Effects of treating *Prosopis juliflora* pods with multienzyme, with and without bacterial cultures on in vitro dry matter digestibility (IVDMD), fermentation kinetics, and performance of growing pigs

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Abstract
This study was conducted to determine the effects of treating *Prosopis juliflora* pods with multienzyme and bacterial cultures on in vitro dry matter digestibility (IVDMD), fermentation kinetics, and performance of growing pigs. Experiment one consisted of a pepsin-pancreatine hydrolysis method to simulate, in vitro, the pig digestive system and was followed by in vitro gas production to assess fermentation kinetics. Samples of ground *Prosopis* pod meal (GPPM) were allocated to four treatments with three replicates each. Treatments included GPPM treated with multienzyme (Natuzyme®) (T1); untreated (GPPM) (T2); GPPM fermented with (*Lactobacillus plantarum* MTD1 Ecosyl ®) (T3), and GPPM treated using natural fermentation (T4). The second experiment assessed the performance of pigs fed the best treatment from experiment 1. Thirty Landrace × large white crosses of 20 ± 2 kg were allotted to five treatments with six pigs each (replicates). The dietary treatments were PC, 0% GPPM + enzyme; NC, 0% GPPM and 0% enzyme; D1, 10% GPPM + enzyme; D2, 20% GPPM + enzyme; and D3, 30% GPPM + enzyme. A randomized complete block design was used for both experiments. Enzyme treatment (T1) and T3 improved the IVDMD of the GPPM compared to T2 by 3.68% and 1.2%, respectively (*p* < 0.05). Cumulative gas was highest and Tmax lowest for T1 but significantly different only to T4 (*p* < 0.05). Average daily gain and intake were highest for pigs fed GPPM up to 10% (PC, D1). Feed conversion ratio increased with the level of GPPM in the diet. The results suggest *Prosopis juliflora* pods treated with enzymes can be added in pig diets up to 30%.

Keywords *Prosopis juliflora* pod meal · Fermentation kinetics · Pig nutrition

Introduction
The animal feed industry faces the challenge of the increased price of cereals and oilseeds that are in high demand for human consumption and bioenergy production, especially in emerging markets (de Vries et al. 2012). These feed resources are critical for pig nutrition since pigs cannot efficiently utilize fibrous feed materials. In developing countries, specifically in Kenya, mainly smallholders with limited resources practice pig farming (Kagira et al. 2010; Kambashi et al. 2014). They face the challenge of the high cost of conventional feed and often resort to the use of locally available feed resources. A survey in Kenya reported that only 30% of pig farmers use commercial feeds while the remaining included some form of alternative feed material in their feeding regime (Mbuthia et al. 2014). Mature *Prosopis* pods from *Prosopis juliflora* tree is such an alternative feed ingredient in Kenya (King’ori et al., 2011).

*Prosopis* is an invasive multipurpose tree species widely distributed within the arid and semi-arid areas of Kenya. *Prosopis* pods have been evaluated by several authors and were found to be of good nutritional quality for both ruminants and nonruminants (Odero-Waititu et al. 2016; Manhique et al. 2017). The energy content of the pods 12.8 MJ/Kg DM is higher than most cereal-based milling by-products (Maize germ and wheat bran) with about 11 MJ/Kg DM and slightly lesser than maize at 13.8 MJ/Kg DM (NRC 2012; Odero-Waititu et al. 2016). However, the pods contain high levels of plant cell wall components that lower the efficiency of nutrient utilization by...
nonruminants because they lack endogenous fiber digestive enzymes (de Vries et al. 2012). Relative to maize, Prosopis juliflora pods contain 69.5%, 53.5%, and 97% more cellulose, hemicellulose, and lignin content, respectively. In poultry, the high fiber content of Prosopis pod meal contributes to reduction in the apparent ileal digestibility and the apparent metabolizable energy relative to maize (Al-Marzooqi et al. 2015). In pigs, poor growth rates and feed intake were reported when Prosopis pod meal replaced 30% maize soybean mixture due to the effects of high fiber (Pinheiro et al. 1993).

Physical, chemical, and biological processing has the potential to increase the utilization of highly fibrous alternative feed materials (de Vries et al. 2012). Processing methods, including the use of particle size reduction, exogenous enzymes, and fermentation, improve digestibility and fermentation kinetics of poor-quality feed materials (Lee et al. 2018; Zangaro et al. 2019). The use of lactic acid bacteria fermentation has not only been utilized as a means of feed preservation over the years but has been associated with a reduction of the total fiber content through hydrolysis of the neutral detergent fiber while improving the crude protein levels (Wang et al. 2018; Odero-Waititu et al. 2020). Lyberg et al. (2006) reported an improvement of organic matter digestibility and crude protein content after lactic acid fermentation of wheat and barley-based diets. This is supported by a recent study that concluded that the use of lactic acid fermentation improved the in vitro dry matter digestibility and the fermentation kinetic of high fibrous rapeseed meal (Jang et al. 2021).

On the other hand, fibrolytic exogenous enzymes have accelerated the use of fibrous feed material due to their ability to break down the soluble and insoluble non-starch polysaccharides. Upon the breakdown of fiber and availability of the encapsulated nutrients, the resultant products are readily accessible for the intestinal microflora, thereby providing multiple beneficial effects on whole animal and animal gut intestinal health (Woyengo et al. 2016). Unfortunately, the lack of empirical evidence on digestibility improvement of Prosopis pod meal subjected to pretreatment methods remains a hindrance to their utilization in pig production in Kenya.

We therefore hypothesized that pre-treatment of Prosopis pod meal with exogenous enzyme or fermentation could improve in vitro digestibility and fermentation kinetics, hence pig performance. The objective of the study was twofold: (i) to evaluate the most suitable pretreatment method of Prosopis pod meal using in vitro digestibility; (ii) to determine the effects of increasing levels of pre-treated Prosopis pod meal in diets of growing pig diets on growth performance.

Materials and methods

Study site

The in vitro experiment was conducted at Egerton University, Animal Nutrition laboratory. The University is situated at 0° 23’ S, 35° 55’ N within Njoro Sub-county, Nakuru County. The area is located at 0° 35’ south and 0° 35 15’ east with an elevation of 2400–3100 m above sea level and an average annual rainfall of 1200–1500 mm.

Experiment 1: in vitro dry matter digestibility

Preparation of experimental treatment and experimental design

Mature Prosopis pods were collected from Marigat Sub-county located 0° 20’ N and 35° 37’ E (FAO 1992) by hand-picking from the ground underneath the Prosopis trees after vigorously shaking. The pods were dried, sorted then milled (Choge et al. 2007a), and used in the preparation of the treatments. There were four treatments with three replicates each, arranged in a completely randomized design; T1, GPPM treated with an enzyme (Natuzyme®); T2, untreated GPPM (control); T3, GPPM fermented with lactic acid bacteria inoculum (Lactobacillus plantarum); T4, GPPM treated using natural fermentation.

Natuzyme® (Bioproton Pty Ltd, Australia) is a multienzyme complexin powder form and contains 12,000 units/g of xylanase, 6,000 units/g of cellulase, 1,500 units/g of phytase, 700 units/g of beta-glucanase, 700 units/g protease, and 400 units/g of alpha-amylases. The enzyme was added to the samples of GPPM in triplicate enzyme at the rate of 350 mg/kg of feedstuff on a dry matter basis following the manufacturer’s instructions and recommendations and mixed thoroughly.

Naturally fermented Prosopis meal was prepared by mixing 1 kg mature GPPM with distilled water at a ratio of 1:2.75 (wt. /vol) in triplicate. The mixture was then incubated at room temperature (+22 °C) for 7 days using 2-kg plastic bottles (Jørgensen et al. 2010). The plastic bottles were sealed tightly to create anaerobic conditions. After 7 days, the pH of individual samples was recorded using a portable pH meter (pH/ORP/Temperature Combo Tester—HI98121 HANNA Instruments), and a sample was
subjected to proximate analysis. A similar procedure was applied for inoculation fermentation but in this case, a single strain of commercial *Lactobacillus plantarum*-MTD1 (Ecosyl® Products, Ltd., Stokesley, England) as the starter culture and applied at the rate of $1 \times 10^6$ cfu/g of GPPM. A sample was also collected for proximate analysis. The pH of individual samples was measured and recorded using a digital hand-held pH meter (pH/ORP/Temperature Combo Tester—HI98121 HANNA Instruments).

**Proximate analysis**

The proximate analysis conducted on the samples included dry matter determination by drying in a hot air oven at 105 °C for 24 h (method 934.01; AOAC, 1990), ash by burning samples in a muffle furnace at 550 °C for 8 h (method 942.05; AOAC, 1990), and ether extract Soxhlet method (using ether) (method 920.39; AOAC, 1990). Total nitrogen for crude protein ($N \times 6.25$) determination was obtained using the micro-Kjeldahl method (method 954.01; AOAC, 1990). Constituents of the cell wall, neutral detergent fiber (NDF) and acid detergent fiber (ADF), were determined using the Van Soest method (Van Soest et al. 1991). Hemicellulose was determined as a difference between the neutral detergent fiber (NDF) and the acid detergent fiber (ADF).

**Enzymatic pre-digestion of ground Prosopis pod meal**

To simulate the digestive process in the pigs’ stomach and intestines, an in vitro digestibility trial was conducted according to Boisen and Fernández 1997. Ground feed sample of 0.4 g was weighed and placed in a 100-ml conical flask. Sodium phosphate buffer solution, 200 ml (0.1 M, pH 6.0), was added to the flask and carefully mixed with the sample by stirring. To simulate the stomach digestive process, 80 ml of 0.2 M HCl was added and the pH adjusted to 2.0 with 1 M HCl or 1 M NaOH solutions. This was followed by addition of prepared 5-mL pepsin porcine grade enzyme with 4x USP activity (pepsin from porcine gastric mucosa powder, ≥ 250 units/mg solid ©Sigma-Aldrich Corp., St. Louis, MO, USA) containing 1-mg pepsin per ml 0.02 M HCl. To each conical flask, 2-ml chloramphenicol C-0378; (Sigma-Aldrich Corp., St. Louis, MO, USA) and 0.5 g/100-ml ethanol were added to inhibit bacterial growth. The flasks were closed and incubated in a water bath at 39 °C and stirred continuously for 2 h. Afterwards, 80 ml of phosphate buffer (0.2 M, pH 6.8) and 20 ml of 0.6 M NaOH were added. The pH was adjusted to 6.8 using 1 M HCl or 1 M NaOH to provide a stable environment for intestinal enzymes to thrive.

To the mixture, 10.6 ml of artificial pancreatin P-1750 Sigma-Aldrich Corp., St. Louis, MO, USA, containing 100 mg/1 L buffer was added and incubated at 39 °C with continuous stirring for 4 h. The residues were filtered through a nylon bag (pores size of (42 µm) washed with distilled water, followed by washing two times using 20 ml, 95% ethanol, and 20 ml, and 99.5% acetone. The residues were dried in an oven at 70 °C for 12 h and weighed.

**In vitro fermentation phase**

The rate of fermentation of the hydrolyzed substrate was assessed in vitro by the cumulative gas production technique adapted to the pig by Bindelle et al. (2007). A 200- mg sample was used for the third step of this procedure involving microbial fermentation.

**Donor animals**

The donor animals were three large white barrows with an average weight of 25 ± 3 kg. Pigs were fed a diet containing *Prosopis* pods for 7 days to allow for the adaptation to the diet (Table 1). The pig’s diet contained no antibiotics. Fecal samples were collected directly from the rectum of the pig and immediately placed in a flask flushed with CO₂ to avoid exposure to aerobic condition and kept in a water bath at 39 °C until the time of use. The feces of the three pigs were pooled together in equal amounts to reduce within animal variation.

**Table 1 Diet containing *Prosopis juliflora* pod meal (g/100 g)**

| Ingredients                        | Quantity in g/100 g |
|------------------------------------|---------------------|
| Maize                              | 60                  |
| Soybean meal                       | 21.4                |
| Fishmeal (Omena)                   | 2                   |
| Ground *Prosopis juliflora* pod meal | 12                |
| Vegetable oil                      | 2                   |
| Di-calcium phosphate               | 2                   |
| Lysine                             | 0.2                 |
| Methionine                         | 0.2                 |
| Vitamin and mineral premix         | 0.1                 |
| Iodized salt                       | 0.1                 |
| Chemical composition               |                     |
| ME(Mj/Kg)                          | 13.2                |
| Crude protein                      | 17.25               |

*Vitamin premix: vitamin and mineral premix: vitamin A 8,000 IU; vitamin D3 2,000; vitamin E 37.5 mg; vitamin K-3 0.925 mg; vitamin B2 8.43 mg; vitamin B12 0.04 mg; nicotinic acid 34.5 mg; pantothenic acid 26 mg; 450 mg Fe; 400 mg Cu; 250 mg Zn; 150 mg Mn; 0.5 mg I; 0.25 mg Se: Omena (Rastrineobola argentea) © Springer
Inoculum preparation and incubation

The fecal mixture was blended to homogenize and then filtered through a double-layered cheesecloth. The filtrate was mixed at a ratio of 1 part filtrate to 20 part buffer solution on a volume/volume basis (Bauer et al. 2001). The buffer solution is composed of micro and macro minerals, reducing solution, and carbonate buffer (Menke et al. 1979). The inoculum prepared was then poured into calibrated 100-ml Poulten & Graf GmbH FORTUNA™ Precision Gas Syringe containing 200-mg substrate in a thermostatically controlled water bath at 39 °C. The syringe pistons were lubricated using oil to allow ease of movement during gas measurement throughout the entire process. The whole process was carried out in anaerobic conditions by continuously bubbling of CO₂. Two syringes containing the inoculum only were used as blanks. Each treatment had three replicates, and the amount of gas produced during fermentation was measured at 0, 2, 5, 8, 12, 16, 20, 24, 30, 36, 48, and 72 h. The total gas produced at a particular period was computed as the total increase in volume minus the volume of gas recorded in the blank. The experimental scheme was as follows: 2 runs × 4 treatment groups × 3 replicate + 2 blanks (contained inoculum only).

Experiment two: in vivo evaluation

Management of experimental animals and experimental design

Experimental animals used were 15 barrows and 15 gilts with an average weight of 20 ± 2 kg which were crosses between Landrace and large white. They were randomly allotted to the five treatment diets in a randomized complete block design with sex being the blocking factor. The experimental animals were placed in pens with concrete floors (3 m × 3 m) that had dry wood shavings as beddings. The pigs used for the experiment were fed on the experimental diets for 7 days for adaptation before the beginning of the data collection period of 35 days.

Experimental diets

The pigs used were allowed ad libitum access to experimental diets and water. The experimental animals were fed from concrete troughs while water was provided ad libitum using drinking nipples. The composition of the experimental diets is shown in Table 1.

These were PC = diet containing 0% GPPM and 0.035% enzyme, NC = diet containing 0% GPPM and 0% enzyme, D1 = diet containing 10% GPPM and 0.035% enzyme, D2 = diet containing 20% GPPM and 0.035% enzyme, and D3 = diet containing 30% GPPM and 0.035% enzyme. The diets were formulated using maize germ, wheat bran, sunflower seed meal, fishmeal-Omen, (Rastrineobola argentea) and ground Prosopis pod meal (GPPM). Natuzyme® was included at the rate of 350 mg/kg of feed in dry form as per the manufacturer’s instructions and recommendations. Diets were formulated to meet the nutrient requirements of growing pigs (NRC 2012) (Table 2).

Data collection on feeding trial

Individual pig body weight from each pen was recorded every week using a digital weighing scale with a portable hanging balance with 10 g accuracy. The weekly weights recorded were used to compute the average daily gain. Feed was offered at 08:00 am

### Table 2 Composition of the experimental diets (g/100 g) and their chemical composition

| Ration composition (g/100 g) | Treatments |
|-----------------------------|------------|
| Wheat bran                  | PC NC D1 D2 D3 |
| Maize germ                  | 15.0 15.0 8.5 4.0 2.8 |
| Vegetable Oil              | 53.165 53.160 48.515 42.475 32.545 |
| GPPM                        | 5.0 5.0 5.0 5.0 5.0 |
| Fishmeal                    | 0.0 0.0 10.0 20.0 30.0 |
| Sunflower seed cake         | 6.0 6.0 5.5 5.0 5.0 |
| Sunflower seed cake         | 17.45 17.49 19.1 20.14 21.22 |
| Lysine                      | 0.9 0.9 0.9 0.9 0.9 |
| Methionine                  | 0.5 0.5 0.5 0.5 0.5 |
| DCP (granular 24%)          | 0.5 0.5 0.5 0.5 0.5 |
| Limestone                   | 0.85 0.85 0.85 0.85 0.85 |
| Iodized Salt                | 0.25 0.25 0.25 0.25 0.25 |
| Vitamin Premix*             | 0.25 0.25 0.25 0.25 0.25 |
| Mycotoxins Binder           | 0.1 0.1 0.1 0.1 0.1 |
| Natuzyme® enzyme            | 0.035 0.0 0.035 0.035 0.035 |

### Nutrients analysis

| Dry matter                   | 88.35 88.35 88.75 88.52 90.47 |
| Crude Protein(CP)            | 17.01 17.01 17.02 16.81 17.01 |
| Ether Extracts               | 7.90 7.90 6.93 5.71 6.51 |
| Ash                          | 8.27 8.32 8.33 12.33 13.11 |
| NDF                         | 32.63 32.63 34.38 35.63 37.13 |
| ADF                         | 11.12 11.10 12.60 13.65 16.94 |
| GE(MJ/kg DM)                 | 17.10 17.10 16.01 16.01 16.10 |

PC = 0% GPPM and 0.035% enzyme per kg of diet; NC = 0% GPPM and 0% enzyme per kg of diet; D1 = 10% GPPM and 0.035% enzyme per kg of diet, D2 = 20% GPPM and 0.035% enzyme per kg of diet; D3 = 30% GPPM and 0.035% enzyme per kg of diet; GPPM ground Prosopis pod meal; *vitamin and mineral premix: vitamin A 8,000 IU; vitamin D3 2,000; 201 vitamin E 37.5 mg; vitamin K 3 0.925 mg; vitamin B2 8.43 mg; vitamin B12 0.04 mg; nicotinic acid 34.5 mg; pantethenic acid 26 mg; 450 mg Fe; 400 mg Cu; 250 mg Zn; 150 mg Mn; 0.5 mg f; 0.25 mg Se. CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; GE, gross energy.
while refusals were collected during the next day prior to feeding. The refusals were weighed using a digital weight balance then used to compute daily feed intake. Average daily gain and daily feed intake were used to compute the feed conversion ratio. The formulae (1, 2, and 3) show the equations used to calculate the production performance:

\[
\text{Average daily gain (ADG) in kg/day} = \frac{(\text{Final Weight} - \text{Initial Weight})}{7 \text{ days}} \quad (1)
\]

Daily feed in take = Feed offered (kg) − Feed refusal (kg) \quad (2)

\[
\text{FCR} = \frac{\text{Feed intake}}{\text{Weight gain}} \quad (3)
\]

### Statistical analysis and calculations

In vitro total tract digestibility (IVDMD) of dry matter (DM) was computed using the following formula (Boisen and Fernández 1997):

\[
\text{DM digestibility} = \left( \frac{\text{DM}_{\text{In}} - \text{DM}_{\text{RS}}}{\text{DM}_{\text{In}}} \right) \times 100
\]

\(\text{DM}_{\text{In}}\) and \(\text{DM}_{\text{RS}}\) are the initial (DM) and residual (DM), respectively.

Gas accumulation curves recorded during the 72 h of fermentation were modified according to the monophasic model (Groot et al. 1996).

\[
G = \frac{A}{1 + \left( \frac{t}{C} \right)^B}
\]

where \(G\) (mL/g DM) is the amount of gas produced per gram of Dry matter (DM) incubated, at time \(T\) after incubation, \(A\) (mL/g DM) is the asymptotic gas production, \(B\) (h) is the time after incubation at which half of the asymptotic amount of gas has been formed, and \(C\) is constant determining the sharpness of the switching characteristic of the profile.

\(R_{\text{max}}\), the maximum rate of gas production (ml g⁻¹ × h⁻¹), and \(T_{\text{max}}\), the time at which \(R_{\text{max}}\) is accomplished, were computed using the formula by Bauer et al. (2001).

\[
R_{\text{max}} = A \times (C^B) \times B \times (T_{\text{max}}^{-B-1})/[1 + (C^B) \times (T_{\text{max}}^{-B})]^2
\]

\[
T_{\text{max}} = C \times \left[ \frac{(B - 1)/(B + 1)}{(B - 1)} \right]^{1/2}
\]

Data for the in vitro experiment was analyzed with on IBM SPSS Statistics version 22. The assumption for normality and homogeneity of variance of the data was checked using Shapiro–Wilk and Levene’s test statistics, respectively, with data assumed to be normal when \(p > 0.05\). The IVDMD during hydrolysis, total gas production, and fermentation kinetics were analyzed using the mixed model procedure. Mean separation was conducted using Tukey’s HSD (honest significant difference) test at 0.05 level of significance (means were considered different if \(p < 0.05\). Application of Excel solver in Microsoft excel was used in curve fitting and in the computation of fermentation kinetics. The statistical model used for the experiment is,

\[
Y_{ijk} = \mu + T_i + G_k + e_{ijk}
\]

where \(Y_{ijk}\) is the observation on the dependent variables, \(\mu\) is the overall mean, \(T_i\) is the fixed effect due to the \(i\)th treatment, \(G_k\) is the random effect due to trial, and \(e_{ijk}\) is the random error.

For experiment two, experiment data was analyzed using SAS 9.0 (2002) using a two-way analysis of variance. Mean separation was conducted using Tukey’s HSD (honest significant difference) test at 0.05 level of significance (means were considered different if \(p < 0.05\). The initial weight of the pigs was fitted as a covariate while sex was used as a blocking factor.

The model was used:

\[
Y_{ijk} = \mu + T_i + B_i + S_j + e_{ijk}
\]

where \(Y_{ijk}\) is the response variable of interest (ADG, FCR), \(\mu\) is the population mean, \(T_i\) is the fixed \(i\)th treatment effect (PC, NC, D1, D2, and D3), \(S_j\) is the fixed effect of sex (gilts and barrows), and \(B_i\) is the fixed effect of initial weight used as a covariate.

### Results

Nutritional composition of GPPM after being subjected to the different treatments is shown in Table 3. Crude protein was higher following fermentation (15.5 g/100 g and 16.4 g/100 g for natural and inoculated, respectively) than that of enzyme treatment and control (14.5). NDF was also higher for fermented GPPM (41.5 and 43.41) for T3 and T4, respectively. The pH values for natural fermented and Lactobacillus Plantarum fermented GPPM were 4.6 and 4.4, respectively.

### In vitro digestibility of untreated, enzyme-treated, and Lactobacillus Plantarum fermented GPPM

Enzyme treatment (T1) and inoculation using Lactobacillus plantarum (T3) resulted to a higher and significant IVDMD compared to natural fermentation (T4). There was a 3.68% increase in IVDMD (Fig. 1) after treating the pods with the multienzyme (T1) (52.81 ± 1.17), relative to the untreated (T2) (49.13 ± 2.14). There was no significant difference in digestibility of T2 and T3.
Fermentation kinetics

The cumulative gas curve shows that T1 (18.4 DM/ML) and T2 (17.8 DM/ML) had the highest gas production, while T3 (14.3 DM/ML) had the lowest. This trend was observed from 8 to 72 h (Fig. 2).

The results of fermentation kinetics of the experimental treatments are presented in Table 4. Treatment 4 (13.429 ± 1.63) had the overall lowest cumulative gas produced G (ml/g DM) after 72 h in contrast to T1 (18.502 ± 1.27) which had the highest cumulative gas produced G (ml/g DM). However, T1, T2, and T3 were not significantly different \( p > 0.05 \) (Table 4). \( R_{\text{max}} \), which represents the rate of gas production, was significantly affected by pre-treatment methods. Enzyme-treated GPPM (T1) had the highest rate of gas production (0.786 ± 0.09), while naturally fermented pod meal T4 had the lowest rate (0.425 ± 0.04). The rate of gas production was similar between enzyme-treated (T1) and control group (T2). On the other hand, \( T_{\text{MAX}} \) representing the time at which the maximum rate of gas production was achieved was significantly different \( p < 0.05 \) with enzyme treatment and untreated Prosopis pod meal being different from natural fermented Prosopis pod meal. The pH was not significantly different across the different treatment \( p > 0.05 \).

Growth performance of the pigs

The diets had a significant effect on FCR \( p < 0.05 \) with D3 being significantly higher than the other treatments. The ADG was significantly \( p < 0.05 \) highest for PC than the other diets. Higher inclusion levels of GPPM in the diet led to an increase in the FCR while pigs fed on PC (2.707) and D3 (3.383) had the lowest and the highest FCR, respectively. Diet significantly affected ADG \( p < 0.05 \) while sex did not affect the ADG \( p = 0.5554 \). The average daily gain of PC, NC, and D1 was not significantly different. However, as the levels of inclusion of GPPM increased, the ADG of the pigs decreased with PC (0.634 kg/day) being significantly different from D2 (0.440 kg/day) and D3 (0.420 kg/day). Diet with 30% GPPM and enzyme had the lowest DFI and differed with T1, NC, and D1 \( p < 0.05 \).

The initial weight was not significantly different across all the experimental diets conversely, sex \( p = 0.122 \) did not affect initial weight. Diet had a significant effect \( p < 0.05 \) on the final weight of pigs (Table 5). Diet with 30% Prosopis pod meal and enzyme was significantly lower final weight compared to NC; however, D3 and D3 had similar final weight.

Weekly body weights were significantly different for the dietary treatments \( p < 0.05 \). Pigs fed on diet D3 had a lower weekly weight relative to other pigs fed on the other diets for the whole feeding period (Fig. 3).
Discussion

Proximate analysis of the treated and untreated Prosopis pod meal

In this experiment, the chemical composition of GPPM was within the expected range reported by several authors (Sawal et al. 2004; King’ori et al. 2011; Manhique et al. 2019). The crude protein and ash content of the GPPM in the current study were however higher than those reported by Manhique et al. (2019) even though GPPM were collected from the same locality. This could be due to the fact that samples from this study were mostly collected from communal grazing lands where local populations carried out livestock grazing. The presence of livestock in the silvo-pastoral systems contributes to a higher total carbon and nitrogen contents on the Prosopis pod tree. This combined with agronomic activities causes a change in the nutritional contents of the various parts of the plant (King’ori et al. 2011; Lira Junior et al. 2020). With regard to relative importance of Prosopis pod meal compared to other feed ingredients, GPPM in this study contained a higher crude protein (14.48%) than maize bran (11%), maize grain (8.25%), and industrial and kitchen swills (8.94–11.9%) which are

| Table 4 Fermentation kinetics of fermented untreated and enzyme-treated Prosopis pod meal |
|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Parameters                    | T1 (mean ± SE)                | T2 (mean ± SE)                | T3 (mean ± SE)                | T4 (mean ± SE)                |
| G (ml/g DM)                   | 18.502 ± 1.27                 | 17.744 ± 1.48                 | 16.535 ± 2.19                 | 13.429 ± 1.63                 |
| A                             | 22.194 ± 2.26                 | 19.722 ± 1.84                 | 23.220 ± 4.63                 | 15.287 ± 2.30                 |
| C                             | 1.488 ± 0.15                  | 1.734 ± 0.22                  | 1.946 ± 0.48                  | 2.091 ± 0.23                  |
| B                             | 20.34 ± 1.53                  | 18.361 ± 1.01                 | 33.988 ± 6.76                 | 22.019 ± 1.74                 |
| $T_{MAX}$ (h)                 | 5.149 ± 1.84                  | 7.911 ± 1.01                  | 13.014 ± 5.68                 | 19.069 ± 1.22                 |
| RMAX                          | 0.786 ± 0.09                  | 0.677 ± 0.04                  | 0.493 ± 0.08                  | 0.425 ± 0.04                  |
| pH                            | 6.89 ± 0.03                   | 6.91 ± 0.04                   | 6.89 ± 0.09                   | 6.95 ± 0.02                   |

The means within a row with the different superscript letters across rows are statistically different $p<0.05$. T1, enzyme-treated Prosopis pod meal; T2, untreated Prosopis pod meal; T3, Lactobacillus plantarum inoculated Prosopis pod meal; and T4, naturally inoculated Prosopis pod meal. G (ml/g DM): total gas produced corrected on dry matter basis; A: the asymptotic gas production; B: the time after incubation at which half of the asymptotic amount of gas has been formed; C: constant determining the sharpness of the switching characteristic of the profile; $T_{MAX}$: the time at which Rmax is accomplished; $R_{MAX}$: the maximum rate of gas production (ml g$^{-1}$ × h).
most commonly used feedstuffs for pig production in Kenya (Muthui 2019). This indicates that GPPM contains a reasonable amount of protein and can be used in place of these ingredients.

Pre-treatment using *Lactobacillus plantarum* and natural fermentation resulted in a lower crude fiber (CF), NDF, and ADF relative to the untreated. The decrease in fiber could be attributed to the hydrolysis of fiber constituents of the enzymatic activity of lactic acid bacteria that in conjunction with the lowering pH during the fermentation process released high lactic acid content in the sample. There is a good consensus that fermentation reduces the NDF content thus improving nutrient availability in feeds for pigs (Kraler et al. 2014; Koo et al. 2018). The results of this study correspond to Wang et al. (2018) and Ni et al. (2014) who reported a decrease in the concentration of hemicellulose and NDF upon ensiling forages and wheat straw because of the acid hydrolysis and the ability of microbial enzymes released by microbes during ensiling to degrade the fiber.

On the other hand, pre-treatment using *Lactobacillus plantarum* and natural fermentation resulted to an increase in the concentration of crude protein as a result of the loss of organic carbon during fermentation. Indeed the values of organic matter from this study after fermentation did decrease compared to the untreated GPPM. Similarly (He et al. 2018) observed an improvement on crude protein after ensiling *Neolamarckia cadamba* leaves with and without lactobacillus inoculum.

**In vitro dry matter digestibility**

Alternative feed resources are the predominant feed materials available for pigs in most parts of Kenya (Muthui 2019). The quality of these alternative feeds depends on their nutrient content.

### Table 5 Effect of experimental diets on growth performance of pigs

| Item      | Dietary treatment | p value | Sex |
|-----------|-------------------|---------|-----|
|           | PC                | NC      | D1  | D2  | D3  |
| ADG       | 0.634±0.05a       | 0.517b±0.07 | 0.516b±0.06 | 0.440b±0.04 | 0.420b±0.05 | <.0001 | 0.55 |
| FCR       | 2.707±0.19        | 2.833b±0.14 | 3.058abc±0.32 | 3.275bc±0.32 | 3.383c±0.33 | 0.037  | 0.77 |
| DFI       | 1.467±0.05        | 1.439b±0.05 | 1.477b±0.06 | 1.330bc±0.08 | 1.271c±0.04 | <.0001 | 0.01 |
| IW        | 22.720±0.58       | 21.593±0.89 | 22.295±0.07 | 22.405±0.80 | 21.688±0.25 | 0.525  | 0.12 |
| FW        | 40.950±0.95       | 37.370b±0.23 | 37.580b±0.42 | 35.650b±0.05 | 34.470b±0.68 | 0.002  | 0.02 |

The means within a row with the different superscript letters are statistically different (p<0.05). Mean±SE. PC =0% GPPM and 0.035% enzyme per kg of diet; NC =0% GPPM and 0% enzyme per kg of diet; D1 =10% GPPM and 0.035% enzyme per kg of diet; D2 =20% GPPM and 0.035% enzyme per kg of diet; D3 =30% GPPM and 0.035% enzyme per kg of diet; GPPM ground *Prosopis* pod meal. ADG, average daily gain (kg/day); DFI, daily feed intake (kg); FCR, feed conversion ratio; IW, initial weight (kg); FW, final weight (kg)
content and the efficiency by which these feed resources are digested and utilized by pigs. However, due to the vastness of these material in the tropics it’s impractical to investigate the digestibility of these materials on the actual animal as it is expensive and unethical. The utilization of in vitro techniques to investigate digestibility and fermentation kinetics provides a viable approach to investigate various alternative feed resources and the various technologies that can be used to improve their utilization and their digestibility (Boisen and Fernández 1997; Coles et al., 2005). The purpose of the first experiment was primarily to screen the three pre-treatment techniques for improving the digestibility of Prosopis pod meal for further animal experiments.

The IVDMD of Prosopis pod meal 49.1% from this study was relatively lower than that of maize grain 75.5% (Park et al. 2016). This could be attributed to a high insoluble crude fiber content in the GPPM in comparison to maize. Consistently to the present study, Al-Marzoqui et al. (2015) reported that despite having 3% more gross energy than corn, the apparent metabolizable energy of Prosopis pod meal was reported to be 30% less than corn due to the high indigestible fiber content (83%) in GPPM suggesting that utilization of Prosopis pod meal is limited due to the high fiber.

The addition of fiber degrading enzyme in this study led to an improvement in the in vitro digestibility of dry matter of the GPPM by 3.68% compared to the control and by 2.51% and 7.21% respectively compared to Lactobacillus plantarum inoculated and naturally fermented GPPM. The multienzyme complex used in this study had a cocktail of fibroytic (xylanase, cellulose, and beta-glucanase) enzyme that potentially had an improvement in fiber degradability. These enzymes have been postulated to cause disruption and hydrolysis of the plant cell wall integrity and consequently led to the release of nutrients encapsulated by the cell wall constituents (de Vries et al. 2012). As a result, more nutrients were made available for digestion by gastric and pancreatic enzymes (Lee et al. 2018). This could explain the increase in digestibility and cumulative gas production on enzyme-treated GPPM.

The results of fermentation using Lactobacillus plantarum (T3) improved the IVDMD by 1.2% compared to the untreated GPPM, while on the other hand, natural fermentation (T4) decreased IVDMD by 3.5% relative to the untreated GPPM. Contrary to the present study, Jørgensen et al. (2010) reported that the ileal digestibility of dry matter after natural fermentation of barley and wheat increased by 6 and 3 percentage units, respectively. The differences in the amount of starch in Prosopis pod meal compared to barley and wheat could explain the differences. Barley and wheat contain a higher amount of starch composition 64.8% and 69.5%, respectively, which are rendered more digestible upon fermentation (Zentek and Boroojeni 2020). The higher ileal digestibility of barley and wheat could therefore be a result of increased starch digestibility. This is unlike Prosopis pods meal that contains a tiny amount of starch as most of the carbohydrates present are majorly free sugars, saccharose (20–25% DM), and reducing sugars (10–20% DM) (Choge et al. 2007b, 2007a) that are usually rapidly lost during the fermentation process. However, this could not explain the lower IVDMD of natural fermentation compared to Lactobacillus plantarum observed in this study. This could probably be explained by the lower carbohydrates and organic matter that was a result of natural fermentation. In fact from this study, natural fermentation had a slightly lower organic matter content than Lactobacillus plantarum fermented GPPM (93.96 vs 94.15%).

Natural fermentation also regarded as spontaneous fermentation is an unreliable type of fermentation as consequence; it is associated with higher nutrient losses due to the longer time it takes to achieve a lower pH that inhibits the growth of organism, such as yeast and clostridia, associated with loss of organic matter through CO2 production (Huyen et al. 2020). It is, therefore, possible that naturally fermented Prosopis pod had less organic matter and sugars available during pepsin-pancreatin digestion thus the low digestibility. On the contrary, fermentation that uses inoculants such as Lactobacillus plantarum is usually associated with less organic matter loss and dry matter loss (Li et al. 2020). This is consistent with Tabacco et al. (2011) who reported a reduction in the dry matter loss after ensiling forage crop treated LB treatment, relative to untreated silage.

Fermentation kinetics

Enzyme supplementation exhibited fast and intense fermentation patterns with the highest cumulative gas productions and rate of fermentation (Rmax) and the lowest time to reach Rmax (Tmax). This is due to the capacity of the multienzyme to break down fiber into short fragments that were highly fermentable by microorganisms in the hindgut of pigs (Woyengo et al. 2016). In contrast, both fermentation (T4) and (T5) did not result in an improvement in cumulative gas production compared to the control. Additionally, the rate of gas produced (Rmax) in natural and inoculated fermentation was significantly lower compared to the control; this is because of the lower amount of crude fiber associated with fermentation. As mentioned earlier, pre-treatment using fermentation could have led to losses of some rapidly fermentable carbohydrates including the saccharose, soluble non-starch polysaccharides, and reducing sugars present in the Prosopis pod meal as evident by the reduction of the total carbohydrates, hemicellulose, and increased ash. Indeed, with hemicellulose values of 18.15%, and 16.90% for untreated and inoculated fermentation respectively, fermentation of Prosopis pod meal did not supply more highly fermentable hemicellulose than the enzyme pre-treated and untreated GPPM diet at 20.28%. These rapidly fermentable components are usually the first to be hydrolyzed or used up to provide energy during microbial fermentation (Koo et al., 2018). This, therefore, meant that the residues subjected
to the hindgut fermentation comprised of slowly fermentable and non-fermentable components whose rate of degradation was lower. Similarly, Ferrer et al. (2021) reported a decrease in soluble organic components (sugars and soluble fiber) while ash and insoluble fibrous content increased as a result of fermentation of dried orange pulp.

Production performance of grower pigs

Feed intake is influenced by many factors including animal characteristics such as body weight, genetic adaptations, and feed factors such as nutrient density, digestibility, and the presence of anti-nutritive factors (Choc et al. 2010). Similarly, factors that affect feed intake also influence feed conversion ratio.

In this study, there was a significant decrease in feed intake and feed efficiency as measured by increasing FCR when GPPM was included at 30% + enzyme (D3) a diet with 371.3 g/kg DM NDF and 169.40 g/kg DM ADF. Several reasons could have contributed to the reduction. Firstly, the physical characteristics of GPPM are usually highly fibrous with a high amount of ADF and NDF. Diets with a high amount of fiber are associated with an increased bulkiness and water holding capacity in the gut that eventually leads to gut fill, thus depressing feed intake (Ndou et al. 2013; Jang et al. 2021). Our results correspond to Ndou et al. (2013) who reported that the gut capacity was attained when weaners pigs were offered a diet containing an NDF of 367 g/kg DM and ADF of 138 g/kg DM above which feed intake starts to reduce. It is interesting to note that values for NDF and ADF of the diet with 30% GPPM had surpassed the values by (Ndou et al. 2013) where feed intake starts decreasing. However, this could not in entirety explain the decrease in feed conversion ratio and feed intake as the effect of fiber on gut fill diminishes as the pig ages (Ndou et al. 2013).

Other factors such as the level of tannins could have resulted in decreased feed intake. Although effects on tannins found in GPPM on performance were not investigated, tannins are usually bitter and astringent and thus affect feed intake (Odero-Waitituh et al. 2016). In fact, tannins have been attributed to reduced dietary protein digestibility (Annor et al. 2017) as a result of their ability to form insoluble complexes with both digestive enzymes and dietary protein thus reducing feed conversion ratio. The low feed intake and feed efficiency from this study are consistent with (Pinheiro et al. 1993) who reported a significant reduction in feed intake and feed conversion when Prosopis pod meal replaced the 30% maize soybean mixture of fatteners pigs. Other authors have also reported that nutrient intakes were depressed in animals that consumed diets containing more than 200 g/kg of Prosopis Juliflora pods due to large amounts of fiber and tannins (Abdullah and Abdelhafes 2004; Obaidat et al. 2008; Odero-Waitituh et al. 2016).

The results from the average daily gain (ADG) of NC (Control without enzyme) compared to D1 10%, D2 20%, and D3 30% were not significantly different though there was a decrease in the feed intake as the level of GPPM increased across the diets. This is in contrast to Pinheiro et al. (1993) who reported a reducing ADG as Prosopis gradually replaces a maize-soybean mixture in the diet of finishing pigs, at a 30% level of inclusion. A number of reasons could explain this, first as evident from the in vitro trial multi-enzyme complex increases IVDMD of Prosopis pod meal. This is due to the fact that multienzyme mixtures have been known to improve the digestibility of protein, energy, and fats by reducing the viscosity of the digesta and hydrolyzing the non-starch polysaccharides (Torres-Pitarch et al. 2017). This increases the contact between the feed and digestive enzymes as well as the digestive surfaces.

Secondly, the addition of multienzyme has been reported to improve fiber digestibility as a result of fermentation of the fiber in the hindgut with the volatile fatty acids produced contributing to the net energy requirements of the pigs needed for growth (Nkosi et al. 2020). This could therefore explain why the inclusion of Prosopis pod meal up to 30% of the diet had no negative effect on growth rate as the enzyme probably counteracted the effects of increasing fiber in the diets. Similarly, Kwon and Kim (2015) reported higher inclusion levels of palm kernel meal (PKM) for growing-finishing pigs of 12% compared to a conservative recommended inclusion level of 10% due to supplementation of β-mannanase enzyme to diets containing PKM.

The addition of enzyme to the diet without GPPM (PC) compared to NC (without the enzyme and GPPM) did not result in an improvement in ADG. This was expected since PC and NC had met the nutrient requirements of pigs while having minimal fiber content. The growth of animals fed these diets reached the commercial expectation, and thus, significant improvement by multienzyme over that which had already been achieved would have been unlikely. This finding is consistent with other studies (e.g., l’Ansonet al. 2014; Nkosi et al. 2020) who reported that the extent to which enzyme supplementation improves nutrient digestibility tends to be low when using highly digestible ingredients thus improvement in growth rates are not observed.

In conclusion, based on this study, pre-treatment of Prosopis pod with exogenous enzyme improved in vitro digestibility compared to untreated, natural, and Lactobacillus plantarum induced-fermentation while cumulative gas produced improved slightly though not significant. Naturally fermented Prosopis pod meal however results in low digestibility and fermentability; therefore, it is not a suitable pre-treatment method for improving the digestibility of the pods for pigs. Further, overall feed efficiency (as measured by FCR) and feed intake decreased at 30% + enzyme GPPM; however, growth rates remained
unaffected at these levels. The study demonstrated that Prosopis pod meal with the multienzyme complex can be included up to 30% in grower’s pig diets without effects on the average daily weight gain.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Ethics approval The materials and procedures of this study had been approved by Egerton University Research Ethics Committee with approval No. EUREC/APP/109/2021 and National Commission of Science and Technology of Kenya under the permit No: NACOSTI/P/20/6926.

Consent to participate Not applicable.

Consent for publication Not applicable.

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