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ROLE OF LIVE MICROBES FOR FERMENTATION AND ENHANCEMENT OF FEEDING VALUE OF WHEAT STRAW AS ANIMAL FODDER

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ABSTRACT

Nowadays, a resource terrible and technologically hungered farmhand within the developing nations of tropical zones faces intense challenges of their livestock farming and products due to tremendously and seriously increments of the human populace in this century. These challenges create this potential to feed human food security and not meet this sector's 2050 human population demand. The farmer faces the challenges of creating better value and sufficient harvests. Their livestock's low-fine feedstuff-like crop-residues with low dietary due to shrinking grazing land shifted to farming land. Hence, our farmers want the generation that tackles this hassle through biological treatment to get without difficulty digested, nicely evolved flavor and nutritionally in shape, extra protein content in flip offers proper milk and red meat in-phrases of high-class besides capacity. This study aimed to determine the nutritional worth of wheat chaff treated biologically by bacterial and fungal Lactobacillus Casei Shirota and Aspergillus Niger strain. It also analyzed the physical and chemical structure of fermented chaff to obtain the numerical values that indicate the increments straw value and enhance feed intake of the individual livestock.

Contribution/Originality: This study uses a new estimation methodology of Protein estimation by the Lowry method by detecting Optical Density which aids us to verify the result gotten by calculating and analyzing the percentage of CP and CF of the treatments which makes us fully confident of analysis.

1. INTRODUCTION

With the rapid increase in human population and increasing demand for food, foraging domains are steadily shrinking, being converted to arable lands, and are restricted to areas with little value or agricultural potentials, such as hilltops, swampy areas, roadsides, and other marginal lands [1]. It causes many cattle are suffering from a shortage of feeds in quality and quantity, and the final product is decreased. Even the available roughage and concentrate nourishing livestock can't meet the daily cattle feed requirement for production and reproduction. In tropical regions globally, ruminants depend on year-round foraging on usual fallows, or the cattle are fed with cut-carry forage and straw [2, 3]. Thus, low-value nutrition is one of the significant production restraints in small-holder systems, particularly in Africa. Much research has been carried out to advance the quality and accessibility of fodder resources, including work on sown forages, fodder conservation, the use of multi-purpose trees, fibrous yield residues, and strategic supplementation rather than treating biologically safe microbes [4, 5]. Also, the accessibility of fodder sources and the nutritious value of the accessible feedstuffs are the most vital factors determining cattle yield. Feedstuff scarcities and nutrient deficiencies become further acute in the dry time for farmers in both the moorlands and swamps [6, 7]. The ruminant animals in many tropical countries subsist mainly on crop residue-based diets which are readiness of husks [8] is closely related to the agricultural system, the type of yields produced, and intensity of cultivation. In integrated crop/livestock systems, the possibility of using chaff as cattle feed is the most significant [9]. Over the last few years, the increasing expansion of the agro-industrial movement has led to the accumulation of many lignocellulosic residues of wheat straw worldwide. Such high fiber contents are believed to be negatively correlated with voluntary intake, organic matter (OM) fermentation rate, microbial cell yield per unit OM fermented, and propionate: acetate ratio in fermentation end products [10]. The deprived nutritious value and metabolism of wheat chaff can be attributed to its truncated nitrogen and high fiber content. The plant's cross-linking cover and structure are covalently encrusted with lignin, preventing biodegradation in the rumen [11]. Improve the access of microbial hydrolytic enzymes to cellulose and hemicellulose for digestibility enhancement, and it is required to break lignin-carbohydrate linkages in the plant cell wall [12]. Removing lignin from the wheat straw using physical, chemical, and biotic methods recovers its digestibility for ruminants. Nevertheless, biological processes are preferred over other methods because they produce minimum harmful by-products and have low energy requirements [12]. Consequently, numerous physio-chemical dealings have been tried, which are recognized to advance feed value by increasing digestibility or enhancing deliciousness. Recently, the biological treatments of crop straw to improve the accessibility of cellulosic fractions. Thus, improving their metabolism, absorption, and feeding value have attracted the all-embracing interests among researchers and farmers to satisfy their cattle production by contributing cattle reliable feed [13, 14]. Hence, the objectives of this study were to discover the process of bioconversion of agricultural wastes into a digestible and nutrient-rich animal feed using a particular lignin-degrading fungus, *Aspergillus Niger*, and lactic acid-producing bacterium, *Lactobacillus casei Shirota.*

2. MATERIALS AND METHODS

2.1. Sample collection

Two kg of PBW-343 wheat straw were procured from a nearby farmer's farm and reduced the size of procured straw into axed three-four (3-4cm) by scissors then stuffed for use. Two kg of wheat straw has been procured from a nearby farmer's farm. It reduces the scale of procured straw into axed three-four (3-4cm) using scissors to ease the utilization of straw during measuring, boiling, and putting the straw flask for fermentation then crammed for use.

2.2. Experimental Treatments

The trial was intended and applied by randomized with two treatments and three replications. The factor *lactobacillus casei* was the natural lactic acid cultures (Bacteria). At the same time, *Aspergillus Niger* was the biotransformation and production of extracellular enzymes to see the outcome of biological treatment (which was pretreated before actual treatment). The fermentation of wheat straw was modified into performed in a 250 ml borosilicate glass flask. 4-gram wheat straw turns out to be added in each flask. The flak is inoculated with 5ml from organized inoculum, which contains 10^5 CFU/ml, from microbial traces inoculum. The microbial inoculum holding flask was incubated at 37°C and 28°C as same to Sutula, et al. [15]; Colombo, et al. [16]; Nanno, et al. [17] for the subsequent four weeks. Afterward of this trial, fermented wheat straw has been dried through the oven at sixty-five degrees Celsius till a very remaining weight and kept at 4°C for the chemical willpower of pattern.

2.3. Microbial

Different microorganisms were used in this look, namely the *Lactobacillus Casei* strain of *Shirota* and *Aspergillus Niger* [18]. *Lactobacillus Casei* was remoted from Yakult probiotics, and *Aspergillus Niger* is acquired from the microbiology faculty of Sharda University. The fermentation time reflected microbial product formation [19].

Strains had been well-maintained on their appropriate media [15]. *Lactobacillus Casei* was cultivated in *Lactobacilli* MRS broth [19] liquid media for 24 hrs /37°C, *Aspergillus Niger* turned into enabled PDB potato dextrose broth media for five days at 28°C as a guide by Kabir, et al. [20].

2.4. Microbial Actions

Bio-treatments apply the living principle [21] to alter diet stuff into more palatable nutritional or staple feed; it has the latent to advance the nutritional value of fibrous agricultural by-products. *Aspergillus Niger* carries out 5 Enzymatic hydrolyses of cellulose was 2984.88μmol/min^{-1} and Lactobacillus casie 2456.84μmol/min^{-1}. This indicates those microbes can degrade wheat straw.

2.5. Chemical Analysis

NDF and acid detergent fiber (ADF) were gritty by the detergent system as described by Settuba [22]; Zayed [23]. All the data was documented on a dry matter basis. Organic matter (OM) and crude fiber (CF) content in wheat straw were resolute by the standard methods described by AOAC Official Methods of Analysis 17th ed. Association of AOAC [24] and in wheat straw were strongminded is defined, and the amount of crude protein (CP) was calculated (Nx6.25).

3. RESULTS AND DISCUSSION

3.1. Assessment of microbial activities on physical change of straw

The evaluation was performed by using lactic acid-producing bacteria viz: *Lactobacillus casei* and a ligninolytic fungus *Aspergillus Niger* like *Saccharomyces cerevisiae* [23, 25] *bacteria (Cellulomonas uda NRRL-404)* [26] *brown rot fungi*, *white-rot fungi*, and *soft rot fungi* [23, 27] such studies are not taking place by these specific strains. Significant variation was recorded among the applied microbial strains regarding their capabilities for dignifying and cellulase activities observed from the fermented straw Figures 1 and 2. The Data shows that *Lactobacillus casei* and *Aspergillus Niger* could be bioconversion cellulose to palatable and nutritional dietary feed. The reliable and visible change was obtained from bacterial inoculum lactobacillus casei. These results agree with that reported by Zayed [23]. Different material sources indicated that the abilities of microorganisms made a significant change in the physical structure of straw depending on the species of microorganism and the growth condition of microbes [28].

3.2. Physical Appearance Observation of Fermented Straw

A bacterial strain inoculated wheat straw exhibits a significantly more significant color change than a fungal implanted strain Figures 1 and 2. We have a softness that increases the palatability of straw so that feed intake of the animal will be enhanced (Figure 1). Our findings are similar to how the feed intake of animals will be improved [14].

Figure-1. Bacterial inoculated, fermented, and oven-dry *Lactobacillus Casei.*

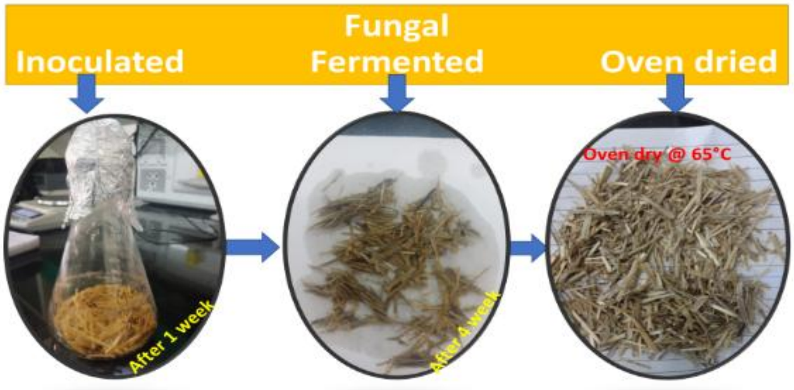

Figure-2. Fungal inoculated, fermented, and oven-dry *Aspergillus Niger*.

3.3. Chemical analysis of Fermented Straw

Each treatment interaction between microbial treatment and physical pre-remedies of wheat straw in Table 1 indicates a significant decrease in natural depend on organic matter percentage while managing one 81.36%. The wild swings on the content material of boiled/pretreatment treated wheat straw inoculated with *Lactobacillus casei* as natural lactic acid tradition and *Aspergillus niger* as selective lignin-degrading fungus 79.67% and 78.42%, respectively (Table 1). Previous works of wheat straw support these results treated wheat straw with white-rot *fungus Agaricus bisporus* referred to a sharp decrease within the content material of natural count crude fiber (40.6 vs. 11.6%) [29-31].

The crude fiber (Cf %) documented from bacterial and fungal remedies suggests a widespread discount. In contrast to the manipulation of 40.41%, a significant drop in crude fiber was achieved 33.16 % and recorded with a boiled or pre-dealt straw pattern injected with bacterial inoculation, and 33.16% is fungal treatment (Table 1).

Our results agree with the previous works reported on animal feed nutritive values and floristic range ecology [32]. The crude protein percentage analyzed a considerable increment in both treatments. A compared to control via 5.33, there is a treasured result of distinguishing between bacterial and fungal inoculated and fermented samples 10.01% and eight 64%, respectively [33] which suggested the exact that impartial detergent fiber NDF and ADF reduced by using 45.1% and 31.5%, respectively (Table 2).

The pre-treated sample's NDF % and ADF % compared to control and visible variations among bacterial and fungal inoculated were obtained (Table 2). Hence, the slight massive reduction in NDF was 61.14%, 63.71% ADF, 45.86%, and 44.92%, respectively (Table 2). Our results are supported by the previous works on rice straw treatments an excellent and suited lower in organic increment in crude protein and reduced crude fiber in the first-rate good-sized [23].

It also recorded a high-quality decrease in Om in wheat straw and barley straw handled with microbial inoculants compared to untreated materials [14]. The composition of wheat straw fungally treated with Agaricus bisporus decreased slightly utilizing the dry matter content material compared with those in un-boiled wheat straw. As a result, the bacterial and yeast strains are greater advice in a position and proper because without difficulty ferment handled wheat straw, resulting in surprisingly reducing dry matter percentage [34]. At the same time, compared to the manipulated one [35].

The digestibility of feed and productivity of animals is altered by the impact of acid detergent fiber and nutrient digestible fiber level in the feed, which measure quality in the forage of the individual animal intake [36]. The following (Table 3) data indicate the percentage level of NDF and ADF of wheat straw fermented by specific bacterial and fungal fermented straw strains.

Table-1. The actual chemical analysis of wheat straw on a dry matter basis, fermented by *bacterial* strain *Lactobacillus Casei and fungal strain Aspergillus Niger.*

Parameter obtained	**Untreated wheat straw**		
	OM%	**CF%**	**CP%**
Control	81.36	40.41	5.33
Microbial treatments	Treatments + Pre-treatments		
Lactobacillus casie Shirota	79.67	33.98	10.01
Aspergillus Niger	78.42	33.16	8.64

Note: *The values are the mean of 3 replicates: Treatments are shown significant change in OM %, CF % & CP% when compared to control one.

Table-2. ADF % and NDF% Bacterial *and Fungal* treated Straw.

Parameter	**Control**	**Treatments**	
		Bacterial inoculated	**Fungal inoculated**
		Lactobacillus Casei Shirota	***Aspergillus Niger***
ADF %	48.24	45.86	44.92
(NDF %	69.18	61.14	63.71

Note: *The values are the average of three replicates: Compared to the control, there is a substantial difference in ADF% and NDF% for bacterial and fungal inoculation treatments.

3.4. The OD Reading of Treatments (Optical Density)

The OD studying and protein attention within the handled samples are performed utilizing protein utilizing folin reaction [37, 38]. The most typically used approach is estimating and determining quantity proteins already in answer or easily soluble in dilute alkali in organic samples, which might be bacterial and fungal treated wheat straw samples [39]. The whole protein concentration gift in the dealt with a model of bacterial fungal and the untreated control one was illustrated underneath by way of exceptional tables and charts. It suggests the terrific significance of protein concentration in the bacterial inoculated remedy fungal and managing one [40].

Table-3. BSA standard stock solution.

Test tube marker	**Vol. of BSA (ml)**	**Concentration of BSA (mg/ml)**	**Vol. of Distilled water (ml)**	**Vol. of reagent I (ml)**		**Vol. of Reagent II (ml)**		**OD At 750nm/Response**
1	0	0	1000	**5 ml**	**Incubation for 1hr**	**1ml**	**Incubation for 30 minutes**	0.00
2	20	20	980					0.195
3	40	40	960					0.391
4	60	60	940					0.568
5	80	80	920					0.629
6	100	100	900					0.833
7	120	120	880					0.931

Note: *BSA standard 1mg/ml [38].
Protein estimation Using Y = 0.0072x + 0.0847.

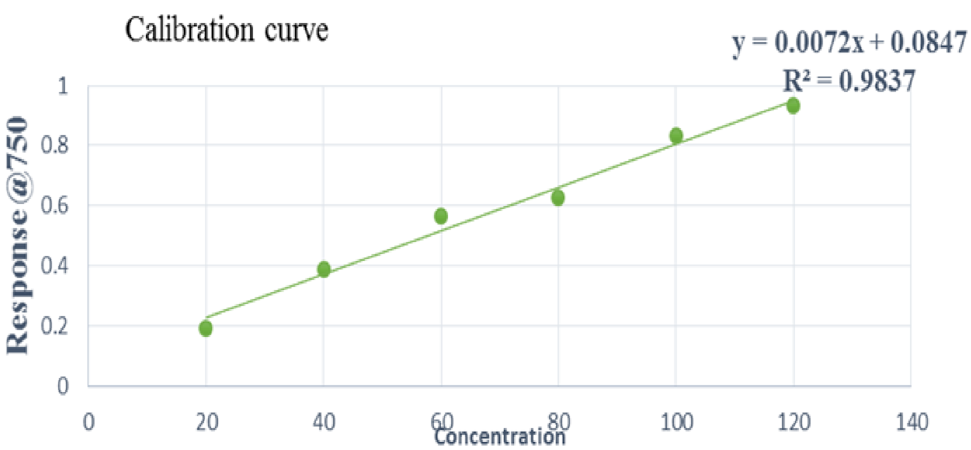

Figure-3. Standard curve result of sample concentration.

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Table-4. Standard Equation.

Parameters	Y = mX + C	X= (Y-C)/m	Intercept= 0.0847	Slope= 0.0072

Table-5. Protein Concentration.

Sample concentration µg/ml	Sample	Response	Concentration from Calibration Curve value (Y-C)/m	µg/ml (Ccc X Df) Df= total Vol ÷ sample con	mg/ml
20	Control	0.105	2.803	140.16	0.140157
40		0.196	15.390	384.74	0.38474
60		0.267	25.210	420.25	0.420247
20	Bacterial	0.325	33.232	1661.60	1.661595
40		0.496	56.883	1422.08	1.422084
60		0.762	93.675	1561.55	1.561554
20	Fungal	0.152	9.304	465.19	0.465191
40		0.204	16.496	412.40	0.412402
60		0.481	54.809	913.66	0.91366

Table-6. Calculated unknown concentration.

SN	Parameters	Control	Bacterial	Fungal
1	20	0.140157	1.661595	0.465191
2	40	0.38474	1.422084	0.412402
3	60	0.420247	1.561554	0.91366
4	Average	0.31505	1.54841	0.597083333
5	Standard Deviation	0.152496	0.1202994	0.27543109
6	Standard Error	0.088044	0.0694549	0.159020214

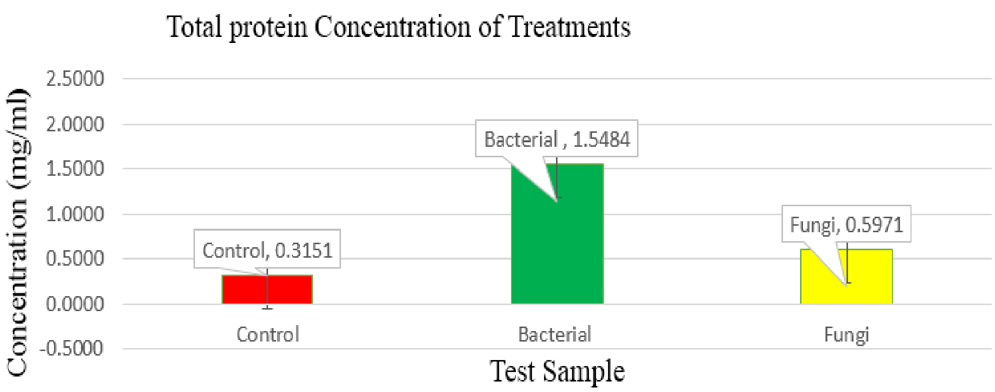

Figure-4. The total Protein concentration.

4. CONCLUSION

This study clarifies the possibility of enhancing the nutritional value of wheat straw using selected microbial inoculants and some pre-treated treatment. Results were shows that it upgraded the palatability, flavor and reduce fibrous content. Bacterial inoculation increased the total protein concentration in fermented wheat straw without using chemical substances to provide safe, non-infected, and practical animal feeds for our valuable resource, terrible farmer. Distinctive microbial inoculants with specific enzymatic activities can be recorded exclusive nutritional values for dealing with wheat straw. Therefore, the kind of microbial inoculant that has to be used to enhance the dietary value of the residues should be decided on the principle of the nutritional content material of the residue used. They contain animal feed, pasteurization, or boiling, which are readily suggested for treating wheat straw prevented contamination and better-great fiber digestion limits in livestock nutrition. In the future, I suggest that any researcher have to research the different bacterial strains and different wheat variety straw. In these inferences, we have advised the crop residue covers more percent of livestock feeds worldwide. The researcher must intentionally create this crop's residue by using various bacterial strains for easy to edible for animals.

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REFERENCES

[1] W. Mekuria, "The link between agricultural production and population dynamics in Ethiopia: A review," *Advances in Plants & Agriculture Research*, vol. 8, pp. 348-353, 2018. Available at: https://doi.org/10.15406/apar.2018.08.00336.

[2] C. Sarnklong, J. Cone, W. Pellikaan, and W. Hendriks, "Utilization of rice straw and different treatments to improve its feed value for ruminants: A review," *Asian-Australasian Journal of Animal Sciences*, vol. 23, pp. 680-692, 2010. Available at: https://doi.org/10.5713/ajas.2010.80619.

[3] H. T. Diressie, "Crop residue management and farm productivity in smallholder crop-livestock system of dry land North Wollo, Ethiopia," Doctoral Dissertation, Wageningen University, and Research Centre, 2011.

[4] P. K. Thornton, "Livestock production: Recent trends, future prospects," *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 365, pp. 2853-2867, 2010. Available at: https://doi.org/10.1098/rstb.2010.0134.

[5] G. C. Mandal, "The role of agricultural diversification in rural development: A case study of mountain livelihood systems in the himalayan region of West Bengal," Doctoral Dissertation, University of North Bengal, 2018.

[6] H. Kubkomawa, H. Olawuye, L. Krumah, E. Etuk, and I. Okoli, "Nutrient requirements and feed resource availability for pastoral cattle in the tropical Africa: A review," *Journal of Agricultural and Crop Research*, vol. 3, pp. 100-116, 2015.

[7] J. Smith, K. Sones, D. Grace, S. MacMillan, S. Tarawali, and M. Herrero, "Beyond milk, meat, and eggs: Role of livestock in food and nutrition security," *Animal Frontiers*, vol. 3, pp. 6-13, 2013. Available at: https://doi.org/10.2527/af.2013-0002.

[8] A. J. Escribano, "Organic feed: A bottleneck for the development of the livestock sector and its transition to sustainability?," *Sustainability*, vol. 10, p. 2393, 2018. Available at: https://doi.org/10.3390/su10072393.

[9] Z. Wondatir and Y. Mekasha, "Feed resources availability and livestock production in the central rift valley of Ethiopia," *International Journal of Livestock Production*, vol. 5, pp. 30-35, 2014. Available at: https://doi.org/10.5897/ijlp2013.0158.

[10] Saunders, Christopher Scott, "Growth Performance, Ruminal Fermentation Characteristics, and Economic Returns of Growing Beef Steers Fed Brown Midrib, Corn, Silage-Based Diet" (2015). All Grad te Theses and Dissertations. 4162. https://digitalcommons.usu.edu/etd/4162

[11] B. Ahring, N. Murali, and K. Srinivas, "Fermentation of cellulose with a mixed microbial rumen culture with and without methanogenesis," *Fermentation Technology*, vol. 7, p. 152, 2018. Available at: https://doi.org/10.4172/2167-7972.1000152.

[12] B. Shrivastava, P. Nandal, A. Sharma, K. K. Jain, Y. Khasa, T. K. Das, V. Mani, N. Kewalramani, S. Kundu, and R. Kuhad, "Solid state bioconversion of wheat straw into digestible and nutritive ruminant feed by Ganoderma sp. rckk02," *Bioresource Technology*, vol. 107, pp. 347-351, 2012. Available at: https://doi.org/10.1016/j.biortech.2011.12.096.

[13] Z. Zheng, J. S. Lauritzen, E. Perlman, C. G. Robinson, M. Nichols, D. Milkie, O. Torrens, J. Price, C. B. Fisher, and N. Sharifi, "A complete electron microscopy volume of the brain of adult Drosophila melanogaster," *Cell*, vol. 174, pp. 730-743. e22, 2018. Available at: https://doi.org/10.1016/j.cell.2018.06.019.

[14] N. A. Abdel-Aziz, A. Z. Salem, M. M. El-Adawy, L. M. Camacho, A. E. Kholif, M. M. Elghandour, and B. E. Borhami, "Biological treatments as a mean to improve feed utilization in agriculture animals—an overview," *Journal of Integrative Agriculture*, vol. 14, pp. 534-543, 2015. Available at: https://doi.org/10.1016/s2095-3119(14)60829-7.

[15] J. Sutula, L. Coulthwaite, and J. Verran, "Culture media for differential isolation of Lactobacillus casei Shirota from oral samples," *Journal of Microbiological Methods*, vol. 90, pp. 65-71, 2012. Available at: https://doi.org/10.1016/j.mimet.2012.03.015.

[16] M. Colombo, A. E. Z. de Oliveira, A. F. de Carvalho, and L. A. Nero, "Development of an alternative culture medium for the selective enumeration of Lactobacillus casei in fermented milk," *Food Microbiology*, vol. 39, pp. 89-95, 2014. Available at: https://doi.org/10.1016/j.fm.2013.11.008.

[17] M. Nanno, I. Kato, T. Kobayashi, and K. Shida, "Biological effects of probiotics: What impact does Lactobacillus casei shirota have on us?," *International Journal of Immunopathology and Pharmacology*, vol. 24, pp. 45S-50S, 2011.

[18] X. Hu, Z. Huang, Y. Zhang, Y. Hong, and Y. Zheng, "Effects of a probiotic drink containing Lactobacillus casei strain Shirota on dental plaque microbiota," *Journal of International Medical Research*, vol. 47, pp. 3190-3202, 2019. Available at: https://doi.org/10.1177/0300060519853655.

[19] Ray, R.C., & Didier, M. (Eds.). (2014). Microorganisms and Fermentation of Traditional Foods (1st ed.). CRC Press. https://doi.org/10.1201/b17307.

[20] M. S. Kabir, Y.-H. Hsieh, S. Simpson, K. Kerdahi, and I. M. Sulaiman, "Evaluation of two standard and two chromogenic selective media for optimal growth and enumeration of isolates of 16 unique Bacillus species," *Journal of Food Protection*, vol. 80, pp. 952-962, 2017. Available at: https://doi.org/10.4315/0362-028x.jfp-16-441.

[21] Editor SP, Edogbanya OP, Kutshik JR. Cellulase activity of Aspergillus niger in the biodegradation of rice husk. *MOJ Biol Med.* 2018;3(2):49–51. DOI: 10.15406/mojbm.2018.03.00075

[22] A. Settuba, "Nutritional evaluation of rice straw from different rice cultivars," Doctoral Dissertation, Makerere University, 2021.

[23] M. S. Zayed, "Enhancement the feeding value of rice straw as animal fodder through microbial inoculants and physical treatments," *International Journal of Recycling of Organic Waste in Agriculture*, vol. 7, pp. 117-124, 2018. Available at: https://doi.org/10.1007/s40093-018-0197-7.

[24] AOAC, *Official methods of analysis*, 17th ed. Gaithersburg, MD: Association of Official Analytical Chemists, 1990.

[25] O. B. Chukwuma, M. Rafatullah, H. A. Tajarudin, and N. Ismail, "A review on bacterial contribution to lignocellulose breakdown into useful bio-products," *International Journal of Environmental Research and Public Health*, vol. 18, p. 6001, 2021. Available at: https://doi.org/10.3390/ijerph18116001.

[26] C. M. Da Silva, D. L. da Silva, L. V. Modolo, R. B. Alves, M. A. de Resende, C. V. Martins, and Â. de Fátima, "Schiff bases: A short review of their antimicrobial activities," *Journal of Advanced research*, vol. 2, pp. 1-8, 2011. Available at: https://doi.org/10.1016/j.jare.2010.05.004.

[27] M. Mahesh and M. Mohini, "Biological treatment of crop residues for ruminant feeding: A review," *African Journal of Biotechnology*, vol. 12, pp. 4221-4231, 2013. Available at: https://doi.org/10.5897/ajb2012.2940.

[28] M. Zayed, M. Szumacher-Strabel, D. El-Fattah, M. Madkour, M. Gogulski, V. Strompfová, A. Cieślak, and N. El-Bordeny, "Evaluation of cellulolytic exogenous enzyme-containing microbial inoculants as feed additives for ruminant rations composed of low-quality roughage," *The Journal of Agricultural Science*, vol. 158, pp. 326-338, 2020. Available at: https://doi.org/10.1017/s0021859620000611.

[29] B. Kokić, D. Palić, D. Ivanov, J. Lević, N. Spasevski, O. Đuragić, and I. Čabarkapa, "Modification of in vitro multi-enzymatic method for determining the organic matter digestibility of feeds," *Agro Food Industry Hi Tech*, vol. 24, pp. 59-61, 2013.

[30] S. Zuo, D. Niu, D. Jiang, P. Tian, R. Li, W. Wu, and C. Xu, "Effect of white-rot fungal treatments on the in vitro rumen degradability of two kinds of corn stover," *BioResources*, vol. 14, pp. 895-907, 2019.

[31] S. K. Singh, "Biological treatment of plant biomass and factors affecting bioactivity," *Journal of Cleaner Production*, vol. 279, p. 123546, 2021. Available at: https://doi.org/10.1016/j.jclepro.2020.123546.

[32] H. Teka, I. C. Madakadze, A. Angassa, and A. Hassen, "Effect of seasonal variation on the nutritional quality of key herbaceous species in semi-arid areas of Borana, Ethiopia," *Indian Journal of Animal Nutrition*, vol. 29, pp. 324-332, 2012.

[33] F. Kong, N. Lu, Y. Liu, S. Zhang, H. Jiang, H. Wang, W. Wang, and S. Li, "Aspergillus oryzae and aspergillus niger co-cultivation extract affects in vitro degradation, fermentation characteristics, and bacterial composition in a diet-specific manner," *Animals*, vol. 11, p. 1248, 2021. Available at: https://doi.org/10.3390/ani11051248.

[34] K. Ni, Y. Wang, H. Pang, and Y. Cai, "Effect of cellulase and lactic acid bacteria on fermentation quality and chemical composition of wheat straw silage," *American Journal of Plant Sciences*, vol. 5, pp. 1877-1884, 2014. Available at: https://doi.org/10.4236/ajps.2014.513201.

[35] W. Khota, S. Pholsen, D. Higgs, and Y. Cai, "Comparative analysis of silage fermentation and in vitro digestibility of tropical grass prepared with Acremonium and Tricoderma species producing cellulases," *Asian-Australasian Journal of Animal Sciences*, vol. 31, p. 1913, 2018. Available at: https://doi.org/10.5713/ajas.18.0083.

[36] Z. B. Khanday, P. Cauhan, D. Dey, R. Malik, D. Pradhan, and C. Goyal, "A review on fermentation quality of paddy straw silage," *Journal of Entomology and Zoology Studies*, vol. 6, pp. 1184-1887, 2018.

[37] Scientific, T. (2009). Thermo scientific pierce crosslinking technical handbook. *Google Scholar There is no corresponding record for this reference.*

[38] O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, "Protein measurement with the Folin phenol reagent," *Journal of Biological Chemistry*, vol. 193, pp. 265-275, 1951. Available at: https://doi.org/10.1016/s0021-9258(19)52451-6.

[39] G. Legler, C. M. Müller-Platz, M. Mentges-Hettkamp, G. Pflieger, and E. Jülich, "On the chemical basis of the Lowry protein determination," *Analytical Biochemistry*, vol. 150, pp. 278-287, 1985. Available at: https://doi.org/10.1016/0003-2697(85)90511-1.

[40] J. Thierie, "A method for exploring suspended particles interactions by means of optical density measurements: Application to bacterial floc and solid nutrients," *Journal of Microbial and Biochemical Technology*, vol. 6, pp. 279-285, 2014. Available at: https://doi.org/10.4172/1948-5948.1000157.