EFFECT OF FEEDING CHEMICALLY TREATED COTTON SEED CAKE ON FEED INTAKE, GROWTH PERFORMANCE AND BLOOD METABOLITES OF DRY NILI RAVI BUFFALOES

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ABSTRACT: An experiment was conducted to investigate the effect of feeding chemically treated cotton seed cake (CSC) on the intake, body condition score, urine pH, and serum mineral concentrations of dry buffaloes. Twenty dry Nili Ravi buffaloes weighing 600 ± 20 kg and of approximately 5 years of age were selected. Animals were divided into five groups having four buffaloes in each group. Dietary treatments based on the concentrate ration were: A) normal concentrate; B) un-treated CSC; C) CSC treated with 0.25% calcium (Ca) propionate; 4) CSC treated with 0.5% Ca propionate, and E) CSC treated with 0.75% Ca propionate. In addition to the concentrate, corn silage and wheat straw were fed at the rate of 2.5 and 0.9% of BW on DM basis, respectively. The CSC had an aflatoxin contamination level of at least 100 ug/kg. The buffaloes fed untreated CSC (Group B) had higher total DMI compared with all other treatment groups. The buffaloes fed either "normal" concentrate (Group A) or CSC treated with 0.75% Ca propionate (Group E) had lower total DMI compared with the other treatment groups. However, average daily gain did not vary between the groups. The feed efficiency was better in group consuming 0.5% treated CSC as compared to other groups. Neither urine pH nor serum Ca, P, K and Na concentrations were affected by the source of concentrate or the treatment of CSC with Ca propionate.

Key Words Cotton seed cake, Aflatoxin, Dry period, Calcium propionate, Nili Ravi buffalo.

(Received 27.09.2025 Accepted 01.12.2025)

INTRODUCTION

Aflatoxin is an emerging problem nowadays, originating from the intake of *Aspergillus* species infected feed or feed ingredients. The most commonly affected feeds are corn, peanuts, and cotton seed, including cotton seed byproducts (Sulzberger *et al*. 2017). Aflatoxin B_1 is the most toxic form of the aflatoxins, being immunosuppressive (Mohsenzadeh *et al*. 2016) and carcinogenic (McKean *et al*. 2006) in both animals and humans. Additional health impacts of aflatoxins include teratogenicity (Wangikar *et al*. 2005), genotoxicity (Gross-Steinmeyer and Eaton 2012), hepatotoxicity (Lu *et al*. 2013), and cytotoxicity (Zhang *et al*. 2015; Ismail *et al*. 2018). Aflatoxins are strongly linked with growth impairment, including stunting and wasting in humans (Khlangwiset *et al*. 2011; Magoha *et al*. 2014), animals, and poultry (Pimpukdee *et al*. 2004; Han *et al*. 2008). Clinical signs of chronic aflatoxin intoxication in cattle include decreased appetite, weight loss, decreased feed efficiency, and decreased milk production (Queiroz *et al*. 2012).

Cotton seed cake (CSC) is a readily available protein source in Pakistan. However, in recent years, concerns have been raised over contamination by aflatoxins as a consequence of inadequate processing and storage (Yunus *et al*. 2015). The Commission of European Communities (EC 2003) set a maximum accepted/residue levels of 20 ug/kg ppb for aflatoxin in animal feeds and most of the feedstuffs for cattle, sheep, goats, pigs and poultry, while it is 5 ppb in complete feedstuffs for dairy animals and 10 ug/kg for complete feedstuffs for calves and lambs. Results from various feed samples analyzed for AFB_1 at the Nutrition Division, Buffalo Research Institute (BRI) revealed AFB_1 concentrations above 100 ug/kg, which may have significant impacts on animal health and production.

Calcium (Ca) propionate can be used to inhibit mycotoxin production and has been shown to be effective in suppressing the germination, growth rate and aflatoxin production of *Aspergillus flavus* (Alam *et al*. 2010). The antimicrobial properties of Ca propionate involve the uncoupling of microbial substrate transport and oxidative phosphorylation from the electron transport system (Saftner *et al*. 2003). The aim of this study was to investigate the effectiveness of various concentrations of Ca propionate in mitigating any adverse effects of feeding aflatoxin-contaminated CSC to dry buffaloes.

The parameters assessed included dry matter intake (DMI), average daily live weight gain (ADG), body condition score (BCS), urine pH, and serum mineral concentrations.

MATERIALS AND METHODS

Animals: This experiment was carried out at Buffalo Research Institute, Pattoki, Pakistan. Twenty Nili Ravi dry buffaloes, weighing 600 ± 20 kg and of approximately 5 years of age were selected. They were randomly allocated to one of five dietary treatments in a randomized complete blocks design, with each dietary treatment group having four buffaloes. The animals were kept under similar management conditions and fed individually. The feeding trial was conducted over a total period of 60 days, including 07 days of dietary adaptation period.

Experimental design, treatments, and feeding: All the buffaloes were fed at a level to meet maintenance requirements. They were all fed a concentrate (either normal or CSC) with corn silage fed at the rate of 2.5% of BW (on DM basis) and wheat straw fed at the rate of 0.9% of BW (on DM basis). The CSC had an aflatoxin contamination level of at least 100 ug/kg. The dietary treatments based on the concentrate ration were: A) normal concentrate (Table 1); B) un-treated CSC; C) CSC treated with 0.25% Ca propionate; 4) CSC treated with 0.5% Ca propionate; and E) CSC treated with 0.75% Ca propionate. Treatment A represented a negative control, while treatment B represented a positive control. Nutrient composition of different dietary components is presented in Table 2. The daily feed allocations (for roughages and concentrate) were split into two equal portions, one fed at 0900, and the other fed at 1630 h. Feed refusals were recorded daily to determine the feed intakes (on DM basis). The animals had ad libitum access to fresh and clean drinking water round the clock. In addition to feed intake, data relating to live weight and BCS were also collected. The animals were weighed on a digital balance YH-T6 Shanghai Yaohua on fortnightly basis. The BCS of the animals was assessed every 2 weeks by two evaluators and scores were averaged (for each animal).

Measurement, sample collection, and preparation: Samples of concentrate, cotton seed cake, wheat straw, and corn silage were taken on weekly basis and subsequently pooled. Samples of feed were subjected to drying at 65 ^{o}C in hot air oven to determine dry matter (DM) at Nutrition Laboratory of Buffalo Research Institute, Pattoki, District Kasur, Pakistan. During the last week of the feeding trial, blood samples were collected from the coccygeal vein of each buffalo using a plain vaccum tube (Bio-Vac) and 18 gauge needles. Serum samples were obtained by centrifugation of the tubes at 2,500 × g for 15 min at 4 ºC and stored at -20ºC for later analysis. The serum samples were sent to Provincial Diagnostic Laboratory for analysis of Ca, potassium (K), sodium (Na) and chloride (Cl) concentrations. The urine was collected in midstream in a bucket and then transferred to a small beaker. The PH was measured using M1 151 pH meter (Martini instruments).

Calculation and Statistics: Feed efficiency for each of the treatment groups was calculated by dividing total feed intake by the total live weight gain of the animals. Data were analyzed using MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Treatment differences were declared significant if $P \leq 0.05$ and a trend toward significance if $0.05 < P \leq 0.10$.

RESULTS

Dry matter intake: The average DMI of the concentrate, roughages, and total DMI are presented in Table 3. The buffaloes fed untreated CSC (Group B) had higher total DMI compared with all other treatment groups ($P < 0.01$). Buffaloes fed either the "normal" concentrate (Group A) or CSC treated with 0.75% Ca propionate (Group E) had lower total DMI compared with the other treatment groups ($P < 0.05$). There was no difference in total DMI of those buffaloes fed CSC treated with either 0.25% (Group C) or 0.5% (Group D) Ca propionate ($P > 0.05$).

Table 1 Composition of the normal concentrate (without cotton seed cake)

Item	Inclusion (% of DM)
Maize	15.0
Rape seed meal	10.0
Canola meal	6.50
Maize gluten meal (30%)	20.0
Wheat bran	34.0
Molasses	12.5
Mineral mixture	0.50
Di calcium phosphate	1.00
Marble powder	0.50

Live weight gain and body condition score: Weight gain and BCS results are presented in Table 4. The source of concentrate or the level of feeding of Ca propionate had no effect on the ADG of the dry buffaloes ($P > 0.10$). The BCS of group D and E were highest with lowest BCS of group A ($P < 0.01$). Feed efficiency was affected by the dietary treatments as group A and E had the highest, whereas group D had the lowest feed efficiency values ($P < 0.01$). Dietary treatments had no effect on urine pH of the buffaloes ($P > 0.10$).

Serum mineral concentrations: Effects of feeding cotton seed cake treated with different levels of calcium propionate on serum calcium, potassium, and sodium and chloride concentrations are presented in Table 5. Dietary treatments had no effect on serum mineral concentrations in this study (*P* > 0.10).

Table 2 Nutrient composition of dietary ingredients

Feed ingredient	Dry matter (%)	Crude protein (% DM)	Fat (% DM)	Fibre (% DM)	Ash (% DM)
Normal concentrate	89.6	16.3	2.96	4.74	3.88
Cotton seed cake	91.9	23.4	8.80	27.4	6.80
Corn silage	72.5	8.50	1.80	27.1	9.80
Wheat straw	90.5	3.00	0.10	41.8	10.9

Table 3 Effects of feeding cotton seed cake treated with different levels of calcium propionate on the dry matter intakes (kg/day) of dry buffaloes.

Feed	Group A	Group B	Group C	Group D	Group E	*P*-value
Concentrate	4.00[c]	5.74[a]	5.22[b]	5.36[b]	4.2[c]	<0.01
Corn silage	7.84	7.67	7.90	7.84	7.55	0.37
Wheat straw	4.52	4.31	4.22	4.25	4.33	0.55
Total	16.3 ± 3.02[c]	17.7 ± 1.31[a]	17.3 ± 1.18[b]	17.4 ± 1.10[b]	16.1 ± 1.15[c]	<0.01

Values are mean ± standard deviation. Values within rows with varying superscripts differ significantly.

Table 4 Effects of feeding cotton seed cake treated with different levels of calcium propionate on live weight and body condition score of dry buffaloes.

Parameter	Group A	Group B	Group C	Group D	Group E	*P*-value
Initial live weight (kg)	583	612	605	595	615	-
Final live weight (kg)	613	654	644	649	646	-
Live weight gain (kg/d)	0.50 ± 0.15	0.70 ± 0.44	0.65 ± 0.25	0.90 ± 0.11	0.51 ± 0.34	0.39
Initial BSC	3.00	2.90	3.10	3.00	3.15	-
Final BCS	3.20[a] ± 0.24	3.25[b] ± 0.20	3.37[c] ± 0.14	3.63[d] ± 0.16	3.56[e] ± 0.23	<0.01
Feed efficiency	32.7[a] ± 0.23	25.3[b] ± 0.15	26.7[b] ± 0.16	19.4[c] ± 0.20	31.5[a] ± 0.22	<0.01
Urine pH	6.90 ± 0.61	6.97 ± 0.41	6.97 ± 0.63	6.96 ± 0.14	6.97 ± 0.42	0.86

Values are mean ± standard deviation. Values within rows with varying superscripts differ significantly.

Table 5 Effects of feeding cotton seed cake treated with different levels of calcium propionate on serum calcium, potassium, sodium, and chloride concentrations.

Serum	Group A	Group B	Group C	Group D	Group E	*P*-value
Calcium (mg/dL)	10.3	10.3	9.72	10.4	10.7	0.36
Potassium (meq/L)	5.50	5.50	4.75	4.00	4.75	0.51
Sodium (meq/L)	77.3	87.3	82.5	71.5	83.0	0.75
Chloride (meq/L)	85.3	98.0	95.8	99.3	98.8	0.05

DISCUSSION

Dry matter intake: With the exception of 0.75% Ca propionate treatment (Group E), the feeding of CSC resulted in a significant increase in total DMI compared with the feeding of the "normal" concentrate ration. Thus an aflatoxin contamination level of at least 100 ppb (as found in the CSC) had no adverse effects on DMI. Similar results were reported by Quiroz *et al.* (2012) for dairy cattle fed an AFB_1-contaminated diet (75 ppb). In contrast, Choudary *et al.* (1998) reported significant reduction in DMI in a dose dependent manner in cattle dosed with 10 to 109 ppb of aflatoxin. The apparent resistance to any adverse effects of aflatoxin in the CSC may be due to the mature age of the buffaloes, with younger ruminant animals typically more susceptible to aflatoxin intoxication (Baines *et al*. 2013; Kaleibar and Helan 2013; Atherstone *et al*. 2016).

Relative to the groups fed CSC, treatment with any level of Ca propionate resulted in a decrease in total DMI (compared with the untreated CSC). Aside from its potential to detoxify aflatoxins, for ruminants, Ca propionate is effectively a gluconeogenic precursor and addition to the diet has improved animal performance in some situations (Overton and Waldron 2004). McNamara and Valdez (2005) found Ca propionate supplementation (0.125 kg/d) increased DMI by 11% prepartum and by 13% postpartum in dairy cows. In contrast, Lee-Rangel *et al.* (2012) found supplementation of 10 g/kg DM of Ca propionate had no effect on feed intake of finishing lambs. Similarly, Zhang *et al.* (2017) found no differences in DMI in Jersey calves with the different feeding levels of Ca propionate. However, the reason(s) for the adverse effects of Ca propionate on total DMI of the dry buffaloes fed CSC is not readily apparent and warrants further research.

Live weight and body condition score: Despite differences in DMI, neither the type of concentrate ration fed nor treatment of CSC with Ca propionate affected the ADG of dry buffaloes, with all animals gaining weight over the feeding period. This is in agreement with other researchers who found that neither aflatoxin challenge nor the use of aflatoxin binders affected weight gain in dairy cows (Kutz *et al*. 2009; Queiroz *et al*. 2012; Sulzberger *et al*. 2017). However, Abdel-Latif *et al.* (2016) found that supplementation of Ca propionate in primiparous buffalo cows during late gestation and early lactation significantly improved the body weight. Zhang *et al.* (2017) found that although there were no differences in DMI, the addition of Ca propionate (10%) improved the growth performance of Jersey calves, likely as a result of increased supply of glucose precursors (Liu *et al*. 2010). The low level of Ca propionate supplementation used in this study would likely have made minor difference to the supply of glucose precursors and account for the lack of differences in ADG between the treatment groups.

Feed efficiency and urine pH: The feed efficiency was better in group consuming 0.5% treated cotton seed cake compared to other groups. The feed efficiency was higher in group A and C. The best results were taken in 0.5% treated cotton seed cake. Lee-Rangel *et al.* (2012) found that supplementation of 10 g/kg DM of Ca propionate had no effect on the feed conversion ratio of finishing lambs. In contrast, in studies using broiler chickens, Bintvihok and Kositcharoenkul (2006) found that supplementing Ca propionate in an aflatoxin-contaminated diet resulted in improved feed conversion ratio. However, limited studies are available in dairy cows or buffaloes. Nevertheless, broiler chickens are much more susceptible to aflatoxins that ruminants and the detoxifying effects of the Ca propionate would account for the improvement in the feed conversion ratio.

Urine pH is a useful method for predicting urine Ca concentrations, particularly in periparturient dairy cows (Constable *et al*. 2019). No change in serum Ca concentrations indicates that feeding of Ca propionate had no effect on Ca homeostasis of the dry buffaloes in this study.

Serum mineral concentrations: The source of concentrate or the treatment of CSC with Ca propionate had no effect on serum Ca, K, Na, and Cl concentrations. Calcium propionate can be absorbed across the rumen and increase the ionized Ca concentration in the blood (Zhang *et al*. 2020). However, Stokes and Goff (2001) also found no change in plasma Ca concentrations of dairy cows administered Ca propionate at calving.

In conclusion, the feeding of CSC contaminated by aflatoxins had no adverse effects on DMI or ADG of dry buffaloes. Treatment of the CSC with Ca propionate (to detoxify the aflatoxins) resulted in decreased DMI with no effects on ADG, resulting in an increased feed efficiency.

Acknowledgments The authors are thankful to Nutrition laboratory BRI Pattoki lab technicians, Mr. Bilal Mansha and Mr. Muhammad Umar Hayat. They helped for searching of AF infected CSC and AF analysis.

Funding The authors acknowledge the financial support provided by the department of Livestock and Dairy Development, Government of the Punjab, Pakistan.

Conflict of Interest The authors declare no conflicts of interest.

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