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Evaluation of Detoxified Karanja (*pongamia sp.*) and Neem (*azadirachta indica*) Cakes in Total Mixed Ration (tmr) for Dairy Cattle–Effect on Nutritional, Biochemical Profiles

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Abstract

Three total mixed rations (TMR) were prepared namely T_1-control where soybean meal was incorporated at 9.6 % of TMR, T_2-detoxified neem cake (dNC) was incorporated at 3.85 % of TMR and T_3-detoxified karanja cake (dKC) was incorporated at 5.85 % of TMR and fed to three groups of six crossbred cows in each group for 90 days. Milk yield (kg/day) and FCM yield (kg/day) increased in all the groups. The average milk fat was found to be lower in ($P<0.05$) in T_3-dKC group (5.03±0.21) compared to T_1-control (6.13±0.25). Nutrient intakes and digestibilities were found to be same. Blood profiles like albumin, glucose, LDH and ALP concentrations were higher ($P<0.05$) and globulin and urea concentration were lower ($P<0.05$) at the end of experiment in all the groups. Serum cortisol (nM/L) and IGF-1 (ng/ml) concentration were also found to be same in all groups. However, final IGF-1hormone concentrations were found to be higher ($P<0.05$) compared to initial values in all the groups. In can be concluded that feeding of TMR containing dNC and dKC have improved milk yield in 90 days lactation period without adversely affecting milk composition, milk production efficiency. Similar effects were observed even nutrient digestibility and nutritive value of diets indicating these unconventional protein supplements can be included in TMR of dairy cattle. Positive effects on serum albumin, glucose, LDH and ALP concentrations and IGF I concentrations were observed at the end of feeding period of 90 days in both treatment groups compared to control.

Keywords: Biofuel by-products; Karanja cake; Neem cake; Milk production; Digestibility; Dairy cattle

Abbreviations: GNC: Ground Nut Cake; SBC: Soybean Cake; TMR: Total Mixed Rations; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; LDH: Lactic Dehydrogenase; ALP: Alkaline Phosphatase; dNC: Detoxified Neem Cake; dKC: Detoxified Karanja Cake

Introduction

Protein is an important macro nutrient and an expensive constituent of animal diet. Price escalation of protein sources such as ground nut cake (GNC), soybean cake (SBC) will have a bearing on profitability of dairy farmers. In the present study, two alternatives for un-conventional protein sources such as Karanja (*Pongamia sp.*) cake and Neem (*Azadirachta indica*) seed cake have been investigated as potential replacement of conventional cakes in the total mixed rations (TMR) of dairy cattle. However, the use of these cakes are limited due to anti-nutritional factors such as karanjin, pongamol in case of karanja [1] and azadirachtin, salannin in case of neem making unpalatable [2]. Various methods, namely refluxing with 2% HCl [3] water leaching, solvent extraction, acid and alkali treatment, autoclaving were adopted to improve palatability and nutritive value of detoxify karanja cake. Among all the methods, solvent extraction was found to be more efficient to remove karanjin [4] and is being commonly used as a method to detoxify karanja cake. In case of neem, water washing of cake resulted in wholesome protein substitute [5-9] due to problem such as loss of 22% DM due to washing. To avoid such loss, processing the cake in alkaline medium without water washing either by soaking it in water (1:5 w/v) or containing either NaOH (2% w/w) for 24 hrs or by ensiling with 2.5% urea (w/w) for 5-6 days [10,11]. The detoxification methodology was upscaled for neem and karanja cake for its bulk production. In the present study, effect of feeding these cakes on its exerted effects on milk production, nutritional and blood profiles was evaluated in crossbred cattle in total mixed rations (TMR).

Material and Methods

Lactation trial was conducted in a field dairy farm close to Department of Animal Nutrition, College of Veterinary Science, Tirupati, India. The experimental protocols were approved in Animal Ethical Committee. Locally available maize fodder and concentrate feed ingredients (Maize, Soybean meal, Deoiled Rice bran) were procured from the local market of Tirupati, India. The maize fodder was dried, chaffed and ground in a chaffer cum grinder and mixed into complete rations in a horizontal mixer and concentrate mixtures were prepared in feed mixing plant at Department of Animal Nutrition, College of Veterinary Science, Tirupati. Detoxification process on Neem seed cake and Karanja cake modifying the method descripted by Saxena et al. [12] for neem cake; and the method proposed by Ravi kanth et al. [13] for Karanja so as to get resultant product with less oil and minimum anti-nutritional factors.

Control TMR (T_1-Control) was formulated using dried maize fodder (roughage source) and concentrate feed ingredients (maize grain, deoiled soybean meal, urea, molasses, mineral mixture and salt). All the ingredients were ground in a Chaffer cum Grinder. dNC and dKC were incorporated in the diets replacing 50% of protein content of soybean meal. In control diets, soybean meal was used at 9.6 % of diet. To achieve replacement level of 50%, dNC was used at 3.78 % of TMR (T_2-dNC) whereas dKC was used at 5.85 % of TMR (T_3-dKC) (Table 1).

Items	T_1 (Control)	T_2 (dNC)	T_3 (dKC)
Ingredients (% as fed basis)			
Dry maize fodder	60	60	60
Maize grain	10.8	16.8	5.55
Deoiled soybean meal	9.6	4.8	4.8
Detoxified Neem seed cake	-	3.78	-
Detoxified Karanj seed cake	-	-	5.85
Deoiled rice bran	8.4	3.42	12.6
Urea	0.3	0.3	0.3
Mineral mixture[a]	0.6	0.6	0.6
Salt	0.3	0.3	0.3
Molasses	10	10	10
Total	100	100	100
Crude Protein	12.02	13.02	11.53
Chemical Composition (%)			
Dry Matter	91.1	90	92.2
Organic Matter	89.64	88.25	87.93
Crude Protein	12.32	12.16	12.06
Ether Extract	1.4	1.37	1.44
Crude Fiber	24.24	26.61	25.27
Total Ash	10.36	11.75	12.07
Neutral Detergent Fiber	54.55	66.58	55.67
Acid Detergent Fiber	28.5	34.36	33.26
Hemicellulose	26.05	32.22	22.41

Table 1: Ingredients and chemical composition of total mixed rations used in the experiments.

(T_1-Control), without any unconventional protein supplements; (T_2-dNC) detoxified neem cake was used at the expense of 50 % of soybean meal protein; (T_3-dKC), detoxified karanja cake was used at the expense of 50 % of soybean meal protein.

[a]Commercial mineral mixture with the composition per kg: Cobalt-150mg, copper-1200mg, iodine-325mg, iron-5000mg, magnesium-6000mg, potassium-1500mg, selenium-100mg, sodium-10mg, sulphur-5.9mg, zinc-0.942%, DL Methinonine-9600mg, L-lysine, mono hydrochloride-4400mg, calcium-24%, phosphorous-12% was used.

Eighteen crossbred cows (Jersey or Holstein-Fresian cross with local cattle) with a mean BW of 334.56±12.76 kg and average milk yield of 5.67±0.29 kg/d were used for conducting the lactation trial. As it was mentioned that the experiment was conducted in field farm, it was understood from the farmer that the cows are in first to second lactation with mid lactation stage. They were divided into three experimental groups in completely randomized design. All the animals were housed in well ventilated katcha shed under uniform management conditions. Three total mixed rations and clean drinking water were offered *ad libitum* level for four times a day. The diets were moistened before feeding to prevent dustiness and faster consumption. To find out the effect of feeding the TMR containing unconventional detoxified cakes a digestion trial of six days duration was conducted after 60 days of feeding. During this period, representative samples of feed offered, residues left and faeces voided were collected, sub-sampled and preserved at -20^{o}C.

Animal body weights were calculated from their heart girth and body length measurements by Shaffer's formula. Body weight (lb) = (G2 x L)/300 where, G is heart girth and L is the body length from shoulder point to pin bone in inches. The factor 0.4536 used to convert these body weights into kilogram. Milk samples were analyzed for milk fat by Gerber's Method (ISI 1977 IS: 1224 Part I), SNF by Gravimetric method (ISI 1982 IS: 10083), Total Solids by the addition of fat and SNF content, 4% FCM Milk Protein by Kjeldahl method [14].

Proximate components and cell wall constituents were determined as per AOAC [14] and Van Soest et al. [15] respectively. Initial and final blood samples were collected by jugular vein puncture in clean test tubes and centrifuged at 3000 rpm for 10 min to separate the serum and stored at -20°C till analyzed. Biochemical parameters such as total protein, albumin, globulin (g/dl), glucose, urea (mg/dl), enzymes such as AST (aspartate aminotransferase), ALT (alanine aminotransferase), LDH (lactic dehydrogenase) and ALP (alkaline phosphatase) were estimated using biochemical kits purchased from Span Diagnostics Limited, Surat, Gujarat, India. The globulin concentration was measured as difference between total protein and albumin. Hormone concentrations were estimated using the kits (Immunotech SAS, Marsille, France). Serum cortisol (nM/L) and IGF-1 (ng/ml) are estimated following as per the procedure specified by the immunotech, France.

Statistical analysis

Data from individual animals were averaged for each parameter and the values were subjected to statistical analysis [16] using SPSS. Statistical significance among groups was determined using two ways ANOVA and Tukey's post-hoc test was employed to assess statistical significance. For blood parameters, treatment, period and interactions were found out using SPSS. The values were expressed as mean ± SE. Differences were considered significant if $P<0.05$.

Results and Discussion

Milk yield, composition and efficiency of milk production

Milk yield (kg/day) and FCM yield (kg/day) was found to be more in dNC compared to dKC groups (Table 2). Over a period of 90 days feeding, both milk yield (kg/day) and FCM yield (kg/day) increased in all the groups. The average milk fat was found to be lower in ($P<0.05$) in T_3-dKC group (5.03±0.21) compared to T_1-control (6.13±0.25). With the progression of experiment, milk fat remained equivalent among all treatment groups, however, total fat yield (g/d) was found to be higher ($p<0.05$) in T_2-dNC. Similarly, compared to initial values, total fat yield increased ($P<0.05$) in all the treatment groups. The average milk SNF was found to be equivalent among all three groups and as experiment progressed, milk SNF also remained same in all the groups. Total solids was found to be lower in ($P<0.05$) in T_2-dNC and T_3-dKC groups compared to T_1-control. Total solids (%) among different groups remained similar during different fortnights. Total solid yield (g/d) found to be higher in T_2-dNC compared to T_3-dKC and T_1-control. Total solid yield (g/d) was increased from initial to final stage of experiment across all the treatments. Density and milk protein was found to be equal among dNC and dKC groups compared to T_1-control. Milk density, milk protein also remained same across all the fortnights. DMI (kg) required for kg FCM was found to be same (P>0.05) in all the groups (1.38±0.08 in T_1-control; 1.30±0.07 in T_2-dNC and 1.66±0.20 in T_3-dKC) (Figure 1). Significant improvements in milk yield, FCM yield was recorded in neem seed cake containing group compared to control and karanja cake fed groups. This can be attributed better protein availability from neem seed cake. Rangaiah and fed water washed neem seed cake to lactating Murrah buffaloes and observed similar milk yield and quality compared to control. In the present study, even though all the three rations having similar protein, the inclusion of neem seed cake showed better conversion efficiency into milk yield without showing any adverse effects.

Attributes	T_1 (Control)	T_2 (dNC)	T_3 (dKC)	SEM	Trt	Period	Trt* Period
Milk yield (kg/d)	4.95^b	6.23^a	5.11^b	0.15	0.001	0.014	0.972
4 % FCM yield (kg/d)	6.46^{ab}	7.47^a	5.91^b	0.2	0.004	0.003	0.921
Fat (%)	6.13	5.33	5.03	0.14	0.006	0.28	0.985
Fat yield (g/d)	291.99	332.6	257.52	10.26	0.008	0.003	0.935
SNF (%)	9.37	9	9.24	0.09	0.208	0.918	0.35
SNF yield (g/d)	465.1	559.83	471.5	14.54	0.011	0.016	0.994
Total solid (%)	15.5	14.33	14.26	0.17	0.003	0.244	0.61
Total solid yield (g/d)	763.09	892.43	729.84	22.88	0.006	0.003	0.969
Milk density	30.84	31.18	31.25	0.16	0.578	0.878	0.764
Milk Protein	3.66	3.68	3.6	0.01	0.096	0.525	0.992

Table 2: Milk yield and composition in treatment groups.
Values bearing different superscripts in a row (a,b) differ significantly (P<0.05)
(T_1-Control), without any unconventional protein supplements; (T_2-dNC) detoxified neem cake was used at the expense of 50 % of soybean meal protein; (T_3-dKC), detoxified karanja cake was used at the expense of 50 % of soybean meal protein.

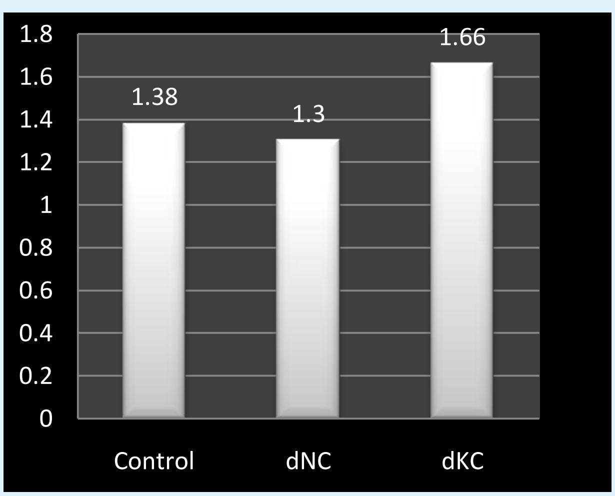

Figure 1: Efficiency of milk production (Kg DM per Kg FCM) different graphs: (T_1-Control) without any unconventional protein supplements. (T_2-dNC) detoxified neem cake was used at the expense of 50 % of soybean meal protein. (T_3-dKC) detoxified karanja cake was used at the expense of 50 % of soybean meal protein.

The milk production of dairy cows can be increased by manipulating post ruminal digestibility of rumen degradable protein and amino acid balance rather than rumen degradable protein alone. Palmquist and Beaulieu have discussed various feed factors like grain intake, undegradable protein intake, fat supplements and energy intake influence milk fat composition. Thus, the differences in milk yield and composition observed in the present study were attributed to quality and quantity of degradability of protein supplements and minor changes in composition of TMR. The results pertaining to the milk yield, milk fat, SNF, milk protein, total solids, density and milk production efficiency revealed that the 50% replacement of soybean meal with neem seed cake and karanja cake did not show any adverse effect. The efficiency of milk production also DM intake (kg/d; % BW; g/Kg $W^{0.75}$), Digestible OM intake (% BW; g/Kg $W^{0.75}$), CPI (kg/d; g/Kg $W^{0.75}$), DCPI (kg/d, g/Kg $W^{0.75}$), TDNI (kg/d, g/Kg $W^{0.75}$) were similar in all the treatment groups with some numerical improvement in dNC supplemented groups (T_2-dNC). DMI (%BW) were 2.53 (T_1-control), 2.77 (T_2-dNC) and 2.61 (T_3-dKC). The percent CP was made around 12% to meet the nutrient requirements as per ICAR taking into consideration the dry matter intake as well as milk yield and stage of lactation. At this level, the DCP and TDN intakes (kg/day) recorded were well above the requirements given by ICAR (1998). Nutrient digestibility (%) of DM, OM, CP, EE, NDF and ADF were found to be same in all treatment groups indicating comparative utilization. The DCP % and TDN % of the diets was also found be the same in all groups (Table 3). The reason can be attributed to low level of incorporation of neem (3.78 %) and karanjaa cake (5.85 %). Similarly Pandya et al. [17] reported that similar dry matter digestibility in crossbred calves fed on complete diets made with sugar cane bagasse. In recent study, Raj Kishore et al. [18] found that nutrient digestibility was improved in buffalo bulls fed on complete rations compared to conventional system of feeding. Similarly, feeding of unconventional feed roughage sources like sweet sorghum bagasse in the form of processed complete rations improved dry matter intake and digestibility in ram lambs 19] and nutrient utilization, microbial N supply in ram lambs [20].

Ingredient	T_1 (Control)	T_2 (dNC)	T_3 (dKC)	SEM	P Value
Body Weights During Trial Period					
(kg)	355.35	354.58	351.79	12.57	0.99
(kg $W^{0.75}$)	81.55	81.52	81.22	2.18	1
DM intake (kg/day)	8.72	9.63	9.16	0.35	0.59
DMI (%BW)	2.53	2.77	2.61	0.13	0.75
DMI (g/kg $W^{0.75}$)	108.57	119.49	112.84	4.92	0.69
DOMI (%BW)	1.61	1.73	1.61	0.09	0.81
DOMI (g/kg $W^{0.75}$)	69.06	74.57	69.61	3.41	0.79
CPI (kg/d)	1.07	1.17	1.1	0.04	0.66
(g/kg $W^{0.75}$)	13.38	14.53	13.61	0.6	0.73
DCPI (kg/d)	0.68	0.72	0.66	0.03	0.85
(g/kg $W^{0.75}$)	8.46	8.96	8.16	0.49	0.81
TDNI (kg/d)	5.2	5.57	5.24	0.26	0.84
(g/kg $W^{0.75}$)	64.43	69.29	64.52	3.4	0.82
Digestibility (%)					
DM	69.93	67.75	68.79	1.35	0.82
OM	71.76	69.49	70.26	1.26	0.78
CP	64.21	59.88	60.12	1.99	0.64
EE	73.78	75.07	75.24	1.59	0.93
NDF	66.02	70.36	66.19	1.39	0.38
ADF	56.08	63.2	61.15	1.67	0.21
Nutritive Value of Diet (%)					
DCP	7.91	7.28	7.25	0.25	0.49
TDN	60.13	56.64	57.3	1.42	0.6

Table 3: Feed intake and nutrient digestibility in different treatments.

Total protein, albumin and globulin concentrations (g/dl) were found to be similar in all treatment groups.

Higher albumin and lower globulin concentrations were (P<0.05) and globulin concentration was less (P<0.05) at the end of experiment. Blood glucose, urea (mg/dl) concentrations were similiar in all the treatment groups. Higher concentration of blood glucose and lower concentration of urea were recorded at the end of experiment. Blood enzymes such as aspartate amino transferase (AST) alanine amino transferase (ALT), alkaline phosphatase, (ALP) and lactic dehydrogenase (LDH) did not show any differences between treatments. However, LDH and ALP concentrations were increased (P<0.05) at the end of feeding trial (Table 4). Serum cortisol (nM/L) and IGF-1 (ng/ml) concentration were also found to be identical in all the groups. However, IGF-1 was more (P<0.05) at the end of experiment in all the treatments. Serum cortisol (nM/L) and IGF I concentrations were found to be same (P>0.05) in all the groups. Cortisol concentrations before and at the end of the experiment were found to be equivalent in all treatment groups. However, IGF-1 concentrations increased (P<0.05) by the end of the experiment irrespective of treatment (91.33±4.53 to 102.99±3.61 in T_1-control; 88.28±1.74 to 104.19±1.18 in T_2-dNC and 89.15±2.40 to 108.00±2.45 in T_3-dKC) (Table 5). Though, we have not studied any reproduction parameters of these animals, increased IGF I concentrations could be due to ovulation of cows due to high plane of nutrition. Kawashima et al. [21] observed increased in IGF I concentrations in ovulatory cows during post-partum.

Attributes	T_1 (CNL)	T_2 (dNC)	T_3 (dKC)	SEM	Trt	Period	Trt* Period
Total Protein (g/dl)							
Initial	7.95	7.94	8.32	0.25	0.81	0.633	0.921
Final	8.25	8.16	8.3	0.21			
Albumin (g/dl)							
Initial	3.76	3.05	3.69	0.17	0.119	0.001*	0.352
Final	2.12	2.08	2.36	0.08			
Globulin (g/dl)							
Initial	4.19	4.89	4.63	0.29	0.752	0.001*	0.652
Final	6.13	6.08	5.94	0.19			
Glucose (mg/dl)							
Initial	46.19	39.17	41.22	2.31	0.301	0.001*	0.644
Final	52.95	49.84	54.01	1.24			
Urea (mg/dl)							
Initial	14.19	16.65	18.89	0.97	0.451	0.001*	0.359
Final	11.27	10.25	10.98	1.05			

Table 4: Blood biochemical profiles in treatment groups.
(T_1-Control), without any unconventional protein supplements; (T_2-dNC) detoxified neem cake was used at the expense of 50 % of soybean meal protein; (T_3-dKC), detoxified karanja cake was used at the expense of 50 % of soybean meal protein.

Attributes	T_1 (CNL)	T_2 (dNC)	T_3 (dKC)	SEM	Trt	Period	Trt* Period
AST (IU/L)							
Initial	101.56	100.24	110.32	4.11	0.362	0.61	0.989
Final	104.05	104.43	112.56	3.81			
ALT (IU/L)							
Initial	32.01	31.55	32.81	1.76	0.913	0.878	0.999
Final	31.77	31.1	32.37	1.45			

LDH (IU/L)							
Initial	737.38	750.19	754.3	46.97	0.909	0.001	0.902
Final	1251.22	1310	1212.9	71.92			
ALP (IU/L)							
Initial	53.56	51.53	54.69	2.12	0.547	0.01	0.359
Final	60.8	61.7	67.8	2.89			
Hormone Profiles Serum cortisol (nM/L)							
nitial	29.87	38.1	28.03	2.58	0.281	0.721	0.471
Final	30.95	31.66	30.09	1.66			
Serum IGF-1 (ng/ml)							
Initial	91.33	88.28	89.15	1.72	0.759	0.001	0.527
Final	102.99	104.19	108	1.8			

Table 5: Blood enzyme and hormone profiles in treatment groups
(T_1-Control), without any unconventional protein supplements; (T_2-dNC) detoxified neem cake was used at the expense of 50 % of soybean meal protein; (T_3-dKC), detoxified karanja cake was used at the expense of 50 % of soybean meal protein

The total protein, albumin and glucose values recorded in the present study are within the range (5.7 to 8.1 g/dl for protein, 2.1 to 3.6 g/dl for albumin and 45 -75 mg/dl for glucose) as suggested by Radostits et al. [22]. The total protein content in the serum changes depending on the amount of protein and energy supplied with the diet, as well as with the cow's age and feeding season [23-25]. In the present study, the protein contents of the diets were kept almost same resulted in similar serum total protein in all the treatment groups. The blood glucose values have significantly improved over a period of 90 days. The blood glucose concentrations is one of the biochemical indicators on the basis of which one may draw conclusions pertaining to body energy supply [26,24]. Glucose is necessary to produce lactose in the mammary gland [27]. The increased glucose levels in the serum of the animals at the final stage when compared to initial stage of the experiment suggest a better nutrition for lactating animals without any adverse effect of feeding detoxified neem seed cake and karanja seed cake. Serum urea concentrations have significantly reduced over a period of 90 days indicative of better protein utilization due to feeding of total mixed rations in all the treatment groups. Blood enzymes like ALT, AST, LDH, ALP (IU/L) were estimated to find out any elevation following the feeding of the unconventional cakes. During the course of 90 days, there was only elevation of LDH and ALP in all the treatment groups indicating normal physiological trends. The concentrations observed for enzymes fall well within the physiological ranges (ALT: 11 to 40, AST: 78 to 132, ALP: 0 to 500 U/L) suggested by Radostits et al. [22] and (LDH: 692 to 1445 IU/L) suggested by Kaneko et al. [28]. Blood enzyme profile showed that there are no deleterious effects of inclusion of neem seed cake and karanja cake in the complete rations in lactating animals. Cortisol is the regulator of glucose in ruminants which acts to increase gluconeogenesis from amino acids. In starving ruminants the gluconeogenesis is maintained by elevated levels of glucocorticoids [29]. In lactating ruminants the rate of hepatic gluconeogenesis and the relative concentrations of glycogenic precursors regulate the level of milk production. However, cortisol is an indicator of stress and pain. In the current study the serum cortisol concentrations were reduced at the end of the experiment when compared to the initial concentrations indicating that the animals in all the treatments were not in stress or pain. Insulin like growth factor (IGF-I) is a potential indicator of the nutritional effects on reproduction [30,31]. The IGF-I concentrations in the peripheral blood of lactating cows have been related directly to their energy status with higher concentrations being positively associated with Body Condition Score [32] and nutrient intake [33]. The results in the present study revealed that there was significant increase in the IGF-I concentrations at the end of experiment in comparison with initial stage of the experiment indicating that the animals in all the treatments were better nutritional status, good energy balance and body condition score.

Conclusion

We conclude that, detoxified Neem cake (dNC) and detoxified Karanja cake (dKC) can be included in total mixed rations of dairy cattle producing (5-8 liters of milk per day) replacing standard soybean meal. Feeding of TMR containing dNC and dKC have improved body weights, milk yield in 90 days lactation period without adversely affecting milk composition, milk production efficiency of cows. Hence, it can be recommended to include detoxified Neem cake and detoxified Karanja cake in rations of medium producing dairy cattle replacing standard soybean meal up to 50% level.

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