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Effect of Soaking, Malting and Fermentation on Nutritional and Phytochemical Properties of Red Sorghum as A Livestock Feed Ingredient

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ABSTRACT

The study was designed to investigate the effects of soaking, malting and fermentation on the nutritional and phytochemical properties of red sorghum as a livestock feed ingredient. A completely randomized design with four treatments (unprocessed, soaked, malted, fermented) and 12 units were used in the study. Significant differences ($p<0.05$) were noted in % value of ash among the treatments. Fermented grains had the highest (4.35%) ash content and malted grains had the lowest (0.95%). Processing methods had a significant effect ($p<0.05$) on % value of the crude protein. However, there were no significant differences ($p>0.05$) between % value of crude protein of the unprocessed and soaked grains. Treatment methods significantly ($p<0.05$) reduced the phytate content. No significant differences ($p>0.05$) were noted on the alkaloid content of sorghum grains. The oxalate content decreased from 33.66mg/100g in unprocessed grains to 9.26mg/100g in soaked grains and, 3.68mg/100g in malted grains. In conclusion, processing techniques such soaking, malting and fermentation can be employed to improve nutritive value of grains with high antinutrient content. Therefore, processed sorghum grain can be an alternative to maize grain in the production of livestock feeds especially in arid and semi-arid areas where maize is not very productive.

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INTRODUCTION

The increased demand for animal-based protein coupled with the advent of climate change calls for alternative sources of feed that can sustainably support livestock production and productivity. Sorghum is a drought resistant crop adaptable to different agro-ecological zones and is tolerant to low input levels compared to other cereal crops (Hossain et al., 2022). The grain can be incorporated in livestock diets as a primary source of energy when processed correctly and balanced with other feed ingredients (McCuistionet al., 2018a). Sorghum has a higher protein content and similar digestible amino acid profile to maize grain (Yusriani et al., 2024). However, it contains antinutritional factors such as tannins, phytates and other phytochemicals that limit its use as an energy source (Masenya et al., 2021). Different processing methods can employed to greatly improve its feed value (Ronda et al., 2018) and inclusion in feed formulations. A greater understanding of the effect of these processing methods on the nutritional and phytochemical profile of sorghum is crucial.

There is a gradual increase in the use of sorghum in livestock feed formulations as a measure for cost reduction (Sun et al., 2018). The disadvantage, however, is that phytochemicals or anti nutritional factors in sorghum can modify the nutritional value of the individual grain. This results in reduced protein digestibility in livestock feed (Wu et al., 2012), thereby negatively affecting the livestock's overall performance and productivity. Polyphenols such as tannins can bind with proteins, form complexes which may reduce availability of nutrients and in turn cause growth depression and increased faecal nitrogen in livestock (Besharati et al., 2022).

Reduction of antinutrients in sorghum increases the nutritional quality and its inclusion level in livestock diets as a replacement or substitute of the conventional cereal grains. The use of sorghum in livestock feed can significantly reduce the competition for maize between humans and livestock especially in the wake of prevailing suboptimal growing conditions for maize in Sub Saharan Africa (Masenya et al., 2021). Price instability and changing availability of maize have raised interest in use of alternative and non-conventional feed ingredients such as sorghum grain (Ciurescu et al., 2023).

A greater understanding of key antinutritive factors such as phytates and other phenolic compounds in sorghum is critical to improve the crop's feeding value (Xiong et al., 2019). Wet processing techniques such as soaking, malting and fermentation are among the cost-effective methods that may be used to reduce antinutritional contents of sorghum. These techniques can enhance sorghum grain functional properties, nutrient composition and availability as well as sensory qualities (Feyera, 2021). The need to reduce antinutritional factors, minimise the loss of micronutrients and improve the palatability of sorghum grain has led to increasing interest in investigation of mechanical, thermal, and biological processing methods. This study was therefore initiated to investigate the effects of soaking, malting and natural fermentation on the nutritional and phytochemical properties of an improved red sorghum variety as a livestock feed ingredient. It was hypothesized that processing treatments would reduce the concentration of phytochemical compounds and improve the nutritional composition of sorghum grains.

MATERIALS AND METHODS

The red sorghum (Macia variety) used in this study was grown in Zimbabwe and bought at a local market in Harare. The grains were cleaned and sorted by removing any foreign material, damaged and broken seed.

Experimental Design

The experimental design used in the study was a completely randomized design (CRD) with four treatments (unprocessed, soaked, malted, and fermented). Each treatment was replicated three times.

Processing Techniques

The unprocessed grains for the control treatment were cleaned and subjected to nutritional and phytochemical analyses. For the soaking treatment, grains were washed with tap water and steeped in water for 48 hours at room temperature as described by Bertin and Carly, (2023) at a grain to water ratio of 1:5 (w/v). The soaking water was replaced every 12 hours and 1 hour air rest was allowed at each refill. The grains were dried in an oven at 50℃ for 24 hours after the steeping period. The steeping procedure for the malted grains is as described for the soaking treatment. After steeping, the grains were placed in sterilized wet hessian and stored in a dark room to sprout at room temperature for 48 hours. The fermented grains were cleaned and steeped in water for 5 days in an airtight container to ferment at room temperature at a grain to water ratio of 1:5 (w/v). Grains were dried at 50°C for 24 hours after the fermentation period.

Proximate Analysis

The AOAC method 930.15 (AOAC, 2019) was used to determine moisture content of grains. Crude protein content was determined by Kjeldahl method of nitrogen content analysis after digestion of 1.0 g of sample as described in AOAC method 2001.11 (AOAC, 2000). The Soxhlet extraction method 920.85 (AOAC, 2019) was followed to determine crude fat content of samples. The crude fiber content was determined following AOAC method 978.10 (AOAC, 2019). Percentage of ash was calculated using the following formula:

$\% \, Ash = \frac{weight\ of\ ash \times 100}{weightof\ sample}$ (AOAC, 2000).

The total carbohydrate of the samples was calculated using the difference method (Gwekwe et al., 2024) by subtracting the value of protein, crude fat, fiber, ash, and moisture content from 100. Gross energy (GE) was determined by bomb calorimetry (Puntigam et al., 2021).

Mineral Analysis

The following minerals: calcium, magnesium, zinc, iron, potassium and sodium were determined by Flame Atomic Absorption Spectrophotometer as described by Gwekwe et al. (2024). Two grams of sample were weighed in clean dry crucible and placed in a muffle furnace for 4 hours at 550°C. Samples were cooled and 10 ml of 3NHCl was added after which the sample was boiled gently for 10 minutes. The contents were then diluted with distilled and filtered through Whatman filter paper No 4. The mineral concentration for each sample was determined by absorption using the calibration curves. Zinc, iron, calcium, sodium and magnesium were determined at the following wavelengths respectively; 213.90 nm, 522.00 nm, 550.00 nm, 589.00nm, 766.50 nm and 610.00 nm.

Phytochemical Analysis

Tannins

The method described by Maxson et al. (1972) was used for determination of condensed tannin content. A weighed sample (1g) was mixed with 10 ml of 1% HCl solution in methanol in a screw cap test tube, agitated for 24 hours at room temperature on a mechanical shaker and then centrifuged at 1,000 rpm for 5 minutes. One ml of supernatant was subjected to the vanillin assay method. A spectrophotometer was used to measure absorbance at 500 nm against a blank prepared with 1% HCl in methanol. Results were expressed as mg per 100g.

Oxalates

The AOAC method 974.24 (AOAC, 2000) was followed to determine the oxalate contents. Samples weighing 2g were digested in 6M HCl at 100°C for 1 hour and diluted to 250 ml and filtered. Four drops of methyl red indicator were added to 125ml of the filtrate and subjected to the dropwise test using NH_4OH solution until the test solution changed from salmon pink color to a faint yellow color (pH 4– 4.5). Samples were heated to 90°C, filtered and reheated to 90°C after adding 10 ml of 5% $CaCl_2$ solution. Samples were cooled overnight and then centrifuged at 2,500 rpm for 5 min. The supernatant was decanted and completely dissolved in H_2SO_4, solution. Total filtrate was diluted, and aliquots of the filtrate were heated then titrated with standard 0.05M $KMnO_4$ solution to a faint pink color which persisted for 15 seconds. The oxalate content was expressed mg per 100g.

Phytates

Phytate content of samples was measured following the method described by Vaintraub and Lapteva, (1988). Weighed samples were extracted with 10 ml of 2.4% HCl and placed in a mechanical shaker for 1 hour at room temperature. This was followed by centrifugation at 3,000 rpm for 30 minutes. The supernatant was mixed with wade reagent and agitated on a vortex mixer for 5 seconds. A spectrophotometer was used to measure the absorbance of the sample. The phytate content was reported in mg /100g.

Alkaloids

The method described by Harborne, (1973) was followed to determine the alkaloid content of the grain. Weighed samples were placed in 50 ml of 10 % acetic acid solution and shaken thoroughly. Samples were left to stand for 4 hours followed by filtration followed by evaporation of the filtrate. Dropwise discharge of concentrated ammonium hydroxide was used to precipitate the alkaloids. Weighed filter papers were used to precipitate the alkaloids, with a facilitated washing using one percent ammonium hydroxide solution. Alkaloid content was calculated as the difference between the filter paper containing the residue and weight of unused filter paper, expressed as a percentage of the sample weight.

Statistical Analyses

Data was analysed using SAS version 9.4, 2020. Analysis of variance (ANOVA) was used to compare means and Fisher's least significant difference (LSD) was used to identify significant differences among means. The model: $Y_{ijk} = \mu + P_i + R_j + \varepsilon_{ijk}$, was used to analyse the data where Y_{ijk} =response variable (nutritional and phytochemical profile); μ =overall mean common to all observations; P_i = effect of the ith processing method (*P = malting, fermentation, soaking*), R_j = effect of the jth replicate, ε_{ijk} = residual error. Significance was considered at the 5 % level of probability.

RESULTS

Effect of Processing Method on Proximate Composition of Macia Sorghum

Treatment method had no significant effect (p>0.05) on moisture, carbohydrate content and gross energy (GE) of the sorghum grain samples (Table 1). The highest (p>0.05) moisture content (13.65%) was recorded for fermented grain, and the lowest (10.05%; p>0.05) was from the control (unprocessed) grain whilst the highest (72.46%: p>0.05) carbohydrate content was from the unprocessed grain and the lowest (61.13%: p>0.05) was from fermented grains. There was a decrease in GE although not significant (p>0.05). Gross energy ranged between 363.47 kcal/100g in unprocessed grain and

342.29 kcal/100g in fermented grain. Significant differences ($p<0.05$) were noted in % ash among the treatments. Fermented grain had the highest (4.35%) ash content while malted grain had the lowest (0.95%). Processing method had a significant effect ($p<0.05$) on crude protein, however, there were no significant differences between crude protein of the unprocessed and soaked grain. Processing method significantly ($p<0.05$) affected the crude fat content of the grain. Malted grain had the highest (6.91%) crude fat content compared to the soaked grain which had the lowest (3.31%) crude fat content. The processing method significantly ($p<0.05$) reduced the crude fiber content. Fermented grain had the lowest (0.93%) crude fibre content, followed by unprocessed grain (1.99%), with the highest (4.63%) value recorded from malted grain.

Table 1. Proximate composition (g/100g) of sorghum as affected by different treatments (soaking, fermentation, and malting)

Treatment	Moisture	Ash	Crude Protein	Crude Fat	Carbo-Hydrate	Crude Fiber	GE (kcal/100g)
Unprocessed	10.05	1.42^{bc}	10.71^{b}	3.42^{cd}	72.46	1.99^{cd}	363.47
Soaked	10.73	1.29^{c}	10.94^{b}	3.31^{d}	71.16	2.32^{b}	359.89
Fermented	13.65	4.35^{a}	14.99^{a}	4.2^{b}	61.13	0.93^{d}	342.29
Malted	10.76	0.95^{d}	7.93^{c}	6.91^{a}	66.35	4.63^{a}	359.25
LSD	0.90	0.85	1.77	0.89	2.83	0.93	5.29
CV	6.05	14.11	7.00	16.21	15.14	10.03	6.05

*Note: Mean values with different superscript(s) in the same column are significantly different ($p\leq0.05$).

Effect of Processing Method on Mineral Composition of Macia Sorghum

Processing method showed a significant ($p<0.05$) effect on the mineral content of samples (Table 2), except for copper which showed no significant ($p>0.05$) differences among the treatments. The results showed a decrease in zinc content for soaked and fermented samples and an increase in zinc content for malted samples compared to the unprocessed grain. Unprocessed samples had significantly higher (9.39mg/100g) iron content than the treated samples while fermented grain had the least (1.69mg/100g) iron content. Processing method significantly ($p<0.05$) decreased manganese content of the grain, however, there were no significant differences in manganese content between fermented and malted grain. Results showed that soaking significantly reduced (10.42mg/100g) the calcium content whilst fermented grain had the highest (27.4 g/100g) calcium content. There was a significant ($p<0.05$) decrease in magnesium content with magnesium decreasing from 185.38 to 68.91mg/100g for soaked grain. The potassium content was highest (289.27mg/100g) in malted grain, followed by fermented grain with 266.1mg/100g and soaked grain had the least (168.32mg/100g) potassium content. The study showed a significant decrease in sodium content from 7.93mg/100g (unprocessed), to 6.30mg/100g (malted), 4.50mg/100g (soaked) and 3.86mg/100g (fermented).

Table 2. Mineral composition (mg/100g) of sorghum as affected by different treatment (soaking, fermentation, and malting)

Treatment	Zn	Fe	Mn	Ca	Mg	K	Na	Cu
Unprocessed	3.33^{b}	9.39^{a}	1.10^{ab}	26.40^{bc}	185.38^{ab}	252.23^{c}	7.93^{ab}	0.03
Soaked	1.89^{cd}	3.34^{c}	0.06^{c}	10.42^{d}	68.91^{d}	168.32^{d}	4.50^{cd}	0.02
Fermented	1.69^{d}	1.69^{d}	0.92^{b}	27.4^{abc}	130.53^{c}	266.1^{bc}	3.86^{d}	0.02
Malted	3.84^{ab}	3.6^{b}	0.95^{b}	25.87^{c}	184.38^{b}	289.27^{ab}	6.30^{b}	0.08
LSD	0.54	1.92	0.26	4.24	29.12	30.24	1.02	0.02
CV	11.01	9.00	17.00	4.03	15.03	5.00	8.00	12.10

*Note: Mean values with different superscript(s) in the same column are significantly different ($p \leq 0.05$)

Effect of Processing Method on Antinutrient Composition of Macia Sorghum

The current study showed that processing method significantly ($p<0.05$) decreased oxalates content (Table 3). The oxalate content decreased from 33.66mg/100g in unprocessed grain to 9.26mg/100g (soaked), 3.68mg/100g (malted) and 2.36mg/100g (fermented) respectively. Processing method significantly reduced the tannin content of grain from 53.58mg/100g in unprocessed grain to 38.11mg/100g (soaked), 30.43mg/100g (malted) and 26.33mg/100g (fermented). The different treatment methods significantly ($p<0.05$) reduced phytate content in sorghum grain. The phytate content was significantly reduced after soaking, malting, and fermenting from 160.10mg/100g to 90.23mg/100g, 30.44mg/100g and 23,68mg/100g respectively. Results of the study show that processing method had no significant ($p>0.05$) effect on the alkaloid content of sorghum grain.

Table 3. Antinutritional composition (mg/100g) of sorghum as affected by different treatments (soaking, fermentation, and malting)

Treatment	Oxalates	Tannins	Phytates	Alkaloids
Unprocessed	33.66^{a}	53.58^{a}	160.10^{a}	0.03
Soaked	9.26^{b}	38.11^{b}	90.23^{b}	0.01
Fermented	2.36^{d}	26.33^{d}	23.68^{d}	0.02
Malted	3.68^{cd}	30.43^{cd}	30.44^{cd}	0.01
LSD	4.24	3.18	7.82	0.02
CV	19.40	11.03	15.01	3.00

*Note: Mean values with different superscript(s) in the same column are significantly different ($p \leq 0.05$.

DISCUSSION

Wet processing techniques such as soaking, germination and fermentation may be employed to improve the bioavailability of minerals and reduce phytochemicals in cereals (Afify et al., 2012). The same processing methods can be employed to increase sorghum inclusion levels in livestock diets (McCuistion et al., 2018b), improve feed palatability and intake thereby improving overall performance and productivity of livestock (Saka et al., 2020). Results from the study showed an increase in moisture content as a result of the different processing methods. These results are consistent with findings by Eburuche et al. (2019) who reported an increase moisture content of malted grain with increase in malting time. Increased soluble matter content due to an increase in enzymatic hydrolytic activities may have resulted in the observed rise in moisture content (Uvere et al., 2014). These findings were however contrary to results by Afify et al. (2012) and Keyata et al. (2021) that showed a decrease in moisture content of soaked and malted grain. The low moisture content may be attributed to modification and hydrolysation of starch granules during malting, resulting in reduced water binding capacity.

Crude protein content of unprocessed grain in the study was higher (10.42g/100g) than 6.90g/100g reported by Tamilselvan and Kushwaha, (2020). The study observed the highest crude protein content in fermented grain which increased from 10.71 to 14.99g/100g and was within the range of 10.42 to 18.301g/100g observed by Eburuche et al. (2019) and Tasie and Gebreyes, (2020). These results are however contrary to findings by Davana et al. (2021) who observed the highest (11.37g/100g) from 72 hour malted grain. Another study (Usman and Bolade, 2017) also found that protein content of malted grain ranged between 9.39 and 11.45g/100g, which was higher than 7.93g/100g observed from malted grain in the current study. Differences in malting period may have contributed to the low crude protein observed in the study.

Soaking can decrease the fat content of sorghum grain due to absorption of water which leads to enzyme activation and digestion of food reserve substance (Lamya et al., 2017). Results from the study showed a decrease in fat content of soaked grain from 3.42 to 3.31g/100g. This result was supported by Lamya et al. (2017) who reported a decrease from 3.34 to 2.43% in fat content of sorghum grains soaked in distilled water. A study by El-safy et al. (2013) found that soaking caused only slight changes in the fat content of grains compared to germination which caused a significant decrease in fat content. Literature reports that germination results in hydrolysis of lipids and oxidation of fatty acids, causing a decrease in fat content of malted sorghum grains (Nemzer and Al-Taher, 2023). This study reported an increase in crude fat content from 3.42 to 4.2g/100g and 6.91g/100g in fermented and malted grain respectively contrary to Keyata et al. (2021), Usman and Bolade (2017) and El-safy et al. (2013) who found a significant fat content decrease in malted grain. The observation from the study was however supported by Embashu (2019), who reported that malting decreased crude fat content in all the cereals in their study, except for Macia, a variety similar to that used in this study.

Results from the current study showed that processing method had no significant effect on the carbohydrate content of sorghum grain. This was contrary to results obtained by Lamya et al. (2017) who observed that soaking significantly increased carbohydrate content of sorghum grain. Another study (Keyata et al., 2021) also found that soaking, malting and fermentation increased the utilizable carbohydrate content (UCC) of sorghum grain. The increase in utilizable carbohydrate content may have resulted from the reduction of the moisture, protein, fat and fibre in soaked and malted sorghum since UCC was calculated by the differences method (Keyata et al., 2021).

The increment of fiber content in soaked and malted sorghum grain was consistent with previous observations (Afify et al., 2012; Usman and Bolade, 2017). Increased fibre content after malting may be attributed to the synthesis of structural carbohydrates, such as cellulose and hemicelluloses during germination (Pandey and Awasthi, 2015). Results from the current study showed a decrease in crude fibre in fermented grain. These findings were supported by Afify et al. (2012) who reported a significant reduction in crude fibre of fermented grain. The reduction in crude fibre may be due to enzymatic solubilization of fibre as the seed germinates.

The current study indicated that processing method had no significant ($p>0.05$) effect on the gross energy content. The study observed a decrease in the gross energy content in processed grain although insignificant. A study by Derbew and Moges, (2017) observed a significant decrease in gross energy with a decrease in crude fat and carbohydrate content in 72 hour germinated grain .These results were however, contrary to a study by Keyata et al. (2021) who found that soaking and washing significantly increased the gross energy content of sorghum grain. Keyata et al. (2021) attributed the low gross energy in malted grain to the relatively low fat content obtained in malted sorghum grain. This was contrary to the current study that observed the highest crude fat in malted sorghum. Malted sorghum had similar gross energy content to soaked sorghum that had the lowest crude fat content.

Results from the current study indicated that malting only increased zinc and potassium content. The decrease in the mineral contents during malting may be attributed to use of available minerals by the growing embryo during the germination process (Udeh et al., 2018). The marked decrease in mineral content in processed grain was also reported by Raihanatu et al. (2011). This may be due to removal of the pericarp of where most of the mineral elements are found. Legodimo and Madibela (2013) also found that malted grain had lower iron content than unprocessed grain. This observation is in line with earlier reports by Claver et al. (2011) who reported a reduction in minerals content as malting time increased. Malting of grain liberates iron ions from the phytate complex, making the iron available for use during germination, hence the reduction.

Fermentation only increased calcium and potassium and resulted in a reduction of the rest of the minerals. These findings were however contrary to reports by Makokha et al. (2002) who found an increase in all mineral elements analysed except for sodium and potassium. Observations from the current study found that soaking reduced the entire mineral content under investigation. Soaking may have led to the decrease of minerals such as Zn, Fe, Mn, Ca, Mg and K due to leaching (Lestienne et al., 2005) as water was discarded every 12 hours to prevent fermentation during the 48hr soaking period. This observation was however contrary to other studies (Claver et al., 2011; Keyata et al., 2021) These studies showed an increase in the mineral content in soaked samples but a decrease in malted grain. An increase in mineral content in the soaked grain may be due to the adsorption of minerals during soaking time (Claver et al., 2011).

Phytochemicals have been observed to result in negative effects such as depressed appetites and reduced digestibility, leading to a decline in animal productivity (Iyakutye et al., 2023). The processing techniques, such as soaking, malting and fermentation of sorghum grain result in hydrolysis of starch and proteins, improved amino acid availability which contribute to the reduction of phytochemicals (Davana et al., 2021). This study showed that treatment methods had no significant effect on alkaloid content. Alkaloid content ranged from 0.03g/100mg in unprocessed to 0.01g/100mg in soaked and malted grains. This was slightly lower than 0.05 to 0.03g/100mg found in soaked grains by another study (Zubair et al., 2023).

Fermentation caused the highest reduction of oxalates, tannins, and phytates. This was in line with the results by Atuna et al. (2022) who reported that fermentation was the most effective treatment method for reducing phytic acid content in cereal flours. The reduction in the levels of phytate during malting may be attributed to activation of endogenous phytase during germination (Svanberg and Lorri, 1997). Phytic acid content for unprocessed grain was 160.10mg/100g, which was lower than 191mg/100g and 278.61mg/100g observed by other studies (Davana et al., 2021; Lamya et al., 2017). Results from the study showed that fermented grain had the lowest phytate content (23.68mg/100g) contrary to observations by Davana et al. (2021) who found the least phytate content from 72hr germinated grain.

The finding that fermentation and malting decreased antinutrient contents was consistent with other studies (Ojha et al., 2018). Malting and fermentation reduced tannin content from 53.58 to 30.43mg/100g and 26.33mg/100g, respectively in the current study. Ojha et al. (2018) observed a decrease in tannin content to 2.6 mg/g in malted grains and 0.1 mg/g in fermented grain, respectively. Another study (Davana et al., 2021) reported that tannin content was reduced to 61mg/100g in 72hr malted sorghum. A combination of germination and fermentation was found to significantly reduce tannin content more than malting, soaking and fermentation alone (Raihanatu et al., 2011). The decrease in the condensed tannin during malting may be due to leaching of water-soluble tannins which are mainly concentrated in the seed coat.

Processing treatment showed a significant reduction in the oxalate content of sorghum grains. Brudzyński and Salamon, (2011) also reported that soaking and malting reduced oxalate content of barley grain. Malting was the most effective treatment that reduced oxalate concentration in sorghum grain (Keyata et al., 2021). These findings were not congruent with reports from this study as fermentation was reported as the most effective processing method to reduce oxalate content.

CONCLUSIONS

Soaking, malting, and fermentation were found to significantly reduce the phytochemical content of sorghum grain. The decrease in phytochemical content implies an increase in mineral bioavailability and improved nutritive value of sorghum grain. Results from the study show that processing techniques such soaking, malting and fermentation can be employed to improve nutritive value of grains with high antinutrient content. The processed grain can be an alternative to maize grain in production of livestock feeds without any adverse effects on animal performance. Inclusion of processed sorghum in diets can positively influence performance and productivity of livestock. Therefore, sorghum-based feeds may be used to lessen competition for maize grain between humans and livestock, especially in marginal areas where maize is not very productive.

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Authors Contribution

N Rugwete contributed to the project idea, designed the methodology, conducted the research and wrote the first draft of the article. T Mutibvu, TE Halimani and E Nyakudya supervised the study and edited the manuscript.

Conflict of interest statement

All authors certify that there is no conflict of interest with any financial, personal, other people or organizations related to the material discussed in the manuscript.

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