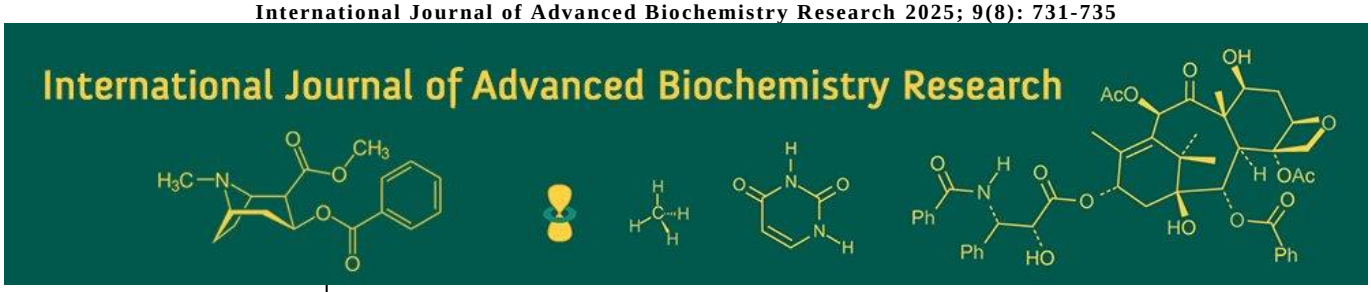

ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; 9(8): 731-735
www.biochemjournal.com
Received: 01-05-2025
Accepted: 05-06-2025

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Influence of De-oiled palm kernel cake inclusion in concentrate mixture on serum biochemistry and hormonal status in lactating Murrah buffaloes

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DOI: https://www.doi.org/10.33545/26174693.2025.v9.i8j.5302

Abstract
This study examined the effect of incorporating deoiled palm kernel cake (DPKC) into the concentrate ration on the blood biochemical profile and serum cortisol levels of early-lactation Murrah buffaloes. Twelve multiparous buffaloes (average body weight: 586.58±12.36 kg) in the first two months of their second lactation were equally divided into two groups: a control diet without DPKC (T_1) and a treatment diet where DPKC replaced 15% of the concentrate mixture (T_2). Both rations, provided with ad libitum green fodder, were formulated to meet ICAR (2013) nutrient requirements.
No significant differences ($p>0.05$) were observed between groups in 6% fat-corrected milk (FCM) yield. Serum total protein, albumin, globulin, albumin-to-globulin ratio, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, glucose, total cholesterol, HDL and LDL cholesterol, triglycerides, urea, creatinine, and cortisol remained within normal physiological ranges, with no treatment effects ($p>0.05$).
These results demonstrate that inclusion of DPKC at a 15% level does not adversely affect metabolic health, hepatic and renal function, lipid status, or stress hormone levels in lactating Murrah buffaloes. Given its neutral impact on biochemical and hormonal parameters as well as milk productivity, DPKC can be effectively used as a cost-reducing alternative feed ingredient in concentrate mixtures for early-lactation buffaloes without compromising health or performance.

Keywords: De-oiled palm kernel cake, serum biochemical profile, serum cortisol

Introduction
Persistent shortage as well as the high cost of conventional feedstuffs for livestock feeding in developing countries like India is a result of inadequate production of farm crops to meet the needs of humans and of their domestic animals. This has forced the animal nutritionists to intensify research into the feeding values of potentially useful, attractive, cheaper and readily available protein and energy sources from unconventional crop products. Palm kernel cake (PKC) is widely recognized as a viable alternative feed ingredient for ruminants due to its high crude protein content (14.5-19.24%) and good palatability, functioning as both a protein and energy source.
Previous research has shown oil palm by-products can successfully replace maize or soybean in ruminant diets without compromising carcass or milk quality (Wan Zahari, 2003) [23]. Feeding PKC can also reduce ration costs while maintaining nutrient utilization in animals such as sheep, dairy cattle, and beef cattle (Ribeiro *et al.*, 2013) [21].
Palm kernel cake (PKC) is the solid residue that remains after the oil is extracted from the palm fruit kernels. It is a by-product of palm oil milling. As a medium-grade protein feed on its own, PKC is frequently seen as appropriate for ruminant feeding due to its high fibre content.
De-oiled PKC, with reduced fat content, offers additional benefits by providing a balanced source of fiber, protein, vitamins, and minerals, making it suitable for fattening and dairy cattle without compromising animal performance. (Alimon and Wan Zahari, 2003) [23]
Blood metabolites are now commonly utilised to evaluate an animal's nutritional condition (Ndlovu *et al.*, 2007) [14]. According to Chester-Jones *et al.* (1990) [6], blood metabolite concentrations serve as a comprehensive indicator of the sufficiency of nutritional supply in

connection to buffaloes' nutrient utilisation. According to Pambu-Gollah *et al.* (2000) [16], they provide a quick evaluation of an animal's nutritional state at that specific time. The analysis of blood metabolites reflects the adaption of animals to locally available feed resources and aids in tracking their health state, according to several authors (Caldeira *et al.*, 1995; Eicher *et al.*, 1999; Mapiye *et al.*, 2010) [3, 8, 12]. The amount of energy, protein, and other nutrition metabolism in animals is also indicated by blood metabolite levels (Maurya and Singh, 2015) [13]. The nutritional suitability of feed given to high-producing ruminant animals during lactation has reportedly been assessed using metabolic profile tests with success (Payne, 1978) [17]. The objective of this study was to ascertain if the addition of an unconventional protein supplement, such as de-oiled palm kernel cake, to the diet of lactating Murrah buffaloes would affect the concentrations of blood metabolites.

2. Materials and Methods

2.1 Experimental animals and housing

Twelve healthy Murrah buffaloes in early lactation (1-2 months post-calving, 2nd lactation) and of similar milk yield, lactation number, and body weight were randomly allotted to two dietary treatments (n = 6 per group). Animals were kept in a well-ventilated shed with individual feeding and watering arrangements, and proper hygiene management was maintained.

2.2 Diets

Both groups received chopped Super Napier green fodder ad libitum and a twice-daily concentrate mix to meet ICAR (2013) [10] nutrient specifications. The control concentrate (CM-1) contained no DPKC, whereas the treatment mix (CM-2) included DPKC at 15% of the formulation, replacing part of maize, DORB, and cottonseed meal. Concentrates were iso-nitrogenous.

2.3 Sampling and analysis

Blood samples were collected from the jugular vein middle of the trial. Serum was separated and stored at-20 °C until analysis. Biochemical indicators assessed were:

- Total protein, albumin, globulin, albumin: globulin ratio
- Liver enzymes (ALT, AST)
- Total bilirubin
- Glucose, total cholesterol, HDL, LDL, triglycerides
- Urea, creatinine
- Calcium, phosphorus, magnesium

Commercial diagnostic kits were used to estimate different biochemical parameters (M/s. Excel Diagnostics Pvt. Ltd., Hyderabad, India) using Multiskan GO spectrophotometer (Thermo Scientific). The serum cortisol (µg/dL) was estimated using commercial diagnostic kit (Bovine cortisol ELISA kit) based on Competitive-ELISA detection method procured from M/s. Wuhan Fine Biotech Co, Limited with product no EB0062.

2.4 Statistics

Data were analysed by independent t-test (Snedecor & Cochran, 1994) [22] using SPSS v24, and results were reported as means ± SE. The significance level was set at $p<0.05$.

3. Results and Discussion

3.1 Proximate and ingredient Composition of Feed Ingredients and Concentrate Mixtures

The chemical composition and cell wall components of the concentrate mixtures with and without de-oiled palm kernel cake (DPKC), green fodder (Super Napier) and DPKC were assessed. Table 1 provides the average proximate percentages of the green fodder (Super Napier) and DPKC. While Table 2 provides the ingredient composition of the concentrate mixtures, provided to lactating Murrah buffaloes. Both the concentrate mixtures were made iso-nitrogenous.

3.2 Dry Matter Intake (DMI)

The mean daily intake of dry matter, including both forage and concentrate, was similar between the groups (14.2kg for T1 versus 13.8 kg for T2, $p>0.05$). This concurs with earlier findings by Pimentel *et al.* (2015) [18], who found no decrease in dry matter intake when palm kernel cake comprised up to 15% of the total diet.

3.3 Milk Yield and 6% Fat-Corrected Milk (FCM) Yield

The average daily milk yield (kg) and 6% fat-corrected milk (FCM) yield (kg/day) in lactating Murrah buffaloes were recorded as 7.86±0.48 and 7.96±0.22 for the T1 group, and 9.52±0.63 and 10.38±0.30 for the T2 group, respectively. Incorporating 15% deoiled palm kernel cake (DPKC) in the concentrate mixture resulted in a numerical increase in 6% FCM yield compared to the control group; however, the difference was not statistically significant ($p>0.05$).

Similarly, Carvalho *et al*. (2006) [4] observed that increasing the proportion of solvent-extracted palm kernel meal (SPKM) in the concentrate mixture from 5% to 15% had no significant impact ($p>0.05$) on milk yield or 4% FCM yield in lactating Holstein cows when compared with the control diet.

Furthermore, research on lactating goats indicates that moderate inclusion of palm kernel cake (PKC) — up to 80 g/kg DM — can sustain milk production while lowering feed nutrient costs (Ribeiro *et al*., 2013) [21].

3.4 Serum Biochemical Profile

The influence of incorporating deoiled palm kernel cake (DPKC) in the concentrate mixture on the serum biochemical parameters of lactating Murrah buffaloes is summarised in Table 3.

Total protein concentrations are generally lower in young animals and increase with maturity, whereas albumin levels are low at birth and rise over time (Otto *et al.*, 2000) [15]. Both total protein and albumin are indicators of protein availability, with reduced concentrations signalling deficiency. Alanine aminotransferase (ALT) serves as a marker of skeletal muscle mass and tissue injury following intense activity, while aspartate aminotransferase (AST) reflects skeletal muscle growth and potential hepatic damage (Otto *et al.*, 2000) [15].

In the present study, inclusion of DPKC at 15% in the concentrate mixture did not produce significant changes ($p>0.05$) in serum total protein, albumin, globulin, albumin-to-globulin ratio, ALT, AST, or total bilirubin levels when compared to the control group. Serum total protein values remained within the normal reference range (6.7-7.4 g/dl; Kaneko *et al.*, 2008) [11], indicating adequate dietary protein supply and absence of tissue damage. These findings are

consistent with Devika (2019) [7], who reported no significant effect of palm oil decanter cake (PODC) at 10-20% inclusion on similar biochemical indices in Nellore brown ram lambs.

Blood glucose, a moderately reliable indicator of nutritional status in buffaloes, can decline with insufficient nutrient intake due to reduced availability of dietary propionate and other gluconeogenic precursors (Reynolds *et al.*, 2003) [19]. In this study, serum glucose, total cholesterol, HDL-and LDL-cholesterol, and triglyceride concentrations were unaffected (p>0.05) by 15% DPKC inclusion, suggesting no adverse impact on nutritional status. Comparable results have been documented with PODC in ram lambs (Devika, 2019) [7] and with PKC in goats (Chanjula *et al.*, 2011) [5], Thai native cattle (Seephueak *et al.*, 2011) [20], and crossbred lactating cows (Silva *et al.*, 2013) [21].

Similarly, no significant differences (p>0.05) were observed in serum urea and creatinine levels between the treatment and control groups, indicating no detrimental effects on renal function. These results align with previous reports on PODC in ram lambs (Devika, 2019) [7] and PKC in goats and cattle (Chanjula *et al.*, 2011; Seephueak *et al.*, 2011) [7, 20].

Mineral profiles, including calcium, phosphorus, and magnesium concentrations, also remained unaffected (p>0.05) by dietary DPKC inclusion, corroborating the findings of Devika (2019) [7] in ram lambs fed PODC.

Overall, incorporating DPKC at a 15% level in the concentrate mixture did not impair liver, kidney, or muscle function, nor did it affect the nutritional or mineral status of lactating Murrah buffaloes.

3.4 Serum cortisol content

The mean serum cortisol concentrations in lactating Murrah buffaloes were 2.26±0.07 ng/ml in the T1 group and 2.40±0.15 ng/ml in the T2 group. Inclusion of deoiled palm kernel cake (DPKC) at a 15% level in the concentrate mixture did not significantly influence (p>0.05) serum cortisol levels compared to the control.

Cortisol, a catabolic hormone, facilitates the conversion of amino acids to glucose, suppresses immune responses, and reduces insulin sensitivity in extra-hepatic tissues during stress periods (Brockman & Laarveld, 1986) [2]. In the present study, blood sampling was conducted in the afternoon during mid-summer, yet cortisol concentrations in both groups remained within the reported physiological range for Murrah buffaloes (2.60-4.80 ng/ml; Aggarwal *et al.*, 2010) [1].

These results suggest that the good management practices implemented at the farm, including maintaining favourable shed temperatures, likely helped minimise environmental and nutritional stress—despite the inclusion of DPKC, considered an unconventional feed ingredient for livestock.

Table 1: Chemical composition (% DMB) of green Fodder (Super Napier) and de-oiled palm kernel cake (DPKC)

Nutrient	Green Fodder (Super Napier)	De-oiled palm kernel cake (DPKC)
Dry Matter	23.16	89.11
Organic Matter	89.23	93.95
Crude Protein	11.91	20.06
Ether Extract	1.72	0.91
Crude Fibre	47.99	19.97
Nitrogen Free Extract	27.61	53.01
Total Ash	10.77	6.05
Neutral Detergent Fibre	79.82	70.12
Acid Detergent Fibre	49.69	40.56
Hemi-Cellulose	30.13	29.56
Cellulose	23.23	17.48
Acid Detergent Lignin	25.67	17.89
Silica	2.02	0.9
Calcium (%)	0.68	0.33
Phosphorous (%)	0.45	0.69

Table 2: Ingredient composition of concentrate mixtures fed to lactating Murrah buffaloes during the digestion trial

Ingredient	CM-1	CM-2
Maize	33	28
DORB	39	33
Cotton seed meal	25	21
De-oiled PKC	0	15
Mineral mixture	2	2
Salt	1	1
Total	100	100

Table 3: Serum Biochemical Profile of lactating Murrah buffaloes fed diets without and with DPKC in the concentrate mixture

Parameter[NS]	Control	Treatment
Total protein in g/dl	6.93±0.09	7.22±0.21
Albumin in g/dl	4.03±0.22	3.80±0.12
Globulin in g/dl	2.90±0.24	3.42±0.27
Albumin to Globulin ratio	1.45±0.18	1.15±0.12
ALT IU/l	26.81±2.30	23.58±1.92
AST (IU/L)	79.80±1.97	88.35±2.03
T. bilirubin (mg/dl)	1.47±0.18	1.18±0.09
Glucose (mg/dl)	37.54±2.87	43.64±1.37
Total Cholesterol (mg/dl)	118.33±6.91	120.83±3.96
HDL (mg/dl)	10.45±0.32	9.13±0.19
LDL (mg/dl)	93.56±4.67	98.21±3.76
Triglycerides (mg/dl)	14.33±4.53	13.50±2.54
Urea (mg/dl)	16.35±2.93	18.91±3.63
Creatinine (g/dl)	1.25±0.22	1.59±0.08
Calcium (mg/dl)	7.35±0.21	7.17±0.33
Phosphorus (mg/dl)	5.98±0.20	5.82±0.58
Magnesium (mg/dl)	2.30±0.15	2.32±0.24

NS-Non significant (P>0.05)

4. Conclusion

This study reported that inclusion of DPKC at 15% level in the concentrate mixture had no effect on serum biochemical and hormonal profile of Murrah buffaloes as observed with DMI and 6% FCM yield (kg/d). Hence, it is concluded that DPKC can be used in the concentrate mixture of lactating Murrah buffaloes without any adverse effects to reduce the cost of production.

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