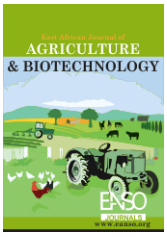

East African Journal of Agriculture and Biotechnology
eajab.easno.org
Volume 8, Issue 1, 2025
p-ISSN: 2707-4293 | e-ISSN: 2707-4307
Title DOI: https://doi.org/10.37284/2707-4307

EAST AFRICAN NATURE & SCIENCE ORGANIZATION

Original Article

Impact of Fermentation and Enzyme-Treatment on *In-vitro* Dry Matter Digestibility, Proximal Chemical Composition, Amino Acids and Tannin Content of *Prosopis juliflora* Pod Meal

Duncan Maina Wanjohi[1], Anthony Macharia King'ori[2], Mary Kivali Ambula[2] & Irene Thiguku Kamanja[2]*

[1] Tharaka University, P. O. Box 193-60215, Marimanti, Kenya.
[2] Egerton University, P. O. Box 536-20115, Egerton, Kenya.
* Author for Correspondence ORCID ID; https://orcid.org/0000-0003-0844-2607; Email: duncanwanjohi09@gmail.com

Article DOI : https://doi.org/10.37284/eajab.8.1.2936

Date Published:
01 May 2025

Keywords:
Amino Acids, Anti-nutritive Factors, Aspergillus niger, Crude Protein, Enzymes, Prosopis juliflora.

ABSTRACT

In vitro and proximal chemical analyses are important ways of determining new potential feed resources. *Prosopis juliflora* pods contain high crude fibre and tannin levels that entrap nutrients and limit utilization. This study evaluated the effect of unfermented pods (treatment 1), spontaneously fermented pods for 3 (treatment 2), 6 (treatment 3), and 9 days (treatment 4) and *Aspergillus niger-induced* fermentation for 3 (treatment 5), 6 (treatment 6), and 9 days (treatment 7) days) on *in vitro* dry matter digestibility (IVDMD), crude protein, amino acids and tannin content and tannin content of Prosopis pods. The effect of Natuzyme®-treated unfermented pods (treatment 8) on IVDMD was also determined. Results indicated that treatment 1 had lower ($P < 0.05$) dry matter content as compared to treatments 2, 5 and 6. Treatments 3 and 6 increased ($P < 0.05$) crude protein content compared to other treatments. Treatment 3 resulted in higher ($P < 0.05$) amino acid content (tryptophan; methionine and lysine) than pods in other treatments. There were reduced ($P < 0.05$) tannin levels in all treatments apart from treatment 1. Treatments 3, 4, and 8 had higher ($P < 0.05$) IVDMD compared to treatments 1 and 5. The study concluded that 6 days of spontaneous fermentation (treatment 3) had a significant effect on the improvement of crude protein content, amino acids, IVDMD and reduced tannin levels. The use of Natuzyme® was found to improve the IVDMD of pods. The results recommended 6 days of spontaneous fermentation as the method of processing pods as an ingredient in formulating poultry diets.

APA CITATION

Wanjohi, D. M., King'ori, A. M., Ambula, M. K. & Kamanja, I. T. (2025). Impact of Fermentation and Enzyme-Treatment on *In-vitro* Dry Matter Digestibility, Proximal Chemical Composition, Amino Acids and Tannin Content of *Prosopis juliflora* Pod Meal. *East African Journal of Agriculture and Biotechnology*, *8*(1), 243-257. https://doi.org/10.37284/eajab.8.1.2936

CHICAGO CITATION

Wanjohi, Duncan Maina, Anthony Macharia King'ori, Mary Kivali Ambula and Irene Thiguku Kamanja. 2025. "Impact of Fermentation and Enzyme-Treatment on *In-vitro* Dry Matter Digestibility, Proximal Chemical Composition, Amino Acids and Tannin Content of *Prosopis juliflora* Pod Meal". *East African Journal of Agriculture and Biotechnology* 8 (1), 243-257. https://doi.org/10.37284/eajab.8.1.2936

Article DOI : https://doi.org/10.37284/eajab.8.1.2936

HARVARD CITATION

Wanjohi, D. M., King'ori, A. M., Ambula, M. K. & Kamanja, I. T. (2025) "Impact of Fermentation and Enzyme-Treatment on *In-vitro* Dry Matter Digestibility, Proximal Chemical Composition, Amino Acids and Tannin Content of *Prosopis juliflora* Pod Meal", *East African Journal of Agriculture and Biotechnology*, 8(1), pp. 243-257. doi: 10.37284/eajab.8.1.2936.

IEEE CITATION

D. M. Wanjohi, A. M. King'ori, M. K. Ambula & I. T. Kamanja "Impact of Fermentation and Enzyme-Treatment on *In-vitro* Dry Matter Digestibility, Proximal Chemical Composition, Amino Acids and Tannin Content of *Prosopis juliflora* Pod Meal", *EAJAB*, vol. 8, no. 1, pp. 243-257, May. 2025.

MLA CITATION

Wanjohi, Duncan Maina, Anthony Macharia King'ori, Mary Kivali Ambula & Irene Thiguku Kamanja. "Impact of Fermentation and Enzyme-Treatment on *In-vitro* Dry Matter Digestibility, Proximal Chemical Composition, Amino Acids and Tannin Content of *Prosopis juliflora* Pod Meal". *East African Journal of Agriculture and Biotechnology*, Vol. 8, no. 1, May. 2025, pp. 243-257, doi:10.37284/eajab.8.1.2936

INTRODUCTION

The production of broiler feed contributes up to 70% of the total production cost (Wanjohi *et al*., 2017). Due to increases in the cost of feed ingredients and unsustainable feed supply for chickens, there is a tendency in the poultry industry to formulate feed from non-conventional feed ingredients. In order to maximize animal growth performance and maintain body health (Wu *et al*., 2020), there is a need to evaluate feed treatment techniques that will ensure that new feed ingredients will address the bio-economic challenges and ensure sustainable strategies to increase feed efficiency (Heyer et al., 2022).

Evaluation and use of alternative feed resources are important in addressing the challenge of feeds in poultry production (Volpato et al., 2023). Since *in vivo* experiments are expensive, *in vitro* studies are vital (Zaefarian et al., 2021) especially when there are potential feed resources in formulating rations. In animal nutrition, an important bio-economical challenge is the simultaneous development of sustainable strategies and increasing feed efficiency (Heyer et al., 2022).

Prosopis juliflora pod meal (PPM) is a feed ingredient for both ruminants and non-ruminants (Wanjohi et al., 2017). It is a xerophytic evergreen tree that thrives on most soil types under variable climatic conditions (Ruiz-Nieto et al., 2020). The tree is typical of those growing in arid and semi-arid regions. It has a tap root system to access subsurface water; stems are greenish-brown, sinuous and twisted (Sawal et al., 2004). Prosopis pod production peaks at 15-20 years of age. Prosopis starts fruiting at 3-4 years of age; 10-year-old plants may yield up to 90 kg pods annually (Ruiz-Nieto et al., 2020), however, annual pod yield ranges up to 100 kg/tree (Kavila et al., 2020) with a high annual yield of 169 kg/tree having been reported in India (Al-Soqeer et al., 2023).

Prosopis pods are underutilized feed resources for livestock (Zhong et al., 2022). Prosopis pod meal, a byproduct of the Prosopis tree, is a potential feed ingredient for livestock due to its availability and nutritional composition (Zhong et al., 2022). However, it contains high levels of tannins (Sawal et al., 2004) which can negatively impact its nutritive value and limit its utilization (Bhatta et al., 2005). In addition, crude fibre entraps nutrients in an insoluble complex which it forms in the cell wall of plants and this resists the digestion by the endogenous enzymes in the gastrointestinal tract (GIT) of poultry and other non-ruminant animals (Singh and Kim 2021; Jha and Mishra, 2021; Tejeda and Kim, 2021). Viscosity-promoting potential of crude fibre also reduces overall digestive and absorptive efficiency by preventing nutrients from being available at the absorptive site in the intestinal mucosa (Han et al., 2023). Various biotechnologies and processing methods, such as fermentation and enzyme treatment, have been explored to mitigate the anti-nutritional effects of tannins and enhance the nutritive value of poor-quality feeds (Maud et

Article DOI : https://doi.org/10.37284/eajab.8.1.2936

al., 2023). Spontaneous and microbial fermentation processes have shown potential in reducing tannin levels and improving the nutritional quality of feed ingredients (Singh et al., 2023). Fermentation involves the action of indigenous microorganisms or specific microbial inoculants on the substrate. Studies have reported that fermentation of Prosopis pod meal can decrease tannin content and enhance protein digestibility (Maud et al., 2023), leading to improved nutrient utilization by animals (Sharma et al., 2020). Enzymes, including tannases and carbohydrases, have been used to degrade tannins and enhance the nutritional value of various feed ingredients (Jiménez et al., 2014). Enzyme treatment can break down tannin-protein complexes and improve protein digestibility (Misquitta et al., 2023). Additionally, carbohydrases can break down complex carbohydrates, releasing more fermentable substrates and improving overall nutrient availability (Zhang et al., 2020; Valente Junior et al., 2024). The application of enzyme treatments to Prosopis pod meal has the potential to reduce tannin content and enhance its nutritive value.

There is therefore the need to render the non-starch polysaccharides in PPM utilizable by non-ruminants through fermentation or the addition of exogenous enzymes. The study determined the effect of spontaneous and *Aspergillus niger*-induced fermentation of *Prosopis juliflora* pod meal on proximal parameters, tryptophan, methionine, lysine and tannin content. The *in vitro* dry matter digestibility of fermented and enzyme-treated *Prosopis julifora* pod meal was also determined. The hypothesis that spontaneous fermentation and *Aspergillus niger*-induced fermentation of *Prosopis juliflora* pod meal had no significant effect on proximal parameters, tryptophan, methionine, lysine and tannin content and *in vitro* dry matter digestibility of fermented and enzyme-treated pods was tested.

MATERIALS AND METHODS

Study Site

The experiment was conducted at Egerton University, Department of Animal Sciences, Animal Nutritional Laboratory. Prosopis pods were collected in the Marigat sub-county, Baringo County in Kenya, which is located at the latitude 0^0 10' South and longitude 35^0 30'West (Ezenwa et al., 2018). The area experiences rainfall in the months of March to August and November to December and has a mean temperature of 32.8 ^{0}C ± 1.6 °C (Muriithi et al., 2018). Soils are mainly clay loams with alluvial deposits and contain high levels of P, K, Ca and Mg and low levels of N and C (Choge et al., 2006). Ripened brownish-yellow mature pods were collected by shaking the prosopis tree. Pods with any blackening, discolouration, or evidence of browsing or attack by insects or moulds were discarded to avoid infection with mycotoxins (Choge et al., 2007). Pods were air-dried for a minimum of three days to avoid stickiness during milling. Milling was done initially without a sieve then milled for a second time to pass through a 2 mm sieve (Choge et al., 2006). The Prosopis pod meal (PPM) was then stored in airtight containers to prevent the absorption of moisture from the atmosphere.

Fermentation and Enzyme-treatment of Prosopis Pod Meal

Solid state fermentation (SSF) was used to ferment PPM by employing two methods- spontaneous and *Aspergillus niger*-induced fermentation. Samples were also treated with Natuzyme enzyme and then the determination of dry matter (DM), crude fibre (CF), ash, crude protein (CP), amino acids (tryptophan, methionine and lysine) and tannin level was carried out. The treatments are indicated in **Table 1**.

Article DOI : https://doi.org/10.37284/eajab.8.1.2936

Spontaneous Fermentation of Prosopis Pod Meal

Thirty (30 g) grams of PPM was mixed with distilled water (1:1 W/V) in airtight flasks according to the modified procedure by Sarasvati et al. (2014). The substrate was then left to undergo anaerobic fermentation for 3, 6, and 9 days. The fermented substrate was then solar-dried until a constant weight was achieved (Odero-Waitituh et al., 2020).

Fermentation of Prosopis Pod Meal Using *Aspergillus niger*

Slants of *A. niger* were obtained from the Microbiology Department at the University of Nairobi. They were sub-cultured on 2% malt extract agar. The medium was sterilized at 121 °C for 15 minutes. Spore suspensions were then prepared with distilled water. About 30 g of PPM samples were added to flasks in triplicates. All flasks and contents were autoclaved and aseptically inoculated with *A. niger* using spore suspension in distilled water (1:1 W/V) (Sarasvati et al., 2014). The substrate was then left to undergo anaerobic fermentation for 3, 6, and 9 days. The fermented substrate was then solar-dried until a constant weight was achieved (Odero-Waitituh 2020).

Preparation of Enzyme-treated Prosopis Pod Meal

Natuzyme® was added to 30 g of PPM in triplicates at the rate of 350 mg/kg of PPM according to the manufacturer's instructions and recommendations.

Experimental Treatments

Prosopis pod meal with different processing methods was used in the preparation of treatments (Table 1).

Table 1: Composition of Treatments Used in *in-vitro* Dry Matter Digestibility Experiment

Technique used in PPM	Days fermented	Treatment code	Treatment
Control	0	UF0	Treatment 1
Spontaneous fermentation	3	SF3	Treatment 2
	6	SF6-	Treatment 3
	9	SF9-T	Treatment 4
Aspergillus niger-induced fermentation	3	ANF3	Treatment 5
	6	ANF6	Treatment 6
	9	ANF9	Treatment 7
*Enzyme-treated	0	Enzy	Treatment 8

PPM=Prosopis pod meal; UF=unfermented; SF=spontaneous fermentation; ANF = *Aspergillus niger* fermentation; 3, 6, and 9 days = fermentation period; Enzy = enzyme; *Natuzyme® provided 2,000 units/g of xylanase, 6000 units/g of cellulase, 1500 units/g of phytase, 700 units/g of beta-glucanase, 700 units/g of protease and 400 units/g of alpha-amylase

Three-way in vitro Digestion of Prosopis Pod Meal

The *in vitro* digestion model used in the study was based on available literature publications, with minor modifications, and the assay was performed for eight different experimental diets in triplicates (Latorre et al., 2015). The treatments were untreated PPM, enzyme-treated PPM, and samples of spontaneously fermented and *Aspergillus niger*-induced fermentation for 3, 6 and 9 days (Table 1). Additionally, all tube samples were held at an angle of 30° inclinations to facilitate the proper blending of feed particles and the enzyme solutions in the tube.

Article DOI : https://doi.org/10.37284/eajab.8.1.2936

The first gastrointestinal compartment to be simulated was the crop, where 5 g of feed and 10 ml of 0.03 M hydrochloric acid (HCl) were placed in 50 mL polypropylene centrifuge tubes and mixed vigorously reaching a pH of around 5.2. Tubes were then incubated for 30 min after which, all tubes were removed from the incubator.

To simulate the proventriculus, 3000 U of pepsin enzyme with 4x USP activity (derived from porcine gastric mucosa powder, with a minimum of 250 units/mg solid, provided by Sigma-Aldrich Corp St. Louis, MO, USA) was added at a rate of one gram per gram of feed. Additionally, 2.5 mL of 1.5M HCl was included in each tube to achieve a pH range of 1.4-2.0. All tubes were then incubated for an additional 45 minutes.

The final stage simulated the intestinal segment of the gastrointestinal tract. There was an addition of 6.84 mg of pancreatin enzyme (obtained from P-1750 Sigma-Aldrich Corp St. Louis, MO, USA) to 6.5 mL of 1.0M sodium bicarbonate. The pH of the mixture was adjusted to 6.4 to 6.8 using 1.0 M sodium bicarbonate. All tube samples were then incubated for an additional 2 hours. The complete *in vitro* digestion process took a duration of 3 hours and 15 minutes. The *in vitro* dry matter digestibility (IVDMD) was computed using the formula according to Boisen and Fernández (1997).

$$DM\ digestibility = \frac{(DM_{initial} - DM_{residual})}{DM_{initial}} \times 100$$

(where DM refers to dry matter)

Chemical Analyses, Amino Acid and Tannin Analyses of Prosopis Pod Meal

The fermented Prosopis pod meal samples were analyzed for dry matter, ash, ether extract, crude fibre and crude protein using proximate analysis following the procedures of AOAC (1990). Dry matter (DM) content was determined by subjecting the samples to drying in a hot air oven at 105 °C for 24 hours. Ash content was determined by complete burning of the samples in a muffle furnace at 550 °C for 8 hours. Ether extract (EE) was determined using the Soxhlet method. Crude fibre (CF) content was determined by chemically digesting and solubilizing the other materials present in the feed samples. Total nitrogen, required for crude protein (CP) determination using the factor N× 6.25, was obtained through the micro-Kjeldahl.

For amino acid analysis, the samples were precipitated at pH 4.5 and then hydrolyzed by constant boiling in a mixture of 1 ml HCL at 110^{o}C for 24 hours. The phenylthiocarbamyl derivatives obtained from the amino acids were separated by reversed-phase HPLC using a dual pump system and a Bondak C-18 reversed-phase column and detected using a spectral UV detector. To detect amino acids, a standard mixture of phenylthiocarbamyl derivatives was used (Marangoni and Alli, 1988). Total Tannin determination was done according to the procedure by Abdulrazak and Fujihara (1999).

Statistical Analyses

The proximate analysis data from samples derived through various fermentation methods and fermentation durations were subjected to analysis of variance (ANOVA) using the General linear model procedure of statistical analysis system (SAS 2002) version 9.0. Mean separation was done using Tukey's HSD (Tukey's Honestly Significant Difference Test) at a significance level of 5%. The statistical model employed for analyzing this experiment was as follows:

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$$Y_{ijk} = \mu + A_i + B_j + (A^*B)_{ij} + \varepsilon_{ijk}$$

Where Y_{ij} = dependent variables, μ= Overall mean, A_i= Effect due to i^{th} fermentation method (i=1, 2), B_j =effect due to j^{th} period of fermentation (j=1…3), $(A*B)_{ij}$=effect due to interaction of i^{th} fermentation method and j^{th} fermentation period (ij=1,..6) and ε_{ij_k} = random error term.

RESULTS

The Proximal chemical composition, amino acid and tannin content of treatments are shown in Table 2. The DM was similar among all treatments in spontaneous and *A. niger*-induced fermentation. However, there was higher (P<0.05) DM for 3-day spontaneously fermented PPM, 3-day and 6-day *A.* niger-induced PPM as compared to unfermented PPM.

Ether extract was lower (P < 0.05) in unfermented PPM as compared to 3 and 6-day spontaneously and 9-day *A.* niger-induced fermentation in PPM. Six-day *A. niger*-induced fermented PPM resulted in higher (P < 0.05) crude protein than 9-day *A. niger*-induced PPM and unfermented PPM. Crude fibre content was lower (P < 0.05)) in 6 days spontaneously fermented pods as compared to unfermented, 3 and 9 days spontaneous and 3-day *aspergillus niger* fermented PPM.

Table 2: Mean Crude Protein, Amino Acids and Tannin Content Values for Prosopis Pod Meal Fermented Spontaneously and Induced with *Aspergillus niger* at Different Periods

	Treatments								
	Unfermented	**Spontaneous fermentation**			***Aspergillus niger*-induced fermentation**				
Parameters (%)	**Control (T1)**	**3 d (T2)**	**6 d (T3)**	**9 d (T4)**	**3 d (T5)**	**6 d (T6)**	**9 d (T7)**	**SEM**	**P**
Dry matter	90.5^{b}	93^{a}	92.1^{ab}	91.8^{ab}	93.5^{a}	92.5^{a}	92.2^{ab}	0.236	0.001
Ether extract	4.29^{b}	4.37^{ab}	4.63^{a}	4.65^{a}	4.39^{ab}	4.54^{ab}	4.62^{a}	0.035	0.001
Crude fiber	20.8^{a}	17.3^{b}	13.3^{d}	16.5^{b}	16.1^{bc}	14.8^{cd}	18.9^{bc}	0.488	<.0001
Ash	4.01	5.1	5.32	5.36	4.9	5.27	5.33	0.151	0.0371
Crude protein	14.6^{b}	15.4^{ab}	16^{ab}	15.1^{ab}	15.8^{ab}	16.7^{a}	14.4^{b}	0.193	0.0075
Tryptophan	0.27^{d}	0.5^{abc}	0.6^{a}	0.54^{ab}	0.5^{abc}	0.45^{bc}	0.4^{c}	0.0214	<.0001
Methionine	1.1^{e}	1.64^{b}	1.96^{a}	1.77^{b}	1.64^{b}	1.49^{c}	1.32^{d}	0.0686	<.0001
Lysine	0.81^{f}	1.37^{c}	1.64^{a}	1.5^{b}	1.37^{c}	1.24^{d}	1.11^{e}	0.0594	<.0001
Tannin	0.075^{a}	24.6^{bc}	0.025^{de}	0.021^{e}	0.026^{b}	0.023^{cd}	0.022^{de}	0.0028	<.0001

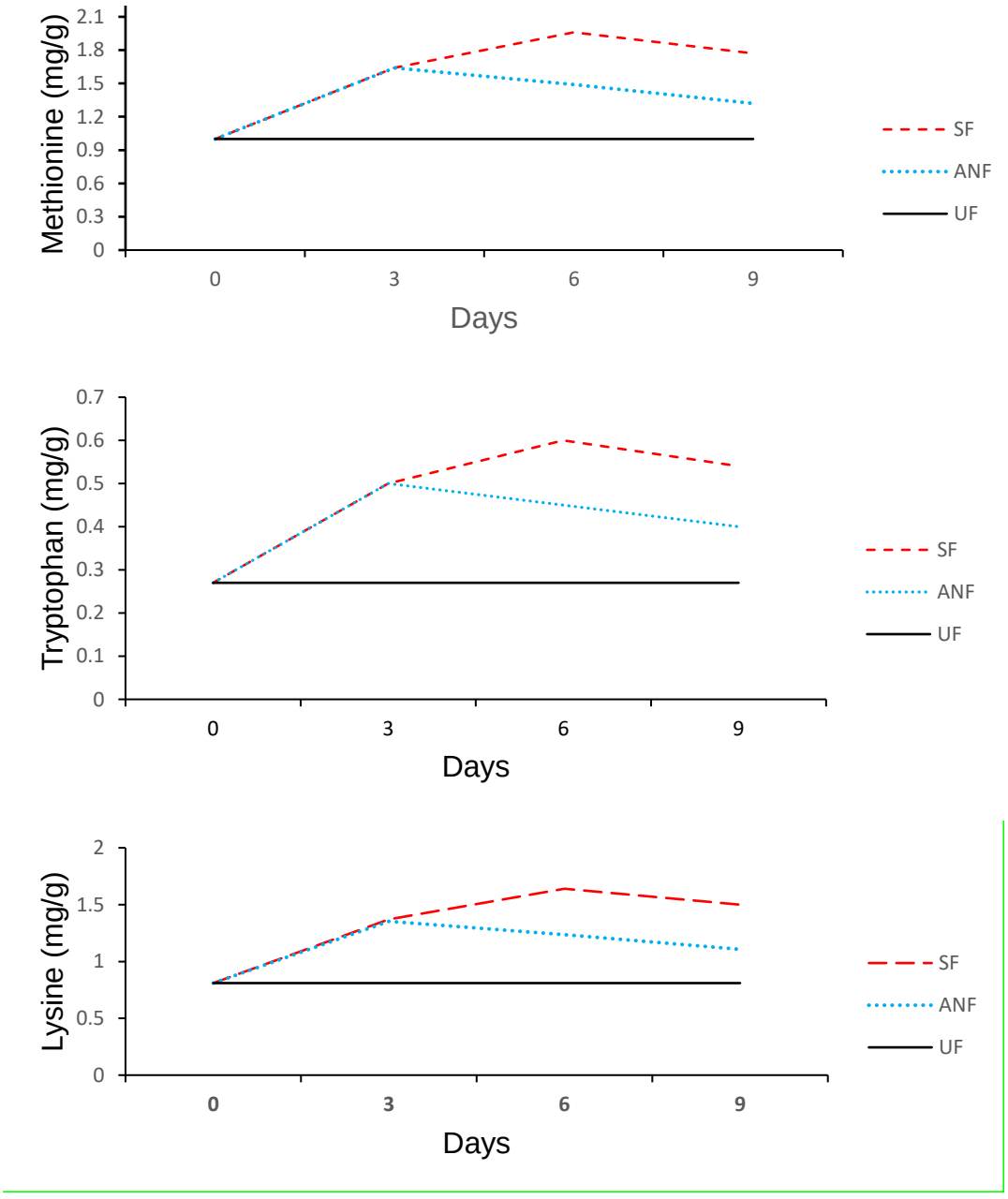
T = Treatment; d= days; SEM= standard error of means; ppm = parts per million; ^{abc} Means in the same row without common letter are different at P < 0.05; ppm = parts per million

Article DOI : https://doi.org/10.37284/eajab.8.1.2936

Effect of Prosopis Pods Meal Fermentation on Amino Acid Composition

The effect of the fermentation period and fermentation methods results are indicated in Table 2 and Figure. 1.

Figure 1: Interaction Plots between Fermentation (SF= Spontaneously Fermented, ANF = *Aspergillus niger*-Fermented, UF = Unfermented) Methods and Fermentation Periods (3, 6, and 9 Days) on Methionine, Tryptophan and Lysine Mean Values.



Methionine Content Post-fermentation

At three days, both fermentation methods showed a positive influence on the methionine content of the pods as compared to unfermented pods where the was no interaction between days and fermentation method. The methionine content was statistically

Article DOI : https://doi.org/10.37284/eajab.8.1.2936

higher ($P < 0.05$) in the two methods as compared to unfermented pods.

At six days of the fermentation period, increased and reduced effects on methionine content were observed for spontaneous and *A. niger*-induced fermentation methods respectively. Although the methionine content was similar ($P < 0.05$) in both, it was statistically lower as compared to content in unfermented pods. Methionine content in unfermented PPM did not show any relationship with the fermentation period.

The nine days in both spontaneous and *A. niger*-induced fermentation methods had the effect of reducing the methionine content. Considering the same fermentation period, there is no effect with methionine content remaining unchanged in unfermented PPM. The methionine content was statistically different ($P < 0.05$) in the three forms of PPM.

Tryptophan Content Post-fermentation

At three days, both fermentation methods showed a positive influence on the tryptophan content of the pods as compared to unfermented pods. The tryptophan content was statistically higher ($P < 0.05$) in the two methods as compared to unfermented pods.

At six days, spontaneously fermented pods showed an increase in tryptophan content while at the same period, there was reduced tryptophan content in *A. niger* induced fermented pods. Tryptophan content was unchanged in unfermented pods. The three PPM were statistically different ($P < 0.05$) from each other.

The 9-day period of fermentation in both spontaneous and *A. niger*-induced fermentation methods showed the effect of reducing tryptophan content in PPM. At the same fermentation period, there was no change in tryptophan in unfermented PPM. The three mean tryptophan values for PPM were statistically different ($P < 0.05$).

Lysine Content Post-fermentation

A positive effect in lysine content at 3 day-fermentation period for both spontaneous and *A. niger*-induced fermentation methods was recorded with the values being statistically similar ($P > 0.05$). During the same fermentation period, there was no effect on the lysine content of unfermented PPM. This observed value was statistically lower ($P < 0.05$) compared to the lysine content recorded in fermented PPM.

At 6 days of the fermentation period, increased and reduced lysine content was observed in both spontaneous and *A. niger* fermentation methods respectively. Lysine values were statistically different ($P < 0.05$) in all three cases of PPM. Unfermented pods showed no relationship with the fermentation period.

There was reduced lysine content at 9 days' fermentation for both the spontaneous fermentation method and for *A. niger* fermentation method. Considering the same fermentation period, there was no change in methionine content in unfermented PPM. The three lysine contents were statistically different ($P < 0.05$).

Effect on *Prosopis juliflora* Tannin Percentage Post-Fermentation

At 3 days, both the spontaneously and *Aspergillus niger* induced fermented pods had similar ($P > 0.05$) tannin percentages but which were lower than the control. Both 6 and 9 days in spontaneously and *Aspergillus niger* induced fermented pods were similar but significantly lower ($P > 0.05$) than in both fermentation biotechnologies and at 3 days fermentation period.

In vitro Dry Matter Digestibility of Enzyme–treated, Spontaneously Fermented and Aspergillus Niger-induced Fermented Prosopis Pod Meal.

The IVDMD results are shown in Figure 2**.** Enzyme-treated PPM (Enz) and fermented PPM (SF6, SF9 and ANF9) resulted in highly significant ($P < 0.05$) IVDMD compared to unfermented (UF0)

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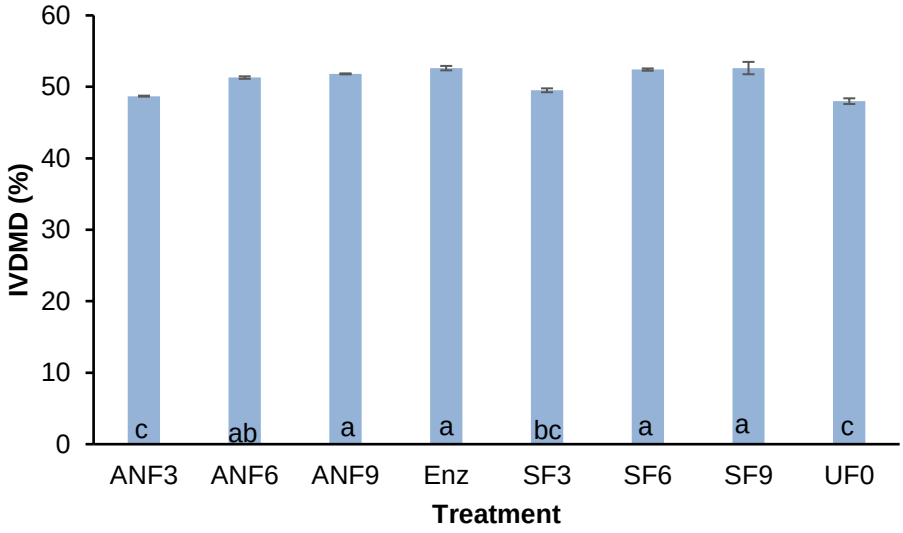
PPM. There was a 4.6% increase in IVDMD with enzyme treatment (Enz) (52.6%) and spontaneous fermentation (SF9) (52.6%) relative to unfermented PPM (UF0) (48%). There was also an increase in IVDMD in both fermented PPM of 4.42% (SF6) (52.42%) and 3.83% (ANF96) (51.82%) relative to unfermented PPM (UF0) with 48.00%.

Figure 2: Effect of Fermentation and Enzyme Treatment of Prosopis Pod Meal on *In vitro* Dry Matter Digestibility. Error Bar Represents Mean (± SE) *in vitro* Dry Matter Digestibility of PPM. [abc] Means in Different Bars without Common Letter are Different at $P < 0.05$.



Enzyme-treated (Enz), 6-day (SF6) and 9-day (SF9) spontaneous and 9-day (ANF9) *A. niger*-induced fermented PPM had higher and significant IVDMD compared to unfermented (UF0) and 3-day *Aspergillus* niger-induced PPM (ANF3). There were no significant differences in digestibility of unfermented (UF0) (48%) and *A. niger*-induced fermentation (ANF3) (48.7%).

DISCUSSION

Effect of Fermentation on Crude Protein

The chemical composition of PPM is within the results reported by similar studies (Wanjohi et al., 2017; Odero-Waitituh et al., 2020). The CP results for unfermented and 3-day spontaneous and *Aspergillus niger*-induced fermentation of PPM are consistent with results reported by Girma et al. (2011) and Wanjohi et al. (2017) on unfermented pods. This indicates that there are no advantages in fermenting either spontaneously or with *A. niger* for a 3-day period. Similarly, the results for 9-day fermentation for both fermentation methods were also within the range reported by the same authors. However, from this study, 6-day fermentation results indicated higher values which can be attributed to the breakdown of fibre that enhances protein biosynthesis (Sugiharto and Ranjitkar 2019).

Fermentation is therefore a means of improving the CP of the PPM specifically for 6 days. The increase at 6 days could be a result of the activity of fermentative microbes and protein hydrolysis (Fadahunsi et al., 2010). The increase concurs with the finding of Balogun (2011) in the production of traditional condiments from *Prosopis africana* seeds. Sirajo and Sani (2015). Previous studies have reported an increase in CP in Prosopis pods when they were fermented (Odero-Waitituh et al., 2020; Marii et al., 2021). Various factors, ranging from the processing method, length of fermentation and the activities of micro-organisms, could be responsible for the differences obtained in studies involving

Article DOI : https://doi.org/10.37284/eajab.8.1.2936

fermentation (Yusuf et al., 2008). Solid-state fermentation of wheat bran using *A. niger* S14 (a mangrove isolate) resulted in a substantial increase in crude protein level (57 to 66%) as compared to that of raw wheat bran (Imelda and Bhatnagar 2008). The positive results observed in the increase in CP content in this study could be linked to a decrease in the concentrations of non-structural carbohydrates (Wu et al., 2020). With regard to reduced CP values at 3 and 9 days, this could be attributed to microbial mechanisms which indicates the optimum fermentation pattern that should be harnessed at six days. At early fermentation the values were low and this could be related to reduced activity due to high strong bonds and complex chains. Fermentation at 9 days also recorded reduced CP values and this could be attributed to microbes utilizing pod protein as the carbon source for the growth of micro-organisms during fermentation (Sarasvati et al., 2014) or utilizing the products of fermentation for their use (Kumar et al., 2020). The results imply that fermentation significantly affects CP content and is greatly dependent on fermentation time (Sarasvati et al., 2014).

Amino Acid Changes with Fermentation

According to Mejia et al. (2022), fermentation increased the protein content by 43.5% with a significant increase in amino acid content by up to 131%, particularly Lysine which increased 93.9% - 131.5% in the different corn cultivars. In other studies, the increase was better for lysine with fungal fermentation (2.31-4.01%) with an improvement in methionine and tryptophan in response to fermentation and an improved essential amino acid index (EAAI) in the fermented ingredients (Jannathulla et al., 2017). According to Odero-Waitituh et al. (2020), there was a significant increase in essential amino acids in fermented Prosopis pods as compared to unfermented Prosopis pods. These findings agree with the results of this study. Dietary fibre can also adsorb amino acids and peptides, thereby withholding them from digestion, therefore reducing the fibre ensures that there is available protein for chicken.

Effect of Fermentation on Crude Fiber

The use of fermentation and enzyme treatment techniques has been reported as key in reducing the fibre content which is regarded as an anti-nutritive factor in feeding of chicken. A study by Jannathulla et al. (2017) reported a significant reduction in fibre contained in rapeseed as an ingredient. According to Jahromi et al. (2010), *the* reduction of lignocelluloses content in the rice straw was attributed to the activities of cellulase, beta glucosidase and xylanase enzymes of the *A. niger*. According to Sarasvati et al. (2014), fermentation resulted in a maximum decrease in total fibre (up to 30%) and in hemicelluloses (up to 17. 67%). In a study by Sirajo and Sani (2015), there was reduced crude fibre in fermented (21.83 ± 0.76%) as compared to unfermented (26.50 ± 1.00%) *Prosopis juliflora* pods. *A. niger* has the ability to produce lignocellulolytic enzymes that can be used for biological treatment of low-quality biomass, such as PPM as reported by fermenting rice straw for animal feed (Jahromi et al., 2010).

According to Hu et al. (2021), dietary cellulase is an important enzyme when the SSF technique is used to improve the nutritional value of feed. The enzyme can be produced from both the fermentation processes as well as from exogenous enzymes like Natuzyme. Fermentation of Prosopis pod meal resulted in reduced crude fibre content in this study. As the fermentation days increased, the fibre in the PPM decreased but as the fermentation days increased, there was an increase in the amount of crude fibre. The results of this study concur with what Sarasvati (2014) reported, where there was an initial decrease in early fermentation followed by an increase later in fermentation. In this study, a 9-day fermentation period for both spontaneous and *A niger*-induced fermentation recorded increased CF while at 6 days there were lower values compared to other treatments. The reduced fibre in early periods can be attributed to the solubilization of

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fibre while the increase at later stages can be due to the accumulation of bacterial cell walls (Saravasti et al., 2014). This proves that 6 days of fermentation either spontaneously or with *A. niger* can be used to decrease the dietary fibre thereby improving the digestibility of feeds.

Effect of Fermentation on Tannin Content

According to Maud et al. (2023), 72 hours of fermentation of the Prosopis pods was the best in reducing the anti-nutritive factors. Mejia et al. (2022) reported that the phytic acid content of the fermented corn decreased by 24.3% suggesting that fermentation may also improve the bioavailability of minerals by lowering the levels of anti-nutritional factors, such as phytates and decreasing the level of mycotoxins corn. The significant reduction of anti-nutrients such as trypsin inhibitor, phytic acid, saponin, tannin, glucosinolate and guar gum were found to be lower in natural fermentation than other methods. The results indicated that fungal fermentation is more suitable for improving the nutritional quality (Jannathulla et al., 2017). Odero-Waitituh et al. (2020), reported reduced levels of tannin content in fermented prosopis pods. In this study, there was a significant reduction in tannin content at 6 and 9 days of fermentation as compared to unfermented and PPM fermented for 3 days.

***In vitro* Dry Matter Digestibility**

The new feed resources are a potential solution to the challenges that have been facing non-ruminant feeding. The nutrients found in these feed resources are usually not readily available to non-ruminants due to high crude fibre. *Prosopis julifora* pods have in the recent past been researched extensively as a feed for non-ruminants. The inclusion levels have been found to be 20% without any processing with a potential increase to above 30% with treatments such as fermentation and the use of exogenous enzymes. *In vitro* digestion and fermentation models are methods that simulate the digestion and/or fermentation processes that occur in the animal's gastrointestinal tract (GIT) (Heyer et al., 2022). *In vitro* dry matter digestibility technique is an easy way to investigate the alternative feed resources that have been processed or treated to improve their use as chicken feed. The IVDMD experiment was meant to evaluate the interaction between the three fermentation periods and two fermentation techniques in PPM to come up with the potential way of preparing the pods as a feed ingredient for non-ruminants.

The IVDMD of PPM at 6 and 9 days for both *A. niger* fermentation, as well as enzyme-treated, were similar but significantly higher than 3-day *A. niger* and unfermented PPM. Marii et al. (2022) reported IVDMD of 49.1% in untreated pods compared to 48% in untreated PPM. In this study, the use of multienzyme resulted in higher values of IVDMD (52.8%) as compared to 52.6%, 52.42% and 52.63% in Natuzyme treated, 6-day naturally fermented and 9-day naturally fermented pods.

CONCLUSIONS

Six days of spontaneous fermentation significantly improved the nutritional profile of *Prosopis juliflora* pod meal by increasing crude protein, decreasing fibre, *in vitro* dry matter digestibility and increasing tryptophan, methionine and lysine contents. This method significantly improved the amino acids that are limiting in maize-based feed formulations.

Recommendations

The study recommended fermenting *Prosopis juliflora* pod meal for six days for optimal benefits, including reduced fibre, increased protein, and essential amino acids. Enzyme-treated pod meal was also recommended as an alternative to maize in broiler diets.

Acknowledgements

The authors would like to acknowledge Egerton University's Centre of Excellence in Sustainable Agriculture and Agribusiness Management (CESAAM), which provided funds for this study. The Technologists in the Animal Nutrition

Article DOI : https://doi.org/10.37284/eajab.8.1.2936

Laboratory in the Animal Science Department, Egerton University are also appreciated.

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