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Analysis of cellulose, hemicellulose, and lignin in fermented complete feed with different fortification materials and microorganism sources

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Abstract

This study aims to analyze: 1) The content of cellulose, hemicellulose and lignin of fermented complete feed made from sorghum with the use of various fortification materials and sources of microorganisms. 2) The interaction of the use of fortification materials and sources of microorganisms on the content value of fermented complete feed made from sorghum. This study used a completely randomized design (CRD) 3x2 factorial pattern consisting of factor A fortification materials (Indigofera leaves and moringa leaves) and factor B sources of microorganisms (mole of rumen contents, mole of cow feces and MA-11) so that there are 6 treatment combinations and repeated 3 times. The variables studied are the content of Cellulose, Hemicellulose and Lignin. The results showed that the fortification materials did not significantly affect the content of cellulose, hemicellulose, and lignin, while the source of microorganisms had a very significant effect on the content of cellulose and lignin, except for the content of hemicellulose. There was no significant interaction between the two factors on all complete feeds. The resulting fermented complete feed was of excellent quality, with Indigofera leaves superior to moringa leaves and MA-11 more effective than other microbial sources. The best combination was A1B3 (Indigofera with MA-11), which reduced the fiber content of the complete feed.

Keywords: Complete Feed; Sorghum Straw; Fortification Materials and Source of Microorganisms

1. Introduction

Demand for livestock in Indonesia, including Gorontalo, continues to increase in line with population growth and rising awareness of the importance of animal protein. One example is beef, which remains a commodity that is consistently needed, both for daily consumption and for special occasions such as holidays and traditional ceremonies. In Gorontalo, the demand for livestock comes not only from the local market but also from other regions in Sulawesi and even outside the island. Factors such as urbanization, increased income, and changing lifestyles also contribute to this rising demand. In addition, government programs such as meat price stabilization and the development of the livestock industry influence the market. However, challenges such as fluctuations in feed prices and limited supply sometimes hinder the fulfillment of demand.

One of the biggest challenges in livestock farming is the availability of feed ingredients. This issue is becoming more complex due to the conversion of forage land into industrial areas, housing, and large-scale plantations. Moreover, unpredictable climate change also has a significant impact on forage availability. During the rainy season, forage production is generally abundant, allowing livestock needs to be met well. However, during prolonged dry seasons, farmers often face great difficulties in providing sufficient feed for their animals.

To address feed availability problems, sorghum (Sorghum bicolor L. Moench) has emerged as a highly promising solution. This plant has several advantages that make it suitable for development in Indonesia, including high tolerance

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to drought and waterlogging, the ability to grow on marginal and less fertile land, and resistance to pests and diseases. Sorghum is also relatively easy to cultivate and requires low input. In terms of nutrition, sorghum biomass has a good composition for livestock feed. Proximate analysis shows that sorghum biomass (consisting of stems and leaves) contains about 10.8 percent moisture, 6.70 percent ash, 8.79 percent crude protein, 1.20 percent crude fat, 27.88 percent crude fiber, and 49.83 percent Total Digestible Nutrients (TDN) [1]. All parts of the sorghum plant, from the stems and leaves to the grains, can be optimally utilized both for human consumption and as high-quality livestock feed.

To further improve the nutritional value of sorghum-based feed, several processing strategies can be applied. Fortification or enrichment of feed by adding high-nutrient ingredients such as Indigofera (Indigofera sp.) and moringa (Moringa oleifera) has been proven effective in improving feed quality. Indigofera contains up to 27.9 percent crude protein and 15.25 percent crude fiber, along with essential minerals such as calcium (0.22 percent) and phosphorus (0.18 percent) [2]. Meanwhile, moringa leaves are known to be rich in antioxidants, vitamins, and other bioactive compounds that support livestock health and productivity.

Fermentation technology also plays an important role in improving livestock feed quality. The fermentation process, which involves various microorganisms such as lactic acid bacteria, can increase feed digestibility by breaking down crude fiber components, reducing lignin levels, and lowering anti-nutritional compounds present in feed materials. In this study, sorghum biomass was used at a level of 65 percent, with the expectation that this proportion would optimally enhance the nutritional quality of the feed. The more complete nutrient content of sorghum biomass, combined with fortification ingredients and microorganism sources, is also expected to improve the quality of the fermented complete feed. Feed quality assessment depends not only on the components used but also on nutrient availability and utilization by livestock. Therefore, an alternative approach that can maintain feed nutritional value is needed, one of which is fermented complete feed. However, information regarding the optimum nutritional value from combinations of sorghum biomass with various fortification ingredients and added microorganism sources during fermentation is still unclear. Thus, an analysis of the nutritional content of fermented complete feed based on sorghum with different fortification ingredients and microorganism sources needs to be conducted.

2. Material and Methods

This research was conducted from June 2024 to March 2025, with sorghum planting carried out on agricultural land in Tambo Village, Tilongkabila District, Bone Bolano Regency. The preparation and fermentation process of complete feed silage were conducted at the Ruminant and Non-Ruminant Livestock Laboratory, Faculty of Agriculture, Gorontalo State University, while the chemical characteristic tests were carried out at the Animal Nutrition and Feed Laboratory, Faculty of Animal Science, Hasanuddin University, Makassar.

The tools used in the study included a forage chopper machine, scoop, glass jars, tape, black plastic, jerry cans, used plastic bottles, containers, mixers, a silo scale, and other laboratory equipment. The materials used in this study were sorghum biomass harvested at 100 days after planting, Indigofera leaves, and moringa leaves. The concentrate ingredients consisted of corn flour, fine rice bran, tofu waste, molasses, and the microbial sources used were rumen fluid mol, cattle feces mol, and MA-11. The treatment ration was formulated at a ratio of 75 percent forage to 25 percent concentrate. The method used in this study was an experimental method with a completely randomized design (CRD) in a factorial pattern with three replications, consisting of two factors: factor A, fortification ingredients, which included Indigofera and moringa; and factor B, microbial sources, which included rumen fluid mol, cattle feces mol, and MA-11. The parameters measured were cellulose, hemicellulose, and lignin content in fermented complete feed based on sorghum with various microbial sources, using the Van Soest method for each treatment.

The initial stage of the study involved the preparation of local microorganisms. Rumen contents and cattle feces totaling ½ kg were mixed with 500 ml of coconut water, ¼ block of palm sugar, and 1 liter of rice-washing water in a container and placed inside a jerry can. The jerry can was tightly closed, and a small hose was inserted through a hole in the lid, connected to a bottle filled with water, then fermented for 14 days. The next stage involved collecting fresh forage such as sorghum, Indigofera leaves, and moringa leaves, which were cut into 3–5 cm pieces using a forage chopper. The materials were then wilted for 12 hours (overnight) in an open room to reduce moisture content. The forage was then mixed and homogenized with the concentrate ingredients (fine rice bran, ground corn, squeezed tofu waste, and diluted molasses at a ratio of 1:10 for every 1 kg of feed) and supplemented with 50 ml of microbial source per kilogram of feed. The mixed silage was placed into glass jars and compacted to create anaerobic conditions, then covered with black plastic before being sealed with tape to prevent oxygen exchange. Once the jars were filled and tightly closed, the silage materials were fermented for 21 days. Samples from each silage treatment were taken for fermented complete feed analysis in the laboratory. The data obtained were analyzed using analysis of variance (ANOVA) and further tested using Duncan's Multiple Range Test (DMRT) according to [3].

3. Results and discussion

3.1. Kan Dungan Setulose

The average cellulose content in fermented complete feed made from sorghum with various fortification materials and sources of microorganisms is presented in the figure below:

Table 1 Cellulose Content of Complete Feed Silage Based on Sorghum Straw with Various Fortification Materials and Microorganism Sources

Fortification Materials	Sources of Microorganisms			Mean ± Stev
	B1	B2	B3	
A1	24.50±0.90c	24.05±0.70b	21.37±0.32a	23.30±1.58a
A2	23.87±1.16b	24.78±0.26c	22.87±1.11b	23.84±1.16a
Mean± Stdev	24.18±0.99b	24.42±0.62b	22.12±1.10a	

Description: A1 = Indigofera leaves; A2 = Moringa leaves
B1 = Moles of Rumen Content; B2 = Moles of Cow Feces; B3 = MA-1
Different superscripts in the same row indicate highly significant differences (P<0.01).

The analysis of variance showed that the fortification materials did not have a significant effect (P>0.05) on the cellulose content of the complete feed, with the mean value for each treatment (A1) using Indigofera leaves (23.30%). This result is higher than that reported in [4] for banana stem (Musa paradisiaca) silage combined with Indigofera leaves as ruminant feed, which had an average value of 13.21%. On the other hand, the treatment using Moringa leaves (A2) produced an average value of 23.84%, which is higher than the findings of the study [5] on complete feed for beef cattle supplemented with Moringa leaves, which reported an average value of 17.08%. These results indicate that the lower the percentage of fortification material in the complete feed, the lower the cellulose content, which is more beneficial for livestock. This may mean that although Indigofera leaves (A1) and Moringa leaves (A2) have different chemical characteristics, their individual contributions to the fortified parameters tested were not large enough to produce differences between fortification materials. Fortification using Indigofera leaves (A1) has also been widely studied as a promising green protein source, especially in livestock and feed fortification, because of its complete amino acid profile and good digestibility, enabling it to reduce the cellulose content in complete feed.

This is in line with the opinion of [6] stating that cellulose is the main component of plant cell walls. Cellulose is almost never found in a pure state in nature, but is always bound to other substances such as lignin and hemicellulose. Cellulose content is also found in plants as a component of the cell wall and plant fibers. Meanwhile, the treatment using Moringa leaves (A2) showed a slightly higher mean value than Indigofera leaves. This is presumed to be due to the high crude fiber content in Moringa leaves, which lowers the amount of energy that can be metabolized by livestock. On the other hand, Moringa leaves are also rich in minerals, vitamins, and especially protein, containing 8 essential amino acids that can improve livestock growth due to the essential nutrients they contain [7]. This is consistent with the opinion of [8], which explains that Moringa leaves have a high protein content along with various vitamins and minerals, such as vitamin A, vitamin C, calcium, and iron, making them suitable as a fortification material to increase the nutritional value of food products.

The similarity in nutrient content between the two types of leaves can be explained by the similarity of their chemical compositions and physiological functions in the plants, even though they come from different species. The protein, amino acids, fiber, and bioactive compounds such as flavonoids and polyphenols in Indigofera and Moringa leaves are reported to have relatively comparable proportions, which may be the reason why there was no significant difference in the average fortification values obtained. These results are consistent with several previous studies reporting that both Indigofera and Moringa leaves are potential sources of protein and other important nutrients for food and feed fortification [9].

The analysis of variance showed that the use of various sources of microorganisms had a highly significant effect (P<0.01) on the cellulose content of complete feed. Further tests indicated that the type of microbial inoculant played a key role in reducing the fiber content of complete feed during fermentation. Treatment using cattle rumen content molasses inoculant (B1) produced a mean cellulose content of 24.18%, which is lower than the value reported in [10] on the effect of mixing odor grass with cattle rumen content on the fiber fraction of complete feed silage, which had an average of 25.231%. Meanwhile, cattle feces molasses inoculant (B2) recorded a cellulose content of 24.42%, lower than the findings of [11] on the fiber fraction of sugarcane bagasse fermented with different inoculate (27.70%). This was followed by treatment using MA-11 (B3), which resulted in a mean value of 22.12%, lower than the findings of [12] on cellulose, hemicellulose, and lignin content in rice straw fermented with various probiotics, which reported an average value of 36.26%.

These results indicate that the lower the proportion of microbial sources in the complete feed, the lower the cellulose content, which is more favorable for livestock. This is presumably because complete feed using MA-11 (B3) tends to be better for livestock productivity compared to feed relying solely on rumen content inoculant (B1) or cattle feces inoculant (B2). MA-11 is a standardized, controlled inoculant formulated specifically to improve feed fermentation. MA-11 generally contains selected superior microbial strains capable of breaking down fiber, increasing digestibility, improving the aroma and quality of feed, and suppressing the growth of pathogenic microbes. Thus, MA-11 provides more consistent, safe, and efficient fermentation results, producing feed with more stable quality. This aligns with the opinion of [13], stating that MA-11 also acts as an activator that can rapidly break down all organic materials and increase the nutritional content within them, helping to reduce cellulose levels in feed.

Meanwhile, treatments using rumen content molasses inoculant (B1) and cattle feces molasses inoculant (B2) have a large microbial diversity, but not all microbes directly contribute to cellulose degradation. Their cellulolytic activity may be lower and inconsistent because they originate from natural, non-standardized sources. Additionally, the fermentation conditions in natural molasses inoculants are often unstable, preventing optimal growth of cellulolytic bacteria. As a result, fiber breakdown in sorghum materials is not maximized, and more cellulose remains after fermentation compared to feed treated with MA-11. This is supported by the opinion of [14] that bacteria play an important role in cellulose digestion, with their degradation products usable by Rhizobium sp., which functions in nitrogen fixation.

The combination of fortification materials and microorganism sources showed no interaction ($P>0.05$) on cellulose content in the feed. Although the mean value of the combination (A1B3) was lower than the other combinations, this occurred because the two factors work independently and do not influence each other's effectiveness in degrading cellulose. This means that the influence of fortification materials, such as nutrient or energy addition, does not strengthen or weaken the performance of microorganisms in breaking down fiber including cellulose. Likewise, the type of microorganism used does not alter the way the fortification materials function during fermentation. This condition may occur when the cellulase enzyme-producing ability of microbes is already specific and relatively stable, so the presence of fortification materials does not significantly affect their activity. Thus, fortification materials and microorganism sources do not have a synergistic relationship in reducing cellulose content.

These results clearly indicate that the effectiveness of a microbial source in degrading cellulose is not universal but highly dependent on the substrate or fortification material used. This is known as substrate-inoculum specific interaction, which has been reported in various fermentation studies. One such study, [15], explains that the chemical composition, physical structure, and bioactive compounds present in the substrate can selectively influence the activity and functional composition of the microbial community in the inoculum. In Indigofera fortification, a synergistic interaction likely occurs between the distinct bioactive components of Indigofera leaves such as tannins and saponins at certain levels and the dominant microbial population in MA-11. The reduction in cellulose content in complete feed occurs because of the loosening of lignocellulose bonds during the ensiling process. This corresponds with the statement of [16], explaining that fermentation can reduce cellulose content into simpler polymers because lactic acid bacteria grow during ensiling. Lactic acid bacteria are cellulolytic bacteria.

3.2. Hemicellulose Content

The average hemicellulose content of fermented complete feed based on sorghum biomass with various fortification materials and microorganism sources is presented in the figure below

Table 2 Hemicellulose Content of Complete Feed Silage Based on Sorghum Straw with Various Fortification Materials and Microorganism Sources

Fortification Materials	**Sources of Microorganisms**			**Mean ± Stdev**
	B1	B2	B3	
A1	15.27±1.70	13.94±2.07	13.75±3.29	14.32±2.24
A2	15.37±0.16	15.38±0.51	15.75±1.23	15.49±0.70
Mean± Stdev	15.32±1.08	14.66±1.56	14.74±2.47	

Description: A1 = Indigofera leaves; A2 = Moringa leaves
B1 = Moles of Rumen Content; B2 = Moles of Cow Feces; B3 = MA-11
Different superscripts in the same row indicate highly significant differences (P<0.01).

The analysis of variance showed that the treatments did not significantly affect the fortification materials (P>0.05) on the hemicellulose content of the complete feed, with the mean value for treatment (A1) using Indigofera leaves being 14.32%. This value is lower than the result reported in [4] for banana stem (Musa paradisiaca) silage combined with Indigofera leaves as ruminant feed, which had an average value of 20.39%. Meanwhile, treatment (A2) using Moringa leaves produced an average value of 15.49%, which is higher than the result reported in [5] for complete feed for beef cattle supplemented with Moringa leaves, which had an average hemicellulose content of 9.73%.

These results indicate that the lower the percentage of fortification materials in complete feed, the lower the hemicellulose content, which is more beneficial for livestock. This difference may be caused by variations in nutrient content and bioactive components present in each type of leaf. Indigofera leaves (A1) are thought to have more diverse nutrient compositions among samples, while Moringa leaves (A2) are known to have a more stable and generally higher nutrient composition, resulting in higher mean values with relatively small variation. This is because Indigofera leaves are more effective at reducing hemicellulose content compared to Moringa leaves due to differences in fiber composition and their responses to the fermentation process. Additionally, the bioactive compounds in Indigofera, such as tannins in moderate amounts, may support the activity of certain microorganisms that contribute to fiber degradation. During fermentation, microorganisms utilize hemicellulose components as an energy source, and Indigofera leaves provide a more supportive environment for this process. This is consistent with [17], which states that lignocellulosic bonds are limiting factors in feed utilization because they reduce digestibility and subsequently decrease feed nutritional value.

Conversely, Moringa leaves have a relatively more complex fiber structure and are more resistant to microbial degradation, resulting in a smaller reduction in hemicellulose compared to Indigofera. This indicates that Indigofera naturally has more favorable characteristics for reducing hemicellulose during feed fermentation. The lack of significant difference between the two fortification materials supports previous findings from [18], which state that both Indigofera and Moringa leaves have comparable potential as fortification sources in certain applications. Thus, although Indigofera leaves show better stability, both plants can still be considered viable alternatives in fortification strategies depending on resource availability and specific application goals.

The analysis of variance also showed that the use of different microbial sources did not significantly affect hemicellulose content in complete feed (P>0.05). The average value for rumen content molasses inoculant (B1) was 15.32%, which is lower than the findings of [10] on the effect of mixing odor grass with cattle rumen content on fiber fraction in complete feed silage, which had an average of 34.148%. Meanwhile, cattle feces molasses inoculant (B2) recorded a hemicellulose content of 14.66%, lower than the findings of [11] for sugarcane bagasse fermented with different inoculate (6.03%). This was followed by MA-11 (B3), which had an average value of 14.74%, lower than the results of [12] on cellulose, hemicellulose, and lignin content in rice straw fermented with various probiotics, which averaged 19.44%.

These results indicate that the lower the proportion of microbial sources in the complete feed, the lower the hemicellulose content, which is beneficial for livestock. Hemicellulose is a fiber component that is generally degraded by specific microorganisms, especially those producing hemicellulose enzymes. Moreover, uniform fermentation conditions such as temperature, moisture content, and fermentation duration may have led to hemicellulose decomposition occurring with similar effectiveness across all treatments. As a result, no type of molasses inoculant exerted a more dominant effect, and the hemicellulose content in the fermented complete feed remained within a statistically similar range.

This variability suggests that other factors besides the type of microbial source may have influenced the experimental results. These factors may include variation in environmental conditions, differences in biological sample responses, or imperfections in experimental control. However, the cattle feces molasses inoculant showed the lowest mean value, indicating its ability to reduce hemicellulose content during fermentation, even though rumen content molasses and MA-11 showed no statistically significant differences. This may be due to the fact that cattle rumen content inoculant contains a highly complex and diverse microbial consortium including cellulolytic, proteolytic, and amylolytic bacteria, as well as protozoa and fungi. This diversity enables more efficient fiber degradation through synergistic and complementary enzymatic activity. This is in line with [19], which explains that functional diversity in rumen microbial communities allows role-sharing and efficient resource use, resulting in a higher rate of organic matter degradation. This is also supported by [20], who reported that natural microbial consortia from cattle feces produce more complete and stable profiles of fibro lytic enzymes such as cellulase, xylanase, and β-gluconate compared to those produced by commercial microbial cultures. These findings are consistent with [21], which states that in biological experiments, natural variation in living materials such as microorganisms often leads to data fluctuations reflected in relatively large standard deviations. In other words, the dynamic nature and complexity of biological systems make experimental results prone to variability even under controlled conditions. From an analytical perspective, all three microbial sources can be considered to have comparable potential.

The combination of fortification materials and microbial sources did not significantly affect (P>0.05) hemicellulose content in complete feed because the two factors did not influence each other in the hemicellulose degradation process. Each fortification material produced relatively similar responses across all microbial sources, and vice versa, resulting in no significant differences between combinations. Although the mean value for the Indigofera–MA-11 combination (A1B3) was lower than the others, this condition typically occurs because hemicellulose breakdown is more strongly determined by the general enzymatic capacity of microorganisms and the initial characteristics of sorghum biomass rather than by specific synergy between additives and particular microbes. This aligns with [22], which explains that the effectiveness of microorganisms depends greatly on their compatibility with a given substrate. The performance of a microbial consortium is influenced not only by its metabolic capacity but also by environmental suitability, nutrient availability, and the chemical-physical properties of the fortified substrate. The decrease in hemicellulose content in fermented complete feed based on sorghum with various fortification materials and microbial sources can be explained by the biological processes occurring during fermentation or ensiling, in which hemicelluloses one of the main structural components of plant cell walls is degraded by enzymatic activity produced by microorganisms.

3.3. Lignin Content

The average lignin content in fermented complete feed based on sorghum biomass with various fortification materials and microorganism sources is presented in the figure below:

Table 3 Lignin Content of Complete Feed Silage Based on Sorghum Straw with Various Fortification Materials and Microorganism Sources

Fortification Materials	Sources of Microorganisms			Mean± Stdev
	B1	B2	B3	
A1	$7.05±0.68^{ab}$	$8.05±0.60^{c}$	$6.62±0.22^{a}$	$7.24±0.79^{a}$
A2	$7.78±0.17^{bc}$	$7.51±0.50^{b}$	$7.01±0.29^{a}$	$7.43±0.45^{a}$
Mean± Stdev	$7.41±0.60^{b}$	$7.78±0.47^{b}$	$6.82±0.31^{a}$	

Description: A1 = Indigofera leaves; A2 = Moringa leaves
B1 = Moles of Rumen Content; B2 = Moles of Cow Feces; B3 = MA-11
Different superscripts in the same row indicate highly significant differences (P<0.01).

Analysis of variance showed that the fortification treatments had no significant effect (P>0.05) on the lignin content of the fermented complete feed. The average lignin content in the treatment using Indigofera leaves (A1) was 7.24%, which is higher than the results reported in [4] for banana stem silage (Musa paradisiaca) combined with Indigofera leaves as ruminant feed, which had an average value of 3.71%. Meanwhile, the treatment using moringa leaves (A2) produced an average lignin content of 7.43%, which is higher than the findings of [5] in complete feed for beef cattle supplemented with moringa leaves (8.25%).

These results indicate that the lower the proportion of fortification material in the complete feed, the lower the lignin content, which is beneficial for livestock. This suggests that the two leaf types have relatively comparable nutritional compositions after formulation and fermentation processes. In other words, the fortification variations using Indigofera or moringa leaves were not substantial enough to produce significant differences in lignin levels. The fermentation process tends to equalize nutrient availability and quality, minimizing the natural differences between the two raw materials. Additionally, feed homogeneity, mixing techniques, and fermentation efficiency may contribute to the uniformity of the final values, which explains why the statistical analysis shows that any differences remain small and fall within a non-significant range.

These findings are consistent with the report in [23], which states that Indigofera leaves have high protein and mineral content, supporting their use not only as feed but also as feed supplements. Similarly, research [8] reported that moringa leaves also have a rich nutritional profile, including protein, vitamins, and minerals, making them highly potential as fortification materials. The comparable nutrient content between Indigofera and moringa leaves is likely due to the similarity of their major nutrient components, particularly protein and minerals. Both plants are known to contain bioactive compounds and essential amino acids required to support livestock health and productivity. Therefore, either Indigofera or moringa leaves can substitute or be combined in feed formulations without causing significant changes in the nutritional value of the final product.

The analysis of variance on the various microbial sources showed a highly significant effect ($P<0.01$) on lignin content in the fermented complete feed. Further analysis indicated that the type of microbial inoculant plays a key role in reducing fiber during fermentation. The treatment using cattle rumen liquor molasses (B1) produced an average cellulose content of 7.41%, which is lower than the results of [10] on the effect of mixing odor grass and rumen liquor on the fiber fraction of complete feed silage (10.246%). Meanwhile, cattle feces molasses (B2) recorded a cellulose content of 7.78%, which is lower than the findings of [11] on the fiber fraction of sugarcane bagasse fermented with different inoculum types (27.70%). The MA-11 inoculum (B3) produced an average value of 29.50%, which is still lower than the results of [12] on cellulose, hemicellulose, and lignin content of rice straw fermented with various probiotics (3.93%).

Microbial sources have different capacities to produce ligninolytic enzymes such as ligase, peroxidase, and lactase, which play important roles in degrading lignin structures. In sorghum-based fermented complete feed, the type of microorganism determines how effectively lignin can be broken down during fermentation. The MA-11 treatment (B3) showed better performance in reducing lignin compared to rumen liquor and feces molasses because MA-11 generally contains more selected and stable microorganisms with stronger ligninolytic enzymatic activity. The microbes in MA-11 usually originate from pure cultures or controlled consortia chosen specifically for their ability to produce ligase, lactase, and peroxidase, which directly break down lignin structures. With its more targeted microbial composition, MA-11 promotes more optimal lignin degradation, resulting in lower lignin content than the other molasses types. This is supported by [24], which stated that MA-11 produces lignin's, an enzyme important for reducing lignin antinutritional compound that protects plant cell walls thereby improving microbial access to intracellular nutrients.

Cattle rumen liquor molasses (B1) and cattle feces molasses (B2) showed slightly higher variability in lignin content due to the diverse microbial compositions present in these inoculants. Microorganisms in rumen liquor and feces are derived from natural digestive processes, meaning their populations fluctuate depending on physiological conditions, diet, age, and animal health. These variations lead to inconsistent enzymatic activity, especially ligninolytic enzymes, across different fermentations. The presence of non-ligninolytic or competing microbes may also reduce the effectiveness of lignin degradation, resulting in more fluctuating reductions. Therefore, the heterogeneity of microbial populations in rumen liquor and feces molasses is the main factor contributing to their greater variability compared to MA-11. This is supported by reports [25] and [26], which state that microbial consortia with broader enzymatic activity can accelerate lignin depolymerization and improve feed nutritional quality. The findings are also consistent with [27], which reported that microbial consortia from cattle feces showed higher enzymatic activity, including lignin peroxidase production, compared to single microbial cultures.

The combination of fortification materials with different microbial sources did not show a significant interaction ($P>0,05$) on lignin content in fermented complete feed. Specifically, the combination of Indigofera leaves (A1) and MA-11 (B3) produced the lowest lignin content (6.62%), indicating that Indigofera leaves combined with MA-11 (A1B3) were more effective in reducing lignin compared to the other combinations. This is consistent with previous studies reporting that certain microorganisms can reduce lignin or crude fiber through lignocellulose degradation. For example, fermentation of rice straw using MA-11 resulted in a significant decrease in lignin content [28].

The absence of interaction suggests that both factors the fortification material and microbial source—work independently in influencing lignin content. In other words, changing the type of leaf used for fortification does not alter how microorganisms affect lignin, and vice versa. This may occur because lignin degradation during fermentation is more strongly influenced by the inherent resistant nature of lignin and the enzymatic capabilities of the microorganisms, which are relatively similar under the fermentation conditions applied. Thus, the results indicate that neither fortification variation nor inoculum source provides a synergistic effect on lignin levels in the fermented complete feed.

This also suggests that the effectiveness of the microbial source is highly dependent on its type and origin, with MA-11 being one of the most effective. This aligns with [13], which stated that MA-11 likely contains specific microbes such as Bacillus subtilis, Trichoderma spp., and Aspergillus spp., all known to produce various hydrolytic enzymes essential for fermentation and feed quality improvement. Meanwhile, molasses from rumen liquor and cattle feces contain more diverse rumen-derived microbial populations, such as cellulolytic, proteolytic, and ligninolytic bacteria that are naturally adapted to degrading plant structural components.

4. Conclusion

Fermented complete sorghum-based feed fortified with Indigofera leaves or moringa leaves had no significant effect on cellulose, hemicellulose, or lignin content. Both ingredients have relatively similar nutritional composition and fiber structure, so there were no significant differences after the ensiling process. In contrast, the source of microorganisms proved to be a significant influence, particularly the MA-11 culture, which consistently produced the highest reduction in structural fiber cellulose, hemicellulose, and lignin compared to rumen contents and cow feces. The superiority of MA-11 stems from a selected microbial consortium with high enzymatic capabilities, particularly in breaking down lignocellulose. The absence of interaction between the fortification material and the source of microorganisms indicates that both factors work independently in the fiber degradation process. Overall, 21 days of fermentation reduced fiber fractions, especially the most resistant lignin, thereby increasing the digestibility and nutritional value of the feed, making it more optimal for consumption by ruminant livestock.

Compliance with ethical standards

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Disclosure of conflict of interest

There are no conflicts of interest to disclose.

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