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Improvement of the Feedstuffs Nutritional Value by Synergistic Microbial Fermentation Techniques

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ABSTRACT

Synergistic microbial fermentation represents a promising approach to enhancing feedstuff nutritional value, with significant implications for animal health, productivity, and environmental sustainability. The scaling up of production,refining microbial consortia, and integrating fermentation technologies are a considerable part of circular bioeconomy frameworks. This study investigated the aerobic bacterial populations in sheep rumen and dung, revealing counts of 10^7 CFU/mL and 10^9 CFU/g, respectively, with dominant genera including *Streptococcus*, *Lactobacillus* and *Bacillus* genera. Standard forage was fermented using synergistic bacterial isolates from rumen (Rumen $_{syn}$), dung (Dung $_{syn}$), their combination (Mix $_{RD\text{-}syn}$), and a mixed consortium supplemented with commercial strains of *Saccharomyces* and *Lactobacillus* (Mix $_{RDA\text{-}syn}$). All fermentation treatments significantly altered nutritional composition by increasing ash, crude protein, electrolytic balance, and increasing the energy metrics such as digestible energy (DE), metabolizable energy (ME), protein digestible in the intestine based on nitrogen (PDIN), and feed conversion unit (UFV), and decreasing dry matter, moisture, crude fiber, crude fat, carbohydrates, and protein digestible in the intestine by enzymes (PDIE). Rumen $_{syn}$ fermentation increased crude protein by 11.3% and reduced fiber by 9.1%, while Dung $_{syn}$ enhanced protein by 12.9% and energy availability. The mixed consortium (Mix $_{RD\text{-}syn}$) balanced these effects, improving fiber degradation (8.2%) and protein content (12.2%). The supplementation with commercial strains of *Saccharomyces* and *Lactobacillus* (Mix $_{RDA\text{-}syn}$) yielded the highest crude protein increase (24.1%) and sugar utilization (46.9% reduction), alongside the increase of DE (8.4%), ME (3%), PDIN (10.7%). These findings indicate that tailored microbial consortia, particularly mixed supplements, can markedly enhance forage nutritional quality for ruminants. Confirmatory *in vivo* studies are warranted to establish their effectiveness in real-world feeding systems.

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INTRODUCTION

The global livestock industry faces increasing pressure to meet the rising demand for animal feed while addressing sustainability challenges, including forage efficiency, environmental impact, and meet conversion in animal production (Makkar, 2018). However, the growing demand for sustainable and high-quality animal feed has driven research into innovative methods to enhance the nutritional value of feedstuffs. Microbial fermentation, particularly through synergistic consortia of microorganisms, has emerged as a promising approach to improve digestibility, nutrient bioavailability, and functional properties of feed ingredients (Lu *et al*., 2025). While conventional feed processing methods often fail to maximize nutrient availability, particularly in plant-based ingredients that contain anti-nutritional factors and hard digestible fibers, lipid and proteins in addition to enriched feedstuffs with probiotics, prebiotics, enzymes, and bioactive peptides (Franco *et al*., 2022). To overcome these benefits and limitations, microbial fermentation has emerged as a transformative bioprocessing strategy by animal microbiomes that enhance feed digestibility, nutrient bioavailability, and functional properties (Xu *et al*., 2023). Recent advancements in synergistic microbial fermentation employing consortia of yeast, bacteria or probiotics have demonstrated superior efficacy compared to single-strain fermentation (Papakonstantinou *et al*., 2024). This technique leverages metabolic interactions between microorganisms to degrade complex substrates, synthesize essential nutrients, and produce bioactive compounds that benefit animal health (Perwez & Al Asheh, 2025). For instance, co-cultures of *Lactobacillus plantarum, Bacillus subtilis* and *Saccharomyces cerevisiae* have been shown to significantly improve protein digestibility in forage composite from soybean meal, corn, wheat straw and wheat brain, while improved the digestibility and reduced oligosaccharides and proteases inhibitors (Wang *et al*., 2024). On other hand, the latest developments in microbial fermentation techniques for feed enhancement, focusing on the mechanisms of nutrient improvement of protein fiber balance and energy supplements by optimization of multi-strain fermentation and fermentation method for maximum efficiency (Senanayake *et al*., 2023). Also, the key advantage of synergistic microbial fermentation is its ability to transform unqualified agro-industrial byproducts into high-value feed ingredients. For example, solid-state fermentation (SSF) of soybean meal with *Bacillus subtilis* and *Saccharomyces cerevisiae* has been shown to increase crude protein content by up to 20% while reducing indigestible oligosaccharides (Wang *et al*., 2024). Similarly, co-cultures of *Lactobacillus plantarum* and *Saccharomyces cerevisiae* enhance the bioavailability of essential amino acids in plant-based feeds, making them more suitable for complex gastric animals (Perwez & Al Asheh, 2025). Beyond macronutrient improvement, fermentation also generates functional metabolites such as short-chain fatty acids and antioxidants which contribute to gut health and immune modulation in livestock (Senanayake *et al*., 2023). Despite these benefits, challenges remain in optimizing fermentation conditions for large-scale production, including strain selection, process standardization, and cost efficiency. Advances in biotechnology, adapted new microbes, nutritional programs and AI-driven fermentation modeling were hold promise for overcoming these barriers (Lu *et al*., 2025). Furthermore, the environmental benefits of fermented feedstuffs such as reduced methane emissions from ruminants and decreased reliance on synthetic additives align with global sustainability goals (Senanayake *et al*., 2023).

Due to the challenges facing the production of high-quality animal forages that meet the needs of producers, the current study aims to utilize aerobic bacteria isolated from the sheep rumen and dung to improve the nutritional composition of forages through solid-state fermentation. Additionally, the rumen (R) and dung (D) microorganisms were used individually, in combination (R+D), and both (R+D) supplemented with commercial strains (*Saccharomyces* and *Lactobacillus*) to ferment the forage, and evaluate their effect on nutritional composition of synergistic fermented forage, such as crude protein, crude fiber, fatty acids, electrolytic balance, and energy metrics analysis like protein digestible in the animal intestine (PDIA $_{\text{Ruminants INRA}}$), digestible energy for ruminants (DE $_{\text{Ruminants INRA}}$), etc.

MATERIALS AND METHODS

1-Sampling and Cultivation Protocols:

1.1-Collection of Rumen Samples:

Through the period from November, 2023 to July, 2024, rumen liquor samples were collected from six full healthy Menoufia (Menoufia Governorate, Egypt) sheep (3 males - 3 females) from sheep barn. The ages were 7-8 months and weighed average was 36.6 ± 2.4 kg. About 10 ml of rumen samples were gathered every time by stomach tube in sterile bags according to the method devolved by (Babayemi & Bamikole, 2006).

Samples were transferred immediately to the microbiology lab, faculty of agriculture, Menoufia University, Egypt. Fresh samples were used as starter inoculates or source for isolation of specific microorganisms by streaking on specific minerals culture media supplemented with filtrated rumen, while the cellulolytic bacteria was isolated on carboxymethyl cellulose (CMC) agar with orange die. All plates incubated aerobically at 37 ± 2°C for 24 hours according to (Deli *et al*., 2022).

The growth media contained 15 ml mineral solution I ($(NH_4)_2SO_4$ 6.0 g, NaCl 6.0 g, KH_2PO_4 3.0 g, $MgSO_4$ 0.6 g, $CaCl_2.2H_2O$ 0.795 g per/liter), 15 ml mineral solution II (K_2HPO_4 3 g/liter) in addition to 1 g tryptone, 0.25 g yeast extract, 0.5 g microcrystalline cellulose, 0.1 g cellobiose, 0.4 g sodium carbonate, 0.1 ml resazurin (0.1%), 20 ml clear rumen liquor, and 50 ml distilled water, the final pH was 7.2 and sterilized by autoclave at 110°C/30 min.

1.2-Isolation of Specific Rumen Microorganisms:

All single colonies appeared on specific media were picked up, purified, re-incubated at 37°C and kept after growth at 5°C for further studies. While for cultures preservation, nutrient agar slant was inoculated with the purified isolated yeast or bacteria and incubated at 37°C for 24 hours, then 15% glycerol was added in the top of culture tubes which were kept at -20°C until use.

1.3-Characteristic of Rumen Associated Microorganisms:

The morphological and biochemical characteristics were checked using Enterobacteriaceae diagnostic kits, catalase, and oxidase enzyme tests, moreover, proteolytic, lipolytic, and cellulolytic hydrolysis were used to roughly identify different major genera according to (Hemraj *et al*., 2013).

The isolated gram-positive long rod bacterial species with the most cellulolytic, lipolytic, and proteolytic activities selected from the rumen samples were given laboratory names CAB-R, LAB-R, and PAB-R, respectively. Likewise, the selected isolates from the dung samples were given laboratory names CAB-D, LAB-D, and PAB-D, respectively. The six selected isolates were used as starter inocula [250 mL (10^6 CFU/mL)/ kg forage] in the standard forage synergistic fermentation by each bacterial strain.

2-Feedstuff Composition and Forage Analysis:

Control forage (14% crude protein, crude lipids 4% and 4.5% crude fiber) is the same unfermented formula of commercial forage with usual ingredients. The feedstuffs treatment included 20% of

fermented substrates mixture of complete fermented forage. Forage was dried by exposure to non-elevated temperature for 48 hours at 40°C, sieved and stored at sealed bags until usage. Ingredient composition and proximate analyses of the experimental feedstuffs are mainly determined by NIR system (Near-Infrared Spectroscopy), and the chemical analyses of the various substrates were performed according to the methods of AOAC (2005).

3-Experimental Feedstuffs and Fermentation Protocols:

Feedstuff mixture composed of soybean meal (SBM), a common protein source for ruminants (34%), wheat bran (WB), a fibrous material used as a carbohydrate source (64%) and other potentially incorporates other ingredients (2%) such as activated charcoal. Weigh each ingredient based on the desired formulation. Feedstuff was solid state fermented with individuals or mixed isolates microorganisms' formula (*Streptococcus*, *Lactobacillus* and *Bacillus*) or obtained strains of *Lactobacillus plantarum* and *Saccharomyces cerevisiae* from Angela Company, China. While solid-state fermentation (SSF) with rumen liquor inoculant, the individual aerobic proteolytic and cellulolytic bacteria (wild type cultivars) and the superior isolates bacteria mixed with rumen liquor inoculant led to high production of carbohydrates and total protein, increased *in vitro* protein digestibility with a reduction of fiber content. Additionally, complete SSF with this bacterial species was chosen for diet production and fermented with individual *Lactobacillus plantarum* and/or *Saccharomyces cerevisiae* to modify the diet composition (Chebaibi *et al*., 2019).

For that purpose, 400 g of various substrates were autoclaved at 121°C/15 min and inoculated with 100 mL of bacterial suspension at a concentration of 1.0 x10^6 CFU/mL the humidity was adjusted to 50% (w/w), fermentation was done at 35°C for 48 hours. To ensure good aeration of the substrate it was mixed at least four times every day for the duration of the fermentation process according to the protocol of Vandenberghe *et al*. (2021).

After the fermentation process, the fermented forage samples were subjected to analyses of dry matter, moisture, ash, total sugars, starch, crude protein, crude fibers, lignin, acid detergent fiber (ADF), neutral detergent fiber (NDF), electrolytic balance, crude fats, fatty acids, saturated fatty acids, and unsaturated fatty acids using NIR system (Near-Infrared Spectroscopy) according to the official methods and procedures for animal feed noted by the association of official analytical chemists (AOAC, 2005). Furthermore, the energy metrics analysis including digestible energy (DE), metabolizable energy (ME), protein digestible in the intestine based on nitrogen (PDIN), feed conversion unit (UFV), protein digestible in the intestine by enzymes (PDIE), and PDIA protein digestible in the animal intestine according to the national agriculture research institute, France, of the fermented forage were performed using NIR system (AOAC, 2005; Choi *et al*., 2014).

Statistical Analysis:

Each experiment was carried out in triplicate and the values averages were recorded with its standard divisions. Probability value for the statistical test was 0.5% was used to compare the differences of the variables. On other side, the results were analyzed according to a completely randomized design where treatments were considered as fixed effects, testing linear and quadratic effects of microbial levels (Brandao *et al*., 2020).

RESULTS AND DISCUSSION

1. Total Aerobic Rumen and Sheep Dung Counts:

The total aerobic bacterial count in sheep rumen liquor samples were ranged from 10^6 - 10^8 CFU/mL with average of 10^7 CFU/mL. This value indicates a significant population of aerobic bacteria, which play a crucial role in rumen fermentation and nutrient metabolism. Although the rumen microbiome is a complex ecosystem

dominated mainly by anaerobic bacteria, the aerobic and facultative anaerobic bacteria also contribute to microbial activity. The presence of aerobic bacteria in the rumen may be attributed to oxygen ingress during feeding or microbial interactions. The recent studies have reported similar findings by Papakonstantinou *et al.* (2024) who observed that aerobic bacteria, including genera of gram positive *Streptococcus*, *Lactobacillus* and *Bacillus* species are mainly present in the rumen. These bacteria may assist in initial oxygen scavenging, creating a favorable environment for anaerobes. Also, Lu *et al*. (2025) found that aerobic bacterial populations fluctuate with diet, with higher counts in sheep fed high-fiber diets due to increased microbial diversity. In the same concept, Xu *et al*. (2023) highlighted that some aerobic bacteria in the rumen exhibit cellulolytic, lipolytic and proteolytic activities, contributing to fiber degradation. The aerobic bacterial count in this study aligns with previous research, suggesting that these microbes play a supplementary role in rumen function. Further studies using metagenomics could better characterize their metabolic contributions. While anaerobic bacteria dominate the rumen, aerobic bacteria are consistently present and may influence microbial dynamics. Future research should explore their functional roles under different dietary and physiological conditions. Also, the total aerobic bacterial count in sheep dung samples were ranged from 10^8 - 10^{10} CFU/g with average of 10^9 CFU/g.

The high density reflects the active microbial ecosystem involved in fermenting organic matter in the gastrointestinal tract. These higher counts (up to 10^{10} CFU/g) were linked to grain-rich diets, while forage-fed sheep showed slightly the dominant genera of culturable isolates included high number of *Enterobacter*, *Escherichia* as gram negative short rods and *Bacillus* and *Lactobacillus* as gram positive long rods with presence of essential facultative anaerobic short rods which represent in high proportions. These results in line with the results obtained by Franco *et al*. (2022) who reported total counts between 10^8- 10^{10} CFU/g in sheep dung, noting that aerobic bacteria aid in lignin degradation and nutrient cycling. However, previous investigation on the typical of herbivores revealed that dung harbors facultative anaerobic proteobacteria more than strict aerobes (Xu *et al*., 2023). However, Papakonstantinou *et al*. (2024) indicate that fiber-rich diets favor for *Bacillus* and *Lactobacillus* genera while high-protein diets increase the Enterobacteriaceae as factors influencing bacterial load in sheep intestinal which later contribute to soil fertility upon environmental decomposition reservoir with counts comparable to other ruminants. Our findings displayed that the most dominant bacterial species isolated from rumen liquor were lactic acid-producing bacteria, such as *Streptococcus* and *Lactobacillus*, which were few in number, but metabolically active, while the bacterial species in the dung were *Bacillus* and Enterobacteriaceae including *E. coli* are mainly dominate in dung due to oxygen exposure, which agreed the results reported by Lu *et al*. (2025). On other hand, yeast count in rumen liquor samples under aerobic condition were ranged from 10^3 - 10^5 CFU/mL with average of 10^4 CFU/mL. While the count in sheep dung samples ranged from 10^5 – 10^7 CFU/g with average of 10^6 CFU/g. It is clear that, yeasts are minor but metabolically active in the rumen and proliferate post-excretion in dung. However, the grain-fed sheep show higher yeast counts (~10^7 CFU/g) than pasture-fed (~10^5 CFU/g) due to soluble carbohydrate availability (Senanayake *et al*., 2023). In this context, the averages of cellulolytic, lipolytic, and proteolytic total count under aerobic conditions were 10^2, 10^3, and 10^6 CFU/mL, respectively in rumen liquor samples, while in the dung samples were 10^4, 10^5 and 10^8 CFU/g, respectively. These results are in line with those obtained by

Papakonstantinou *et al*. (2024), who reported that *Bacillus subtilis* dominant in dung, while *Bacillus subtilis* and *Paenibacillus polymyxa* dominate in the rumen (with cellulose and hemicellulose degradation activity) despite anaerobic conditions due to micro-oxide zones. By the way, *Bacillus megaterium* mainly secretes proteases that accelerate nitrogen recycling, *Bacillus cereus* secretes alkaline proteases, and *Bacillus licheniformis* isolated from dung secretes thermostable lipase as reported by (Deli *et al*., 2022). In addition, *Streptococcus bovis* with *Lactobacillus* sp. in rumen and dung were dominant and linked to lactic acid production with lipolytic and proteolytic super actions. While, most yeast species belong to *Saccharomyces* and *Candida* contributed to lipid and carbohydrates metabolisms (Lu *et al*., 2025)**.**

2. Synergistic Fermentation of Standard Forage by Isolated Bacteria:

The data presented in Figures (1, 2, and 3), discuss the results of the main parameters obtained from NIR system analysis for the standard fermented forage using the synergetic bacterial isolates from rumen, dung, rumen-dung, and rumen-dung supplemented with commercial *Saccharomyces* and *Lactobacillus* strains.

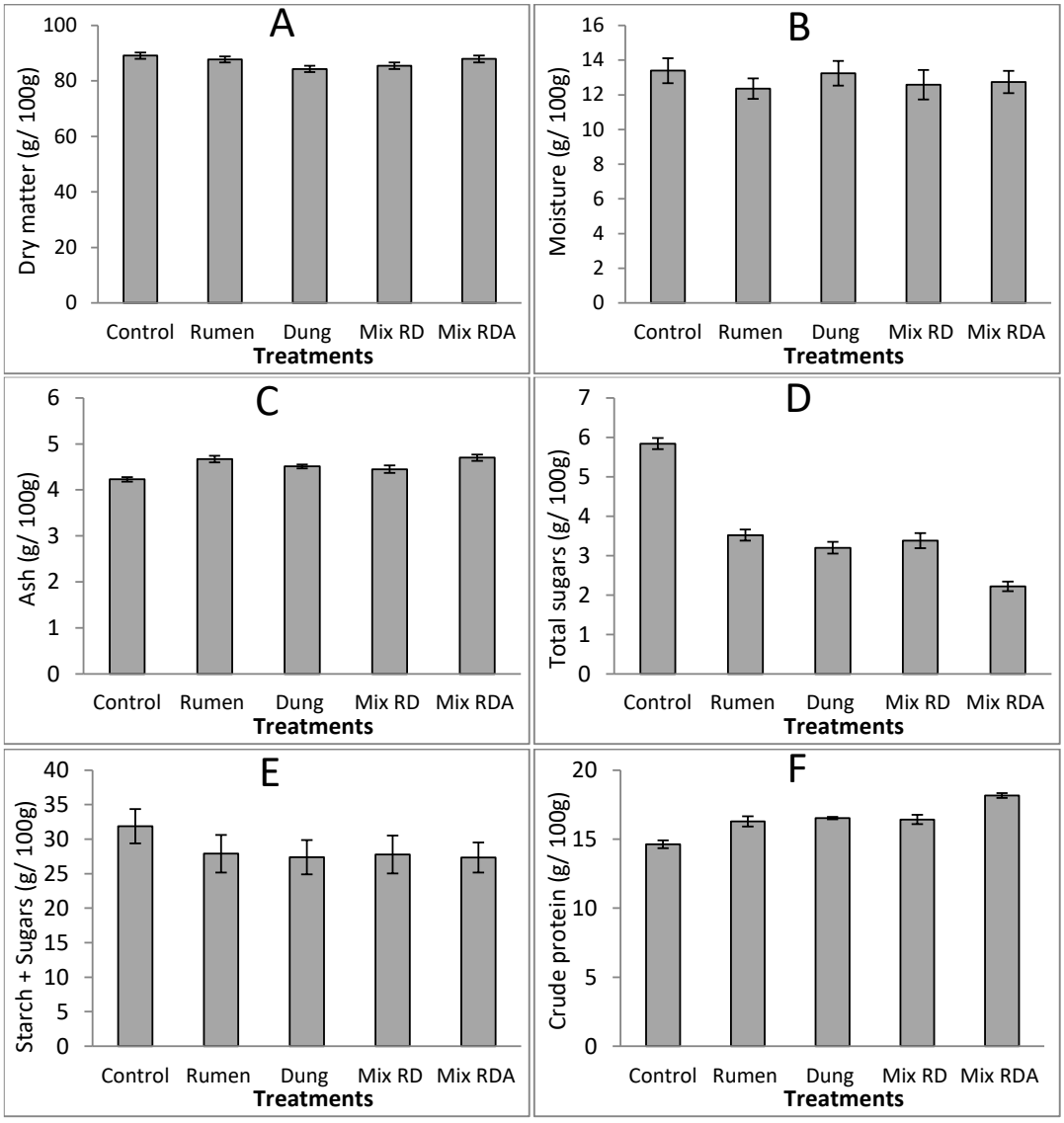

Fig. 1. The main parameters analysis including, dry matter (A), moisture (B), ash (C), total sugars (D), starch+ sugars (E), and crude protein (F) for the synergistic fermented forage.

2.1. Forage Fermentation by Synergistic Rumen Bacterial Isolates:

The study evaluated the effects of fermenting forage using synergistic bacteria isolated from rumen liquor ($Rumen_{syn}$) on its nutritional composition (Figs. 1, 2, and 3). The non-fermented forage served as the control, and the results revealed significant changes post-fermentation. Fermentation with $Rumen_{syn}$ bacteria reduced dry matter content by 1.5% and moisture by 7.7% (Figs. 1A and B), indicating microbial activity that likely degraded some organic components according to Jeon *et al*. (2024).

Moreover, a notable increase in crude protein (11.3%) (Figure 1F), and ash content (10.3%) (Fig. 1C), was observed, suggesting microbial synthesis of protein and mineralization during fermentation matched with Osorio-Doblado *et al*. (2023). The crude fiber content was decreased by 9.1% (Fig. 2A), while total sugars, and starch + sugars declined by 39.7% and 12.5%, (Figs. 1D and E), respectively. This reflects the bacteria's ability to break down complex fibers and sugars, as documented in studies on rumen microbiota (Weimer, 2015). Saturated fatty acids decreased significantly (42.5%) (Figure 3D), whereas unsaturated fatty acids showed minimal changes (Fig. 3C), aligning with findings that microbial fermentation alters lipid profiles in matching with Hiller (2014).

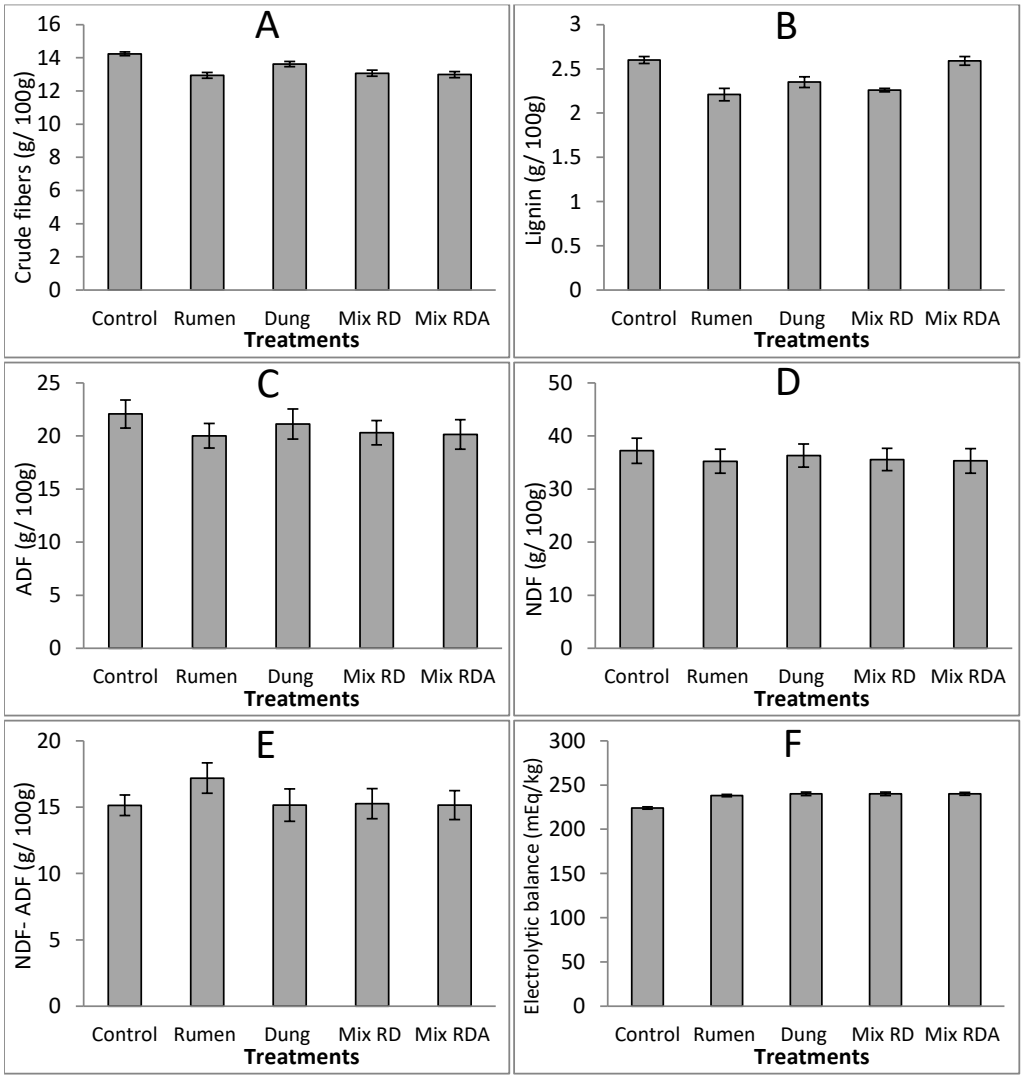

Fig. 2. Fibers analysis includes crude fibers (A), lignin (B), ADF (Acid detergent fiber) (C), NDF (Neutral detergent fiber) (D), NDF- ADF (E), and electrolytic balance (F) for the synergistic fermented forage.

2.2. Forage Fermentation by Synergistic Dung Bacterial Strain:

The fermentation of forage using synergistic bacteria isolated from dung ($Dung_{syn}$) resulted in notable changes in nutritional composition compared to the non-fermented control. The dry matter decreased by 5.4% (Fig. 1A), indicating microbial degradation of organic components. Moisture content showed a minor reduction (1.1%) suggesting less water loss compared to rumen-based fermentation (Fig. 1B). Crude protein increased by 12.9%, highlighting microbial protein synthesis (Fig. 1F). Ash content rose by 6.5%, likely due to mineral release during fermentation (Kung *et al*., 2008). While crude fiber decreased by 4.3%, NDF and ADF declined by 2.4% and 4.2%, respectively, indicating partial fiber breakdown (Figure 2A, C, and D). Total sugars and starch + sugars dropped sharply (45.2% and 14.1%, respectively), confirming microbial sugar utilization (Weimer, 2015). The crude fat decreased by 6.2%, with saturated fatty acids declining by 32.3% as illustrated in Figure (3A, and D), suggesting microbial lipolysis (Jenkins *et al*., 2008).

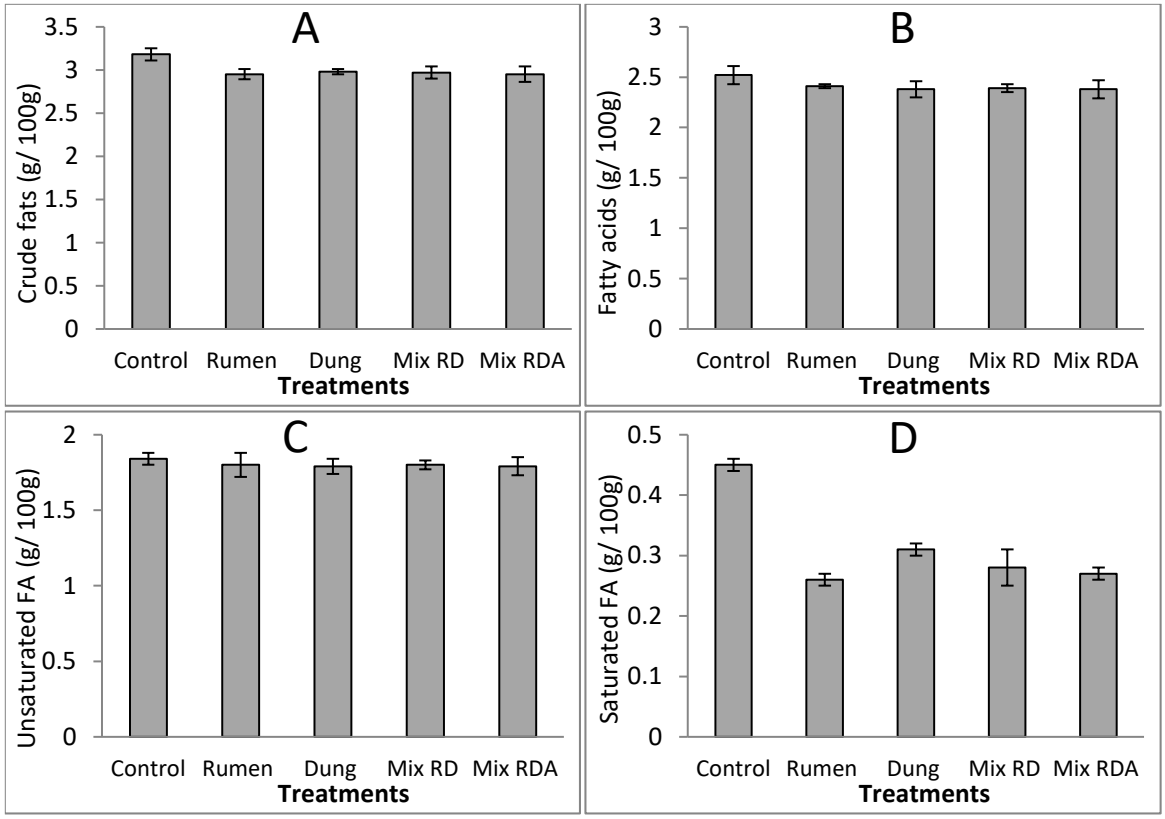

Fig. 3. Fats analysis including, crude fats (A), fatty acids (B), unsaturated fatty acids (C), and saturated fatty acids (D) for the synergistic fermented forage.

2.3. Forage Fermentation by Synergistic Rumen and Dung Bacterial Strain:

The forage fermentation using rumen and dung isolated bacteria (Mix RD-syn) caused intermediate changes in nutritional composition comparing to rumen-only. The dry matter decreased by 4.1%, slightly less than dung-only fermentation (5.4%) but more than rumen-only (1.5%). Moisture declined by 6.1%, indicating moderate water loss due to microbial activity. Crude protein increased by 12.2%, slightly lower than dung-only (12.9%) but higher than rumen-only (11.3%). Ash content rose by 5.2%, suggesting balanced mineral retention between the two bacterial sources. The crude fiber decreased by 8.2%, more than

dung-only (4.3%) but similar to rumen-only (9.1%), indicating enhanced fiber degradation (Fig. 2A). NDF and ADF declined by 4.4% and 8.0%, respectively, showing synergistic fiber breakdown. Total sugars and starch + sugars dropped by 42.1% and 12.9%, respectively, confirming efficient microbial carbohydrate utilization (Fig. 1D, and E). Crude fat decreased by 6.6%, with saturated fatty acids declining sharply (37.5%), similar to rumen-only fermentation. The mixed bacterial consortium (Mix $_{RD\ syn}$) demonstrated synergistic effects, particularly in fiber degradation (crude fiber: −8.2%) and protein enhancement (crude protein +12.2%). While rumen bacteria excel in fiber breakdown (Weimer, 2015), dung bacteria contribute to protein synthesis (Osorio-Doblado *et al*., 2023)**,** explaining the intermediate but balanced improvements.

2.4. Forage fermentation by RD Synergistic Bacteria Supplemented with Commercial Strains:

The forage was fermented using synergistic rumen and dung bacteria supplemented with *Saccharomyces cerevisiae* and *Lactobacillus plantarum* (Mix $_{RDA\ syn}$). The addition of commercial yeast and lactic acid bacteria to the mixed rumen-dung bacterial consortium (Mix $_{RDA\ syn}$) further enhanced forage fermentation, yielding the most pronounced improvements in nutritional quality among all treatments. The dry matter decreased modestly (-1.3%), the smallest reduction among all treatments, suggesting efficient microbial activity with minimal dry matter loss. Moisture declined by 4.9%, indicating stable fermentation with controlled water loss. Crude protein increased dramatically (24.1%), the highest among all treatments, highlighting the protein-enhancing effects of *Saccharomyces* and *Lactobacillus*. Ash content rose by 11.1%, suggesting enhanced mineral solubilization due to microbial activity. Crude fiber decreased by 8.8%, comparable to Mix $_{RD\ syn}$ (-8.2%), confirming sustained fiber degradation (Fig. 2A). NDF and ADF declined by 5.1% and 8.7%, respectively, reinforcing efficient fiber breakdown. Total sugars dropped sharply (-46.9%), the highest reduction observed, due to vigorous microbial sugar utilization by supplemented strains. The crude fat decreased by 7.1%, similar to other treatments. Saturated fatty acids declined significantly (-39.8%), while unsaturated fatty acids remained stable (-2.4%). About the energy metrics, digestible energy (DE) increased by 8.4%, slightly higher than Mix RD syn (7.8%). The metabolizable energy (ME) rose by 3.0%, consistent with other treatments. Feed unit (UFV) improved by 10.2%, the highest among all groups, indicating superior energy utilization. Also, protein digestibility, protein digestible in the intestine based on nitrogen (PDIN) increased by 10.7%, the highest gain, attributed to enzymatic activity from *Saccharomyces* and *Lactobacillus*. PDIE decreased by 7.4%, but the high PDIN suggests better microbial protein synthesis (Table 1). Electrolytic balance improved (+8.0%), supporting rumen health. The supplemented microbes play a vital role in amino acid synthesis with superior protein enhancement with 24.1% increase in crude protein in Mix $_{RDA\ syn}$ far exceeds other treatments, likely due to *Saccharomyces,* which contributes to microbial protein synthesis and amino acid production (Jeon *et al*., 2024). While *Lactobacillus* enhanced proteolysis and peptide availability (Osorio-Doblado *et al.*, 2023). Also, from the results recorded efficiency on fiber and sugar utilization. More than 46.9% reduction in total sugars (the highest among all groups) indicates vigorous fermentation by the supplemented strains. Fiber degradation (-8.8% crude fiber) remained strong, though not significantly better than Mix $_{RD\ syn}$, suggesting rumen bacteria still dominate fiber breakdown (Weimer, 2015).

3. The Energy Metrics of Synergistic Fermentation of Standard Forage:

From the presented data in Table (1)**,** digestible energy (DE) and metabolizable

energy (ME) for the rumen bacterial strain increased by 8.8% and 3.1%, respectively, enhancing the forage's nutritional value for ruminants (Jeon *et al*., 2024). The feed converts unit (UFV) also improved by 8.3%, indicating better energy utilization. Protein digestible in the intestine (PDIE) increased by 11.5%, while PDIA decreased by 11.2%, highlighting shifts in protein availability due to microbial action. Fermentation with $Rumen_{syn}$ bacteria enhanced the forage's protein and energy content while reducing fiber and sugars, making it more digestible for ruminants. These findings align with established research on microbial fermentation's role in improving forage quality (Osorio-Doblado *et al*., 2023).

While, dung bacterial strain, (DE) increased by 11.3%, and (ME) by 2.7%, improving forage energy availability. The UFV rose by 10.0%, indicating enhanced nutritional value. About protein digestibility, PDIN increased by 7.2%, while PDIA decreased by 5.0%, reflecting microbial modification of protein fractions. It is clear that dung-derived bacterial fermentation improved protein and energy availability while reducing fiber and sugar content, similar to rumen-based fermentation but with distinct efficiency in fiber degradation. These findings align with previous studies on microbial forage enhancement (Jeon *et al*., 2024).

Table 1: The energy metrics analysis for the synergistic fermented forage**.**

Forage Composition % ± SD	Forage N-Fermention	Rumen $_{syn}$ Fermention	Dung $_{syn}$ Fermention	Mix $_{RD\ syn}$ Fermention	Mix $_{RDA\ syn}$ Fermention
PDIA $_{Ruminants\ INRA}$ (g/Kg)	0.80±0.00	0.87±0.00	0.88±0.00	0.88±0.00	0.88±0.00
PDIE $_{Ruminants\ INRA}$ (g/Kg)	51.9±0.01	45.96±0.01	49.31±0.01	48.94±0.01	48.06±0.01
PDIN $_{Ruminants\ INRA}$ (g/Kg)	96.1±0.01	107.15±0.01	103.19±0.01	105.61±0.01	106.38±0.01
PDIA/PDIE (g/Kg) 0.45:0.50	104.0±0.01	99.51±0.01	101.48±0.01	100.26±0.01	97.66±0.01
PDIN/PDIE (g/Kg) 0.85-0.95	0.54±0.01	0.43±0.01	0.48±0.01	0.46±0.01	0.45±0.01
DE $_{Ruminants\ INRA}$ (Kcal/Kg)	2924±1.86	3181±4.66	3254±3.97	3152±2.88	3168±4.16
ME $_{Ruminants\ INRA}$ (Kcal/Kg)	2482±4.53	2558±3.44	2549±2.07	2554±4.02	2556±4.83
ME/DE (Kcal/Kg) 0.820-0.825	0.849±0.04	0.804±0.02	0.783±0.06	0.810±0.03	0.806±0.08
UFV $_{Ruminants\ INRA}$ (UF/Kg)	0.80±0.00	0.87±0.00	0.88±0.00	0.88±0.00	0.88±0.00

DE $_{Ruminants\ INRA}$: Digestible energy for ruminants according to National Agriculture Research Institute, France.

ME $_{Ruminants\ INRA}$: Metabolizable energy for ruminants according to National Agriculture Research Institute, France.

UFV $_{Ruminants\ INRA}$: Feed unit for meat for every kilogram according to National Agriculture Research Institute, France.

PDIA $_{Ruminants\ INRA}$: Protein digestible in the animal intestine according to National Agriculture Research Institute, France.

PDIE $_{Ruminants\ INRA}$: Protein digestible in the intestine by enzymes according to National Agriculture Research Institute, France.

PDIN $_{Ruminants\ INRA}$: Protein digestible in the intestine with based on nitrogen according to National Agriculture Research Institute, France.

Also, (DE) increased by 7.8% when the synergistic rumen and dung which slightly lower than dung-only (11.3%) but still significant. (ME) rose by 2.9%, comparable to both individual treatments. While the feed convers unit (UFV) improved by 10.1%, reinforcing the combined bacteria's ability to enhance forage quality. Also, PDIN increased by 9.9%, higher than dung-only (7.2%) and closer to rumen-only (11.5%). PDIA decreased by 5.7%, similar to dung-only (5.0%), suggesting microbial protein modification. The higher PDIN increase (9.9%) compared to dung-only suggests rumen bacteria's enzymatic role complements dung microbes' proteolytic activity. However, the lower DE increase (7.8%) than dung-only (11.3%) implies competition between microbial groups for substrates, slightly reducing energy yield. This aligns with studies showing mixed cultures may have variable efficiency (Kung *et al.*, 2008). The Mix $_{RD\ syn}$ approach leverages the strengths of both bacterial sources, offering a balanced improvement in fiber digestibility, protein content, and energy availability. For applications prioritizing fiber breakdown over maximal protein or energy gain, this mixed fermentation is highly effective. In this context, the supplementation by commercial strains has a perfect metrics, the energy and digestibility showed highest UFV (10.2%) which confirms the improved energy availability, likely due to better starch and sugar conversion by *Saccharomyces*. The PDIN increase (10.7%) suggests superior protein digestibility, critical for ruminant performance (Jeon *et al*., 2024).

Conclusion

This study provides a comprehensive analysis of the aerobic bacterial populations in sheep rumen and dung, revealing their critical roles in nutrient metabolism, fiber degradation, and microbial ecosystem dynamics. The aerobic bacterial counts in rumen liquor (10^6-10^8 CFU/mL) and dung (10^8–10^{10} CFU/g) highlight the presence of metabolically active microbial communities, including key genera such as *Streptococcus*, *Lactobacillus*, and *Bacillus*. The present study demonstrated that the aerobic solid-state fermentation using synergistic bacterial isolates from rumen and dung (individually or in combination) of sheep significantly improves forage quality by increasing crude protein, reducing fiber content, and improving energy availability. Moreover, the addition of *Saccharomyces* and *Lactobacillus* to the mixed rumen and dung bacteria resulted in the most substantial nutritional enhancements, including a 24.1% increase in crude protein, 12.99 % reduction in fibers, 46.9% reduction in sugars, and increase digestible energy. Implementing such microbial solutions could lead to sustainable advancements in animal nutrition, reducing reliance on synthetic additives while improving livestock productivity and health. These results motivate the animal feed sector to seek affordable ways to incorporate fermented foods into the diet.

Declarations:

Ethical Approval: Not applicable.

Authors Contributions: Amr M.A. Elmasry, Adel Elsayed Elbeltagy, Sameh F. Fahim and Dina R. ElSharkawy. conceptualized the study. Wafaa Hanafy, Ali Abdelmoteleb, Amr M.A.Elmasry, Sameh F. Fahim and Dina R. ElSharkawy carried out the methodology, data collection, and analysis. Ali Abdelmoteleb, Amr M.A. Elmasry Sameh F. Fahim and Dina R. ElSharkawy prepared the initial manuscript draft. Ali Abdelmoteleb, Amr M.A.Elmasry and Dina R. ElSharkawy reviewed and edited the manuscript. All authors approved the final published version.

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authorities at the Agricultural Microbiology and Biotechnology, Botany Department, Faculty of Agriculture, Menoufia University, Shibin El-Kom, Egypt where the work has been carried out, before the work is submitted.

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ARABIC SUMMARY

رفع القيمة الغذائية للأعلاف بواسطة تقنيات التخمير الميكروبي التعاوني

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يُمثل التخمير الميكروبي التعاوني نهجًا تحوليًا لتعزيز القيمة الغذائية للأعلاف، مع ما يترتب عليها من آثار كبيرة على صحة الحيوانات وإنتاجيتها واستدامة البيئة. حيث ينبغي أن تُركز الأبحاث المستقبلية على زيادة الإنتاج، وتحسين التعاون الميكروبي، ودمج تقنيات التخمير في أطار الاقتصاد الحيوي المستمر. بحثت هذه الدراسة في مجموعات البكتيريا الهوائية في كرش وروث الأغنام، حيث كشفت عن التعداد الكلي ١٠^٧ مستعمرة/مل و١٠^٩ مستعمرة/جرام على التوالي، مع تواجد للأجناس السائدة والتي تشمل الباسيلس، واللاكتوباسيلس، والبينيباسيلس. حيث تلعب هذه البكتيريا دورًا رئيسيًا في تحلل الألياف وتمثيل العناصر الغذائية. وقد أدى تخمير العلف القياسي باستخدام عزلات بكتيرية متعاونة معزولة من الكرش (Rumen syn) والروث (Dung syn) ومزيجهما (Mix RD-syn) إلى تغيير كبير في التركيب الغذائي. وأدى التخمير بواسطة ميكروبات الكرش إلى زيادة البروتين الخام بنسبة 11.3% وانخفاض الألياف بنسبة 9.1%، بينما عزز التخمير بواسطة ميكروبات الروث الى زيادة البروتين بنسبة 12.9% ووفرة في الطاقة المقدرة. وقد وازنت المجموعة المختلطة (مزيج RD-syn) هذه التأثيرات، محسّنةً تحلل الألياف بنسبة (8.2%) ومحتوى البروتين الخام (12.2%). وقد حققت الإضافات الميكروبية التجارية من سلالات الخميرة والاكتوباسيلس (المزيج RDA-syn) أعلى زيادة في البروتين الخام (24.1%) وانخفاضًا في استهلاك السكر(46.9%)، إلى جانب إثراء فائق بالأحماض الأمينية. وتُبرز النتائج إمكانات المجموعات الميكروبية المُصممة خصيصًا لتحسين جودة الأعلاف، حيث يُقدم المزيج المُكمل أهم التحسينات الغذائية لأعلاف المجترات.