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Influence of Different Biological Treatments on Fermentation of Soyabean and Sunflower Meals

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Abstract: This study examines fermented soya bean (SBM) and sunflower meal (SFM) with three biological treatments: *Aspergillus Oryza*, *Ruminococcus flavefaciens* and *Lactobacillus plantarum*, as well as the effects of these treatments on chemical composition, cell wall constituents, *in vitro* dry matter disappearance (IVDMD), and enzyme activity of the fermented SBM and SFM. After 2 h of ruminal incubation, all biological treatments (fungi, Bacteria, fungi with Bacteria) improved the IVDMD for Fermented SBM compared to that of the control (19.47, 20.65 and 18.38%, respectively). The maximum improvement values of IVDMD for SBM were recorded after 4 h and 6 h of incubation for all treatments. In addition, SFM after 2 h of ruminal incubation with any examined biological treatment (fungi, Bacteria, fungi with Bacteria), the IVDMD values have improved compared to that of the control group. Moreover, enzyme activities (cellulase, amylase, xylanase, and protease**)** have increased in all treatments. It was determined that SSF with *A. oryzae* and *Ruminococcus* boosted the protein content and digestibility of soybean and sunflower meals.

1 Introduction

Utilizing filamentous fungi and bacteria on industrial byproducts, solid-state fermentation provides a practicable and eco-friendly alternative for the production of technically significant extracellular enzymes. The food and animal feed industries have long regarded soybean meal (SBM), a by-product of soybean oil refinement, as an important source of protein due to its high protein content (40–50%) and balanced amino acid (AA) composition (Steudler et al 2019, Lu et al 2022). However, soybean meal contains antinutritional factors that inhibit nutrient absorption and utilization during digestion, thereby reducing the nutritional value of feeds designed for juvenile animals (Abdel-Raheem et al 2023). Sunflower meal is the fourth-largest oil meal; this protein source is commonly used in animal, poultry, and swine rations. Methionine and cysteine are sulfur-containing, amino acids that are utilized as an alternative protein source for livestock and monogastric animals. SFM contains fewer antinutritional components than other plant-based meals. SFM is a substantially more cost-effective source of protein and a suitable substitute for SBM in feed formulation (Yaqoob et al 2022). Due to its significant amount of crude fiber, SFM can not be

used as a dietary supplement. Utilizing solid-state fermentation, which has been utilized to reduce ANFs, has enhanced the nutritional profile of food items and agro-industrial byproducts (Olukomaiya et al 2020). In solid-state fermentation (SSF), microorganisms degrade macromolecules into smaller compounds. Bacillus species, for example, can improve the nutritional value of soybeans by decreasing the anti-nutritional factor content *via* SSF (Suprayogi et al 2022). The fermentation process increases the nutritional value of SBM by removing antinutritional substances (Draźbo et al 2020). Soybean fermentation resulted in the eradication of 33% of its phytic acid content (Nualkul et al 2022). The genome sequence for the *Aspergillus oryzae* strain has been determined. This strain is capable of producing amylase and protease (Chacón-Vargas et al 2021). In fermentation processes, *Lactobacillus brevis* and *Aspergillus oryzae* are frequently employed. Furthermore, microorganism fermentation can reduce the cytotoxicity of herbal extracts (Chen et al 2022). Lactobacillus sp. has been used for a long time to produce dairy products, vegetation, and feedstuffs in recent usage as a probiotic (Li et al 2022). The present research seeks to increase the nutrient content of certain feedstuffs for safe use in ruminant feeding by fermentation with bacterial and fungal species.

2 Materials and Procedures

2.1 Types of Strains

2.1.1 *Aspergillus Oryza*

(EMCC Number: 163) was obtained from the Laboratory of Microbiology, Department of Microbiology Ain Shams University.

2.1.2 *Ruminococcus flavefaciens*

Ruminococcus flavefaciens is an anaerobic bacterial strain that has been isolated from rumen fluid and obtained from the Animal production department of Ain Shams University.

2.1.3 Lactic acid bacteria

(*Lactobacillus Plantarum)* were obtained from Ain Shams University's Department of Microbiology. has been isolated from a variety of environments, including plants, human gastrointestinal systems, animals, poultry, and insects (Martino et al 2016).

2.2 Solid-state fermentation

Components of soybean meal and sunflower meal were fermented in two steps:

First: 10 g of *Aspergillus Oryza* (10^{10} CFU), or 10 g of *Ruminococcus flavefaciens* (10^{8} CFU) was added to 1000 g feedstuff then water was added to the components until hydration became 45% (100 g feedstuff added to 80 ml water) then fermented at 30° C for 24 hours.

Second: 4 grams of *Lactobacillus Plantarum* (10^{9} CFU) and water were added to the components until the moisture content reached 60 percent (100 grams of feed added to 30 ml of water) and then fermented for 16 hours at 37° C. The mixture was then desiccated at 45°C for 24 hours (Chen et al 2010).

2.3 Proximate analysis

On the basis of (AOAC 2023), a proximate analysis of feed material and its constituents was performed to determine dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF), Ash content and NFE (100-(DM+CP+EE+CF+Ash). The fiber proportion was determined in accordance with Van Soest et al (1991).

2.4 Analysis of enzyme activity

2.4.1 Method for sample preparation

Added 0.5 g of feedstuff (SBM or SFM) to a 250-ml flask and brought the volume to 100 ml by adding distilled water.

2.4.2 Protease activity

Using the method of Chopra and Mathur (1983), the protease activity of the culture supernatant was evaluated.

2.4.3 Amylase activity

The activity of amylase was measured in accordance with Liu et al (2015).

2.4.4 Cellulase activity

The cellulase activity was measured by the method of Ghose (1987) with modifications.

2.4.5 Xylanase activity

Xylanase activity was determined using the Bailey et al (1992) method.

2.5 Characteristics of the *in vitro* fermentation process

The rate of DM loss *in vitro* in fermented feed was determined using fermented feed samples, the effects of *Ruminococcus sp*., *Aspergillus Oryza* and *Ruminococcus sp*. in conjunction with *Aspergillus Oryza* and *Lactobacillus sp*. on the rate of DM extinction were evaluated. Each sample was incubated for two, four, six, twelve, twenty-four, forty-eight, seventy-two, ninety and one hundred hours. Three blank containers were prepared for each incubation time; each sample consisted of three replicates.

2.6 Statistical examination

The experimental data were analyzed using a one-way ANOVA (SPSS V.20) (Verma 2013) to determine the differences between regimens. Using Duncan's multiple range tests and analysis of variance, significant differences (P> 0.05) between regimens were determined. The following model was used to calculate the differences between treatment groups:

$$Y_i = \mu + T_i + e_{ij}$$

Where Y_i: represents the dependent variable, represents the aggregate mean, μ is the overall mean, T_i: represents the treatment effect and e_{ij}: represents the residual error.

3 Results and Discussion

3.1 Soybean meal solid-state fermentation

The chemical structure of control SBM and fermented SBM are shown in **Table 1**. Due to the increased content of fiber in fermented SBM, the CF, NDF, and ADF increased (P<0.05) where fungi were growing on barley grain containing 4% CF. Fermented SBM contained 48.19% more CP than unfermented SBM (44.69%), and the fermentation procedure had a significant effect on the dry matter and ether extract (EE) content of fermented SBM (P<0.05). The most beneficial treatment was fermented soya bean meal containing bacteria that increase CP, EE, and NFE. The results in consistent with those observed by Jazi et al (2018).

In accordance with Sharawy et al (2016), the fermentation process enhanced the chemical composition of SBM. In addition to increasing the concentration of CP, microbial fermentation of SBM significantly reduced the concentrations of phytic acid, trypsin inhibitor, β-conglycinin, and glycinin; these findings are comparable to those of Sharawy et al (2016). Consistent with the results of Draźbo et al (2020), fermented soybean meal with *Aspergillus oryzae* for 48 hours, there is an increase in protein concentration induced by fermentation. The protein increase may have been caused by the fermentation process using the SBM's protein and carbohydrates for microbial growth (Chen et al 2010).

The lactobacillus bacteria make the medium acidic and extend the product's expiration life. During SBM fermentation, Aspergillus species may have secreted protease and carried out protein and amino acid proteolysis to stabilize the pH level but produced no organic acid at this stage. *Aspergillus oryzae* strain can secrete amylase and protease; in addition, SBM infected with *Aspergillus oryzae* enhanced the activity of the enzyme α-galactosidase during early fermentation where their intermediate products serve as a carbon source for the growth of *Lactobacillus sp*. subsequently during fermentation (Chen et al 2010). Fermented SBM has great potential to be a protein source for aquafeed due to its higher contents of crude protein compared to SBM control (Shiu et al 2015).

3.2 *In vitro* DM disappearance and enzyme activities of SBM

Table 2 illustrates the effect of solid-state fermentation on the *in vitro* disappearance of SBM DM. At 2, 4, and 24 hours of incubation, the results demonstrated a statistically significant difference (P< 0.05), but not at longer times i.e. 48, 72, 90, or 120 hours. After two hours, fermented SBM improved IVDMD values (19.47, 20.65, and 18.38%, respectively) compared to the control diet. *In vitro*, dry matter loss was greatest after 4 and 6 hours for all interventions, with values ranging from 18.42% to 29.12%. After 24 hours, the improvement in IVDMD values was greater than after 2, 4, and 6 hours for all interventions. After 48 to 120 hours of incubation, the IVDMD values for all treatments were high but not statistically significant. Bacteria plus fungi had the highest value after 24 hours (88.25%) while after 48 to 120 hours of incubation, IVDMD values were high but not statistically significant; these results were comparable to what Saeed et al (2018) observed, who stated that *Ruminococcus sp*. participates in the digestion of coarse fibers efficiently.

Table 1. Chemical structure of dry matter (DM basis %) of Soybean meal

Treatment	Chemical Analysis (%)						Cell wall constitutes (%)	
	DM	CP	CF	EE	Ash	NFE	NDF	ADF
Control	89.50^{b}	44.69^{c}	4.39^{d}	5.59^{b}	1.79^{a}	33.04 ^{a}	14.95^{c}	8.19^{b}
Fungi	92.38^{a}	47.50^{ab}	5.56^{c}	7.22^{a}	1.77^{a}	30.32 ^{b}	17.02^{b}	10.78^{a}
Bacteria	92.34^{a}	48.19^{a}	6.8^{a}	7.63^{a}	1.81^{a}	27.91 ^{c}	18.45^{a}	11.80^{a}
Bacteria + Fungi	92.12^{a}	47.43^{b}	6.29^{b}	7.66^{a}	1.77^{a}	28.95 ^{bc}	17.09^{b}	10.85^{a}
Mean	91.58	46.95	5.76	7.02	1.78	30.05	16.88	10.4
SEM	0.618	0.308	0.045	0.227	0.104	0.968	0.369	0.65
SD	1.42	1.44	0.95	0.92	0.11	2.25	1.36	1.56

Control: unfermented soya bean meal, fungi: fermented soya bean meal with *Aspergillus oryzea+ lactobacillus*, bacteria: fermented soya bean meal with *Ruminococcus + Lactobacillus*, bacteria + fungi: fermented soya bean meal with *Aspergillus oryzea+ Ruminococcus + Lactobacillus*

M: moisture, DM: dry matter, CP: crude protein, CF: crude fiber, EE: ether extract, Ash: crude ash, NFE: nitrogen-free extract, NDF: neutral detergent fiber, ADF: acid detergent fiber.

$^{a, b, c, and d}$ Means within the same row with different superscripts significantly different (P<0.05). Where: S.E is the standard error

Table 2. *In vitro* Dry Matter disappearance (DM basis %) of Soybean meal

Treatment	In Vitro DM Disappearance %							
	2 hrs	4 hrs	6 hrs	24 hrs	48 hrs	72 hrs	90 hrs	120 hrs
Control	47.55^{b}	50.15^{c}	63.45^{a}	83.85^{b}	96.99^{a}	97.88^{a}	97.51^{ab}	98.61^{a}
Fungi	56.81^{a}	64.48^{a}	64.62^{a}	86.44^{ab}	95.95^{a}	96.97^{a}	98.67^{a}	98.00^{a}
Improv. %	19.47	22.22	1.8	2.99	-	-	-	-
Bacteria	57.37^{a}	61.48^{b}	64.80^{a}	86.44^{ab}	93.45^{a}	94.34^{b}	94.93^{b}	96.58^{b}
Improv. %	20.65	18.42	2.12	3.18	-	-	-	-
Bacteria + Fungi	56.29^{a}	61.18^{b}	64.36^{a}	88.25^{a}	96.19^{a}	98.55^{a}	97.99^{ab}	99.00^{a}
Improv. %	18.38	21.99	1.43	5.24	-	-	-	-
Mean	54.5	59.32	64.31	86.24	95.64	96.93	97.27	98.04
SEM	1.041	0.749	2.404	2.129	1.522	0.839	1.392	0.5
SD	4.35	5.75	2.57	6.10	2.11	1.89	2.07	1.09

Control: unfermented soya bean meal, fungi: fermented soya bean meal with *Aspergillus oryzea+ lactobacillus*, bacteria: fermented soya bean meal with *Ruminococcus + Lactobacillus*, bacteria + fungi: fermented soya bean meal with *Aspergillus oryzea+ Ruminococcus + Lactobacillus*

$^{a, b, c, and d}$ Means within the same row with different superscripts significantly different (P<0.05). Where: S.E is the standard error

Table 3 displays the enzyme activity of α-amylase, cellulase, protease, and xylanase in SBM. The enzymes were produced by solid-state fermentation; the control SBM enzyme concentrate contained 36333.33 IU/kg α-amylase, 368333.33 IU/Kg cellulase, 182333.33IU/Kg protease, and 392333.33 IU/Kg xylanase. In addition, the enzyme activity in SBM fermented with *Aspergillus oryzea* was 44333.33IU/kg α-amylase, 427333.33 IU/Kg cellulase, 368333.33 IU/Kg protease, and 403333.33 IU/Kg xylanase. All fermented SBM interventions increased the activity of cellulase and xylanase significantly (p<0.05) compared to control SBM.

In the *Ruminococcus sp*. fermented SBM, enzyme activities were 44333.33 IU/kg for α-amylase, 428333.33 IU/kg for cellulase, 362333.33 IU/kg for protease, and 413333.33 IU/kg for xylanase. The results suggest that the rise in IVDMD and CP in fermented SBM may be attributable to the increased proliferation of bacteria and fungi in solid-state soybean meal, which decreased CF by producing cellulase and xylanase enzymes. In the starch processing industries, amylase was used to convert polysaccharides into sugars (Sadh et al 2018).

Table 3. Enzyme activity of Alpha amylase, Cellulase, Protease, and xylanase in Soybean meal

Treatment	Enzymes IU/KG			
	Alpha amylase	**Cellulase**	**Protease**	**Xylanase**
Control	**36333.33^{c}**	**368333.33^{c}**	**182333.33^{d}**	**392333.33^{d}**
Fungi	44333.33^{a}	427333.33^{b}	368333.33^{a}	403333.33^{c}
Improv. %	22.01	16.01	102.01	2.8
Bacteria	44333.33^{a}	428333.33^{b}	362333.33^{b}	413333.33^{a}
Improv. %	22.01	16.281	98.72	5.35
Bacteria + Fungi	42333.33^{b}	428666.67^{a}	304333.33^{c}	408333.33^{b}
Improv. %	16.51	16.38	66.91	4.07
Mean	41833.33	413166.67	304333.33	404333.33
SEM	471.405	471.405	471.405	471.405
SD	3459.725	27044.856	78064.464	8138.945

Control: unfermented soya bean meal, fungi: fermented soya bean meal with *Aspergillus oryzea+ lactobacillus*, bacteria: fermented soya bean meal with *Ruminococcus + Lactobacillus*, bacteria + fungi: fermented soya bean meal with *Aspergillus oryzea+ Ruminococcus + Lactobacillus*

[a, b, c, and d] Means within the same row with different superscripts significantly different (P<0.05). Where: S.E is the standard error

3.3 Fermentation of sunflower meal (SFM) in solid state

Table 4 exhibits the chemical composition of the fermented SFM samples. The values of CP and NFE were significantly higher in fermented SFM than in the control group ($p < 0.05$). The CP and NFE of fermented SFM with fungi increased to 26.32% and 28.94%, while with bacteria they increased to 27.45% and 26.36% respectively. However, with fermented SFM fungi and bacteria, the CP and NFE increased to 27.12% and 30.16% respectively. The values of CF, NDF, and ADF in fermented SFM were significantly lower than in the control group ($p < 0.05$). The CF, NDF, and ADF of fermented SFM with fungi increased by 21.01, 16.52, and 11.24 percent compared to those control, whereas the CF, NDF, and ADF of fermented SFM with fungi and bacteria increased by 20.30, 14.79, and 12.03%, respectively.

Fermented sunflower meal with fungi and bacteria plus fungi had the highest value improvement of CF, NDF, ADF, and CP. Solid-state fermented SFM feedstuff increased cellulase and xylanase activities with increased CP content and decreased CF, NDF, and ADF.

The present study showed that the significant increase in the crude protein content of SFM may be attributable to the presence of fungi and bacteria during the biological production of fermentation. By fermenting soybean meal with *Aspergillus oryzae*, the microorganisms, that have been recognized as a rich source of enzymes, increased its crude protein content (Draźbo et al 2020). *Aspergillus oryzae, Ruminococcus sp*., and *Lactobacillus sp*. contribute to the fermentative effect of SSF. On non-starch polysaccharides and other complex carbohydrate structures within the matrix of sunflower meal, the current study observed a significant decrease in fiber content. The paucity of high molecular weight polypeptides in fermented soybean meals could be due to the proteolytic breakdown of polypeptide chains; this is likely caused by the SSF process, which involves the production of many enzymes that break down the fiber. Our results align with those of Hassaan et al (2017) who found a decrease in the fiber content of *Bacillus sp*. fermented grains, which functioned synergistically as multiple enzymes secreted during the fermentation process.

3.4 *In vitro* DM disappearance and enzyme activities of SFM

In vitro DM elimination of SFM was demonstrated in **Table 5**. At 2, 4, 6, 24, 48, 90, and 120 hours of incubation; the *in vitro* dry matter disappearance (IVDMD) significantly increased ($p < 0.05$). *In vitro* DM disappearance of sunflower meal control after 2 hours was 26.60%, while in fermented SFM was enhanced to 20.82% with fungi, 23.12% with bacteria, and 34.63% with bacteria plus fungi. After 6 hours of incubation, the IVDMD of unfermented SFM (control) increased to 33.55%, while fermented SFM was enhanced with fungi (27.83%), bacteria (22.23%) and bacteria plus fungi (25.15%) compared to control SFM.

Table 4. Chemical structure of dry matter (DM basis %) of Sunflower Meal

Treatment	Chemical Analysis (%)						Cell wall constitutes (%)	
	DM	CP	CF	EE	Ash	NFE	NDF	ADF
Control	88.13^{b}	24.45^{b}	32.65^{a}	2.78^{a}	6.19^{a}	33.91^{a}	63.11^{a}	36.55^{a}
Fungi	91.34^{a}	26.32^{ab}	25.76^{b}	2.48^{a}	7.84^{a}	37.59^{b}	52.68^{c}	32.44^{b}
Improv. %	-	7.64	21.01	-	-	-	16.52	11.24
Bacteria	91.90^{a}	27.45^{a}	27.71^{b}	2.26^{a}	8.11^{a}	34.46^{ab}	56.03^{b}	31.46^{b}
Improv. %	-	12.26	15.13	-	-	-	11.21	14
Bacteria + Fungi	92.34^{a}	27.12^{ab}	26.02^{b}	2.40^{a}	6.64^{a}	37.81^{b}	53.77^{bc}	32.15^{b}
Improv. %	-	10.92	20.3	-	-	-	14.79	12.03
Mean	90.93	26.34	28.03	2.48	7.19	35.94	56.4	33.15
SEM	0.62	1.2	1.82	0.85	0.91	1.53	1.2	0.88
SD	1.84	1.75	3.46	0.91	1.27	2.44	4.42	2.28

Control: unfermented sunflower meal, fungi: fermented sunflower meal with *Aspergillus oryzea+ lactobacillus*, bacteria: fermented sunflower meal with *Ruminococcus + Lactobacillus*, bacteria + fungi: fermented sunflower meal with *Aspergillus oryzea+ Ruminococcus + Lactobacillus*

M: moisture, DM: dry matter, CP: crude protein, CF: crude fiber, EE: ether extract, Ash: crude ash, NFE: nitrogen-free extract, NDF: neutral detergent fiber, ADF: acid detergent fiber.

[a, b, c, and d] Means within the same row with different superscripts significantly different (P<0.05). Where: S.E is the standard error

Table 5. *In vitro* Dry matter disappearance (DM basis %) of Sunflower meal

Treatment	*In Vitro* DM Disappearance %							
	2 hrs	4 hrs	6 hrs	24 hrs	48 hrs	72 hrs	90 hrs	120 hrs
Control	26.60^{c}	30.32^{c}	33.55^{b}	34.56^{c}	35.42^{c}	41.98^{b}	42.08^{d}	29.54^{c}
Fungi	32.14^{b}	37.74^{b}	42.89^{a}	42.99^{b}	43.45^{b}	49.65^{a}	51.09^{c}	64.32^{a}
Improv. %	20.82	24.45	27.83	24.39	22.67	18.26	21.41	117
Bacteria	32.75^{ab}	38.21^{b}	41.01^{a}	45.78^{a}	49.56^{a}	52.45^{a}	55.87^{b}	65.56^{a}
Improv. %	23.12	26.02	22.23	32.46	39.92	24.94	32.77	121
Bacteria + Fungi	35.74^{a}	39.42^{a}	41.99^{a}	46.12^{a}	50.78^{a}	52.56^{a}	58.56^{a}	61.65^{b}
Improv. %	34.63	30.01	25.15	33.44	43.36	25.2	39.16	108
Mean	31.8	36.43	39.86	42.36	44.8	49.16	51.9	55.27
SEM	1.373	0.264	1.958	0.906	1.097	1.225	0.651	0.624
SD	3.73	3.75	4.38	4.96	6.46	4.68	6.59	15.60

Control: unfermented sunflower meal, fungi: fermented sunflower meal with *Aspergillus oryzea+ lactobacillus*, bacteria: fermented sunflower meal with *Ruminococcus + Lactobacillus*, bacteria + fungi: fermented sunflower meal with *Aspergillus oryzea+ Ruminococcus + Lactobacillus*

[a, b, c, and d] Means within the same row with different superscripts significantly different (P<0.05). Where: S.E is the standard error

After 24 hours of incubation, the IVDMD of unfermented SFM (control) increased to 34.56%, while in fermented SFM was 42.99% (fungi), 45.78% (bacteria) and 46.12% (bacteria plus fungi). Although IVDMD values of fermented solid-state SFM were high after 48, 72, 90 and 120 hours, these values were not statistically significant when compared to respective values after 24 hours of incubation. The best (highest) improved values of IVDMD were 43.45 and 50.78% for fermented SFM with fungi or fungi plus bacteria respectively after 48 hrs incubation. This observation was similar to what was observed by Saeed et al (2018) who reported that *Ruminococcus flavefaciens* has a role in the digestion of coarse fiber and can digest fiber rapidly and thoroughly.

Table 6 lists detailed α-amylase, cellulase, protease, and xylanase activities. The enzyme activity of the control was 32333.33 IU/kg for α-amylase, 302333.33 IU/Kg for cellulase, 238333.33IU/Kg for protease and 341333.33 IU/Kg for xylanase, whereas the enzyme activities of the SFM fermented with *Ruminococcus sp.* were for α-amylase 66333.33 IU/kg, cellulase 325333.33 IU/kg, protease 360333.33 IU/kg and xylanase 405333.33 IU/kg; the values were significantly greater than SFM in the control group ($p < 0.05$).

Table 6. Enzyme activity of Alpha amylase, Cellulase, Protease, and xylanase in Sunflower meal

Treatment	Enzymes IU/KG			
	Alpha amylase	Cellulase	Protease	Xylanase
Control	32333.33[d]	302333.33[d]	238333.33[c]	341333.33[c]
Fungi	52333.33[c]	318333.33[c]	366333.33[a]	411333.33[a]
improvement%	61.85	5.29	53.7	20.5
Bacteria	66333.33[a]	325333.33[a]	360333.33[b]	405333.33[b]
improvement%	105.15	7.6	51.18	18.75
Bacteria + Fungi	58333.33[b]	322333.33[b]	361000[b]	405333.33[b]
improvement%	80.41	6.61	51.46	18.75
Mean	52333.33	317083.33	331500	390833.33
SEM	471.405	471.405	816.497	471.405
SD	13137.96	9278.11	56240.55	29963.11

Control: unfermented sunflower meal, fungi: fermented sunflower meal with *Aspergillus oryzea+ lactobacillus*, bacteria: fermented sunflower meal with *Ruminococcus + Lactobacillus*, bacteria + fungi: fermented sunflower meal with *Aspergillus oryzea+ Ruminococcus + Lactobacillus*

[a, b, c, and d] Means within the same row with different superscripts significantly different (P<0.05). Where: S.E is the standard error

In addition, there were 52333.33 IU/kg α-amylase, 318333.33 IU/Kg cellulase, 366333.33 IU/Kg protease, and 411333.33 IU/Kg xylanase in SBM fermented with *Aspergillus oryzea*. All fermented SBM interventions increased the activity of cellulase, and xylanase significantly ($p<0.05$) compared to those of SBM control.

In comparison to the control, the activity of α-amylase and protease in fermented SBM with all interventions was not significant ($p > 0.05$). Enzyme activity was greatest in fermented SFM with fungi and bacteria plus fungi, particularly α-amylase, protease, and xylanase, whereas cellulase enzyme was elevated in fermented SFM with bacteria. The results suggest that the increase in IVDMD and CP in fermented SFM could be attributable to the increased proliferation of bacteria and fungi in solid-state soybean meal, which decreased CF by producing cellulase and xylanase enzymes.

4 Conclusion

Fermentation with bacterial and fungal species enhanced the nutritional value of certain forages for safe use in feeding ruminants. The fermentation of sunflower meal was superior to that of soybean meal.

It can be concluded that fermented soybean and sunflower meal products can be used as a source of protein or enzymes because SSF increased the protein of soybean meal to 48% and sunflower to 27% while the enzyme activity of cellulase, amylase, xylanase, and protease increases significantly.

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