Lactobacillus Fermentation of Jatropha Kernel Cake: Nutritional Composition, Anti-Nutritional Factors, and Mineral Bioavailability

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Abstract

Jatropha kernel cake (JKC), a by-product of biodiesel production, contains valuable nutrients but is limited in use due to toxic phorbol esters and other anti-nutritional factors (ANFs). While previous studies have reported improvements in proximate composition after lactobacillus (LAB) fermentation, limited data exist on ANFs reduction and mineral bioavailability. This study was designed to investigate the effectiveness of LAB fermentation in improving the nutrient composition, degrading ANFs and enhancing mineral concentrations in JKC. Defatted JKC was fermented with Lactobacillus acidophilus-DLB07, Lactiplantibacillus plantarum-DLB13, Lactobacillus rhamnosus-DLB23, and a co-culture of the three strains for 7 days at 37 °C. Phorbol esters were quantified using HPLC. Other ANFs (phytate, tannins, saponins, and trypsin inhibitor) were analysed using standard methods, and mineral concentrations were determined by atomic absorption spectrophotometry. Data were analysed using one-way ANOVA. Lactobacillus fermentation significantly ($p < 0.05$) improved crude protein by 22.41%, and decreased the crude fibre (39.71%), reduced ANFs compared with untreated JKC, with the co-culture showing the greatest reductions in phorbol esters (0.03 mg/g vs. 0.08 mg/g control), phytate (1.32% vs. 6.80%), tannins, saponins, and trypsin inhibitor. Fermentation also enhanced mineral contents, notably calcium, iron, and zinc. Mechanistic insights suggest that microbial phytase and organic acid production contributed to ANFs degradation and mineral release.

Lactobacillus fermentation effectively detoxified JKC and improved mineral bioavailability, with co-cultures being the most efficient. These findings extend earlier reports on proximate composition and highlight the potential of LAB-fermented JKC as a safe, nutrient-enriched feed ingredient.

Keywords: Jatropha kernel cake; lactobacillus; fermentation; nutrient composition; anti-nutritional factors Lactobacillus Fermentation of Jatropha Kernel Cake

Fermentation du Tourteau de Graines de Jatropha par des Lactobacilles : Composition Nutritionnelle, Facteurs Anti-Nutritionnels et Biodisponibilité Minérale

Résumé

Le tourteau de graines de Jatropha (TGJ), un sous-produit de la production de biodiesel, contient des nutriments précieux mais son utilisation est limitée en raison des esters de phorbol toxiques et d'autres facteurs anti-nutritionnels (ANFs). Bien que des études antérieures aient rapporté des améliorations de la composition globale après fermentation par des lactobacilles (LAB), les données sur la réduction des ANFs et la biodisponibilité des minéraux sont limitées. Cette étude a été conçue pour évaluer l'efficacité de la fermentation par des LAB à améliorer la composition nutritionnelle, à dégrader les ANFs et à augmenter les concentrations en minéraux du TGJ. Du JKC dégraissé a été fermenté avec Lactobacillus acidophilus-DLB07, Lactiplantibacillus plantarum-DLB13, Lactobacillus rhamnosus-DLB23, et un co-culture des trois souches pendant 7 jours à 37°C. Les esters de phorbol ont été quantifiés par HPLC. Les autres ANFs (phytate, tanins, saponines et inhibiteur de trypsine) ont été analysés par des méthodes standards, et les concentrations minérales ont été déterminées par spectrophotométrie d'absorption atomique. Les données ont été analysées par ANOVA à un facteur. La fermentation par les lactobacilles a significativement ($p < 0,05$) amélioré la teneur en protéines brutes de 22,41%, et diminué la teneur en fibres brutes (39,71%) et réduit les ANFs par rapport au TGJ non traité, la co-culture montrant les réductions les plus importantes pour les esters de phorbol (0,03 mg/g contre 0,08 mg/g témoin), le phytate (1,32% contre 6,80%), les tanins,

saponines et inhibiteur de trypsine. La fermentation a également augmenté les teneurs en minéraux, notamment le calcium, le fer et le zinc. Des perspectives mécanistiques suggèrent que la production de phytase microbienne et d'acides organiques a contribué à la dégradation des ANFs et à la libération des minéraux. La fermentation par les lactobacilles a efficacement détoxifié le TGJ et amélioré la biodisponibilité minérale, les co-cultures étant les plus efficaces. Ces résultats étendent les rapports antérieurs sur la composition globale et soulignent le potentiel du TGJ fermenté par des LAB comme ingrédient alimentaire sûr et enrichi en nutriments.

Mots-clés : Tourteau de graines de Jatropha ; lactobacilles ; fermentation ; composition nutritionnelle ; facteurs anti-nutritionnels

Introduction

The growing global demand for renewable energy has driven large-scale biodiesel production from oil-bearing crops, including the *Jatropha curcas*, resulting in substantial quantities of residues (Jatropha kernel cake) as a by-product (Che Hamzah *et al*., 2020). This residual material left after oil extraction is rich in protein, with crude protein concentrations reported between 45 – 64% and an amino acid profile that compares favourably with conventional protein sources such as soybean meal and fishmeal (Musa *et al*., 2018; Fawole *et al*., 2022). Its abundance, relatively low cost, and balanced nutrient composition underscore its potential as an alternative protein supplement in livestock feeding systems, particularly in regions where feed resources are limited and expensive.

Despite its nutritional promise, the practical utilisation of Jatropha kernel cake (JKC) is constrained by the presence of toxic phorbol esters (PEs) and a range of anti-nutritional factors (ANFs), including phytates, tannins, saponins, trypsin inhibitors, glycosides, and phenolic compounds. These compounds impair digestibility, interfere with nutrient absorption, and, in the case of PEs, pose direct toxicological risks to animals (Fawole *et al*., 2022). For instance, the PE levels in raw Jatropha seeds (1–3 mg/g) can be fatal to livestock, while phytates chelate essential minerals, reducing their bioavailability, and saponins and tannins disrupt enzyme activity and protein utilisation. As a result, detoxification of JKC is indispensable for its safe incorporation into animal diets.

A variety of detoxification strategies have been investigated to mitigate the limitations of JKC. Physical methods, such as heat treatment, can reduce some heat-labile anti-nutrients but are often incomplete and energy-intensive. Chemical methods, including solvent extraction and alkaline treatment, effectively reduce toxins but may leave residues, alter the nutritive value, and pose environmental concerns (Belewu *et al*., 2010). In contrast, biological approaches, particularly microbial fermentation, offer a more sustainable solution. Among these, lactic acid bacteria (LAB)-mediated fermentation has gained considerable attention due to its ability to degrade toxic compounds, hydrolyse complex anti-nutrients, and simultaneously improve nutrient availability (Wafar *et al*., 2021; Moriconi *et al*., 2024). LAB fermentation enhances the nutritional value of plant-based feedstuffs by producing enzymes such as phytases, tannases, and β-glucosidases, while also generating organic acids that lower pH and optimise mineral solubility.

Evidence from previous studies supports the effectiveness of LAB in enhancing the feed value of JKC. Widiyastuti and Hidayat (2018) demonstrated that fermentation with LAB improved its proximate composition, while Imam *et al*. (2024) reported increased crude protein following LAB inoculation. Beyond JKC, similar benefits of LAB fermentation have been observed in soybean meal, maize, and other oilseed by-products, where microbial activity improved amino acid profiles, degraded anti-nutrients, and increased mineral bioavailability (Chawla *et al*., 2017; Ali *et al*., 2024).

Despite these promising findings, there remains a lack of comprehensive data on the effects of specific LAB strains on both nutrient composition and mineral enrichment in JKC. Such strain-specific insights are crucial, as LAB differ in their enzymatic capabilities and detoxification efficiency, which directly

influence the extent of improvement in feed value.

We hypothesised that LAB fermentation would not only enhance the nutrient profile of JKC but also significantly reduce ANFs and improve mineral bioavailability. This study therefore evaluated the detoxification potential of selected LAB strains: *Lactobacillus acidophilus*-DLB07, *Lactiplantibacillus plantarum*-DLB13 and *Lactobacillus rhamnosus*-DLB23 and their co-culture on JKC, with emphasis on the degradation of toxic factors and the enrichment of mineral availability.

Materials and Methods

Ripe *Jatropha curcas* seeds were obtained from the University of Ilorin plantation. The seeds were sun-dried for three days, cleaned, and dehulled to obtain the kernels. The kernels were milled using a hammer mill fitted with an adjustable screen (Christie-Norris Lab. Mill, Christie-Norris Ltd., Chelmsford, UK), defatted by hydraulic pressing, and further extracted with petroleum ether. The defatted kernel cake was stored in polyethylene bags and sterilised in an autoclave at 121 °C and 15 psi for 25 min to eliminate microbial contaminants, following the procedure of Belewu *et al*. (2010).

Preparation of Starter Cultures

Three strains of Lactobacillus (LAB): *Lactobacillus acidophilus*-DLB07, *Lactiplantibacillus plantarum*-DLB13 and *Lactobacillus rhamnosus*-DLB23 were obtained from the National Biotechnology Centre, Abuja, Nigeria. The strains were used individually and as a co-culture (1:1:1 ratio).

Inoculation and Fermentation of Kernel Cake

Sterilised JKC samples were inoculated with each LAB strain (*L. plantarum*, *L. acidophilus*, *L. rhamnosus*) or with their co-culture at a rate of 1.0×10^6 CFU/g. Control samples were not inoculated. The inoculated substrates were incubated at 37°C for seven days to allow microbial colonisation. Following fermentation, samples were oven-dried at 80°C to terminate microbial activity and stabilise the samples.

Chemical Analyses

Proximate composition

Proximate composition of untreated and fermented JKC was determined following AOAC (2012) procedures. Samples (200 g) were oven-dried at 65°C to constant weight, milled, passed through a 1 mm sieve, and analysed for crude protein using the Kjeldahl method.

Cell wall components

Neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) were determined as described by Van Soest *et al*. (1991). Hemicellulose and cellulose were calculated as:

Hemicellulose = NDF – ADF

Cellulose = ADF – ADL

Determination of Anti-Nutritional Factors

Phorbol esters

Phorbol esters were quantified following Phengnuam and Suntornsuk (2013). Methanol extracts of 5 g JKC were analysed using high-performance liquid chromatography (HPLC), with phorbol-12-myristate-13-acetate (PMA; Sigma-Aldrich) as the analytical standard.

Phytates

Phytates were determined according to Reddy *et al*. (1982). The JKC powder was extracted in 0.2 N HCl, centrifuged, and the filtrate was titrated with $FeCl_3$ solution. Quantification was performed using the spectrophotometric method of Haug and Lantzsch (1983) at 500 nm against phytate standards.

Trypsin inhibitor

Trypsin inhibitor activity was measured following AOCS (2017). Trypsin was preincubated at 37 °C with graded volumes of sample extract before adding Nα-benzoyl-DL-arginine-p-nitroanilide hydrochloride (DL-BAPA) as substrate. Absorbance was recorded at 410 nm (Muhammed and Abubakar, 2016).

Alkaloids

Five grams of powdered JKC was extracted with 10 mL methanol. The filtrate was treated with dilute ammonia and chloroform, followed by acetic acid. Mayer's and Wagner's reagents confirmed alkaloid presence by cream and reddish-brown precipitates, respectively. Quantification was done at 420 nm using a UV–visible spectrophotometer with bromocresol green.

Glycosides

Five grams of JKC was extracted with methanol, concentrated, and re-extracted. One millilitre of the extract was reacted with Baljet's reagent and incubated for 60 min. A deep red colour indicated glycosides, and absorbance was read at 495 nm (Muhammed and Abubakar, 2016).

Saponins

Ten grams of JKC was extracted with 20% ethanol, re-extracted, concentrated, and partitioned with diethyl ether and n-butanol. The butanol layer was washed with 5% NaCl, concentrated, and dried to constant weight for quantification (Obdoni & Ochuko, 2001).

Tannins

Five hundred milligrams of JKC was extracted with water, and 1 mL of the extract was reacted with ferric chloride and potassium ferrocyanide. Absorbance was measured at 120 nm within 10 minutes (Van Burden and Robinson, 1981).

Phenols

Methanolic JKC extract was diluted with distilled water and reacted with ferric chloride and potassium ferrocyanide. After 15 min, absorbance was recorded at 725 nm (Gupta *et al*., 2011).

Mineral Determination

Mineral concentrations (Ca, Na, K, Mg, Mn, Fe, Cu, Zn) were analysed by atomic absorption spectrophotometry (AAS; 230-ATS, BUCK Scientific, USA) following AOAC (2005). Calibration curves were prepared using analytical-grade standards.

Statistical Analysis

Data were analysed using one-way analysis of variance (ANOVA) under a completely randomised design (CRD) with SAS® software (2019). Treatment means were separated using Duncan's Multiple Range Test at $p < 0.05$.

The statistical model was:

$$Yij=\mu+Ti+\varepsilon ij$$

Results

LAB fermentation significantly influenced the proximate composition of Jatropha kernel cake (JKC). As shown in Table 1, crude protein (CP) increased across all treatments compared with untreated JKC, with the highest value recorded in the co-culture treatment (43.48% vs. 35.52% in untreated). Ether extract (EE), crude fibre (CF), and nitrogen-free extract (NFE) decreased significantly ($p < 0.05$) following fermentation, whereas dry matter (DM) and ash showed moderate increases.

Table 1: Nutritional Composition of untreated and Lactobacillus fermented Jatropha kernel cake on DM-basis

PARAMETERS (%)	UJK	LAJK	LPJK	LRJK	CCJK
Dry Matter	$89.76^{e} \pm 0.19$	$91.85^{d} \pm 0.20$	$92.72^{b} \pm 0.16$	$92.22^{c} \pm 0.12$	$93.43^{a} \pm 0.21$
Crude Protein	$35.52^{d} \pm 0.13$	$37.78^{c} \pm 0.05$	$41.15^{b} \pm 0.18$	$37.82^{c} \pm 0.25$	$43.48^{a} \pm 0.20$
Ether Extract	$22.59^{a} \pm 0.29$	$18.28^{b} \pm 0.03$	$14.60^{d} \pm 0.20$	$16.57^{c} \pm 0.21$	$12.66^{e} \pm 0.20$
Ash	$5.48^{b} \pm 0.06$	$5.61^{ab} \pm 0.06$	$5.70^{a} \pm 0.02$	$5.81^{a} \pm 0.04$	$5.62^{ab} \pm 0.16$
Nitrogen Free Extract	$19.31^{a} \pm 0.39$	$16.83^{b} \pm 0.22$	$16.26^{b} \pm 0.62$	$17.13^{b} \pm 0.64$	$18.84^{a} \pm 0.29$
Neutral Detergent Fibre	34.98 ± 0.17	33.00 ± 0.10	32.34 ± 0.28	33.21 ± 0.15	32.84 ± 0.04
Acid Detergent Fibre	$6.91^{a} \pm 0.19$	$4.97^{b} \pm 0.10$	$4.51^{b} \pm 0.17$	$4.98^{b} \pm 0.06$	$4.74^{b} \pm 0.08$
Hemicellulose	29.08 ± 0.02	28.03 ± 0.02	27.83 ± 0.20	28.23 ± 0.17	28.10 ± 0.11
Lignin	$1.39^{a} \pm 0.03$	$1.30^{b} \pm 0.03$	$1.26^{c} \pm 0.09$	$1.34^{ab} \pm 0.07$	$1.21^{c} \pm 0.12$
Cellulose	$4.53^{a} \pm 0.21$	$3.67^{b} \pm 0.21$	$3.25^{b} \pm 0.09$	$3.64^{b} \pm 0.13$	$3.53^{b} \pm 0.15$

^{abcde} means with different superscript across the rows differed significantly ($p < 0.05$); UJK- Untreated *Jatropha curcas* kernel cake; LAJK-*Lactobacillus acidophilus*-DLB07 fermented Jatropha kernel cake; LPJK-*Lactiplantibacillus plantarum*-DLB013 fermented Jatropha kernel cake; LRJK-*Lactobacillus rhamnosus*-DLB023 fermented Jatropha kernel cake; LCCJK-LABs Co-culture fermented Jatropha kernel cake

The effects of LAB fermentation on the anti-nutritional factors of Jatropha kernel cake (JKC) are presented in Table 2. All fermentation treatments significantly ($p < 0.05$) reduced phorbol esters, phytate, saponins, tannins, and trypsin inhibitors compared with the untreated sample. Among the treatments, the co-culture yielded the lowest concentrations of phorbol esters (0.03 mg/g vs. 0.08 mg/g in untreated JKC), phytate (1.32% vs. 6.80%), and trypsin inhibitor (0.35 mg/g vs. 30.32 mg/g). Similar trends were observed for tannins and saponins, where co-culture fermentation was most effective.

Table 2: Anti-nutrient composition of untreated and Lactobacillus fermented Jatropha kernel cake

Parameters	UJK	LAJK	LPJK	LRJK	LCCJK
Phorbol ester (mg/g)	$0.08^{a} \pm 0.00$	$0.06^{b} \pm 0.00$	$0.05^{c} \pm 0.00$	$0.06^{b} \pm 0.00$	$0.03^{d} \pm 0.00$
Phytate (%)	$6.80^{a} \pm 0.12$	$3.23^{b} \pm 0.13$	$2.62^{c} \pm 0.16$	$3.20^{b} \pm 0.13$	$1.32^{d} \pm 0.16$
Saponin (mg/g)	$10.81^{a} \pm 0.00$	$1.23^{c} \pm 0.01$	$1.13^{d} \pm 0.00$	$1.48^{b} \pm 0.100$	$1.15^{d} \pm 0.00$
Tannin (mg/g)	$9.80^{a} \pm 0.00$	$1.81^{b} \pm 0.01$	$1.83^{b} \pm 0.01$	$1.96^{a} \pm 0.02$	$1.79^{c} \pm 0.01$
Trypsin (mg/g)	$30.32^{a} \pm 0.06$	$1.46^{c} \pm 0.14$	$1.46^{c} \pm 0.00$	$1.66^{b} \pm 0.01$	$0.35^{d} \pm 0.01$

^{abcd} means with different superscripts across the rows differed significantly ($p < 0.05$); UJK- Untreated *Jatropha curcas* kernel cake; LAJK-*Lactobacillus acidophilus*-DLB07 fermented Jatropha kernel cake; LPJK-*Lactiplantibacillus plantarum*-DLB013 fermented Jatropha kernel cake; LRJK-*Lactobacillus rhamnosus*-DLB023 fermented Jatropha kernel cake; LCCJK-LABs Co-culture fermented Jatropha kernel cake

The mineral composition of untreated and LAB-fermented JKC is summarised in Table 3. Fermentation significantly ($p < 0.05$) enhanced both macro- (Ca, K, Mg, Na) and micro-minerals (Cu, Fe, Mn, Zn) concentrations. The co-culture treatment recorded the highest levels of calcium (9.43 mg/g), potassium (11.57 mg/g), and zinc (2.36 mg/g) compared with the untreated control. Iron concentration also increased substantially under *L. acidophilus* fermentation (9.65 mg/g) relative to untreated JKC (8.14 mg/g). Although all LAB treatments improved mineral contents, the co-culture generally produced superior enrichment.

Table 3: Mineral composition of the untreated and Lactobacillus fermented Jatropha kernel cake

Parameters	UJK	LAJK	LPJK	LRJK	LCCJK
Macro-minerals (mg/g DM)					
Ca	$8.16^{c} \pm 0.04$	$8.75^{b} \pm 0.03$	$8.86^{b} \pm 0.02$	$8.80^{b} \pm 0.01$	$9.43^{a} \pm 0.06$
K	$10.38^{c} \pm 0.12$	$11.16^{ab} \pm 0.25$	$10.75^{bc} \pm 0.09$	$10.76^{bc} \pm 0.01$	$11.57^{a} \pm 0.13$
Mg	$7.32^{c} \pm 0.05$	$7.40^{bc} \pm 0.01$	$7.41^{b} \pm 0.00$	$7.40^{bc} \pm 0.02$	$7.58^{a} \pm 0.01$
Na	$2.97^{c} \pm 0.01$	$3.84^{a} \pm 0.04$	$3.78^{a} \pm 0.04$	$3.54^{b} \pm 0.02$	$3.55^{b} \pm 0.03$
Micro-minerals					
Cu	$2.17^{d} \pm 0.00$	$2.58^{a} \pm 0.00$	$2.42^{b} \pm 0.00$	$2.54^{a} \pm 0.00$	$2.31^{c} \pm 0.00$
Fe	$8.14^{c} \pm 0.03$	$9.65^{a} \pm 0.18$	$8.35^{bc} \pm 0.18$	$8.13^{c} \pm 0.06$	$8.62^{b} \pm 0.03$
Mn	$2.06^{c} \pm 0.01$	$2.72^{a} \pm 0.00$	$2.21^{bc} \pm 0.01$	$2.29^{b} \pm 0.00$	$1.97^{d} \pm 0.00$
Zn	$2.13^{d} \pm 0.00$	$2.31^{a} \pm 0.00$	$2.21^{c} \pm 0.00$	$2.28^{b} \pm 0.01$	$2.36^{a} \pm 0.01$

^{abcd} means with different superscripts across the rows differed significantly ($p < 0.05$); UJK- Untreated *Jatropha curcas* kernel cake; LAJK-*Lactobacillus acidophilus*-DLB07 fermented Jatropha kernel cake; LPJK-*Lactiplantibacillus plantarum*-DLB013 fermented Jatropha kernel cake; LRJK-*Lactobacillus rhamnosus*-DLB023 fermented Jatropha kernel cake; LCCJK–LABs Co-culture fermented Jatropha kernel cake

Discussion

The increased dry matter (DM) and crude protein (CP) in Lactobacillus-detoxified Jatropha kernel cake (JKC) can be attributed to microbial metabolism during fermentation. The Lactobacillus strains preserve substrate integrity, form protein-rich biomass, and degrade protein-bound anti-nutritional compounds, thereby enhancing protein bioavailability. Enzymatic activity, particularly from amylases, proteases, and xylanases, further contributes by breaking down fibre and lipids (Liu *et al*., 2017; Feng *et al*., 2023).

These results agree with several studies recording improved CP and DM in fermented JKC (Belewu *et al*., 2010; Sanusi *et al*., 2013; Fajingbesi *et al*., 2019). In contrast, some reports have shown reductions in CP when microorganisms with limited proteolytic capacity utilised available

amino acids (Michael *et al*., 2019; de Olmos *et al*., 2022; Ganiyu & Belewu, 2023).

The increase in ash fraction suggests improved mineral concentration during organic matter metabolism, consistent with Bacillus- and Mucor-fermented meals (Hassaan *et al*., 2017; Zhang *et al*., 2020). Meanwhile, decreases in fibre fractions confirm enzymatic degradation of cell wall components, improving nutrient availability (Sanusi *et al*., 2013; Li *et al*., 2018). In this study, co-culturing multiple LAB strains yielded the greatest CP increase, supporting earlier reports that multi-strain systems outperform single strains due to synergistic metabolic activities (Belewu *et al*., 2013; Wu *et al*., 2018).

Fermentation with the selected Lactobacillus species markedly reduced anti-nutritional factors (phorbol esters, phytates, tannins, trypsin inhibitors, glycosides, phenols), in agreement with earlier findings (Musa *et al*., 2018; Alrosan *et al*., 2022). Nonetheless, fermentation efficiency is influenced by factors such as duration, inoculum, and environmental conditions (Okomoda *et al*., 2020).

The Lactobacillus-detoxified JKC showed reduced phorbol ester (PE) levels, likely due to enzyme activity from LAB during fermentation. Similar reductions in PE have been reported with *Aspergillus niger*, *Basidiomycetes*, and *Bacillus* strains (Chin-Feng *et al*., 2014; Handajani *et al*., 2021; Guimarães *et al*., 2022). Given that raw Jatropha contains 1- 3 mg/g PE, which is toxic to animals, the reductions observed bring levels closer to the safe limit of 0.09 mg/g (Gomes *et al*., 2018).

The phytate content was also significantly reduced, reflecting the phytase activity of LAB and the favourable acidic conditions during fermentation (Belewu and Sam, 2010; Zhao *et al*., 2017). Also, LAB effectively reduced saponins, consistent with earlier findings that β-glucosidase activity degrades these compounds (Lai *et al*., 2013). Reductions were also reported wit*h A. niger*, *Bacillus*, *Enterobacter*, and *P. aeruginosa* (Belewu *et al*., 2010; Chang *et al*., 2014; Ghosh *et al*., 2021).

Tannin concentrations decreased due to microbial tannase activity, aligning with earlier reports in corn flour and JKC fermented with bacteria and fungi (Ogodo *et al*., 2018; Zhao *et al*., 2018). Trypsin inhibitors were lowered, supporting prior reports of up to 99% elimination by *Bacillus spp*. and *A. nige*r (Phengnuam and Suntornsuk, 2013; Phulia *et al*., 2018). Glycosides and phenolics also declined, likely through microbial enzymatic hydrolysis (Michael *et al*., 2019; Öztürk, 2024).

The increases in calcium, potassium, magnesium, and trace elements (Fe, Zn, Cu, Mn) highlight the role of LAB fermentation in mineral release. Fermentation enhanced macro- and micro-mineral availability (Ca, K, Mg, Na, Cu, Fe, Mn, Zn), consistent with reports in legumes and oilseed cakes (Chen *et al*., 2021; Ali *et al*., 2024). This enrichment is likely due to hydrolysis of phytate mineral complexes, which liberates bound minerals, and organic acid production (e.g., lactic acid), which enhances mineral solubility and bioavailability (Rollán *et al*., 2019; Raj *et al*., 2022). However, some studies note mineral losses during prolonged fermentation due to leaching (Michael *et al*., 2019). The co-culture fermentation showed the most pronounced effects, suggesting synergistic enzymatic activities among the three LAB strains, resulting in more effective detoxification and mineral release compared with single-strain inoculations.

Conclusion

This study demonstrated that lactic acid bacteria (LAB) fermentation is an effective, eco-friendly strategy for reducing anti-nutritional factors and enhancing mineral bioavailability in *Jatropha curcas* kernel cake (JKC). The co-culture fermentation with *Lactobacillus acidophilus*-DLB07, *Lactiplantibacillus plantarum*-DLB013, and *Lactobacillus rhamnosus*-DLB023 produced the greatest detoxification and most pronounced nutritional improvements, demonstrating its potential for safe dietary inclusion for livestock.

By extending earlier findings limited to proximate composition, this work provides new mechanistic insights into the detoxification and nutritional upgrading of JKC. The results suggest that LAB-fermented JKC, especially when using co-cultures, has strong potential as a safe, mineral-rich ingredient for livestock feed. Future research should include animal feeding trials to validate the nutritional and health benefits observed *in vitro*.

Conflict of Interest

The authors declare no conflict of interest.

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