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Original Article

Proximate Composition of Detoxified *Jatropha curcas* Kernel Meal and its Toxicological Properties

Joel Uyi Ewansiha[1 *], Lilian Sopuruchi Oriji[2], Ljeoma Gladys Oriji[2]

1. Department of Microbiology, Modibbo Adama University of Technology, Yola, Adamawa State, Nigeria.

2. *Bio-resources Development Centre, National Biotechnology Development Agency, Lugbe Abuja, Nigeria.*

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Physic nut (*Jatropha curcas* L.) is a multipurpose drought- resistant small industrial tree or shrub, widespread throughout the arid and semi-arid tropical and subtropical regions of the world whose toxicity is attributed to the antinutritional factors including trypsin inhibitor; lectin, phytate and phorbol esters in the seed and other parts of the plant which restrict its use as animal feed. In this study, the detoxification of *Jatropha curcas* kernel meal (JCKM) was carried out using selected organisms isolated from food products using solid state and submerged fermentations at two separate periods (five and ten days) to determine the proximate composition and toxicity in whistar rats for two weeks using standard laboratory methods. Increase in fermentation duration was found to produce significant increase ($p<0.05$) in protein, content at both day five (46.97±1.08mg/g) and day ten (49.96±1.32mg/g) when compared to the control (40.14±2.12mg/g) while crude fiber, fat and carbohydrate decreased with increase in fermentation time. The moisture content of *J. curcas* kernel fermented with submerged method (9.51±0.26mg/g) and solid state (6.86±0.71mg/g) were significantly higher ($p<0.05$) compared to the control (5.29±0.87mg/g). Detoxified (DTX) and non-detoxified (NDTX) JCKM fed rats showed significant reduction ($p<0.05$) in neutrophil count when compared with the control (rats fed commercial feed). A significant increase ($p<0.05$) in lymphocyte level and non-significant difference ($p<0.05$) in eosinophil count was observed in NDTXJCKM when compared with the control. There was no significant difference ($p>0.05$) in monocyte count between NDTX, DTX and control. Detoxification of JCKM did not affect the haematological indices (platelet, RBC, MCH, MCHC, MCV and PCV levels) of rats except for haemoglobin (Hb) level which showed significant reduction ($p<0.05$) when compared with the control. There was a significant increase ($p<0.05$) in the biomarkers of the hepatic function (AST and ALT level) in rats fed with NDTX JCKM except for ALP level which was significantly lower ($p<0.05$) when compared with the control. Although there was elevation in the total protein (TP) level in DTX JCKM but it was not significantly different ($p>0.05$) from the control. The results revealed that rats fed with DTX and NDTX JCKM had a significant increase ($p<0.05$) in body weight than the control. The results obtained suggest that detoxified JCKM could be used to improve nutritional quality of animal feed.

Keywords: *Jatropha curcas* L, Phorbol esters, Fermentation, Antinutrient, Haematological indices

Jatropha curcas is regarded as a wonder plant because of its numerous attributes; the seeds contain up to 60% oil with a fatty acid pattern similar to that of edible oil, the percentage of essential amino acids and mineral content can be compared to those of other seeds (1). However, the oil and meal

*Correspondence: Department of Microbiology, Modibbo Adama University of Technology, Yola, Adamawa State, Nigeria.
E-mail: ewansihajoel@gmail.com Tell: +2348036206117

of *Jatropha curcas* seed were found toxic to mice calves, goats and sheep (2), rats and fish (1), chicken (3), and humans. The toxicity of *Jatropha* was attributed to the antinutritional factors including trypsin inhibitor; lectin, phytate (4) and phorbol esters (5) in the seed and other parts of the plant which restrict its use as animal feed. The heat-labile antinutrients, protease inhibitors and lectins are easy to inactivate by moist heating (4). Phorbol esters (phorbol-12-myristate - 13- acetate) have been identified as the main toxic agent in *Jatropha* seeds (6). Phorbol esters are toxic since they have biological effects that include skin inflammation, tumor promotion, tissue damage, activation of blood platelets, lymphocyte mitogenesis, prostaglandin production, and stimulation of degranulation in neutrophils in living cells (7). Therefore, detoxification is necessary before *Jatropha* seed meal can be used as animals'feed (7). Heat and chemical treatments were used to detoxify *Jatropha curcas* meal but the combined treatment approach was economically expensive since heat treatment alone could not detoxified phorbol esters (8). *Jatropha curcas* kernel meal (JCKM) is a cost-effective potential source of rich dietary protein supplement in animal feeds whose inclusion in animal feeds is currently hampered by the presence of toxic anti-nutritional components. As the world human population increases, there is concomitant increase in demand and competition for conventional food/feedstuffs used both by man and livestock. This is especially true in underdeveloped and developing countries where food production cannot keep pace with the high growth in population. There is also an increase in the prices of these orthodox feedstuffs as a result of competition between feed industries and man. This has caused developing countries to embark on researches focused on novel feedstuffs that are not staple for human consumption to alleviate the problems of shortage and competition for the available traditional feedstuffs. It is for these reasons that *Jatropha curcas* seeds are considered as alternative feedstuff in this experiment. This study therefore seeks to determine the proximate composition of detoxified *Jatropha curcas* kernel meals and sub-chronic toxicity in Wistar rats.

Materials and methods

The media (potato dextrose agar, nutrient agar) and chemicals used for this research work were standard analytical grade, purchased from reputable company include and the experimental organisms viz: *Aspergillus niger*, *Saccharomyces cerevisiae*, *Candida tropicalis*, *Penicillium album*, *Rhizopus microsporum*, *Bacillus subtilis*, *B alvevi*, *B licheniformis*, *B megaterium*, and *Zymomonas mobilis* were isolated from food products.

Collection and Preparation of *Jatropha curcas* Kernel meal

Seeds of *Jatropha curcas* were purchased from River Basin Farms, Minna, Niger State Nigeria. The seed was identified at the Department of Crop Production, Federal University of Technology, Minna. The seeds were sorted to get rid of stones and other debris, cleaned, weighed and later cracked individually to remove the kernel which was equally weighed. The kernel was milled using a miller and defatted using solvents as described by Belewu (9).

Proximate Composition of *Jatropha curcas* Kernel Meal

The proximate compositions of the samples were determined according to the method described in A.O.A.C (10). The parameters determined include moisture, crude protein, crude fats, crude fibre, and carbohydrate contents.

Determination of Crude Protein

Kjeldahl method used involved three stages. Five grams (5g) of the samples were weighed and placed in the digestion flask; copper and selenium (one tablet each) were used as the catalyst. Twenty-five milliliters (25ml) of sulphuric acid was added to each flask containing the samples and digested until a clear solution was obtained. After cooling, the digested samples were transferred into two hundred and fifty milliliters (250ml) volumetric flask and

made up to the mark with distilled water. The distillation apparatus (Markham semi-micro Kjeldahl apparatus) was connected up with delivery tube dipping below the boric acid in the receiving flask. Ten milliliters (10ml) of two percent (2%) boric acid was used for each sample distilled and five drops of screened methyl red was added as indicator. Ten millilitres (10ml) of the diluted digest was transferred into the digestion flask followed by the addition of fifteen milliliter (15ml) of forty percent (40%) NaOH. After seventy five millilitres (75ml) had been distilled, the tip of the condenser was washed into the distillate. The distillate was titrated with 0.01N HCl and the percentage nitrogen calculated thus: %Crude Protein = 6.25 × %N (Correction factor)

$$\text{Nitrogen (\%)} = \frac{\text{volume of acid (HCl) used } 0.1 \times 0.014 \times 50 \times 100}{\text{Weight of the sample}}$$

Determination of Crude Fibre Content

Five grams of the sample was weighed into one liter (1L)conical flask (W1), followed by the addition of two hundred milliliters (200ml) of boiling 1.25% H_2SO_4. The solution was boiled gently for 30 minutes then filtered through a muslin cloth. Stretched over a Brichner funnel and rinsed with hot distilled water. The residue was scrapped back into the flask with a spatula and two hundred milliliters (200ml) of boiling 1.25% NaOH was added. The solution was allowed to boil gently for 30 minutes and cool thereafter. This was again washed thoroughly with hot distilled water and rinsed once with 10% HCl and twice with industrial methylated spirit. The residue was rinsed finally three times with petroleum ether (40-60°C) and later drained, dried and scrapped into a crucible. The residue was dried overnight at 105°C in the oven cooled in a dessicator and then weighed (W2). The residue was also ashed at 550°C for 90 minutes in a muffle furnace, then allowed to cool in a desiccator and weighed (W3). Percentage of crude fibre was estimated as bellow:

$$\text{Crude fibre (\%)} = \frac{\text{W2} - \text{W3} \times 100}{W1}$$

W1 = weight of sample

W2 = weight of residue

W3 = weight of residue after ashing

Determination of Ash Content

Five grams of each sample were added into a pre- weighed porcelain crucible and placed in a temperature controlled furnace pre-heated to 550°C, which was maintained for two hours. The crucible was transferred directly into desiccator, cooled and reweighed immediately. The ash content was then calculated thus:

$$\text{Ash content (\%)} = \frac{\text{Weight of ash}}{\text{Weight of dried sample}} \times 100$$

Determination of Moisture content

Moisture content was determined using the oven-drying method. A clean and dry dish was weighed and recorded (W1). Five grams of the sample was weighed into the dish and re-weighed (W2). The dish containing the sample was transferred into the oven, dried and maintained at 105°C for 4 hours after which the dish was transferred into the desiccator and allowed to cool before re-weighing. The process of heating, cooling and weighing was continued until a constant weight (W3) was obtained. Moisture content was calculated as follows:

$$\text{Moisture content (\%)} = \frac{W2-W3}{W2-W1} \times 100$$

weight of sample take = W2 – W1

W1 = weight of dish

W2 = weight of dish + sample

W3 = weight of dish + sample after drying

Determination of Fat content

Two grams (2g) each of dried *Jatropha curcas* sample was weighed into a pre-weighed filter paper and folded neatly. This was placed into the soxhlet extractor thimble containing petroleum ether and was extracted for six hours at 40 - 60°C. The filter paper was taken out, air-dried and reweighed.

$$\text{Lipid (\%)} = \frac{\text{loss in weight of sample}}{\text{Original weight of sample}} \times 100$$

Results and Discussion

The nutritional value of JCKM compares favourably with those from conventional seed meals, such as soybean, making it a potential source of dietary energy and protein (11). The kernel meal of *J. curcas* left as a by-product after oil extraction

from whole seed by screw press has a high crude protein content of which reasonable amount is true protein (4). The percentage of essential amino acids and mineral contents is comparable to those of other seed and press cakes used as a fodder (12). Hence, the JCKM has a great potential to complement and substitute soybean meal as a protein source in livestock diets (13). *Jatropha curcas* kernel meal was also reported to contain micro and macro minerals (14). The seed meal and oil from *J. curcas* were however found toxic to calves, goats and sheep (2). The toxicity of *Jatropha* was attributed to the anti-nutritional factors including trypsin inhibitor, lectin, phytate (15) and phorbol esters (5) in the seed and other parts of the plant. However, the presence of anti-nutritional factors restricts the utilization of the JCKM in animal feed (16). This obstacle however can be overcome by detoxifying the kernel through simple inexpensive method known as fermentation (17). The results presented show that irrespective of the process of fermentation as well as the microbes involved, there was significant increase ($P<0.05$) in the moisture and protein contents at the tenth day fermentation process when compared with the fifth day and control. However, fermentation at the fifth day did not affect the moisture and fat contents when compared with the control (Table 1). The significant increase ($p<0.05$) in moisture content of fermented kernel meals could be due to the water absorbed by JCKM during fermentation process (18). Similar observation was made when JCKM were treated with *Aspergillus niger* and *Trichoderma longbrachitum* (19). Increase in protein content was observed as fermentation duration of JCKM was increased. This increase in protein level could be attributed to the ability of the microorganism to secret some extra cellular enzymes (proteins) which degrade the materials during fermentation. This result is similar to the observations when pure strain of *Aspergillus niger* was used to ferment maize cobs by Oseni and Ekperigin (20). Fermentation duration (five or ten days) did not significantly affect crude fiber, ash and carbohydrate compositions in JCKM. The carbohydrate level of the kernel at both the fifth and tenth days were significantly reduced ($p<0.05$) compared to the control. The longer the fermentation duration in JCKM brought about reduction of crude fiber, fat and carbohydrate content in JCKM when compared with the control. Furthermore, fermentation has no effect on ash content of JCKM when compared with the control (unfermented JCKM). The reduction in crude fiber was consistent with the work of Belewu (9), where decrease in crude fibre content was observed for JCKM treated with *A. niger* and *Mucor mucedo*. The decrease might be due to the action of various enzymes (cellulase, xynalase, pectinase, chitin, amylase, hemicellulase and lipase) secreted by microbes during fermentation process (21). The fermentation process involves the breakdown or conversion of materials to the perculiar needs of the microorganisms. Reduction in fat is comparable with the result obtained by Rotimi *et al.* (22) during the fermentation of locust bean. This may be as a result of utilization of lipids by fermentation microbes to obtain energy for their activity when sugars were in short supply. While the reduction in carbohydrate level could be as a result of utilization and transformation by fermentation microorganisms to obtain energy to carry out cellular activities (23). This observation is however similar to that of Oladele and Oshodi (24).

Fermentation has been widely used for the production of a wide variety of substances that are highly beneficial to individuals and industry (Rossi, 2009). Over the years, fermentation processes have gained immense importance due to their economic and environmental advantages. Ancient methods have been further modified and refined to maximize productivity. This has also involved the development of new machinery and processes. Two broad fermentation processes have emerged as a

Table 1. Effect of fermentation time on the proximate composition of JCKM

Nutrient	Control	5th day	10th day
Moisture	5.29±0.87^{a}	7.86±1.43^{a}	8.51±1.21^{b}
Protein	40.14±2.12^{a}	46.97±1.08^{b}	49.96±1.32^{c}
Crude fiber	9.29±0.73^{b}	8.55±0.82^{ab}	7.49±0.91^{a}
Fat	1.58±0.66^{b}	1.52±0.23^{b}	1.24±0.16^{a}
Ash	7.87±1.16^{a}	7.10±1.08^{a}	6.55±1.37^{a}
Carbohydrate	35.83±2.11^{b}	27.76±2.04^{a}	26.26±1.75^{a}

JCKM: *Jatropha curcas* kernel meal.Values are Mean±Standard Error of Mean of duplicate determinations. Values with different superscripts across the row are significantly ($p < 0.05$) different.Control: unfermented *Jatropha curcas kernel meal*.

result of this rapid development: Submerged Fermentation (SMF) and Solid State Fermentation (SSF). Discovery of the beneficial activity of several secondary metabolites produced by microorganisms (bioactive compounds) has resulted in the further exploration of fermentation as a production method for these compounds (25). At the research level, both SSF and SMF had been used; however, some methods yielded better results than others. The result obtained from this study revealed significant increase in the moisture content of submerged fermented JCKM when compared with solid state fermented JCKM and control (unfermented JCKM). Fermentation increased protein content in JCKM generally when compared with the control. The increase yield in protein observed by solid state fermentation method may be as a result of microbial ability to secret special group of cells known as enzymes which aid in the breakdown of complex materials. These observations are however contrary to the reports of Eze and Ibe (26) as they observed decreased in protein content of fermented oil bean. Submerged fermentation increased the moisture content of JCKM significantly when compared with solid state fermented JCKM and the control. This is obviously due to water absorbed by JCKM during the fermentation process. This result compares favourably with the work of Oladele and Oshodi (24) on the effect of fermentation on the nutritive properties of Berlandier Nettle Spurge (*Jatropha cathartica*) and Physic Nut (*Jatropha curcas*). Fermentation generally did not have any significant ($p>0.05$) effect on crude fiber, fat and ash contents of JCKM when compared with the control. It was observed that both of the fermentation processes (submerged and solid state fermentation) had effect on fat, fiber and ash contents of JCKM. Fermentation reduced the carbohydrate level in JCKM and both processes (submerged and solid state fermentation) reduced carbohydrate contents to the same degree. The decrease in carbohydrate level may be due to the fact that carbohydrate content in JCKM was used up as source of energy during fermentation. The same trend was reported by Oseni and Akindahunsi (20) and also by Phengnuam and Suntornsuk (27) who observed that fermentation of *Jatropha curcas* kernel cake led to a reduction in the level of carbohydrate. (Table 2).

Table 2. Effect of fermentation processes on the proximate composition of JCKM Proximate components (mg/g)

Fermentation	Moisture	Protein	Crude fiber	Crude fat	Ash	Carbohydrate
Control	5.29±0.87^{a}	40.14±2.12^{a}	9.29±0.73^{bc}	1.58±0.66^{a}	7.87±1.16^{ab}	35.83±2.11^{b}
Solid state	6.86±0.71^{a}	50.14±1.75^{c}	8.44±0.75^{bc}	1.37±0.15$^{a`}$	7.28±1.02^{ab}	27.74±1.69^{a}
Submerged	9.51±0.26^{b}	46.79±2.06^{b}	7.86±0.64^{ab}	1.40±0.38^{a}	6.37±0.31^{a}	29.26±2.47^{a}

JCKM: *Jatropha curcas* kernel meal,Values are Mean±Standard Error of Mean of duplicate determinations. Values with different superscripts along the column are significantly ($p < 0.05$) different. Control: unfermented *Jatropha curcas kernel meal*.

Microorganisms used in food production are live bacteria, yeasts or moulds. The microorganisms carry out the fermentation process by preserving foods through formation of inhibitory metabolites such as organic acid (lactic acid, acetic acid, formic acid, propionic acid), ethanol, bacteriocins, often in combination with decrease of water activity by drying or use of salt (28). Further, microbes used in fermentation of food help to improve food safety through inhibition of pathogens or removing of toxic compounds (29). Microorganisms also improve the nutritional value and organoleptic quality of the food (30). More than 260 different species of microbe are identified and described for their beneficial use in fermented food products globally, showing the importance of their use (31). Microorganism used in food fermentation can be divided into three major groups: bacteria, yeasts and moulds. The result obtained from this study shows that irrespective of the duration of fermentation as well as the methods of fermentation employed, the microorganisms had no effect in the moisture content when compared with the control except *Penicillium album*, although the increment was not significant. This may be as a result of hydrolytic action of the fermenting microbes or fermentation process which involved water (22). Similar result was also observed by Akinyele and Oloruntoba (2013). The significant ($p<0.05$) increase in protein content of fermented JCKM with *B. subtilis* when compared with other microorganisms and the control could be as a result of addition of microbial protein during the process of fermentation (19). This increment in the protein content of the fermented samples was in agreement with the work of Belewu *et al*. (32). The fibre content in *J. curcas* fermented with *B. licheniforms*, *B. subtilis* and *A. niger* decreased fibre content but this reduction is not significantly different ($p>0.05$) when compared with other organisms performance and the control. The slight reduction observed in crude fibre might be due to the action of various enzymes (cellulase, xynalase, pectinase, chitin, amylase, hemicellulase and lipase) secreted by the fungi during fermentation process (21). The reduction in fibre content confirmed the assertion of Makinde *et al*. (33) on fermented sesame seeds. None of the microorganisms used decreased fat and ash content appreciably when compared with the control. This result is contrary to that obtained during the fermentation of locust bean by Wang *et al.* (34) and Ameen *et al*. (35). *Bacillus subtilis* decreased carbohydrate level significantly ($p<0.05$) when compared with the other organisms and the control. A general decrease in carbohydrate level of JCKM occurred by microorganisms during fermentation when compared to the control. The decrease in the level of carbohydrate may be due to their utilization and transformation by fermentation microorganisms to obtain energy. A similar result was obtained by Abou-Arab and Abu-Salem (36) who reported low carbohydrates in fermented *Jatropha* kernel meal (Table 3).

Blood differential counts are useful indicators in evaluating the toxicity of substances in the mammalian cell. Neutrophils and lymphocytes are biomarkers of immunity, while eosinophil and monocytes are essential indicators of allergic reactions. The results obtained from this study illustrated that although all animals exposed to the treatments showed significant reduction in neutrophil count, the group exposed to non-detoxified (NDTX) *Jatropha curcas* kernel meal exhibited marked reduction in neutrophil count more. This suggests that exposure to non-detoxified *Jatropha curcas* kernel meal may reduce the animals' ability to fight infections, thereby increasing the risk of neutropenia; a condition where normal bacteria from the mouth and digestive tract can cause serious infections. In some cases, low neutrophil counts have been attributed to nutritional deficiencies. *Jatropha curcas* has been found to contain phorbol esters and variety of anti-nutritional factors such as trypsin inhibitors, phytic acid, lectin and saponin (37), implying that animals exposed to NDTX may have suffered marked nutritional

Table 3. Microbiological influence on proximate composition of fermented JCKM Proximate component (mg/g)

Microorganisms	Moisture	Protein	Fiber	Fat	Ash	Carbohydrate
Control	5.29±0.87^{a}	40.14±2.12^{a}	9.29±0.73^{ab}	1.58±0.66^{a}	7.87±1.16^{ab}	35.83±2.11^{d}
Bacillus alvevi	7.01±0.32^{a}	49.72±3.50^{b}	8.80±0.51^{ab}	1.22±0.14^{a}	6.62±0.41^{a}	24.63±3.08^{b}
Bacillus licheniformis	7.75±0.82^{a}	50.21±2.77^{b}	7.69±1.09^{a}	1.05±0.21^{a}	6.55±0.46^{a}	26.75±1.52^{b}
Bacillus megaterium	7.80±0.57^{a}	48.11±0.59^{ab}	8.95±1.22^{ab}	1.40±0.75^{a}	6.81±0.87^{a}	26.93±1.11^{b}
Bacillus subtilis	7.05±0.76^{a}	56.31±1.02^{c}	7.94±0.95^{a}	1.11±0.27^{a}	6.22±1.01^{a}	21.37±0.01^{a}
Aspergillus niger	7.91±0.18^{a}	50.60±3.84^{b}	7.69±0.88^{a}	1.31±0.60^{a}	6.78±0.59^{a}	24.57±1.65^{b}
Saccharomyces cerevisiae	7.67±0.60^{a}	48.84±0.97^{ab}	8.80±0.57^{ab}	1.40±0.31^{a}	6.43±0.95^{a}	26.86±3.52^{b}
Penicillium album	8.18±1.05^{ab}	47.03±2.79^{ab}	7.26±0.94^{a}	1.06±0.25^{a}	7.80±0.58^{ab}	28.65±2.42^{bc}
Rhizopus microsporum	7.03±0.13^{a}	44.88±1.03^{a}	9.48±0.41^{ab}	1.17±0.18^{a}	6.29±0.65^{a}	29.15±4.35^{bc}
Candida tropicalis	7.28±0.68^{a}	47.68±2.99^{ab}	8.90±1.02^{ab}	1.22±1.03^{a}	7.03±0.51^{a}	27.89±2.85^{b}
Zymomonas mobilis	7.22±0.34^{a}	49.03±1.13^{b}	8.18±1.06^{ab}	1.41±0.60^{a}	6.17±1.85^{a}	26.96±3.06^{b}

Values are Mean±Standard Error of Mean of duplicate determinations. Values with different superscripts along the column are significantly ($p < 0.05$) different. Control: unfermented *Jatropha curcas kernel meal*. JCKM: *Jatropha curcas* kernel meal.

deficiency to elicit the significantly low neutrophil level recorded in the group. This finding is consistent with the report of Gibson and Berliner (38), who acknowledged that acquired neutropenia can be observed after nutritional deficiency. The results obtained in this study also revealed a significantly ($p<0.05$) elevated level of lymphocyte in the animal groups that were treated with NDTX *Jatropha curcas* kernel meal. This suggests that exposure to the NDTX *Jatropha curcas* kernel meal, which contains high amount of the toxic compound (phorbol esters), may have compromised the animals' immune system and triggered associated immune response seen in the abnormally elevated level of lymphocytes. This assertion is consistent with the report of Saetae and Suntornsuk (37) that the toxic effect of phorbol esters may cause lymphocyte mitogenesis. The study result revealed that while monocyte levels did not differ significantly ($p>0.05$) between the treated groups and the control, eosinophil level in the animal group exposed to DTX showed significant reduction ($p<0.05$) when compared with the control. Since eosinophil and monocyte are markers of allergic reaction, it suggests that the detoxified *Jatropha curcas* kernel meal did not exert any allergic reaction in the animals fed with it (Table 4).

The examination of hematological parameters especially the erythrocytic indices plays a vital role in the physiological, nutrition and pathological status of an organism (39). Red blood cell and factors relating to it are major indices for evaluating circulatory erythrocytes are significant in the diagnosis of anaemia and also serve as useful indices of the bone marrow capacity to produce RBC in

Table 4. Correlation analysis and linear regression between *G. bimaculatus* count and bulb light intensity

Groups	Neutrophil	Lymphocyte	Eosinophil	Monocyte*
DTX	11.33±2.96^{b}	67.00±5.68^{a}	1.33±0.88^{a}	5.93±0.44^{a}
NDTX	8.00±0.00^{a}	71.00±2.51^{b}	2.00±2.08^{b}	5.80±0.20^{a}
Control	15.66±2.18^{c}	64.33±2.56^{a}	2.00±2.00^{b}	7.70±0.37^{a}

JCKM: Jatropha curcas kernel meal; DTX: Rat fed with detoxified JCKM. NDTX: Rat fed with non-detoxified JCKM.;Control: Rat fed with commercial feed. Values are mean ± SEM for n = 5. Mean data on column carrying different superscripts are significantly different

mammals (40). In the present study, neither detoxified (DTX) nor non-detoxified (NDTX) *Jatropha curcas* kernel meal in the diet of whister rats had any significant ($p>0.05$) effect on PCV, MVC, MCH, MCHC, RBC and Platelet count. Since these parameters are useful indices in elucidating the etiology of anemias (41), it therefore suggests that *Jatropha curcas* kernel meal inclusion in rats' diet does not produce anemic effects. This finding may substantiate earlier claims cited in the report of Nwaka *et al.* (42) that leaf extract of *Jatropha curcas* possess anti-anemic property. Since variations in differential count are a major indicator of white blood cell function, the significant reduction in WBC in the present study is concurrent with earlier results of differential count discussed in this chapter, where marked reduction in neutrophil level is found to reconcile with marked reduction in WBC in animals exposed to NDTX (Table 5).

The biochemical indices monitored in the serum of experimental rats are useful biological markers for assessment of tissue damage. The measurement of activities of various enzymes in the body fluids plays a significant role in disease investigation and diagnosis assault on the organs/tissues and to a reasonable extent the toxicity of the drug (43). The transaminase enzymes (ALT and AST) are markers of liver damage and can thus be used to assess liver cytolysis with ALT being a more sensitive biomarker of hepatotoxicity than AST (44). Consequently, degenerative changes in hepatic tissues were accompanied with enhancement of liver enzymatic activities, which was recorded in thebiochemical analysis in this study. A significant elevation in AST, ALT concentration in the serum of rat fed with diet containing 25 % NDTX *Jatropha curcas* kernel meal than the control were obtained. The significant increase in the liver enzymes activities were attributed to liver damage and cytotoxic effect of *J. curcas* kernel meal on the liver cells leading to leakage of AST & ALT from damaged hepatocytes (45). The concentrations of total protein are a useful 'marker' of synthetic and excretory functioning of the liver and kidney (46). The hyperproteinemia observed in the serum of the same group of rats were attributed to the direct toxic effect of phorbol esters which led to degeneration and necrosis of hepatocytes (8). The increase in total protein in serum of rats fed diet containing NDTX *Jatropha curcas* kernel meal could, lead to dehydration which is detrimental to cellular homeostasis. This will negatively affect the metabolic activities of the liver and consequently the health of the animals. However hyperproteinemia observed in this study disagrees with findings of Nabil *et al.* (47), who reported hypoproteinemia in serum of rat fed *J. curcas* kernel meal (Table 6).

Body weights are commonly used for monitoring the nutritional status and growth of animals (48). The importance of feed intake by animals as a determinant of rat's performance has been strongly emphasized (49). The effect of

Table 5. Changes in haematological indices of rats fed with JCKM

Samples	Hb	PCV*	MCV*	MCH*	MCHC*	RBC*	Platelet*	WBC
DTX	13.86 ±1.08^a	40.00 ±3.60^a	49.66 ±2.18^a	18.33 ±1.33^a	36.33 ±0.88^a	7.96 ±0.49^a	515.67 ±98.82^a	7.03 ±1.70^b
NDTX	13.66 ±0.08^a	39.33 ±0.33^a	49.33 ±0.66^a	17.33 ±1.33^a	34.66 ±0.33^a	7.45 ±0.03^a	528.33 ±67.30^a	6.03 ±0.40^a
Control	20.96 ±8.03^b	39.26 ±8.27^a	49.00 ±1.00^a	17.66 ±0.88^a	35.33 ±0.33^a	7.83 ±0.66^a	564.67 ±42.35^a	7.23 ±0.12^b

Values are mean ± SEM for n = 5, Mean data on column carrying different superscripts are significantly different ($p< 0.05$), *: No significant difference, JCKM: Jatropha curcas kernel meal, DTX: Rat fed with detoxified JCKM. NDTX: Rat fed with non-detoxified JCKM, Control: Rat fed with commercial feed, WBC: White Blood Cells, RBC: Red Blood Cells, PCV: Packed cell volume, Hb: Haemoglobin, MCH: Mean Corpuscular Haemoglobin, MCHC:Mean Corpuscular Haemoglobin Concentration, MCV: Mean Corpuscular Volume.

Table 6. Change in serum biochemical parameters of rats fed JCKM

Groups	AST(U/L)	ALT(U/L)	ALP(U/L)	TP(g/dl)
DTX	53.45±5.68^{a}	55.52±3.46^{a}	157.34±3.69^{a}	26.12±3.45^{ab}
NDTX	67.54±3.45^{b}	66.67±4.32^{b}	165.78±4.32^{a}	28.76±4.56^{b}
Control	58.45±4.32^{a}	56.32±2.34^{a}	203.12±6.89^{b}	24.45±4.32^{a}

Values are mean ± SEM for n = 5, Mean data on column carrying different superscripts are significantly different ($p<0.05$), JCKM: Jatropha curcas kernel meal, DTX: Rat fed with detoxified JCKM, NDTX: Rat fed with non-detoxified JCKM, AST: Aspartate transaminase, ALT: Alanine transaminase, ALP: Alkaline phosphatase, TP: Total protein.

Body weights are commonly used for monitoring the nutritional status and growth of animals (48). The importance of feed intake by animals as a determinant of rat's performance has been strongly emphasized (49). The effect of treatments on rat growth performance in this research findings shows that the level of non-detoxified JCKM inclusion had a negative effect on body weight ($p<0.05$). Weight gain ($p<0.05$) was negatively affected during experimental periods, and these effects were more pronounced during the second week (Table 7). The finding is an indication that the *Jatropha curcas* kernel meal mediate abnormal physiological and adaptational responses, with consequence negative effect on the appetite and caloric intake by the animals (50). These adverse effects on animal performance and health observed in rat due to the level of non-detoxified JCKM inclusion in the diet are consistent with the results presented by other authors (51). Nevertheless, these deleterious effects could be attributed to high level of phorbol esters (2.78±0.32). As stated before, PE are considered to be the main toxic component of JCKM, limiting its use in animal feeding, More recently, other authors have also reported reduced feed intake and growth in rats (8) fed diets containing *Jatropha curcas* with final phorbol esters concentrations varying from 0.015 to 0.021% .

The dietary levels of NDTX had a negative effect on body weight gain, and average feed intake. Therefore, threshold levels of PE in animals' diets must be carefully evaluated as available data on animal toxicity has been basically obtained in trials using force-feeding of feed or its organic solvent/aqueous extracts (52), and not in growth trials. The ameliorative effect of fermentation on toxicity of JCKM as seen in normal growth performance, and biochemical parameters of rat fed with DTX feed could be used as a protein-rich dietary supplement in the animal feed (53). The use of detoxified JCKM in animal nutrition can minimize expenditures on the development of food sources, such as soybean, cotton and wheat meals, without causing undesirable effects on the overall production system (51).

Table 7. Change in body weight gain of rats fed JCKM

Weeks	Control	DTX	NDTX
1*	95.01±1.56^{a}	105.68±0.01^{a}	104.90±0.23^{a}
2	101.54±1.34^{a}	111.57±0.56^{b}	109.56±1.13^{b}

Values are mean ± SEM for n = 5, Mean data on row carrying different superscripts are significantly different ($p<0.05$). *: No significant difference, JCKM: Jatropha curcas kernel meal, DTX: Rat fed with detoxified JCKM. NDTX: Rat fed with non-detoxified JCKM.

Conclusion

Based on the result of this study, it can therefore be concluded that selected microorganism isolated from food product can be employed to detoxify defatted *Jatropha curcas* karnel meal to a safe and acceptable level for livestock use.

Conflict of interest

The authors declared no conflict of interest.

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