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An Integrated Approach to Feed Safety: Using an Organic Additive (Armour®) To Inhibit Mold and Bind Mycotoxin in Cattle Feed

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ABSTRACT: Aspergillus flavus and Aspergillus parasiticus are the primary producers of aflatoxins, strong hepatotoxic and carcinogenic substances; aflatoxin B_1 (AFB_1) is one of those compounds. AFB_1 is consumed by dairy cows through feed; it is converted to aflatoxin M_1 (AFM_1) in milk. Scientists have already set rigorous regulatory limits (0.5 µg/kg AFM_1 in milk and 20 ppb AFB_1 in feed), but it is still difficult to manage contamination in tropical storage conditions. Exposure to aflatoxin causes illness in humans and lowers animal immunity, productivity, and reproduction. After a long time, ARCL ORGANICS LTD developed Armour®, an organic product that prevents mold growth and significantly reduces microbial respiration, absorbs toxins, lower AFB_1 levels, and keep feed quality high. It's also valuable for feed safety, promotes animal health, and safeguards public health at 1.5 kg/ton of feed.

KEYWORDS: Aflatoxin B_1, Cattle feed, Toxin adsorbent, Mould inhibitor, Health promoter.

INTRODUCTION

Mold infestation is a major risk for feed products kept in environments with high levels of moisture and warmth. The production of aflatoxins, particularly aflatoxin B_1 (AFB_1), a very poisonous secondary metabolite, by *Aspergillus flavus* and *Aspergillus parasiticus* is particularly concerning among them. The International Agency for Research on Cancer has designated AFB_1 as a Group I human carcinogen [1]. AFM_1, which is secreted into milk and retains its hepatotoxic and carcinogenic potential, is produced by the hepatic biotransformation of ingested AFB_1 in nursing ruminants [2]. Notably, AFM_1 remains unchanged throughout pasteurization and processing, which raises significant concerns about feed safety [3].

The Bureau of Indian Standards (BIS) recommends a maximum of 20 µg/kg AFB_1 in cattle feeds, while regulatory bodies like the Food Safety and Standards Authority of India (FSSAI) and the United States Food and Drug Administration (USFDA) set a maximum limit of 0.5 µg/kg for AFM_1 in milk to protect consumers. These restrictions are challenging to uphold, as post-harvest fungal growth occurs under less-than-ideal storage conditions commonly contaminates raw materials and compounded feeds [4].

While chronic exposure to AFB_1 may lead to decreased milk yield, impaired reproduction, immune suppression, and increased susceptibility to disease, the acute exposure in dairy cattle can result in reduced feed intake, impaired growth, poor feed efficiency, kidney damage and hepatic injury, even death in extreme conditions [5]. Hepatic fatty degeneration, necrosis, bile duct growth, and neoplasia are examples of pathological symptoms [6]. Additionally, mycotoxins might interfere with rumen microbial activity, which can negatively impact nutrient absorption and digestion [7]. *Fusarium* and *Penicillium sp.,* which contribute extra mycotoxins and lower feed quality through nutritional degradation, clumping, and decreased palatability, may also be present in processed cow feeds [8].

Effective mycotoxin mitigation techniques are crucial for food safety and animal productivity in light of these issues. Among the various methods, adding mold inhibitors and mycotoxin binders to feed is effective. By binding AFB_1 in the gastrointestinal system, substances such as clays, zeolites, and yeast cell wall derivatives can lower the excretion of AFM_1 in milk [9, 10].

With a thorough evaluation of its function in cattle feed preservation, this current study investigates the potential of Armour®, a novel organic mold inhibitor and adsorbent formulation. The study particularly examined the physico-chemical characteristics,

minimum inhibitory concentration (MIC) against common feed spoilage fungi, efficacy in preventing mold growth in cattle feed formulations, reduction of CO_2 generation during storage, binding of aflatoxin B_1 (AFB_1), and maintenance of nutritional integrity. The findings of this study have significant implications for the cattle feed sector, which suffers significant financial losses as a result of mycotoxin contamination and spoilage. Additionally, Armour® may provide indirect benefits to dairy product end users through enhanced milk quality and safety by guaranteeing feed safety and preserving nutritional quality. The study's overall findings demonstrate Armour®'s potential as a dual-purpose organic feed additive offering and eco-friendly solution.

MATERIALS AND METHODS

Physico-chemical Characterization of Armour®

Physico-chemical properties were analyzed following standardized protocols. The pH was measured in a 1:10 (w/v) aqueous solution using a calibrated glass-electrode pH meter (TOSHCON-TOSHNIWALCL54+) with automatic temperature compensation [11]. Volatile content was determined by loss-on-drying at 105 °C to constant weight and expressed as percentage volatile matter [12]. Active aldehyde concentration was determined by hydroxylamine titration, in which carbonyl groups were converted to oximes and the un-reacted hydroxylamine back titrated to calculate aldehyde content [13]. The cation exchange capacity (CEC) was quantified by saturating the material with 1 M ammonium acetate (pH 7.0), displacing the adsorbed NH_4^+ with 1 M KCl, and measuring the released ammonium; values were expressed as cmol(+)/kg [14].

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of Armour® was determined using a modified broth dilution method [15, 16] against common feed spoilage fungi, including *Aspergillus flavus*, *Fusarium* spp., and *Rhizopus* spp. Working concentrations ranging from 100 to 1000 ppm at each 50 ppm intervals were prepared by using sterile distilled water. Equal volumes (1:1, v/v) of each concentration and actively growing fungal inoculums (~10^6 spores mL^{-1}) were incubated at 28 ±2°C for 72 h under static conditions.

Following incubation, 100 µL aliquots from each mixture were aseptically spread onto Potato Dextrose Agar (PDA) plates and further incubated at 28 ± 2 °C for 3–5 days. The MIC was defined as the lowest concentration at which no visible fungal growth was observed. All experiments were performed in triplicate to ensure reproducibility.

Experimental Feed Preparation

A total of 50 kg of mash feed was prepared in accordance with the Cattle Feed Type I formulation [17, 18] ensuring compliance with standard nutritional specifications.

Fungal Growth Monitoring

A portion of the bulk feed was divided equally into two experimental groups each of 1kg. The first served as the Control (basal feed without additives), while the second was designated as the Treatment (supplemented with 0.15% Armour®) and thoroughly homogenized to ensure uniform distribution. Both variants were adjusted to 13% moisture levels and monitored on Days 0, 7, 14, and 21. For fungal counts, 0.1 mL aliquots from suitable dilutions were spread on Potato Dextrose Agar (PDA) supplemented with chloramphenicol (100 mg/l) to inhibit bacterial growth. Plates were incubated aerobically at 28 ± 2 °C for 5–7 days, and colonies were enumerated and expressed as $\log_{10}$ cfu/g [19, 20].

Estimation of CO_2 Release

The evolution of CO_2 from stored feed is a reliable indicator of fungal metabolic activity and associated spoilage [21]. Among various respirometric techniques, the alkaline trap method followed by titrimetric back determination was adopted in this study due to its simplicity, sensitivity, and suitability for feed matrices [22].

A closed jar respirometric assay was conducted on 7, 14, and 21 Days using both control and Treatment samples. Approximately, 50 g sample of each previously prepared Control and Treatment (≤1 mm particle size) were adjusted to 20-25% moisture to promote fungal activity and placed in airtight 1 L glass jars. Each jar contained a trap with 15 ml of 1.0 N sodium hydroxide (NaOH) suspended above the feed surface to absorb the CO_2 evolved during incubation. Blank jars containing only the trap solution were maintained as controls. All jars were incubated at 28 ± 2 °C for 24 h.

At the end of incubation, the NaOH traps were carefully removed, and 1–2 ml of saturated barium chloride ($BaCl_2$) was added to precipitate carbonate ions, thereby enhancing endpoint clarity. The residual alkali was titrated against standardized 1.0 N hydrochloric acid (HCl) using phenolphthalein as an indicator. The amount of CO_2 released was calculated from the difference in titration volumes between blank and sample traps and expressed as milligrams of CO_2 per gram of feed dry matter per 24hr.

Determination of AFB_1 binding ability

Immediately after preparation of the mash feed, a representative subsample was collected and analyzed for Aflatoxin B_1 (AFB_1) to establish the baseline concentration (Day 0) and divided into two variants. Subsequently, Armour® was incorporated into the remaining bulk feed at a dosage level of 0.15% (w/w). Post-incorporation, AFB_1 quantification was carried out at defined storage intervals of 7, 14, and 21 days to evaluate the temporal dynamics of toxin levels in the presence of the additive. Binding efficiency is calculated as the percentage reduction in toxin concentration relative to the initial level.

Aflatoxin B_1 (AFB_1) in feed samples was analyzed by in-vitro batch adsorption assays following AOAC Official Method 991.31 with minor modifications, using an Agilent 1260 Infinity III LC system equipped with a Fluorescence Detector (G7121A).

Approximately 25 g of samples were extracted with 100 mL of methanol–water (80:20, v/v) by shaking for 30 min at room temperature. The extracts were filtered through Whatman No. 1 paper and diluted with phosphate-buffered saline (PBS). For purification, 10 mL of the diluted extract was passed through an aflatoxin-specific immune-affinity column (VICAM, Afla B) at ≤2 mL/min, washed with 10 mL of water, and eluted with 1.5 mL of HPLC-grade methanol. The elute was evaporated under nitrogen at 40 °C and reconstituted in 500 µL of mobile phase for injection.

Chromatographic separation was performed on a Zorbax SB C18 column (150 × 4.6 mm, 5 µm; Agilent Technologies) using an isocratic mobile phase of water, methanol, acetonitrile (60:20:20, v/v/v) at 1.0 mL/min. post-column derivatization with a KOBRA® cell brominating system enhanced fluorescence, which was monitored at 365 nm excitation and 440 nm emission. The injection volume was 50 µL.

Quantification was based on external calibration with matrix-matched standards (0.1–50 ng/mL), showing excellent linearity (R^2 > 0.999). The method achieved an LOQ of 0.5 ng/g. Recovery tests at 2, 10, and 20 ng/g yielded 78–105% recoveries with relative standard deviations <10%.

Nutritional Integrity Evaluation

Proximate nutrient composition of the bulk feed was analysed at Day 0 (baseline). The remaining material was subsequently partitioned into two variants: Control (without additive) and Treatment (supplemented with Armour® at 0.15%). Both were adjusted to 13% moisture and stored under ambient conditions (28 ± 2 °C temperature and >70% relative humidity). Proximate analysis was repeated on Day 21 to assess nutrient stability relative to baseline.

Crude Protein (CP, %) was quantified by the Kjeldahl method [23, Method 984.13], Crude Fat (%) by Soxhlet extraction [23, Method 920.39], and Crude Fibre (%) following the Weende procedure [23, Method 978.10].Calcium (%) was analysed by EDTA titrimetric method [23, Method 968.08], while Phosphorus (%) was determined colorimetrically using the molybdovanadate method [23, Method 965.17].

Gross Energy (GE, kcal/kg DM) was determined using a Toshniwal microprocessor bomb calorimeter - CC01-M3 [23 Method 983.23]. For GE estimation, finely ground and oven-dried feed samples were pelletized, combusted in an oxygen-saturated chamber at constant volume, and the heat released was recorded based on the calibrated temperature rise of the calorimeter's water jacket. Benzoic acid served as the standard reference for calibration to ensure measurement accuracy.

RESULTS

Physico-chemical properties of Armour®

As presented in Table 1. The pH of the aqueous solution (1:10, w/v) was 6.5 ± 0.2, indicating slight acidic to near-neutral stability favourable for feed applications. The total aldehyde content was 39.0 ± 2.0%, highlighting a substantial concentration of active aldehyde functional groups, which are known to exert fungistatic and fungicidal effects by disrupting microbial cell integrity. Furthermore, the cation exchange capacity (CEC) was determined to be 278.0 ± 2.0 cmol/kg, reflecting a high adsorption potential particularly relevant for binding mycotoxins such as aflatoxin B_1.

Table 1. Physico-Chemical parameters of Armour®

Parameters	Results (Mean ± SD)
pH (1:10, w/v)	6.5 ± 0.2
Volatiles (%)	13.0 ± 2.0
Active Aldehyde Content (%)	39.0 ± 2.0
CEC (cmol/kg)	278.0 ± 2.0

Minimum Inhibitory Concentration (MIC) against selected Fungi

The results demonstrated distinct interspecies differences in susceptibility (Table 2). *Aspergillus flavus* exhibited growth suppression at 483.3 ± 28.9 ppm, indicating that it was inhibited at comparatively lower concentrations relative to other fungi. *Fusarium* sp. showed an MIC of 600.0 ± 50.0 ppm, reflecting moderate susceptibility. In contrast, *Rhizopus* sp. required relatively higher concentrations for inhibition, with an MIC of 616.7 ± 57.7 ppm, suggesting a higher tolerance and greater variability among replicates. Taken together, these findings confirm that *Armour®* exerts broad-spectrum antifungal activity against common feed-spoilage moulds, with *A. flavus* being the most susceptible.

Table 2. Minimum Inhibitory Concentration (MIC) values of organisms

Molds	Conc., ppm (Mean ± SD)
Aspergillus flavus	483.3 ± 28.9
Fusarium spp.	600.0 ± 50.0
Rhizopus spp.	616.7 ± 57.7

Fungal growth in feed

Fungal growth in the stored feed exhibited marked differences between the Control and Treatment variants throughout the 21-day storage period (Table 3, Figure 1). On day 0, fungal counts were identical in both groups (5.30 ± 0.00), reflecting uniform baseline conditions. Thereafter, a progressive increase in fungal load was observed in the Control group, reaching 8.70 ± 0.23 by day 21. In contrast, the Treatment group, fortified with Armour®, demonstrated a sharp decline in fungal counts, which decreased steadily and reach as low as 0.50 ± 0.12 by day 21.

Statistical analysis revealed that differences between Control and Treatment variants became significant as early as day 7 ($p = 0.0184$), with the Treatment group showing a significantly lower fungal load compared to Control. This inhibitory effect was more pronounced on day 14 ($p = 0.0003$) and day 21 ($p < 0.0001$), where the Treatment values were substantially reduced relative to Control.

Table 3: Fungal colony forming unit (cfu) in control and treatment groups during storage (13% Moisture)

Day's Interval	*Control cfu (µl/ml) (Mean ±SEM)***	**Treatment, cfu (µl/ml) (Mean ± SEM)	****p-value
0th	5.30 ± 0.00	5.30 ± 0.00	-
7th	5.27 ± 0.54	2.03 ± 0.18	0.0184
14th	6.40 ± 0.25	1.47 ± 0.15	0.0003
21st	8.70 ± 0.23	0.50 ± 0.12	0.0001

*Control: Basal Cattle Feed type I, **Treatment: Basal Cattle Feed Type I + 0.15% Armour®, ***SEM: Standard Error of Mean, ****p-value: Paired t Test

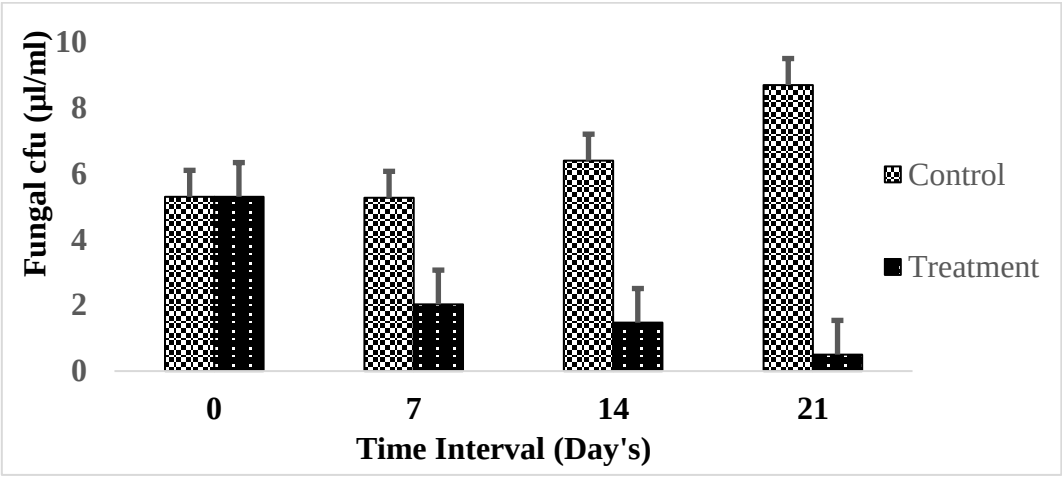

Figure 1. Fungal growth phenomena throughout the Test Period (13% moisture)

CO_2 generation in Feed

The progression of CO_2 release during storage revealed a clear and statistically robust contrast between the Control and Treatment variants (Table 4, Figure 2). On Day 7, the control was recorded a mean release of 9.16 ± 0.05 mg/g, while the Treatment registered only 1.58 ± 0.03 mg/g. This represented an almost six-fold reduction, which was highly significant (p< 0.001), underscoring the early suppressive effect of Armour® on microbial respiration.

As storage advanced, the divergence became more pronounced. By Day 14, CO_2 release in the Control rose to 12.51 ± 0.08 mg/g, whereas the Treatment maintained a markedly lower value of 2.32 ± 0.03 mg/g, corresponding to a reduction of over five-fold. The statistical comparison again confirmed strong significance (p< 0.001).After Day 21, the trend was most striking: the control exhibited 15.86 ± 0.19 mg/g, while Treatment restricted CO_2 release to 2.56 ± 0.08 mg/g, a difference of more than six-fold, which is highly significant (p< 0.001).

Table 4. CO_2 Release Dynamics of Control and Treatment Feed

Day interval	*Control, mg/g (Mean ± ***SEM)	**Treatment, mg/g (Mean ± SEM)	****p-value
7th	9.16 ± 0.05	1.58 ± 0.03	2.04×10^{-7}
14th	12.51 ± 0.08	2.32 ± 0.03	7.98×10^{-6}
21st	15.86 ± 0.19	2.56 ± 0.08	2.89×10^{-5}

*Control: Basal Cattle Feed type I, **Treatment: Basal Cattle Feed Type I + 0.15% Armour®, ***SEM: Standard Error of Mean, ****p-value: Paired t Test

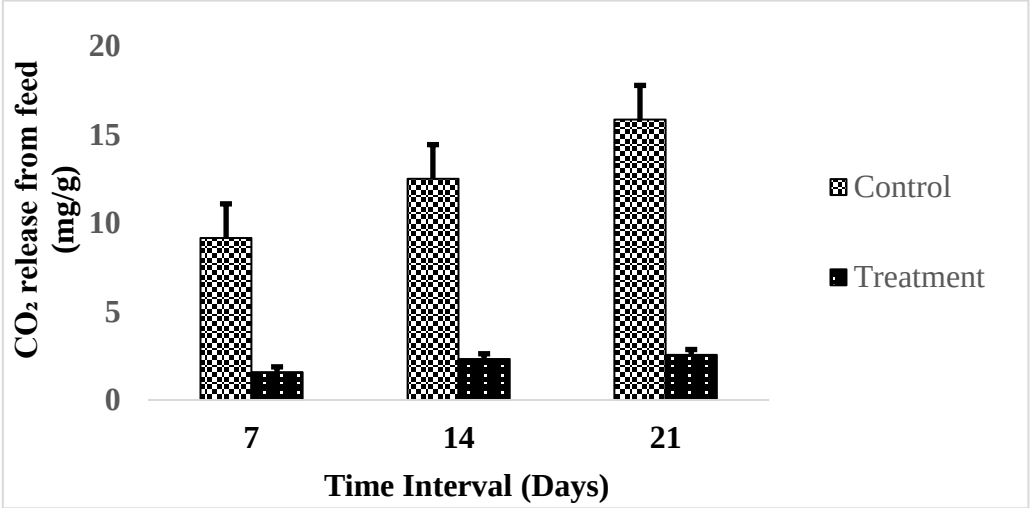

Figure 2. CO_2 Release phenomena throughout the period

AFB_1 binding

The concentration of Aflatoxin B_1 (AFB_1) at the onset of storage (Day 0) was quantified at 28.35 µg/g, markedly surpassing the Bureau of Indian Standards (BIS) permissible threshold of 20 µg/g for safe cattle feed. Following fortification with Armour®, AFB_1 concentrations declined precipitously, falling below the limit of quantification (BDL, <5 µg/g) by Day 7, and remained consistently undetectable throughout the subsequent storage period (up to 21 Days). This represents an effective detoxification of ≥82.4%, with the potential for complete elimination of the toxin burden relative to the baseline concentration (Table 5, Figure 3).

Table 5. Level of Aflatoxin (AFB_1) throughout the test period

Days	*LOQ, µg/g	Aflatoxin B_1, µg/g
0^{th}	5	28.351
7^{th}	5	**BLQ
14^{th}	5	BLQ
21^{st}	5	BLQ

*LOQ: Limit of Quantification, **BLQ: Below Limit of Quantification

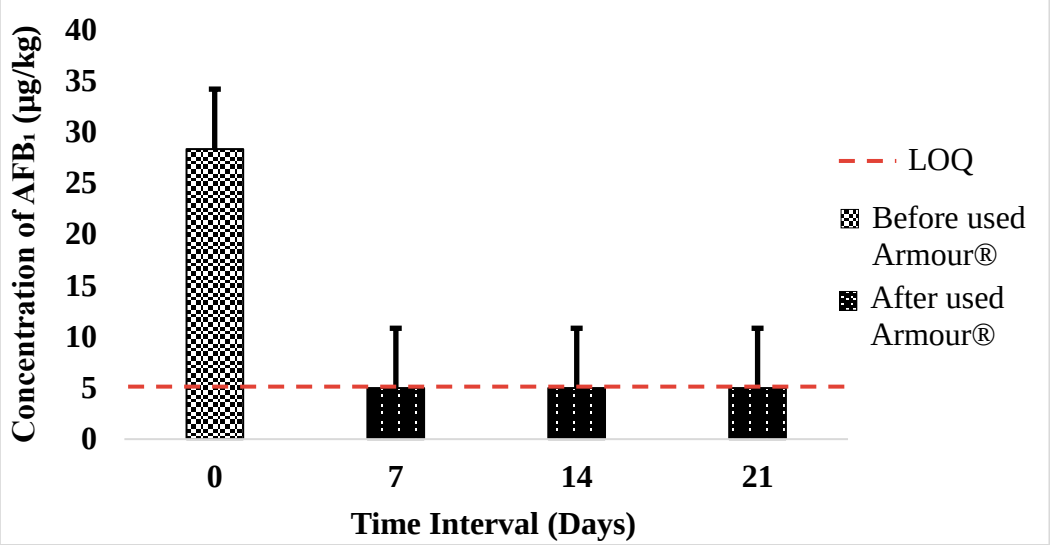

Figure 3. AFB_1 level in feed before and after Armour®

Nutritional Quality

The nutritional integrity comparison between the Control and Treatment variants is presented in Table 6 and illustrated in Figure 4. In the Control variant, a progressive decline was evident across major nutritional parameters. Crude protein decreased significantly from 20.34 ± 0.06% at Day 0 to 18.62 ± 0.01% at Day 21 ($p = 0.0014$), reflecting pronounced protein degradation during storage. Similarly, crude fat content declined from 3.50 ± 0.03% to 3.05 ± 0.01% ($p = 0.0019$), suggesting oxidative deterioration of lipids. A sharp reduction in crude fibre was also noted (7.54 ± 0.06% to 6.53 ± 0.00%; $p = 0.0039$), which may be attributed to microbial utilization of structural carbohydrates. Mineral stability was likewise compromised, as calcium declined from 0.95 ± 0.01% to 0.85 ± 0.00% ($p = 0.0025$), and phosphorus reduced from 0.41 ± 0.00% to 0.38 ± 0.00% ($p = 0.019$). These cumulative nutrient losses were further observation in the gross energy (GE) value, which reduce from 4301.62 ± 2.77 kcal/kg DM at Day 0 to 3928.87 ± 4.30 kcal/kg DM at Day 21 ($p < 0.001$), indicating a significant deterioration of feed energy density over storage.

In contrast, the Treatment group, fortified with preservative intervention, displayed remarkable stability in nutrient composition over the storage period. Crude protein levels remained largely unaffected, showing only a marginal and statistically non-significant reduction from 20.34 ± 0.06% to 20.02 ± 0.03% ($p = 0.054$). Crude fat (3.50 ± 0.03% to 3.41 ± 0.00%) and crude fibre (7.54 ± 0.06% to 7.50 ± 0.01%) exhibited negligible and non-significant differences ($p = 0.082$ and $p = 0.535$, respectively), underscoring effective protection against oxidative and microbial degradation. Calcium levels were also maintained (0.95 ± 0.01% to 0.94 ± 0.00%; $p = 0.308$), while phosphorus showed a minimal but statistically significant decline (0.41 ± 0.00% to 0.40 ± 0.00%; $p = 0.026$). Importantly, the gross energy value demonstrated far greater retention in the Treatment variant, declining only slightly from 4301.62 ± 2.77 to 4222.45 ± 7.58 kcal/kg DM ($p = 0.009$), a change that was significantly less severe compared to the Control.

Table 6. Nutritional quality of feed after storage of 21days (13% Moisture)

Parameter	Control			Treatment		
	Day 0 (Mean ± SEM)	Day 21 (Mean ± SEM)	p-value	Day 0 (Mean ± SEM)	Day 21 (Mean ± SEM)	p-value
Crude Protein%	20.34 ± 0.06	18.62 ± 0.01	0.0014 **	20.34 ± 0.06	20.02 ± 0.03	0.054 ns
Crude Fat%	3.50 ± 0.03	3.05 ± 0.01	0.0019 **	3.50 ± 0.03	3.41 ± 0.00	0.082 ns
Crude Fiber%	7.54 ± 0.06	6.53 ± 0.00	0.0039 **	7.54 ± 0.06	7.50 ± 0.01	0.535 ns
Calcium%	0.95 ± 0.01	0.85 ± 0.00	0.0025 **	0.95 ± 0.01	0.94 ± 0.00	0.308 ns
Phosphorus%	0.41 ± 0.00	0.38 ± 0.00	0.019 *	0.41 ± 0.00	0.40 ± 0.00	0.026 *
GE(kcal/kg DM)	4301.62 ± 2.77	3928.87 ± 4.30	<0.001 ***	4301.62 ± 2.77	4222.45 ± 7.58	0.009 **

ns: Not significant ($p > 0.05$): No meaningful difference between Day 0 and Day 21., *: $p < 0.05$: Significant difference (small but real change)., **: $p < 0.01$: Highly significant difference (strong evidence of change)

***: $p < 0.001$: Extremely significant difference (very strong evidence of change)

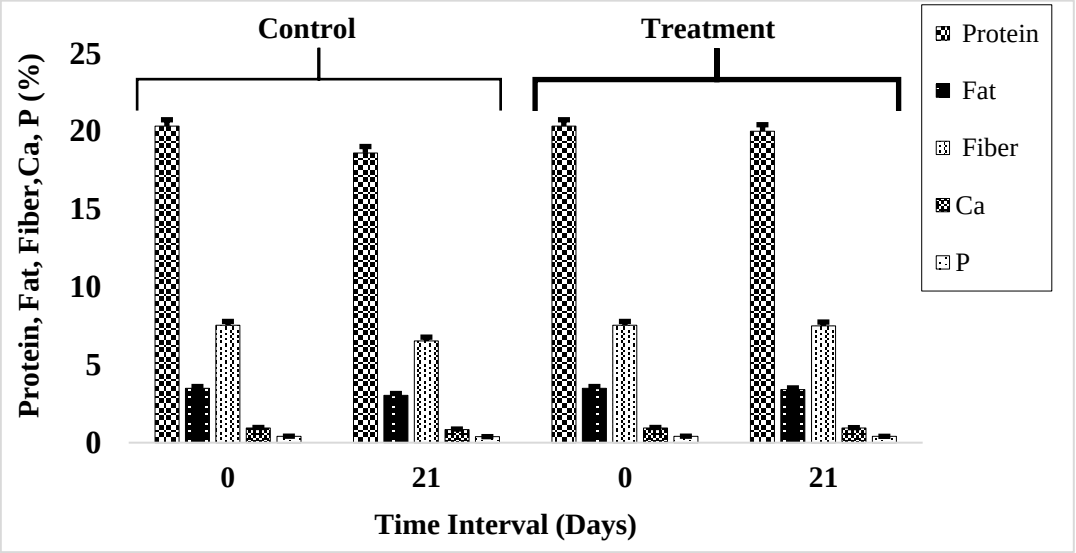

Figure 4. Nutritional value throughout the trial of 21days

DISCUSSION

The current study emphasizes Armour®'s dual function in cattle feed as an aflatoxin-binder and an antifungal ingredient. In line with previous studies on aldehyde-based preservatives and high-CEC adsorbents, its high aldehyde content and cation exchange capacity provided strong fungistatic action and toxin-binding potential [24, 25]. In storage trials, Armour® significantly decreased CO_2 evolution and fungal proliferation, confirming its ability to suppress spoilage, metabolism under tropical conditions where molds like *Aspergillus* flavus typically thrives [26]. Significantly, within 7 days, AFB_1 levels dropped from 28.35 µg/g to below quantification limits, demonstrating the detoxification efficiency superior to many conventional binders [27]. Additionally, proximate composition and energy values remained stable in the treated feed, which contrasted sharply with nutrient degradation in the Control, and consistent with previous observations on fungal-mediated feed deterioration [28].

All of these results point to Armour® as a successful intervention that protects animal health and lowers the possibility of toxin carryover into milk by maintaining nutritional quality of feed while also guaranteeing the feed safety.

CONCLUSION

Armour® significantly detoxified aflatoxin B_1, reduced microbial respiration, limited the growth of mold, and maintained the nutritional integrity of cattle feed that was stored. It provides a long-term way to reduce financial losses and improve feed safety

in tropical storage settings by combining fungus suppression with toxin binding. Its use has the potential to protect public health while increasing the cattle productivity.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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