Asian Journal of Applied Research for Community Development and Empowerment. Vol 10 (2026), No.2

e-ISSN 2581-0405

Asian Journal of Applied Research for Community Development and Empowerment

Journal home page: http://ajarcde-safe-network.org ISSN 2581-0405

Nutritional Stability of Molasses Multinutrient Soft During Storage

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ARTICLE INFO

Article History:
Received: 8 April 2026
Final Revision: 29 April 2026
Accepted: 01 May 2026
Online Publication: 02 May 2026

KEYWORDS

alternative feed, crude protein, molasses, multinutrient soft, nutritional quality, storage duration

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Abbreviations: MMS: molasses multinutrient soft; DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; AIA: acid-insoluble ash; NFE: nitrogen-free extract; NDF: neutral detergent fiber; ADF: acid detergent fiber; Ca: calcium; P: phosphorus

ABSTRACT

Molasses Multinutrient Soft (MMS), an innovative alternative livestock feed formulated from agro-industrial waste products such as molasses, tofu dregs, coconut cake, rice bran, salt, and a mineral mix, was evaluated for nutrient stability during storage. Feed storage was conducted on a laboratory scale. This study employed a completely randomized design across 0, 10, 20, and 30-day storage periods, revealing that storage significantly influenced ($p < 0.05$) key quality parameters, including moisture, dry matter, organic matter, ether extract, and acid-insoluble ash. While crude protein and nitrogen-free extract remained stable, significant changes ($p < 0.05$) were observed in fiber fractions (neutral detergent fiber, acid detergent fiber, and hemicellulose) and phosphorus content. These alterations, predominantly driven by microbial activity and environmental conditions, suggest that MMS maintains safe storage limits for up to 30 days. A strong correlation between moisture and dry matter was observed during storage, a key attribute that determined the feed's nutritional quality. This study offers valuable insights into the post-production quality of alternative feeds, underscoring MMS's potential as a viable and promising feed option for smallholder livestock farmers, particularly under minimal storage conditions. Further investigation is required regarding the effects of prolonged feed storage on the physical and chemical characteristics of MMS.

Contribution to Sustainable Development Goals (SDGs):

SDG 2: Zero Hunger; **SDG 12:** Responsible Consumption and Production; **SDG 15:** Life on Land

1. INTRODUCTION

1.1. Research background

Rising feed ingredient prices pose a significant challenge to the global livestock sector, particularly for smallholder farmers who rely heavily on commercial feeds [1,2]. This financial burden highlights the urgent need for more economical, accessible, and nutritionally superior feed alternatives [3]. Repurposing agro-industrial by-products is a promising strategy, but their variable nutritional quality and potential for contamination during storage present limitations [4]. Therefore, processing these materials, such as into molasses multinutrient soft (MMS), is crucial to enhance their nutritional stability and overall efficacy.

MMS is a highly nutritious feed for livestock made from waste materials, including molasses, tofu dregs, coconut cake, and bran, and enriched with salt and a mineral mix. MMS has a nutritional profile that can address deficiencies in primary livestock feed. In addition, MMS can increase daily feed intake in livestock and stimulate growth of rumen microorganisms, thereby improving feed efficiency [5]. With this composition, MMS can be an environmentally friendly and effective alternative to conventional livestock feed for improving livestock growth.

Ref. [6] showed that providing 5% MMS in the feed can increase body weight gain in Bali cattle. Saipul et al. [7] stated that feeding up to 20% MMS in feed did not have a negative impact on the feed consumption of male Limousin cattle. Therefore, MMS is an innovation in agro-industrial waste-based feed that not only offers a cost-effective alternative but also provides sufficient nutritional content to support livestock growth and production. At the same time, prior studies have explored the benefits of MMS as a ruminant feed for production and reproductive performance [6,8,9]. To date, no studies have specifically evaluated changes in the nutritional content (e.g., crude protein, fibre, minerals, and other components) of MMS during storage, particularly under tropical conditions with high temperatures and humidity, which could accelerate nutrient degradation. Therefore, a scientific study is warranted to comprehensively evaluate the nutritional stability of MMS during storage, thereby ensuring its quality and safety for animal feed applications.

1.2. Research objective

This study aimed to evaluate the effect of storage time on the stability of nutrient content in MMS, including proximate components, fiber fractions, minerals, and phenols. Thus, the findings of this study are expected to provide concrete solutions to optimize the use of MMS as an efficient and economical feed alternative for farmers.

2. MATERIALS AND METHODS

The composition and formulation of MMS were based on the method described by Herfandi et al. [6]. The ingredients used in the MMS production included molasses, tofu dregs, coconut cake, rice bran, salt, and a mineral mix. The ingredients used were sourced from local feed stores or poultry supply shops. These ingredients were thoroughly mixed and stirred until a homogeneous consistency was achieved, with a particle size ranging from 1 to 2 mm, as per the specified formulation. Subsequent drying was performed under direct sunlight for 6–24 hours to reduce the moisture content to 10% DM. The dried MMS was then weighed (in batches of 500 g) and packaged in sealed, labelled plastic bags for subsequent analysis or observation. Table 1 presents the detailed composition and formulation of the MMS.

The study employed a completely randomized experimental design with four treatments and three replications, with each replication consisting of a single MMS plastic bag. The treatments were as follows:

- D0: 0-day storage (control)
- D10: 10-day storage
- D20: 20-day storage
- D30: 30-day storage

MMS was stored in sealed plastic bags at room temperature (25–30°C) with an average humidity of 76%. Samples for analysis were collected on days 0, 10, 20, and 30. However, the sample storage conditions were not experimentally controlled. The parameters analyzed in this study included proximate components (moisture, DM, organic matter, crude protein, ether extract, acid-insoluble ash, and nitrogen-free extract), fiber fractions (neutral detergent fiber, acid detergent fiber, cellulose, and hemicellulose), minerals (calcium and phosphorus), and phenol content. The proximate components of the MMS were determined according to AOAC methods [10]. The fiber fractions of the MMS were analyzed using the Van Soest method [11]. The mineral content of the MMS was analyzed using atomic absorption spectrophotometry. The total phenolic content of MMS was measured by UV-Vis spectrophotometry using the Folin–Ciocalteu reagent, with absorbance measured at 765 nm [12].

Statistical assumptions were rigorously verified prior to analysis. Data normality was confirmed using the Shapiro-Wilk test ($p > 0.05$), and variance homogeneity was established through Levene's test ($p > 0.05$). Furthermore, the data exhibited no visual outliers. The obtained data were subsequently analyzed using analysis of variance (ANOVA) for variables that demonstrated significant effects. A recognized limitation of this statistical evaluation is the relatively small sample size (n=3) and the presence of temporal dependence. When significant differences ($p < 0.05$) were identified among treatment means, a post-hoc Fisher’s test was employed. Pearson's correlation coefficient (r) was used to assess the interrelationships among the nutritional parameters. All data processing was conducted using JASP 0.95.4 software [13].

Table 1. Composition and formulation of MMS [38].

Ingredients	Formulation (%)
Molasses	17
Tofu dregs	30
Rice bran	30
Coconut cake	20
Salt	1
Mineral mix	2

3. RESULT AND DISCUSSION

3.1. Proximate analysis

The moisture content of MMS significantly increased ($p < 0.05$) from 7.51% on day 0 to 8.67% on day 30, with notable increases occurring at 20 and 30 days of storage (Table 2). Conversely, DM and OM components exhibited a significant decrease ($p < 0.05$) with extended storage durations. Storage of MMS for up to 30 days did not significantly affect its crude protein content ($p > 0.05$) (Table 2), indicating that crude protein levels remained relatively stable throughout this period. A statistically significant decrease ($p < 0.05$) in ether extract content was observed over the increasing storage duration; however, it remained within recommended safe limits. AIA showed a slight decrease on day 10 compared to the control, followed by a significant increase ($p < 0.05$) at 20 and 30 days of storage, ultimately surpassing the control level. Finally, no significant change in NFE content was observed during the 30-day storage period ($p > 0.05$).

The rise in MMS moisture content during storage is primarily due to two interrelated factors: environmental moisture absorption and the metabolic activities of microorganisms that produce water as a by-product [14]. These findings align with previous research, such as that by Retnani et al. [15], which also reported increased feed moisture content due to atmospheric water vapour. While increased moisture can heighten the risk of microbial growth, potentially degrading feed nutrients [16], it is crucial to note that, in this study, the MMS moisture content remained within the safe limit of 14%, as recommended by the NRC [17]. This indicates that MMS remains safe for livestock consumption for up to 30 days of storage. MMS is envisioned as a valuable feed resource for smallholder farmers, particularly during dry seasons or periods of forage scarcity, providing essential ruminant nutrients. To further enhance its shelf life, interventions such as efficient feed-out systems and additional feed covers are recommended to minimize exposure to light and other contaminants during extended storage.

The observed stability of crude protein content suggests that MMS maintains good shelf life without compromising this nutritional aspect. Although protein degradation in feed ingredients can occur due to microbial, oxidative, and enzymatic activities [18], the molasses component in MMS likely acts as a natural preservative. Molasses effectively inhibits proteolytic microbial activity and, owing to its high sugar content, reduces pH, thereby suppressing proteolytic microbe proliferation and undesirable fermentation in fermented and molasses-based feeds [19,20,21]. Thus, the consistent protein stability in MMS can be attributed to the synergistic effect of its molasses content and appropriate storage conditions, which collectively prevent the proliferation of protein-degrading microorganisms.

The decrease in ether extract content is most likely due to oxidative processes. Exposure to air (oxygen), light, and temperature during storage initiates these processes, forming free radicals that damage unsaturated fatty acids and reduce the feed ether extract content [22]. This is corroborated by Alabi et al. [23], who found a correlation between minimal light exposure during storage and higher ether extract and ash content. Additionally, the natural lipase activity in the feed ingredients may contribute to this phenomenon [24].

AIA, a component of total ash, comprises inorganic materials such as silica that are poorly absorbed by animal digestive systems [25]. The observed increase in AIA content during prolonged storage is likely due to OM degradation. As OM decomposes, it leads to a reduction in dry matter composition, which, in turn, contributes to a relative increase in the AIA component. While AIA is commonly used to estimate feed digestibility [26,27], there is currently limited documentation on the increase in AIA during storage.

The stability of NFE indicates effective control over storage conditions and that the relatively short storage duration adequately preserved this parameter. This observation is consistent with the findings of Prasetyo et al. [28], who reported no change in NFE levels in complete feed stored for 6 months. The long-term stability of NFE in MMS is crucial to ensuring a consistent supply of nutritious feed, making it an optimal investment for farmers.

3.2. Fiber fraction

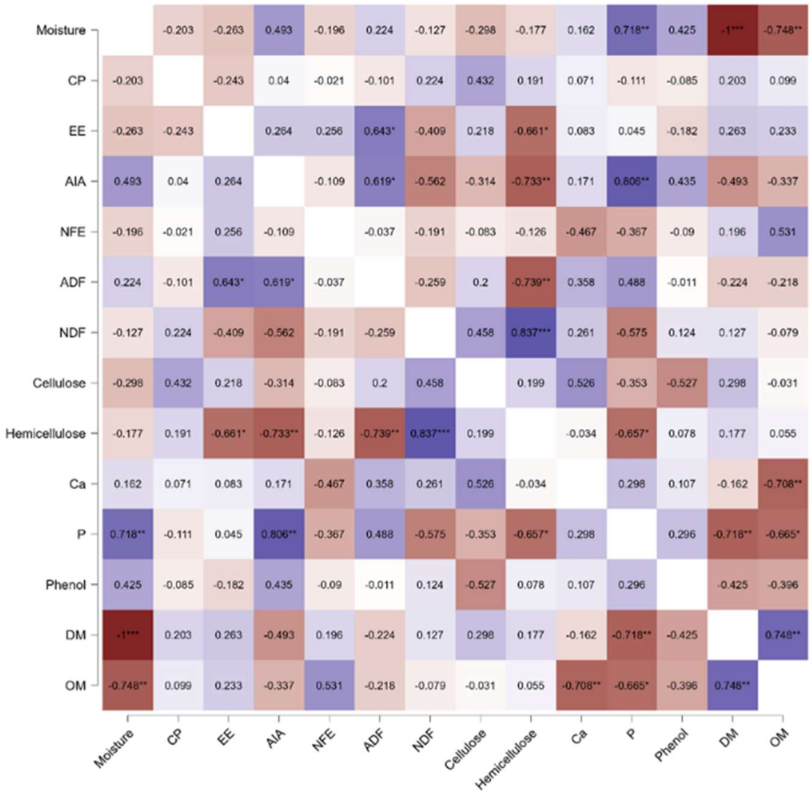
This study revealed a significant increase ($p < 0.05$) in the NDF, and hemicellulose content in the MMS on day 10, followed by a subsequent decrease on day 30 (Table 2). No significant change ($p > 0.05$) was observed in the cellulose content. The increase in fibre components may result from alterations in the physical structure of feed ingredients during storage and may be associated with oxidation and microbial activity. These processes can degrade other feed constituents, elevating the proportion of residual crude fiber [29]. Changes in the fiber fraction can also be influenced by natural fermentation processes in storage environments with increased moisture content. While cellulolytic bacteria can hydrolyze complex compounds, they do not completely degrade the fibre fraction, which remains relatively higher than other degradable components [30].

Microbial activity likely reflects an increase in the fiber fraction by digesting more easily broken-down components, thereby enriching the more resistant fiber fraction. Furthermore, Syafari et al. [31] demonstrated that physical changes in feed ingredients during storage can lead to a higher relative fibre concentration due to faster degradation of other components. Therefore, monitoring livestock feed during storage is essential to control the fibre fraction.

Table 2. Nutrient MMS content with different storage durations.

Parameters	Treatments				SE	SD	p-value
	D0	D10	D20	D30			
Proximate analysis (% dry matter)							
Moisture	7.51^{a}	7.69^{a}	8.20^{ab}	8.67^{b}	0.27	0.46	0.011
DM	92.49^{a}	92.31^{a}	91.80^{ab}	91.32^{b}	0.27	0.47	0.011
OM	76.60^{a}	76.19^{a}	75.94^{ab}	74.68^{b}	0.33	0.57	0.002
CP	10.85	10.86	10.70	10.80	0.22	0.38	0.878
EE	4.26^{a}	3.50^{b}	3.76^{ab}	3.74^{ab}	0.17	0.29	0.014
AIA	6.26^{a}	5.40^{a}	6.36^{a}	6.58^{b}	0.33	0.57	0.033
NFE	53.75	52.93	52.48	52.60	0.63	1.09	0.255
Fiber fraction (% dry matter)							
NDF	41.17^{a}	42.41^{b}	40.88^{a}	41.46^{ab}	0.37	0.64	0.017
ADF	28.55^{a}	27.53^{b}	28.01^{ab}	28.60^{a}	0.30	0.52	0.025
Cellulose	15.68	15.61	14.52	15.52	0.48	0.83	0.126
Hemicellulose	12.52^{a}	14.88^{b}	12.87^{a}	12.86^{a}	0.29	0.50	0.001
Mineral content (% dry matter)							
Ca	1.21	1.21	1.12	1.37	0.08	0.13	0.081
P	0.53^{a}	0.49^{b}	0.60^{c}	0.63^{c}	0.01	0.02	0.001
Phenol (ppm)	160.20	163.60	168.80	170.50	14.42	24.98	0.883

Note: D0: 0-day storage (control), D10: 10-day storage, D20: 20-day storage, and D30: 30-day storage. Different superscript letters within a row indicate significant differences ($p < 0.05$).



	Moisture	CP	EE	AIA	NFE	ADF	NDF	Cellulose	Hemicellulose	Ca	P	Phenol	DM	OM
Moisture		-0.203	-0.263	0.493	-0.196	0.224	-0.127	-0.298	-0.177	0.162	0.718**	0.425	-1***	-0.748**
CP	-0.203		-0.243	0.04	-0.021	-0.101	0.224	0.432	0.191	0.071	-0.111	-0.085	0.203	0.099
EE	-0.263	-0.243		0.264	0.256	0.643*	-0.409	0.218	-0.661*	0.083	0.045	-0.182	0.263	0.233
AIA	0.493	0.04	0.264		-0.109	0.619*	-0.562	-0.314	-0.733**	0.171	0.806**	0.435	-0.493	-0.337
NFE	-0.196	-0.021	0.256	-0.109		-0.037	-0.191	-0.083	-0.126	-0.467	-0.367	-0.09	0.196	0.531
ADF	0.224	-0.101	0.643*	0.619*	-0.037		-0.259	0.2	-0.739**	0.358	0.488	-0.011	-0.224	-0.218
NDF	-0.127	0.224	-0.409	-0.562	-0.191	-0.259		0.458	0.837***	0.261	-0.575	0.124	0.127	-0.079
Cellulose	-0.298	0.432	0.218	-0.314	-0.083	0.2	0.458		0.199	0.526	-0.353	-0.527	0.298	-0.031
Hemicellulose	-0.177	0.191	-0.661*	-0.733**	-0.126	-0.739**	0.837***	0.199		-0.034	-0.657*	0.078	0.177	0.055
Ca	0.162	0.071	0.083	0.171	-0.467	0.358	0.261	0.526	-0.034		0.298	0.107	-0.162	-0.708**
P	0.718**	-0.111	0.045	0.806**	-0.367	0.488	-0.575	-0.353	-0.657*	0.298		0.296	-0.718**	-0.665*
Phenol	0.425	-0.085	-0.182	0.435	-0.09	-0.011	0.124	-0.527	0.078	0.107	0.296		-0.425	-0.396
DM	-1***	0.203	0.263	-0.493	0.196	-0.224	0.127	0.298	0.177	-0.162	-0.718**	-0.425		0.748**
OM	-0.748**	0.099	0.233	-0.337	0.531	-0.218	-0.079	-0.031	0.055	-0.708**	-0.665*	-0.396	0.748**	

Fig. 1 Pearson's correlation among parameters. *** Highly significant, **moderately significant, *significant. Blue and red indicate positive and negative correlations, respectively. The darker the color, the stronger the correlation.

3.3. Mineral content

This study revealed no significant effect ($p > 0.05$) on Ca content throughout the 30-day storage period (Table 2). Conversely, a significant increase in P ($p < 0.05$) was observed on days 20 and 30. The observed changes in phosphorus levels in MMS during storage are not attributable to external accumulation but are primarily influenced by the degradation of DM and OM. This degradation can occur through various mechanisms, including oxidation, enzymatic reactions, or microbial processes. As explained by Rathod and Kumawat [32], both endogenous and exogenous enzymes can alter the chemical composition of feed during storage, leading to quantitative changes in macronutrients and qualitative changes in its physical properties. This understanding underscores the critical importance of implementing storage strategies that effectively minimize OM degradation, thereby preserving the stability of vital nutrients such as phosphorus. Furthermore, incorporating feed additives, particularly antioxidants or enzyme inhibitors, may be essential for maintaining the integrity and nutritional quality of the feed over time.

3.4. Phenol

The changes in phenol levels within MMS over a 30-day storage period were not statistically significant ($p > 0.05$) (Table 2). However, a consistent tendency for increasing MMS phenol levels was observed throughout the storage duration. The observed trend of increasing phenol levels may be attributed to the release of bound phenolic components from the feedstuff matrix. This release can be driven by microbial activity (both degradation and synthesis) and oxidation reactions, particularly in uncontrolled storage environments with ambient temperatures and high humidity. Such factors are known to contribute to the liberation and subsequent quantification of previously bound or undetected phenolic compounds. This finding aligns with observations from other studies, such as Nyong and Olubunmi [33], who reported a similar quantitative increase in certain phenolic groups, including tannin compounds in pelleted feed, over a 0–6-week storage period.

Understanding the dynamics of phenols during storage holds significant implications for ruminant feed formulation. While phenols and their derivatives are recognized as natural antioxidants with positive effects on ruminant production performance [34,35,36], an uncontrolled increase in free phenol levels can also exert antinutritional effects, impacting feed palatability and digestibility. Furthermore, monitoring phenol levels can serve as an early indicator of feed quality.

3.5. Correlation between parameters

Figure 1 depicts a strong negative correlation between DM and moisture content ($r = -1$). Significant positive correlations were observed between P and moisture ($r = 0.718$), P and AIA ($r = 0.806$), and hemicellulose and NDF ($r = 0.837$). Conversely, negative correlations were found between hemicellulose and AIA ($r = -0.733$), and between OM and moisture content ($r = -0.748$). The correlations among the nutritional parameters, as presented in the heatmap analysis, are anticipated to contribute to a deeper understanding of the intricate relationships among feed components. The strong negative correlation between DM and moisture content indicates that an increase in DM levels generally corresponds to a decrease in feed moisture content, and vice versa. This highlights the critical role of DM stability as a key indicator during storage. This is further emphasized by Akakpo et al. [37], who reported that storage of grain-based feed for 120 days resulted in an average DM loss of 24%, accompanied by reductions in crude protein and OM content. Elevated moisture levels can accelerate the risk of contamination during storage. This presents a considerable challenge for farmers in equatorial climate zones, where high environmental humidity necessitates stringent control over feed storage conditions.

It is important to note that a limitation of this study is the relatively small sample size, the absence of microbial detection (e.g., microbial counts and mould growth), and the short storage duration, which could potentially affect the representativeness and comprehensiveness of the findings. Nevertheless, the findings of this study remain valuable for small and medium-scale farmers in understanding the nutritional indicators that change during uncontrolled storage conditions and in implementing appropriate mitigation measures.

4. CONCLUSION

The experimental findings demonstrate that MMS maintains good nutritional stability for up to 30 days of storage. Crude protein and nitrogen-free extract contents remained relatively stable, while moisture content increased, although still within safe limits for daily feed intake. The strong correlation between dry matter and moisture content necessitates strict monitoring as a crucial parameter for controlling MMS quality during storage. Therefore, MMS stored for up to 30 days remains nutritionally acceptable despite modest changes in moisture, fibre fractions, and phosphorus content. Future work is expected to evaluate the nutritional stability over longer storage periods, as well as the effects of storage duration on feed digestibility and the productive performance of various ruminant species.

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