in JM could be eliminated through heat treatment, and extending the treatment duration to 48 h is recommended for transforming the potentially harmful jatropha meal into an alternative protein source for livestock nutrition.

Key words: *Jatropha cucas*, heat detoxification, reproductive performance, quail

2023 Poultry Science 102:103072
https://doi.org/10.1016/j.psj.2023.103072

INTRODUCTION

Jatropha plant is a medicinal herb that has powerful potentials for use both medicinally and nutritionally and belongs to Euphorbiaceae family (Barros et al., 2015; Saleh et al., 2023). The jatropha plant has a lifespan of more than 50 yr, and can grow even in soils that are poor in their nutrient contents (Openshaw, 2000; Saeed et al., 2017). Recently, there has been more focus on using unconventional feedstuffs in poultry diets in developing countries (Hafez and Attia, 2020; Alagawany et al., 2022). Jatropha is used for bio-diesel production, and the oil extraction from its seeds produces enormous amounts of cake or meal as by-products rich in carbohydrates, crude protein, and essential amino acids (except for lysine), which meet the reference proteins of FAO (Harinder et al., 2008; Taufiq-Yap et al., 2011). These characteristics make jatropha meal a promising alternative source of protein for feeding livestock and poultry.

The majority of the poisonous effects of *Jatropha* spp. are caused by its antinutritive ingredients, which include saponins, tannins, proteases, alkaloids, and toxic

Received June 26, 2023.
Accepted August 23, 2023.

[1]Corresponding author: dr.mahmoud.alagwany@gmail.com

substances such as phytate, lectins, and forbol esters, which restrict its usage for feeding animals (Saetae and Suntornsuk, 2011, Pelletier et al., 2015). The seeds of *Jatropha curcas* exhibited some toxicity in broilers (Pasaribu et al., 2010), quails, (Agboola and Adenuga, 2015) livestock (Chivandi et al., 2006; Ferreira et al., 2011) rats and mice (Panigrahi et al., 1984). In mice, rats, and rabbits, raw jatropha oil-induced skin lesions and gastrointestinal problems (Gandhi et al., 1995) and inhibited rat pup delivery (Odusote et al., 2002). Acetonitrile extract from oil or jatropha seeds caused biochemical and histopathological changes (Abd-Elhamid, 2004). Reducing the antinutritive and toxic constituents of jatropha meal is, therefore, crucial before being used as an alternative feed ingredient for poultry and animal (Annongu et al., 2010; Agboola and Adenuga 2015; Salazar et al., 2021).

To be able to utilize the jatropha meal as source of protein in the diet of livestock, the anti-nutritive compounds particularly phorbol esters need to be removed. About 70−75% of phorbol esters are separated from the pressed cake after oil extraction, while 25 to 30% remains in the pressed cake (Devappa et al., 2010). Therefore, its degradation is very important to make the cake safe for using. Several methods including physical and chemical have been tested for their efficiency for the phorbol esters degradation (Devappa and Swamylingappa, 2008). Most treatments used a combination of alkalis, solvent extraction and high temperatures (Zimila et al., 2018) which are neither specific nor environmentally friendly and expensive. Meanwhile, the use of heat treatment only (autoclave) reduced lectin and anti-trypsin in seed meal (Aderibigbe et al., 1997). On the other hand, physical detoxification of Jatropha seed kernel with micro wave treatment showed good results by reducing phorbol esters content to 86.29% (Abou-Arab et al., 2019). Our previous study showed that heat treatment of *J. curcas* meal in oven for 24 or 48 h reduced phorbol esters content (Farag et al., 2018). So, it is hypothesized that heating treatment of *J. curcas* meal is expected to reduce anti-nutritional and toxic components, while the crude protein content is retained and to show beneficial effects on quail health. Although previous studies have utilized detoxification methods for jatropha, these studies have not sufficiently elucidated the repercussions of including raw jatropha in avian diets on reproductive outcomes. Therefore, this study aims to examine the harmful effects of dietary inclusion of raw jatropha meals on the reproductive and productive performances of male Japanese quail and to assess the impact of heat treatments of the meals as an environmentally friendly detoxifying technique.

MATERIALS AND METHODS

Source and Preparation of Jatropha Meal

Seeds of *J. curcas* were exposed to processing to obtain the meal as previously described in Farag et al. (2018). The meal was partitioned into 3 portions. The first part was kept without heating (raw jatropha meal: RJM). The second part was heated for 24 h at 100°C in an oven (JH_{24}). The third part was heated for 48 h at 100°C in an oven (JH_{48}). The AOAC method was used to evaluate the proximate analysis of raw and heated jatropha (AOAC, 2006) (Supplementary Table 1). Phorbol ester (**PEs**) content was determined as mentioned in (Makkar et al., 1997, 2007). Herein, the resulting PEs in a heat-treated meal of jatropha is 0.091 mg/g in JH24 and 0.068 mg/g in JH48, which came in line with that found in non-toxic varieties of *J. curcas* (Makkar et al., 1998).

Experimental Design and Diet Formulation

One hundred and twenty healthy mature male quails (*Coturnix coturnix japonica*) at the age of 12 wk (initial BW: 240.75 ± 1.11 g) were assigned randomly to 4 treatments. Every treatment had 6 replicates, with 5 birds per replicate. For 8 wk (12 to 20 wk old), birds were fed 4 treatment diets (Supplementary Table 2) as follows: 1) control diet without jatropha meal; 2) diet with 3.5% raw jatropha meal (RJM); 3) diet containing 3.5% JM heated for 24 h (JH_{24}); and 4) diet with 3.5% JM heated for 48 h (JH_{48}). According to NRC (1994) recommendations, all tested diets were formulated to provide the nutrients that Japanese quails need. Conventional cages (each floor space /bird = 300 cm^2 and the dimensions of 60 × 50 × 30 cm^3) were used to house the birds under hygienic conditions and at 7 h dark: 17 h light cycle along the experiment. Fresh water and feed in the form of a mash were provided to birds all the time. The drinker and feeder spaces were 4 cm and 3.7 cm, respectively. Daily assessments of the birds' health were monitored throughout the trial.

Data Collection

Data was gathered 8 wk after feeding quails on the experimental diets (12−20 wk old). The body weight (**BW**) of each bird was recorded at the beginning of the feeding trial (initial BW) and the end of the feeding trial (final BW). Feed intake (**FI**) was taken every week and calculated as grams of consumed feed over 7 d divided by the number of birds in each group.

Evaluation of Male Fertility

During the experiment, 120 healthy mature female quails at the age of 12 wk were used to determine the fertility percentage. For evaluating male fertility, the male quails were allowed to have a natural mating with females at 1:1 ratio. Eggs from the last 5 experimental days were collected from each group and kept at 15°C to 18°C. Eggs (n = 100 eggs/treatment) were then incubated in an automatic incubator at 60% humidity and 37.6°C. On the 16th incubation day, the eggs were broken and classified as infertile or fertile based on macroscopic appearances. The formula determined fertility %:

Fertility % = (number of fertile eggs/ total eggs set) × 100.

Preparation of Samples and Biochemical Analyses

At the experimental end, samples (n = 6/treatment) of blood were collected by the brachial vein venipuncture followed by centrifugation for 15 min at (2,100 × *g*), and the obtained sera were kept at −20°C till analyzed. The following parameters were evaluated in serum total cholesterol, triglycerides (**TG**), low-density lipoprotein (**LDL**) cholesterol and high-density lipoprotein (**HDL**) cholesterol albumin, total protein, aspartate transaminase (**AST**), and alanine transaminase (**ALT**) by the use of commercially available biodiagnostic kits from Biodiagnostic Co (29 El-Tahrir St. Dokki, Giza, Egypt). Testes (n = 6/treatment) were dissected and portioned into 2 portions, and the first one was immediately preserved (at −80°C) for detection of Vtg and Erα mRNA expressions. The second one was fixed in buffered neutral formalin (10%) for histopathological examination.

Analysis of Serum Reproductive Hormones

Estradiol (E2) and testosterone concentrations (n=6/treatment) were estimated in serum by ELISA kits (Cat.-No.: DE4399 and DE1559 respectively) (DEMEDITEC Diagnostics GmbH; D-24145 Kiel-Wellsee, Germany), following the directions of the manufacturer.

Extraction of RNA and Quantitative Real-Time PCR of Erα and VTg

Quantitative real-time polymerase chain reaction (**RT-PCR**) was utilized to determine the mRNA expression levels for the Erα and Vtg in frozen testicular tissues (6/treatment) as previously decribed in Khalil et al.(2017) using the following specific primer pairs: ERα F: 50- CTTGCAGACAGAGAATTAGTGCACA-30 and R: 50-GTTAAATCCACAAATCCTGGAACTC-30 (GenBank accession no. AF442965), Vtg F: 50-GAAAACCCTGAGCAACGGATAG-30 and R: 50-TGGAACATCATCATGGAAATCTTG-30 (GenBank accession no. AF199490) and the housekeeping gene (β-actin) F: 50-AAATTGTGCGTGACATCAAGGA-30 and R: 50-GAGGCAGCTGTGGCCATCT-30 (GenBank accession no. AF199488).

Biomarkers of Antioxidant Status

Testes specimens (n = 6/treatment) were homogenized in PBS (potassium phosphate buffer: pH 7.4) (10% w/v) and exposed to centrifugation for 15 min at 3,000 rpm. The obtained supernatants were utilized to measure the activities of SOD (superoxide dismutase) and CAT (catalase) and the content of GSH (reduced glutathione) using the kits from BioMérieux, Marcy l'etoile, France, following the directions of the manufacturer.

Biomarkers of Oxidative Stress

Malondialdehyde (**MDA**) and protein carbonyls (**PC**) were investigated as markers of oxidative damage of lipids and protein in the testes using commercial kits (Cat No. ab118970, ab126287 respectively) from Abcam Co, UK. While 8-OHdG (8 -hydroxy-2-deoxyguanosine) was measured (n = 6/treatment) to assess the DNA oxidation in serum using ELISA kits (Cat No. MBS261211) from MyBiosource.com, San Diego, California following the directions of the manufacturer.

Histopathological Investigation

Specimens from the testes (n = 6/treatment) were gathered and fixed in neutral buffered formalin (10%), followed by dehydration in ethanol (70−100%), clearing in xylene, and embedding in paraffin. Paraffin sections (5-micron thickness) were prepared and stained with HE stain (hematoxylin and eosin) (Bancroft and Gamble 2008) and then microscopically investigated.

Statistical Analysis

Data were subjected to the ANOVA procedure for a completely randomized design using the GLM procedures of SAS (version 14.2, 2016). The replicate was the experiment unit. The normality of data distribution was tested with the Shapiro-Wilks test of normality (SAS, 2016). The differences among treatments were determined using the Post-hoc Newman-Keuls test ($P < 0.05$).

RESULTS

Effect on Production and Reproduction of Birds

Table 1 showed that RJM resulted in a significant ($P < 0.001$) decline in final body weight (**FBW**) and FI than control diets, while heated meals (JH_{24} and JH_{48}) significantly improved FBW and FI, particularly JH_{48}, which restored FBW to control value. RJM recorded the lowest fertility rate when compared with the control or treated jatropha meal groups ($P < 0.001$). At wk 20 of age, the mortality rate was significantly higher in RJM and was reduced in heated groups, especially JH_{48}; however still higher than the control.

Effect on Liver Function Markers

The albumin ($P < 0.001$) and total protein ($P = 0.003$) contents showed a significant reduction in quails fed on RJM compared to the control. At the same time, they were restored to normal in both heated groups (JH_{24} and JH_{48}). The ALT ($P < 0.001$) and AST

Table 1. Effect of dietary jatropha meal on productive and reproductive performance of male Japanese quail at 20 wk of age.

Items	Jatropha meal (3.5%)				
	Control	RJM	JH_{24}	JH_{48}	[1]*P*-value
Productive performance					
IBW (g)	241.20 ± 0.41	241.00 ± 0.28	240.90 ± 0.66	240.56 ± 1.24	0.942
FBW (g)	250.88 ± 0.40[a]	235.20 ± 0.41[c]	246.16 ± 0.60[b]	249.60 ± 1.15[a]	<0.001
DFI (g/bird)	25.60 ± 0.05[a]	21.06 ± 0.28[d]	22.38 ± 0.05[c]	23.98 ± 0.19[b]	<0.001
Reproductive performance, %					
Fertility (%)	84.66 ± 0.61[a]	39.70 ± 0.72[d]	70.33 ± 0.32[c]	80.61 ± 0.15[b]	<0.001
Mortality (%)	1.04 ± 0.03[d]	19.30 ± 0.41[a]	10.45 ± 0.11[b]	3.64 ± 0.06[c]	<0.001

[1]Overall treatment *P*-value. Initial body weight (IBW), final body weight (FBW), daily feed intake (DFI).
[abcd]Different superscripts within a row are significantly different ($P < 0.05$).

Table 2. Effects of dietary jatropha seed meal supplement on liver functions of male Japanese quail at 20 wk of age.

Parameters	Jatropha meal (3.5%)				
	Control	RJM	JH_{24}	JH_{48}	[1]*P*-value
Liver functions[2]					
TP (g/dL)	4.03 ± 0.37[a]	2.45 ± 0.06[b]	3.77 ± 0.15[a]	3.68 ± 0.09[a]	0.003
Albumin (g/dL)	2.25 ± 0.09[a]	1.86 ± 0.17[b]	2.17 ± 0.08[a]	2.28 ± 0.06[a]	<0.001
AST (U/L)	60.22 ± 0.69[b]	79.14 ± 3.88[a]	65.42 ± 3.47[b]	60.41 ± 1.36[b]	0.004
ALT (U/L)	13.75 ± 0.71[b]	39.99 ± 3.39[a]	17.83 ± 2.03[b]	13.87 ± 0.64[b]	<0.001

[1]Overall treatment *P*-value.
[2]Total protein (TP), aspartate transaminase (AST), and alanine transaminase (ALT).
[ab]Different superscripts within a row are significantly different ($P < 0.05$).

($P = 0.004$) activities were significantly increased in the RJM group and returned to the control value in both heated groups (Table 2).

Effect on Lipid Profile Biomarkers

Table 3 showed that triglycerides and vLDL were significantly ($P < 0.001$ and 0.030) increased with 3.5% RJ relative to control while their levels in JH_{24} and JH_{48} groups returned to normal. While, the levels of total, LDL, and HDL- cholesterol did not significantly differ among groups.

Effect on Reproductive Hormones

Data in Table 3 showed that birds fed on an RJM-supplemented diet had the lowest value of testosterone ($P = 0.013$) and the highest estradiol ($P < 0.001$) value than the JH_{24} group. While JH_{24} restored them to control values.

Effect on mRNA Expression of Hepatic ERα and Vtg

The expression of ERα and Vtg were significantly upregulated in the RJM-supplemented group relative to the control. JH_{24} significantly reduced their values, but the JH_{48} returned them to control values (Figure 1).

Antioxidant and Oxidative Stress Status

The CAT ($P = 0.001$) and SOD ($P = 0.003$) activities and the content of GSH ($P < 0.020$) in testicular tissues were significantly decreased by RJM inclusion compared

Table 3. Effects of dietary jatropha seed meal supplement on lipid parameters and reproductive hormones of male Japanese quail at 20 wk of age.

Parameters	Jatropha meal (3.5%)				
	Control	RJM	JH_{24}	JH_{48}	[1]*P*-value
Lipid parameters[2]					
TG (mg/dL)	262.33 ± 8.19[b]	288.00 ± 1.73[a]	262.30 ± 6.33[b]	262.30 ± 6.33[b]	<0.001
vLDL	52.46 ± 2.55[b]	57.60 ± 3.87[a]	52.46 ± 2.98[b]	52.46 ± 2.98[b]	0.030
TC (mg/dL)	271.00 ± 1.53	269.00 ± 4.51	270.00 ± 3.21	270.00 ± 3.21	0.966
HDL (mg/dL)	161.67 ± 5.17	160.33 ± 4.84	158.00 ± 7.02	158.00 ± 7.02	0.979
LDL (mg/dL)	76.11 ± 0.76	76.17 ± 0.99	75.66 ± 1.02	75.66 ± 1.02	0.942
LDL/HDL ratio	0.47 ± 0.01	0.47 ± 0.01	0.47 ± 0.01	0.47 ± 0.01	0.986
Reproductive Hormones					
Testosterone (ng/mL)	2.33 ± 0.17[a]	0.85 ± 0.39[b]	1.60 ± 0.18[ab]	2.22 ± 0.24[a]	0.013
Estradiol (ng/mL)	6.79 ± 0.38[c]	52.18 ± 1.26[a]	40.51 ± 1.26[b]	7.80 ± 0.12[c]	<0.001

[1]Overall treatment *P*-value
[2]Triglycelides (TG), very low density lipoprotein (vLDL), total cholesertol (TC), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol.
[abc]Different superscripts within a row are significantly different ($P < 0.05$).

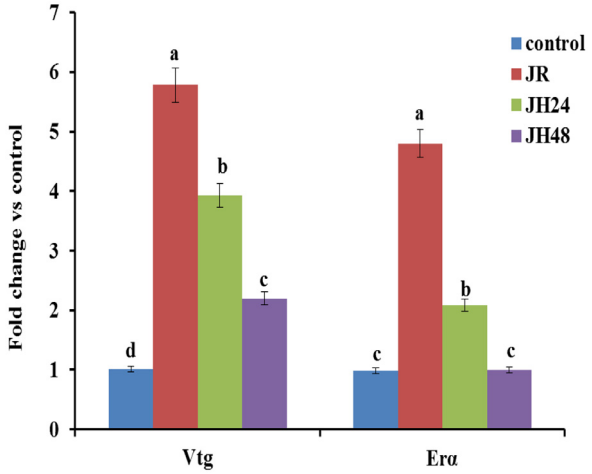

Figure 1. Effects of dietary jatropha meal on Vtg and ERα. The data points without the same superscripts (a-c) are significantly different (1-wayANOVA) at the level of $P < 0$s.05.

to the control. Heating of JM or 24 h succeeded in elevating the SOD and CAT activities; however, they were still lower than the control, while in JH48, their activities were normal. The GSH content was returned to control values in JH_{24} and JH_{48} groups (Table 4).

The PC, MDA, and 8-OHdG levels showed the highest values in the RJM group ($P < 0.001$). Heating of JM for 48 h reduced the 8-OHdG and MDA levels toward the control level better than JH24 and restored PC to normal (Table 5).

Histopathological Findings

Heated Jatropha at 48h induced reproductive recovery and sperm production. The histological effects of Jatropha on the testis germinal epithelium were studied using transverse sections of the testis. Here the control testis appears normal with well-organized seminiferous tubules (**ST**), each investigating the spermatic epithelium layers containing cells in different developmental stages, including; spermatogonia at the peripheral zone (sg), spermatocytes (Sc), and sperms (sp) in the central zone (Figure 2A). On the other side, Raw jatropha has a toxic effect on the general architecture of seminiferous tubules that appeared scattered with significant vacuolation and progressed atrophy with the deletion of germ cells (black stars) (Figure 2B). Heated jatropha at 24h could rescue the formation of the sperm in a few seminiferous tubules. However, other tubules degenerated with disorganized germinal epithelium and hypoplasia (green stars) (Figure 2C). Interestingly, heating for 48 h could effectively break down the toxic effect of raw Jatropha as the testis appeared normal with multiproduced sperms in side tubules (Figure 2D).

DISCUSSION

It has become clear from the current investigation that is adding RJM to quail diets significantly reduced feed consumption and final body weight. In line with these findings, RJM has been shown to reduce FI and live weight increase in both chicks (El Badwi et al., 1992) and laying Japanese quails (Abd El-Hack et al., 2017). Reduced nutritional intake, particularly protein, which is crucial for maintaining the body's protein and promoting the growth of birds, may cause quails' low productivity.

These undesirable effects may be returned to RJM's low nutritional values, which were revealed by proximate analysis. RJM has higher levels of fats and lower levels of crude protein than treated meals, according to our prior research (Farag et al., 2018). Due to the increased concentration of poisonous and antinutritive

Table 4. Effect of dietary jatropha meal on antioxidant biomarkers in testicular tissue of male Japanese quail at 20 wk of age.

	Jatropha meal (3.5%)				
Parameters	Control	RJM	JH_{24}	JH_{48}	[1]*P*-value
Antioxidant biomarkers[2]					
SOD (μg/g tissue)	0.22 ± 0.01^{a}	0.12 ± 0.01^{b}	0.16 ± 0.01^{ab}	0.22 ± 0.01^{a}	0.003
CAT (μg/g tissue)	0.34 ± 0.02^{a}	0.21 ± 0.01^{b}	0.27 ± 0.01^{b}	0.34 ± 0.01^{a}	0.001
GSH (μg/g tissue)	0.23 ± 0.02^{a}	0.16 ± 0.01^{b}	0.22 ± 0.02^{a}	0.23 ± 0.02^{a}	0.020

[1]Overall treatment *P*-value.
[2]Superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH)
[ab]Different superscripts within a row are significantly different ($P < 0.05$).

Table 5. Effect of dietary jatropha meal on oxidative stress biomarkers in testicular tissue of male Japanese quail at 20 wk of age.

	Jatropha meal (3.5%)				
Parameters	Control	RJM	JH_{24}	JH_{48}	[1]*P*-value
Oxidative stress biomarkers[2]					
MDA (nmol/g tissue)[2]	0.16 ± 0.01^{d}	0.45 ± 0.01^{a}	0.30 ± 0.00^{b}	0.22 ± 0.02^{c}	<0.001
PC (nmol/g tissue)[2]	6.13 ± 0.33^{b}	13.53 ± 0.58^{a}	6.04 ± 0.29^{b}	6.00 ± 0.45^{b}	<0.001
8-OHdG (ng/mL)[2]	0.27 ± 0.01^{d}	0.51 ± 0.01^{a}	0.45 ± 0.01^{b}	0.32 ± 0.01^{c}	<0.001

[1]Overall treatment *P*-value.
[2]Malondialdehyde (MDA), Protein carbonyl (PC), 8-hydroxy 2 deoxyguanosine (8-OHdG)
[abcd]Different superscripts within a row are significantly different ($P < 0.05$).

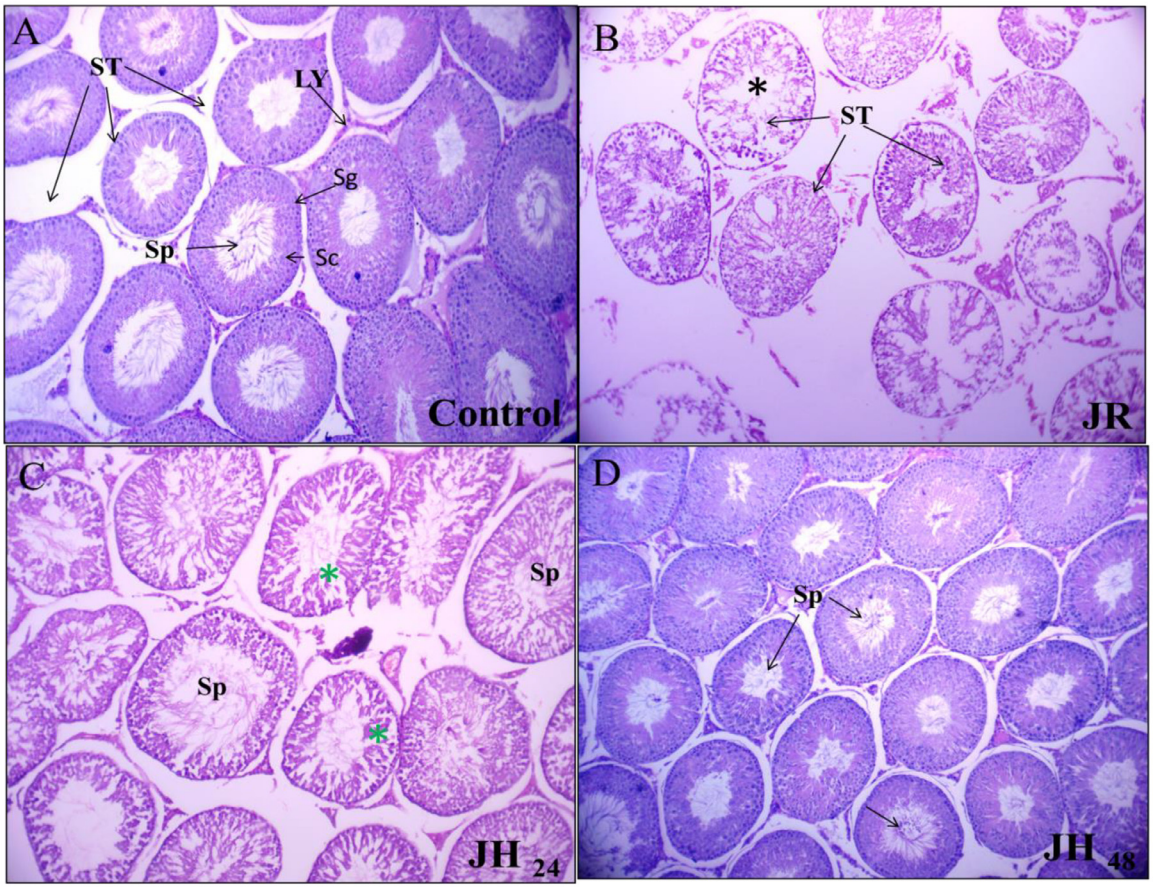

Figure 2. Photomicrograph of Hematoxylin−Eosin (H&E) stained sections of the (A) control group showing that testis appeared normal with well-organized seminiferous tubules (ST) with spermatogonia (sg), spermatocytes (Sc), and sperms (sp), (B) Testis from the JR group showing scattered seminiferous tubules with vacuolation and atrophy with deletion of germ cells (black stars), (C) Testis from the JH24 group showed the formation of sperms in a few seminiferous tubules; however, some tubules still showed degenerated and disorganized germinal epithelium and hypoplasia (green stars), (D) Testis from the JH48 group appeared normal with multiproduced sperms inside tubules.

substances such as phorbol esters, lectin, anti-trypsin, phytate, and curcin, RJM has a low nutritional value (Makkar and Becker, 2009; Barahona et al., 2010). RJM inclusion in the diet resulted in lectin accumulation that negatively affects protein synthesis, whereas anti-trypsins inhibit trypsin and chymotrypsin activity (Barahona et al., 2010). Phytate, however, decreased the availability of nutrients for animals through its binding to proteins, minerals, and carbohydrates (Martinez-Herrera et al., 2006; Saleh et al., 2023). Additionally, it has been noted that the combination of these substances might have detrimental effects on the digestive system, such as gastroenteritis, which reduces nutrient utilization and, as a result, decreases female growth and egg production in females (Burrows and Tyrl, 2001; Salazar et al., 2021).

On the other hand, treating JM by heating in an oven for 48 h enhanced the productive performance of birds; however still under control values, and this could be explained by the findings of (Pasaribu et al. 2010; Abd El-Hack et al., 2017; Farag et al. 2018) who reported that heating of JM resulted in positive changes in the contents of crude lipid and protein than an untreated meal with increasing the time of treatment. The current improvements might be returned to the reduction in the content of phorbol ester in both treated meals (0.091 mg/g in JH24 and 0.068 mg/g in JH48), which is considered to be safe for animals where the permissible limit is 0.09 (Makkar et al. 1998). Additionally, heating as a physical treatment method has been shown to reduce the quantities of heat-labile lectin and anti-trypsin, the 2 poisonous and antinutritive components of raw jatropha that have been shown to have adverse effects (Aderibigbe et al. 1997; Ojediran et al., 2014). Likewise, heat treatment had a good impact on reducing antinutritional factors but had a small effect on saponin and phorbol esters (Ojediran et al., 2014).

According to this study, the fertility rate of the JH_{24} and JH_{48} groups was higher than those of the RJM group. The hazardous component phorbol ester in the residual oil of untreated Jatropha meal may be to blame for the detrimental effects on reproductive characteristics (El Badwi and Adam, 1992). Additonally the lower level of tesosteron in RJM fed group could explain the reduced fertility in this group.

The mortality rate increased when RJM was added to quail meals, but it was reduced by heating the meal for 24 h and a more reduction of mortality was observed after heating for 48 h. As previously demonstrated, 5% heat-treated JM was thought to be the safest level for growing Japanese quail. However, levels beyond 10% of JM enhanced the mortality rate (Agboola and Adenuga

2015). Additionally, according to Gross et al. (1997), adding raw JM to a fish diet resulted in hundred percent mortalities, while chemically treating meals could decrease the mortalities to 8% indicating the high sensitivity of such species to the toxic component of aw jatropha . Likewise, the inclusion of RJM in the diet led to deaths in rats (Rakshit et al., 2008) and broilers (Pasaribu et al.,2010). Earlier studies in broilers, Sumiati et al. (2007); Ojediran et al. (2014) reported reduced feed consumption, 100% mortalities, and liver damages as a result of jatropha's antinutritional components. The buildup of harmful substances in the body is the best explanation for the high mortality rate (Farag et al., 2018).

In both the JH24 and JH48 groups, the albumen and total protein were lowered with RJM but returned to normal after heating. The adverse effects of phorbol ester, the primary toxic compound of raw jatropha meal, caused necrosis and degeneration of hepatic cells, the primary site of synthesis of protein (Areghore et al., 2003), which may be the cause of the hypoproteinemia and consequently reuced albumin level seen in the RJM group. These findings concur with earlier research on laying Japanese quails (Barahona et al., 2010; Farag et al., 2018). Reddy and Salunkhe (1982) similarly link decreases in total proteins to the birds' ineffective protein utilization, which leads to lower digestibility. The physiological indices alteration can affect characteristics related to the health of birds (Emam et al., 2023).

Our findings also showed that RJM in male quail diets causes a notable rise in the liver enzymes ALT and ALT. Increased liver enzyme levels in the serum are a sign of liver injury, indicating that RJM's cytotoxic actions on the liver cells caused these enzymes to leak from injured hepatic cells into the bloodstream (Nabil et al., 2011). Compared to the other liver enzymes, the ALT enzyme is the most sensitive indicator of liver damage and can quantitively evaluate the severity of liver damage (Aniagu et al., 2004). Our findings concur with those made by Kaneko (1989) and Farag et al. (2018), who discovered that jatropha had a significant impact on the ALT enzyme.

The results showed that the 3.5% RJM resulted in significantly higher triglyceride levels than the control group despite no changes in total cholesterol, HDL, or LDL cholesterol contents. According to Farag et al. (2018), the detrimental effects of RJM on liver functions and structure could account for the altered hepatic lipogenesis.

Vitellogenesis (Vtg) is a normal process in mature females, and vitellogenin (Vtg) is a marker of it. In oviparous animals, the liver ordinarily produces and secretes vitellogenin (the precursor of estrogen-induced egg protein) in females. However, male and immature animals lack vitellogenin but it can be induced by estrogen and xenobiotics (Specker and Sullivan, 1994). For this, quantification of Vtg and ERα is of importance during the evaluation of exposure to xenoestrogen such as atrazine, bisphenol A and DDT in wild animals and in in vitro and in vivo studies (de la Casa-Resino et al., 2012; El Gawish et al., 2013; Osachoff et al., 2016) to determine the extent of environmental pollution with estrogenic compounds.

Herein, RJM altered both ERα andVtg. Upon heating of RJM, particularly JH48, the expression of ERα and Vtg returned to the baseline values. The significant induction of these proteins might be returned to the vitellogenesis induction in hepatic tissue following RJM exposure, as previously observed in Farag et al (2018), where xenoestrogen substances can interact with binding sites of estrogen receptors resulting in the transcriptions of estrogen-responsive genes and productions of egg proteins such as Vtg. For this, the present data suggest that RJM may be an estrogenic substance that might disrupt the endocrine systems and affect reproduction.

Besides energy and protein, the hormones of reproduction have a significant role in regulating the reproductivity in birds, including hatchability and fertility (Wingfield, 2005). Herein, the male hormone (testosterone) in RJM and JH24 groups was lower than in other groups, while the opposite was observed for estradiol. The altered hormonal assay could explain the decreased fertility percentages and suggest the harmful effects of RJM on the function of the testes, which is supported by the histological changes in the testicular tissue of the RJM-treated group. It's interesting to note that heating jatropha meal for 48 h significantly reduced the majority of the negative impacts of RJM on reproduction hormones and brought them close to control levels.

Histopathological study of the testes from RJM group exhibited marked alterations and structural disturbances in the RJM group, which could be a primary response to the ROS production and lipid, protein, and DNA oxidation observed in the testicular tissue of males in RJM group and this agrees with Khan et al., (2015) who stated that oxidative damage could alter the testicular membrane leading to the Leydig cells and spermatogenic degeneration, thereby causing disruption of spermatogenesis and reduction spermatic count, and consequently lowering the male fertility.

The histological alterations became less prominent in JH_{24} group and mild in JH_{48} group, indicating that lengthening the time of heat treatments could reduce the negative impacts of RJM on the structure and function of testicular tissue. This suggests that the extended heating period was more helpful in the destruction or lowering of the activity of antinutritive and toxic compounds in jatropha meal without additional treatments to levels making the resulting meal suitable for feeding animals, and this agreed with Farag et al. (2018) with quails and Alatise et al. (2014) with fish, which tolerated substitutions of soybean by boiled jatropha meal to 30%. While disagreed with Areghore et al. (2003), Chivandi et al. (2006), and Martinez-Herrera et al. (2006), where physical treatment with heat required additional chemical treatments to reduce the activity of Saponin, phorbol ester, lectin, and trypsin inhibitors in JM. This difference might be attributed to the small amount of JM for inclusion in the diet of male quails and the

different degrees and times of hearing in the present study. Therefore, more studies were recommended to give a right judgment on the accurate level of JM which would induce only beneficial effects on animal health.

CONCLUSIONS

From the current observation, it is evident that the presence of poisonous and antinutritive substances in the raw meal of *J, curcas* could adversely affect the production and reproduction abilities of male quails and thus restricts its utilization in the feeding of poultry and animals. While, treating *J.s curcas* meal by heating for extended periods (24 or 48 h) could diminish the activity of such undesirable components without adverse effects on production and reproduction abilities of male quails.

ACKNOWLEDGMENTS

This research work was funded by Institutional Fund Projects under the grant no. (IFPIP: 805-155-1443). The authors gratefully acknowledge the technical and financial support provided by the Ministry of Education and King Abdulaziz University, DSR, Jeddah, Saudi Arabia.

Institutional Review Board Statement: Committee of the Department of Agriculture, Faculty of Environmental Sciences, King Abdulaziz University, which regulates the rights and welfare of animals according to government law No. 9 dated 24/8/2010 and institutional approve code ACUC-22-1-2.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data are available upon official request from the principal investigator upon the permission of the funding agent.

DISCLOSURES

The authors declare no conflict of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2023.103072.

REFERENCES

Abd El-Hack, M. E., M. Alagawany, S. A. A. El-Sayed, and J. Fowler. 2017. Influence of dietary inclusion of untreated or heat-treated Jatropha meal on productive and reproductive performances and biochemical blood parameters of laying Japanese quail. Poult. Sci. 96:2761–2767.

Abd-Elhamid, H. F. 2004. Investigation of induced biochemical and histopathological parameters of acetonitrile extract of *Jatropha curcas* in lbino rats. J. Egypt. Soc. Parasitol. 34:397–406.

Abou-Arab, A. A., M. H. Mahmoud, D. M. M. Ahmed, and F. M. Abu-Salema. 2019. Comparative study between chemical, physical and enzymatic methods forJatropha curcas kernel meal phorbol ester detoxification. Heliyon 5:e01689.

Aderibigbe, A. O., C. O. Jonson, H. P. S. Makkar, K. Becker, and N. Foidl. 1997. Chemical composition and effects of heat on organic matter- and nitrogen-degradability and some antinutritional components of Jatropha meal. Anim. Feed Sci. Tech. 67:223–243.

Agboola, A. F., and A. A. Adenuga. 2015. Performance and organ histopathology of growing Japanese quails (*Coturnix coturnix* japonica) fed heat treated jatropha seed cake substituted for soyabean meal. Trop. Anim. Prod. Invest. 18:01–08.

Alagawany, M., M. R. Farag, M. E. Sahfi, S. S. Elnesr, O. Alqaisi, S. El-Kassas, A. S. Al-wajeeh, A. E. Taha, and M. E. Abd E-Hack. 2022. Phytochemical characteristics of Paulownia tree wastes and its use as unconventional feedstuff in animal feed. Anim. Biotechnol. 33:586–593.

Alatise, S. P., M. A. Adedokun, O. B. Adelodun, and G. E. Ajiboye. 2014. Effects of boiled jatropha kernel meal as a substitution for soyabeans meal in diet of African mud catfish (*Clarias gariepinus*) fingerlings. J. Fisheries Aquatic Sci. 9:446–456.

Aniagu, S. O., F. C. Nwinyi, D. D. Akumka, G. A. Ajoku, S. Dzarma, K. S. Izebe, M. Ditse, P. E. C. Nwaneri, C. Wambebe, and K. Gamaniel. 2004. Toxicity studies in rats fed nature cure bitters. Afr. J. Biotechnol. 4:72–78.

Annongu, A. A., J. K. Joseph, D. F. Apeta, A. O. Adeyinka, M. B. Yousf, and K. B. Ogunjimi. 2010. Detoxification of *Jatropha curcas* seeds for use in nutrition of monogastric livestock as alternative feedstuff. Pakistan J. Nutr. 9:902–904.

AOAC. 2006. Official Methods of Analysis Association. 18th ed. Off. Anal. Chem., Washington, DC.

Areghore, E. M., K. Becker, and H. P. S. Makkar. 2003. Detoxification of a toxic variety of *Jatropha curcas* using heat and chemical treatments and preliminary nutritional evaluation with rats. Pak. J. Nat. Sci. 21:50–60.

Bancroft, J. D., and M. Gamble. 2008. Theory and Practice of Histological Technique. 4th ed. Churchill, Livingston, New York, London, San Francisco, Tokyo.

Barahona, E., P. Díaz, V. Castellano, and A. Anadón. 2010. Toxicological profile by *Jatropha curcas* L.Toxicol. Lett. 196:S287.

Barros, C. R., M. A. M. Rodrigues, F. M. Nunes, M. C. M. Kasuya, J. M. R. daLuz, A. Alves, L. M. M. Ferreira, V. Pinheiro, and J. L. Mour∼ao. 2015. The effect of Jatropha curcas seed meal on growth performance and internal organs development and lesions in broiler chickens. Br. J. Poult. Sci. 17:6.

Burrows, G. E., and R. J. Tyrl. 2001. Pages 473−486 in Toxic Plants of North Americas. Iowa State Univ. Press, Ames, Iowa, USA.

Chivandi, E., K. H. Erlwanger, S. M. Makuza, J. S. Read, and J. P. Mtimuni. 2006. Effect of dietary Jatropha curcas meal on percent cell volume, serum glucose, cholesterol and triglyceride concentration and alpha-amylase activity of weaned fattening pigs. Res. J. Anim. Vet. Sci. 1:18–24.

de la Casa-Resino, I., A. Valdehita, F. Soler, J. M. Navas, and P. M. Lopez. 2012. Endocrine disruption caused by oral administration of atrazine in Europeanquail (*Coturnix coturnix* coturnix). Comp. Biochem. Physiol. Part C Toxicol. Pharmacol. 156:159–165.

Devappa, R. K., H. P. Makkar, and K. Becker. 2010. Jatropha toxicity-a review. J. Toxicol. Environ. Health 13:476–507.

Devappa, R. K., and B. Swamylingappa. 2008. Biochemical and nutritional evaluation of Jatropha protein isolate prepared by steam injection heating for reduction of toxicand anti-nutritional factors. J. Sci. Food Agric. 88:911–919.

El Badwi, S. M., and S. E. Adam. 1992. Toxic effects of low levels of dietary *Jatropha curcas* seed on Brown Hisex chicks. Vet. Hum. Toxicol. 34:112–115.

El Badwi, S. M. A., H. M. Mousa, S. E. I. Adam, and H. J. Hapke. 1992. Response of Brown Hisex chicks to low levels of *Jatropha curcas, Ricinus communis* or their mixture. Vet. Human Toxicol. 34:304–306.

El Gawish, R., M. Elshabrawy Ghanem, and T. Maeda. 2013. Effects of bisphenol A and DDT on mRNA expression of vitellogenin II in liver of quail embryos. Iran. J. Vet. Res. 14:237–240.

Emam, A. M., S. S. Elnesr, E. A. El-Full, B. Y. Mahmoud, and H. Elwan. 2023. Influence of improved microclimate conditions on growth and physiological performance of two Japanese quail lines. Animals 13:1118.

Farag, M. R., M. Alagawany, M. M. Badr, S. R. Khalil, and M. S. El-Kholy. 2018. An overview of *Jatropha curcas* meal-

induced productive andreproductive toxicity in Japanese quail: potential mechanisms and heat detoxification. Theriogenology 113:208–220.

Ferreira, O. R., S. S. Brito, F. C. S. Chagas, A. T. Ramos, F. Y. M. Hosomi, F. G. Lima, P. C. Maiorka, V. L. Araújo, J. N. M. Neiva, M. C. S. Fioravante, and V. M. Maruo. 2011. Poisoning of sheep by shells of *Jatropha curcas* seeds. Pages 472−476 in Poisoning by Plants, Mycotoxins and Related Toxins. F. Riet-Correa, J. Pfister, A. L. Schild and T. Wierenga, eds. CABI International, Wallingford, CT.

Gandhi, V. M., K. M. Cherian, and M. J. Mulky. 1995. Toxicological studies on ratanjyot oil. Food Chem. Toxicol. 33:39–42.

Gross, H., G. Foidl, and N. Foidl. 1997. Detoxification of *Jatropha curcas* press cake and oil and feeding experiments on fish and mice. Proc. Jatropha 97: International Symposium Biofuel and Industrial Products from Jatropha curcas and Other Tropical Oil Seed Plants. Managua, Nicaragua, Mexico, 23−27 February 1997. G. M. Gubitz, M. Mittelbach and M. Trabi, eds. Graz University of Technology and Karl-Franzens University Graz, Austria.

Harinder, P. S., G. F. Makkar, and B. Klaus. 2008. Protein concentrate from *Jatropha curcas* screw-pressed seed cake and toxic and antinutritional factors in protein concentrate. J. Sci. Food Agric. 88:1542–1548.

Hafez, HM., and YA. Attia. 2020. Challenges to the Poultry Industry: Current Perspectives and Strategic Future After the COVID-19 Outbreak. Front Vet Sci. 7:516, doi:10.3389/fvets.2020.00516.

Kaneko, J. J. 1989. Clinical Biochemistry of Domestic Animals. Academic Press, San Diego, CA.

Khalil, S. R., A. Awad, and H. H. Mohammed. 2017. Behavioral response and gene expression changes infipronil-administered male Japanese quail (*Coturnix japonica*). Environ. Poll. 223:51–61.

Khan, S., M. H. Jan, D. Kumar, and A. G. Telang. 2015. Firpronil induced spermotoxicity is associated with oxidative stress, DNA damage and apoptosis in male rats. Pestic. Biochem. Physiol. 124:8–14.

Makkar, H. P. S., A. O. Aderibigbe, and K. Becker. 1998. Comparative evaluation of non-toxic and toxic varieties of *Jatropha curcas* for chemical composition, digestibility, protein degradability and toxic factors. Food Chem. 62:207–215.

Makkar, H. P. S., and K. Becker. 2009. *Jatropha curcas*, a promising crop for the generation of biodiesel and value-added coproducts. Eur. J. Lipid Sci. Technol. 111:773–787.

Makkar, H. P. S., K. Becker, F. Sporer, and M. Wink. 1997. Studies on nutritive potential and toxic constituents of different provenances of *Jatropha curcas*. J. Agric. Food Chem. 45:3152–3157.

Makkar, H. P. S., G. Francis, and K. Becker. 2007. Bioactivity of phytochemicals in some lesser-known plants and their effects and potential applications inlivestock and aquaculture production systems. Animal 1:1371–1391.

Martínez-Herrera, J., P. Siddhuraju, G. Francis, G. Dávila-Ortíz, and K. Becker. 2006. Chemical composition,toxic/antimetabolic constituents, and effects of different treatments on their levels, in four provenances of *Jatropha curcas* L. from Mexico. Food Chem. 96:80–89.

Nabil, A. E. A., A. E. Neveen, E. E. Elsharkawy, and E. A. Elmotleb. 2011. Chemical and pathological evaluation of jatropha curcasseed meal toxicity with or without heat and chemical treatment. Aust. J. Basic Appl. Sci. 5:49–59.

NRC. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

Odusote, O. M., A. O. Abioye, and M. O. Rotib. 2002. Jatropha curcas seed oil Linn (Euphorbiaceae) contraceptive activity and on oral formulation. Nig. Quart. J. Hosp. Med. 12:44–47.

Ojediran, T. K., Y. A. Adisa, S. A. Yusuf, and I. A. Emiola. 2014. Nutritional evaluation of processed jatropha curcas kernel meals: effect on growth performance of broiler chicks. J. Anim. Sci. Adv. 4:1110–1121.

Openshaw, K. 2000. A review of jatropha curcas: an oil plant of unfulfilled promise. Biomass Bioener. 19:1–15.

Osachoff, H. L., L. L. Brown, L. Tirrul, G. C. van Aggelen, F. S. Brinkman, and C. J. Kennedy. 2016. Time course of hepatic gene expression and plasma vitellogenin proteinconcentrations in estrone-exposed juvenile rainbow trout (*Oncorhynchus mykiss*). Comp. Biochem. Physiol. Part D. Genomics Proteom. 19:112–119.

Panigrahi, S., B. J. Francis, L. A. Cano, and M. B. Burbage. 1984. Toxicity of Jatropha curcas seeds from Mexico to rats and mice. Nutri. Rep. Inter. 29:1089–1099.

Pasaribu, T., E. Wina, B. Tangendjaja, and S. Iskandar. 2010. Performance of broiler chicken fed physically and chemically treated jatropha (*Jatropha curcas*) seed meal. Indonesian J. Agri. 3:121–126.

Pelletier, G., B. K. Padhi, J. Hawari, G. I. Sunahara, and R. Poon. 2015. Development of a sensitive in vitro assay to quantify the biological activity of pro-inflammatory phorbol esters in Jatropha oil. In vitro cellular and developmental biology. Animal 51:644–650.

Rakshit, K. D., J. Darukeshwara, K. Rathina Raj, K. Narasimhamurthy, P. Saibaba, and S. Bhagya. 2008. Toxicity studies of detoxified Jatropha meal (*Jatropha curcas*)in rats. Food Chem. Toxicol. 46:3621–3625.

Reddy, N. R., and D. K. Salunkhe. 1982. Phytates in legumes andcereals reviews. Adv. Food Res. 28:1–8.

Saeed, M., M. A. Arain, M. Arif, M. Alagawany, M. E. Abd El-Hack, M. U. Kakar, R. Manzoor, and S. Erdenee. 2017. Jatropha (*Jatropha curcas*) meal is an alternative protein source in poultry nutrition. World. Poult. Sci. J. 73:783–790.

Saetae, D., and W. Suntornsuk. 2011. Toxic compound, antinutritional factors and functional properties of protein isolated from detoxified Jatropha curcas seed cake. Inter. J. Mole. Sci. 12:66e77.

Salazar, I., R. Rodríguez, R. Aroche, M. Valdivié, and Y. Martínez. 2021. Phytobiotic effect of Jatropha curcas leaf powder on productivity, egg quality and blood biochemistry of laying quails. Cuban J. Agri. Sci. 55:315–326.

Saleh, Z., A. Abdel Azeiz, A. Mehany, and Z. El-Swaify. 2023. Anticancer and antimicrobial activity of jatropha's leaves extracts. Egypt. J. Botany 63:621–634.

SAS Institute. 2016. SAS. User's Guide: Statistics. Version 14.2 ed. SAS Institute Inc., Cary, NC.

Specker, J. L., and C. V. Sullivan. 1994. Vitellogenesis in fishes: status and perspectives. Pages 304−315 in Perspectives in comparative endocrinology. K. G. Davey, R. E. Petter and S. S. Tobe, eds. National Research Council, Ottawa.

Sumiati, A., I. N. Sudarman, I. Hidayah, and W. B. Santoso. 2007. Toksisitas racun bungkil biji Jarak pagar (*Jatropha curcas* L.) pada ayam broiler (Toxicity of *Jatropha curcas* L. mealtoxins in the broilers). Proceeding Seminar Nasional AINI VI. Bagian Nutrisid Makanan Ternak, Fakultas Peter nakan, Universities Gadjah Mada (pp. 144−150)144–150.

Taufiq-Yap, Y. H., H. V. Lee, M. Z. Hussein, and R. Yunus. 2011. Calcium-based mixed oxide catalysts for methanolysis of Jatropha curcas oil to bio-diesel. Biomass Bioener. 35:827–834.

Wingfield, J. C. 2005. Historical contributions of research on birds to behavioral neuroendocrinology. Horm. Behav. 48:395–402.

Zimila, H. E., J. S. Mandlate, R. S. Chivodze, H. F. Muiambo, V. Skripets, and A. A. Uamusse. 2018. An optimized solid-liquid method for rapid extraction of phorbol esters from Mozambican Jatropha seeds. Ind. Crops Prod. 124:941–946.