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Effect of fermented and enzyme-treated *Prosopis juliflora* pod meal diets on carcass quality and organ weights in broilers

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Abstract
The study was conducted to determine the effect of substituting varying levels of fermented and enzyme-treated prosopis pod meal with maize in broiler diets on carcass characteristics and organ weights. One hundred and eighty Cobb 500 broilers were fed rations containing pod meal at substitution levels of 0, 15, 30 and 45% of maize. At 15%, the fermented pods had significantly higher ($p<0.05$) slaughter weight than both the unfermented and enzyme treated. At 45% fermented pod meal, thigh and drumstick weighed significantly higher than in diets with enzyme treated and unfermented pod meal. Birds receiving fermented pod at 45% had heavier ($p<0.05$) livers and gizzards compared to enzyme-treated and unfermented pods as was with heart compared with unfermented pods. All levels resulted in heavier and longer ($p<0.05$) small intestines weight and length respectively than control diet. It is recommended to ferment the pods and use enzyme to achieve higher inclusion levels and improve chicken performance in terms of carcass weight.

Keywords: Fermented pods, exogenous enzyme, carcass characteristics, organ weights

Introduction
Maize has multiple uses such as human food, livestock feed and also used in biofuel and biogas productions. This is projected to reduce available land for food grains and feed production, leading to a considerable increase in animal production feed costs [21; 24]. This situation creates unpredictable market and it is therefore becoming unsustainable for small and medium scale poultry farmers [16]. In Kenya, chicken farming has a contributes a big share in livestock subsector with an estimated 31.8 million birds [2]. With human population projected to increase [11], reliance on maize is going to be unsustainable. The use of conventional ingredients could be replaced by use of alternative feeds. The new feeds should offer nutrients in a manner that poultry performance is maintained or improved or there is saving on poultry feeding costs [3]. The new ingredients require treatment or processing with the strategies put in place being simple and have the capacity to avail the nutrients in a simple way that can be adopted easily by the poultry farmers. Prosopis pods (PPM) have been used to replace conventional energy ingredient in feeding of non-ruminants [23; 24] owing to their rich energy and protein content [15]. The major limitation is anti-nutritive factors that prevent availability of nutrients from the pods as was reported in broilers [18] and improved indigenous chicken [24] where 20% of pods was the optimum replacement levels. To address this issue, nutritional strategies have been put in place that involve fermentation and use of exogenous enzymes.

Materials and Methods
One hundred and eighty unsexed day old Cobb 500 strain chicks were selected from chicks purchased from Kenchic® Company Limited, Nakuru, Kenya to determine the effect of fermented and enzyme treated on the carcass and organ weights. They were allocated to twelve dietary treatments in triplicates in a completely randomized design. Each replicate had five birds. The birds were vaccinated against Marek's disease at hatching by injection. Vaccination against Newcastle disease was given through eye drop at the age of three days. At seven days, chicks were vaccinated against Gumboro disease through drinking water and a booster given at

18 days of age. The chicks were brooded using 250 watt infrared electric bulbs with gradual height adjustment as sources of heat and light in single-tier deep litter cages. The room temperature was maintained at temperature range of 20-25 °C. The floor was covered with wood shavings. Clean water was offered *ad libitum* throughout the experiment period using the watering troughs. Before commencement of the experiment, the experimental pens, watering and feeding troughs were cleaned thoroughly with liquid soap, disinfected using omnicide®, dusted with Sevin® dust, disinfected and sprayed against external parasites. The Egerton University Institutional Scientific and Ethics Review Committee approved this study with Approval No. EUISEC/APP/193/2022.

Experimental diets

The feeding trial was divided into starter and finisher phases. Maize was substituted with three forms of prosopis pod meal, namely, unfermented, enzyme-treated and fermented at 0, 15, 30 and 45%. Natuzyme® was used and added at the rate recommended by the manufacturer. 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. The pods were spontaneously fermented for six days. The compounded diets were iso-caloric and iso-nitrogenous. The broilers were fed on experimental diet for the first 7 days to background them before the start of data collection.

Carcass evaluation and measurement of internal organs

At 42 days of age, three birds per replicate in each treatment were randomly selected and starved for 12 hours but with free access to water. The birds were weighed immediately before slaughter and then sacrificed (slaughtered) following humane cervical dislocation method [13] and bleeding for 3 minutes, then they were de-feathered manually after scalding at 60-70 °C and dissected into various cuts [14]. Dressed, eviscerated weights, dressed and eviscerated percentages were calculated according to method described by [7] using equations 1, 2, 3 and 4 respectively.

$$\text{Dressed weight} = \text{Thighs} + \text{Wings} + \text{Breast} + \text{Ribs} + \text{Back} + \text{Heart} + \text{Liver} + \text{Gizzard} + \text{Neck} + \text{Feet} + \text{Head} + \text{Viscera (inedible offal)} \quad \text{------Equation 1}$$

$$\text{Eviscerated weight} = \text{Dressed weight} - \text{Viscera} \quad \text{------Equation 2}$$

$$\text{Dressing (\%)} = \frac{\text{dressed weight}}{\text{preslaughter weight}} \times 100 \quad \text{------Equation 3}$$

$$\text{Eviscerated (\%)} = \frac{\text{Eviscerated weight}}{\text{preslaughter weight}} \times 100 \quad \text{------Equation 4}$$

Internal organ weights and length of the small intestines were measured at the end of the experiment. Heart, gizzard, liver, small intestine (SI) were weighed on an analytical scale (0.01g precision), and their weights were calculated relative to live weight, according the equation 5.

$$\text{Relative organ weight (\%)} = \frac{\text{organ weight}}{\text{preslaughter weight}} \times 100 \quad \text{------Equation 5}$$

Statistical analyses: Data was first tested for normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test). As these assumptions were not violated, data was submitted Analysis of variance (ANOVA) on dressed weight, eviscerated weight, dressing percentage, eviscerated percentage, relative organ weight percentage, small intestines weight and length using the general linear model procedure of statistical analysis system version 9.0 [20]. When statistical difference was detected ($p<0.05$), means were compared by Tukey's test at 5% probability. The model for this experiment used for statistical analysis was;

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

Where:

Y_{ij}= dependent variables

μ= overall mean

A_i= Effect due to i^{th} percentage of the prosopis pod meal in the diet (i=1...4)

B_j=Effect due to j^{th} type of treatment for prosopis pod meal (j=1…3), $(A^*B)_{ij}$ =Effect due to interaction of i^{th} percentage of prosopis pod meal and j^{th} type of treatment for prosopis pod meal (ij=1…12) and ε_{ijk} = Random error term.

Results

The carcass yield of broilers is presented in Figures 1, 2 and 3 and Tables 1 and 2. All the carcass parameters indicated that there was significant interaction effect ($p<0.05$) of PPM levels and the technology. The 30 and 45% PPM levels subjected to different biotechnology techniques resulted in significantly different ($p<0.05$) slaughter weights with fermented being the highest followed by enzyme-treated pod diets. At 15% PPM, the fermented pods had significantly higher slaughter weight than both the unfermented and enzyme treated. The eviscerated weight was similar in the broilers fed on the 30 and 45% PPM diets. The fermented pod at 45% PPM had significantly higher thigh and drumstick weights than enzyme treated and unfermented PPM that were also significantly different, with unfermented being lower. The 0, 15 and 30% had similar weights among their different technologies. The percentages of wing in each PPM level were also similar in different technologies with 15% enzyme and 30% unfermented being significantly lower ($p<0.05$) than control treatments. Birds receiving fermented PPM at 45% had heavier ($p<0.05$) livers and gizzards compared to enzyme-treated and unfermented pods as was with heart compared with unfermented PPM. Interaction between *Prosopis juliflora* pod meal levels and biotechnology methods did not affect ($p>0.05$) the small intestines length, weight, esophagus and crop weight. There was no effect of technology as the main effect on the gastro-intestinal parameters but *Prosopis juliflora* pod meal levels affected these parameters. The 15, 30 and 45% PPM levels resulted in heavier and longer ($p<0.05$) small intestines weight and length respectively than the control diet.

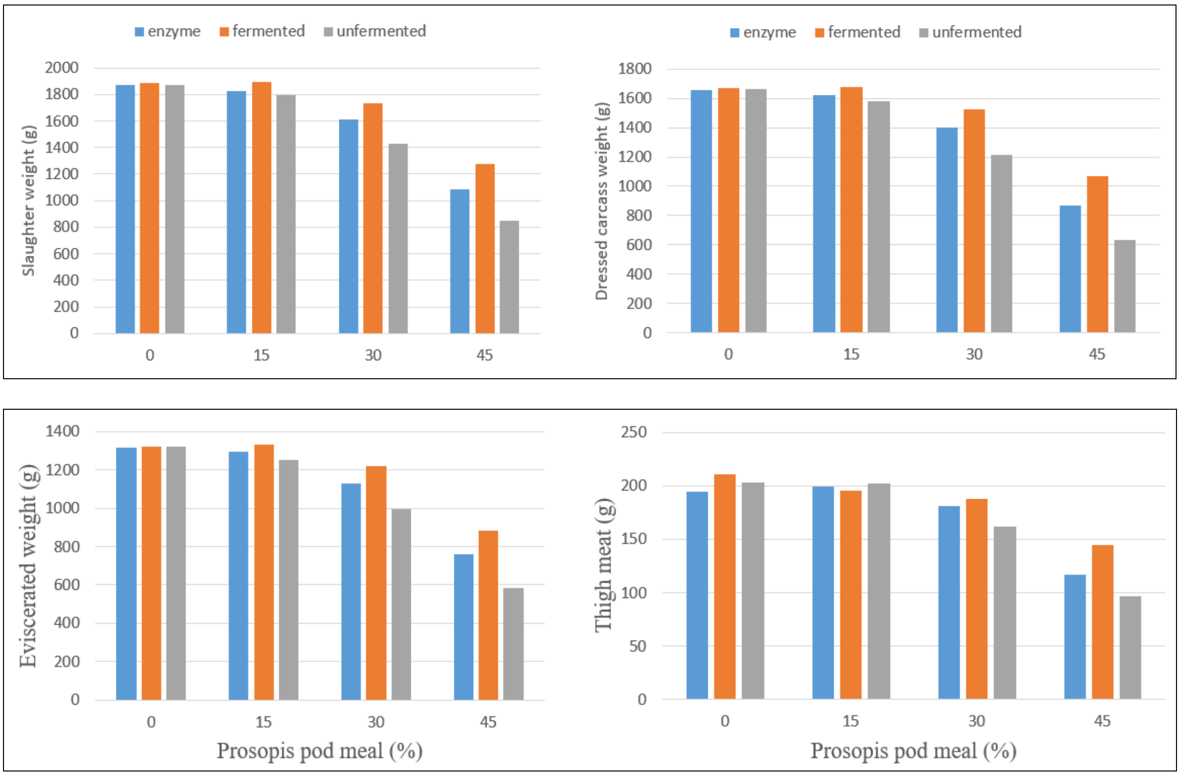

Fig 1: Carcass characteristics interaction graphs for slaughter weight, dressed carcass weight, eviscerated weight and thigh meat of broilers fed varying levels of prosopis pods meal subjected to different biotechnology methods

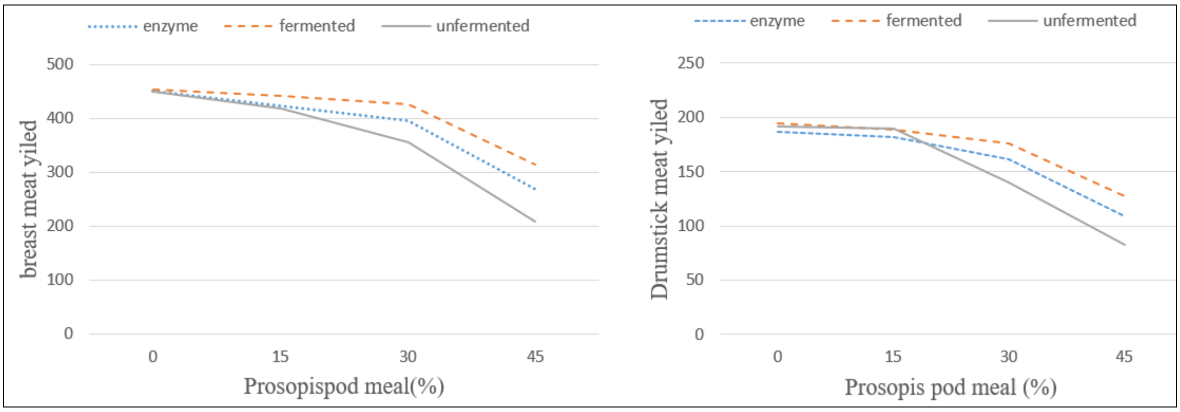

Fig 2: Carcass characteristics interaction plots for breast weight and drumstick weight of broilers fed varying levels of prosopis pods meal treated with different biotechnologies methods

Table 1: Carcass yield characteristics of broilers fed varying levels of PPM subjected to different biotechnologies treatment

Treatments				Parameters								
PPM (%)	**BT**		**Slaughter weight (g)**	**Dressed carcass weight (g)**	**E Wt (g)**	**Thigh (g)**	**Drum-stick (g)**	**Breast (%)**	**Wing (%)**	**Heart (%)**	**Liver (%)**	**Gizzard (%)**
0	0		1872.4^{ab}	1656.5^{a}	1319.3^{a}	202.6^{a}	191.6^{a}	450.4^{abcd}	4.50^{a}	0.31^{de}	2.30^{cd}	1.96^{d}
15	UNF		1792.3^{cd}	1582.2^{bc}	1251.8^{ab}	201.5^{a}	188.8^{a}	418.3^{bcd}	3.97^{ab}	0.39^{ab}	2.35^{bc}	2.12^{c}
15	ENZ		1825.5^{bc}	1618.2^{ab}	1296.4^{ab}	199.5^{a}	181.9^{ab}	418.3^{d}	4.17^{ab}	0.33^{de}	2.30^{cd}	1.99^{d}
15	FER		1895.9^{a}	1679.3^{a}	1328.9^{a}	194.6^{ab}	188.9^{a}	440.2^{cd}	3.90^{b}	0.33^{de}	2.30^{cd}	1.99^{d}
30	UNF		1430.2^{f}	1214.1^{e}	992.8^{d}	161.6^{bc}	140.0^{cd}	354.5^{a}	3.82^{b}	0.41^{ab}	2.39^{b}	2.47^{a}
30	ENZ		1608.8^{e}	1398.7^{d}	1127.1^{c}	180.2^{ab}	160.5^{bc}	394.6^{abc}	4.17^{ab}	0.33^{de}	2.31^{cd}	2.00^{d}
30	FER		1730.9^{d}	1523.7^{c}	1219.8^{b}	187.8^{ab}	175.8^{ab}	425.2^{abc}	4.11^{ab}	0.34^{cd}	2.34^{bcd}	1.99^{d}
45	UNF		850.2^{i}	633.6^{h}	584.2^{g}	96.4^{e}	82.4^{f}	207.5^{abcd}	3.78^{b}	0.43^{a}	2.45^{a}	2.54^{a}
45	ENZ		1083.9^{h}	867.8^{g}	761.2^{f}	117.0^{d}	108.2^{e}	267.5^{ab}	4.03^{ab}	0.39^{ab}	2.34^{bc}	2.23^{b}
45	FER		1277.5^{g}	1067.6^{f}	881.4^{e}	144.85^{c}	126.87^{d}	313.3^{abc}	4.10^{ab}	0.37^{bc}	2.35^{bcd}	2.24^{b}
	SEM		57.44	57.65	41.20	6.17	6.19	13.14	0.04	0.001	0.001	0.033
ANOVA												
Source		**df**		---------------*p-values*---------------								
PPM		3	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.011	<0.001	<0.001	<0.001
	BT	3	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.067	<0.001	<0.001	<0.001
PPM*BT		5	<0.001	<0.001	<0.001	0.0002	<0.001	<0.001	<0.001	<0.001	0.0002	<0.001

SEM= standard error of mean; FCR= feed conversion ratio; E Wt. =Eviscerated weight; BT= biotechnology; PPM= prosopis pod meal; Enz= enzyme technology used in treating the PPM; FER=fermented pods; UNF=unfermented pods; ANOVA=analysis of variance; ^{NS}= not significant; the figures in parentheses are percentage values; ^{a,b,c} means within a column with different superscripts are significantly different

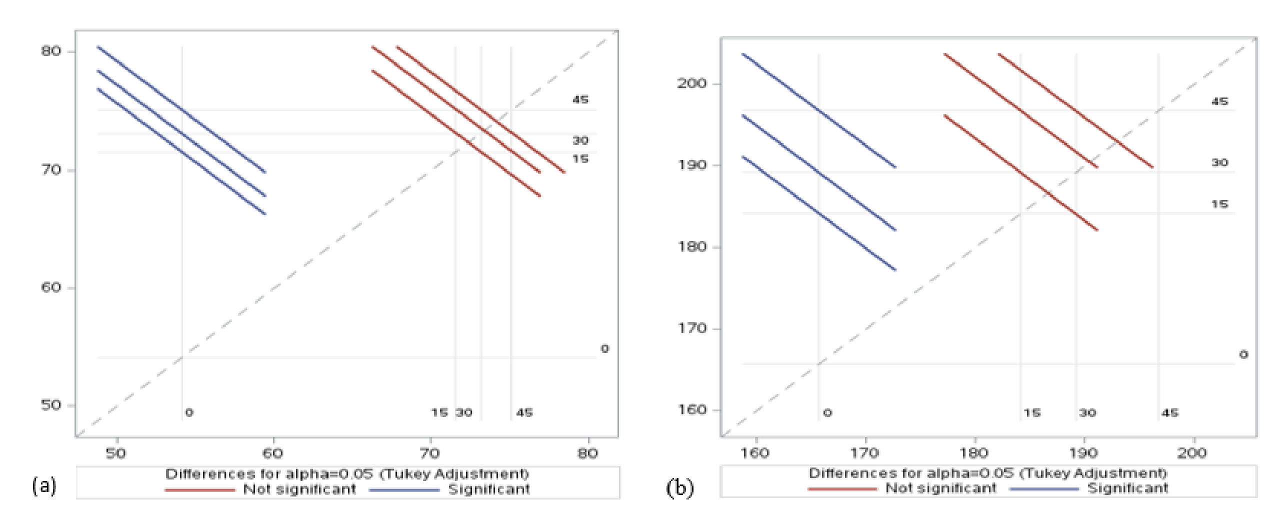

Fig 3: Diffogram showing mean comparisons for different levels of PPM on (a) weight of small intestines in grams and (b) length of small intestines in centimeters

Table 2: Small intestines, esophagus and crop weights and small intestines length in broilers fed varying levels of PPM subjected to different biotechnological treatments

Treatments			--	Parameters	---------------------------
PPM	**Biotechnology**		**Small intestines weight (g)**	**Small intestines length (cm)**	**Esophagus and crop weight (g)**
0	None		58.66	176.67	14.33
15	Unfermented		76.00	186.67	15.33
15	Enzyme		69.00	184.00	12.67
15	Fermented		69.67	182.00	11.00
30	Unfermented		80.00	195.33	14.33
30	Enzyme		77.00	183.00	13.67
30	Fermented		62.33	189.33	13.33
45	Unfermented		81.33	212.33	13.67
45	Enzyme		74.00	188.00	12.33
45	Fermented		70.00	190.00	12.33
Pooled SEM			2.01	2.75	0.45
Main effect means					
0			54.11^{b}	165.78^{b}	12.78
15			71.56^{a}	184.22^{a}	13.00
30			73.11^{a}	189.22^{a}	13.78
45			75.11^{a}	196.78^{a}	12.78
ANOVA					
Source		**df**	--------------------------------	***p-values***	-------------------------
PPM	BT	6	0.187	0.183	0.968
PPM		2	<0.001	<0.001	0.8702
	BT	3	0.156	0.053	0.164

SEM= standard error of mean; FCR= feed conversion ratio; BT= biotechnology; PPM= prosopis pod meal; Enz= enzyme technology used in treating the PPM; FER=fermented pods; UNF=unfermented pods; ANOVA=analysis of variance; [NS]= not significant; [a,b,c] means within a column with different superscripts are significantly different

Discussion

Slaughter and eviscerated weight

The significant interaction between *Prosopis juliflora* pod meal (PPM) inclusion levels and biotechnological treatments on slaughter weight was indicative of the enhanced nutrient digestibility and availability from fermentation and enzyme treatments. The highest slaughter weights were observed in broilers offered diets with 30% and 45% fermented PPM, followed by those with enzyme-treated PPM diet. This trend agrees with a study showing that fermentation improves nutrient bioavailability and reduces anti-nutritional factors which include tannins and crude fibre, thereby promoting growth [1; 6]. The consistency in eviscerated weights across diets with 30% and 45% PPM levels with slaughter weights concurs with the argument that the improved nutrient profile from fermented and enzyme-treated PPM translates to better overall carcass yield. At 15% PPM, the fermented treatment resulted in significantly higher slaughter weights compared to unfermented and enzyme-treated PPM diet, highlighting the effectiveness of fermentation even at lower inclusion levels.

Thigh and drumstick weight

The significantly higher thigh and drumstick weights in broilers fed diets with 45% fermented PPM compared to enzyme-treated and unfermented PPM suggest that fermentation is vital in enhancing muscle development. This effect is likely due to improved protein quality and amino acid availability, which are crucial for muscle growth [11, 19]. A study by [5] reported that gradual increase in lysine resulted in increased breast weight compared to other muscles. The observed differences among treatments at 45% PPM level indicated that the benefits of fermentation are more pronounced at higher inclusion levels as compared to enzyme-treated and unfermented pods.

The diets with 0, 15 and 30% PPM levels had similar weights among different biotechnological treatments. This suggested that the effects of fermentation and enzyme treatments on these specific muscle groups were more noticeable at higher PPM inclusion levels. This indicates that while the overall effect of biotechnological treatments on wing development is minimal, specific combinations of PPM level and treatment can have adverse effects. This is supported by research showing that anti-nutritional factors can disproportionately affect certain body parts, including wings [4].

Internal organ weights

The significantly heavier livers, gizzards, and hearts in birds fed diets with 45% fermented PPM indicated enhanced metabolic activity and nutrient processing capabilities. Fermentation is known to improve gut health and nutrient absorption, leading to increased organ weights [10; 17]. The increased weights of these organs suggest that fermented PPM optimizes nutrient utilization, supporting overall growth and health.

Small intestines length and weight of small intestines and crop and esophageal

The significant increase in the small intestines' length and weight at higher PPM diets (15%, 30%, and 45%) indicates that the inclusion of PPM in the diet enhances the development of the gastrointestinal tract. This increase can be attributed to the increased fiber content in PPM, which has been reported to stimulate gut development in poultry. Dietary fiber can increase the size and weight of small intestines as a way of establishing an environment for fibre digestion [9]. The increased intestinal length and weight may be beneficial for absorption processes for nutrient derived from digestion process and this would impart more benefit especially in feeds high in fibre. A study by [8], reported that high-fiber diets result in increased intestinal length and weight consequently enhancing the digestive capacity of broilers and increasing the potential benefit of high fibre diets in the birds' nutrition.

Lack of significant effects on the esophagus and crop weight in the biotechnological treatments or their interaction with PPM levels points to the fact that these components of the digestive system are to a less extent influenced by dietary changes than the small intestines. This could arise from the primary role that the esophagus and crop play in transportation and storage of feed temporarily, respectively, as compared to complex digestion and nutrient absorption. Studies have shown that nutritive modifications typically have a more pronounced impact on the lower gastrointestinal tract, such as the intestines, where most digestion and absorption occur [22] than in upper gastrointestinal tract. The absence of significant changes in the esophagus and crop weights is congruent with this interpretation and points to the fact that PPM levels primarily affected the parts of the digestive system involved in nutrient extraction and absorption rather than digestion functions. The results that the biotechnology used (fermentation, enzyme treatment, or unfermented control) did not significantly alter gastrointestinal parameters as a main effect is notable. This suggests that while biotechnological treatments can enhance nutrient availability and overall growth performance, they did not in a big way alter the anatomical development of the gastrointestinal tract. This could be due to the fact that these technologies primarily affect the chemical composition and digestibility of the feed rather than its physical structure.

Research by [4] supports this, showing that enzyme treatments improve nutrient digestibility without significantly affecting the physical development of the digestive organs. In the same manner, fermentation primarily enhances feed quality by reducing anti-nutritional factors, crude fibre and enhances nutrient bioavailability [10], rather than changing the anatomy of the gastrointestinal tract.

Conclusion and Recommendations

This study determined the effect of substituting maize in broiler diets with varying levels of fermented and enzyme-treated *Prosopis juliflora* pod meal on performance and carcass quality.

Conclusion

1. The broilers receiving diet with 30 and 45% fermented PPM had significantly higher thigh and drumstick weights than enzyme treated and unfermented PPM at the same level. Control and 15% level had no effect on thigh and drumstick weight.
2. Birds receiving diet with unfermented PPM at 45% had heavier livers and gizzards compared to enzyme-treated and unfermented pods as was with the heart compared with unfermented PPM.
3. Interaction between all PPM levels and biotechnology methods had similar effect on the small intestines' length, weight, esophagus and crop weight. However, inclusion of *Prosopis juliflora* pod meal at 15, 30 and 45% increased the weight and the length of the small intestines as compared to the broilers offered control diet.

Recommendations

1. The study recommended the use of 45% fermented *Prosopis juliflora* pod meal for higher broiler thigh and drumstick weights as compared to enzyme treated and unfermented PPM.
2. The study recommended that fermentation and enzyme treatment had better performance than unfermented diets with *Prosopis juliflora* pod meal substituting maize portion
3. The study recommended further research on processing of *Prosopis juliflora* pod meal to reduce further the antinutritive factors like crude fibre to enhance the broiler performance

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Conflict of Interest

Not available.

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