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EFFECT OF EXTRUSION COOKING VARIABLES ON *IN VITRO* DRY MATTER DIGESTIBILITY OF SORGHUM [*Sorghum Bicolor* (L.) Moench] EXTRUDATE

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ABSTRACT
Sorghum grain can potentially be a high-quality energy source in non-ruminant diets. However, its digestibility is constrained by endogenous anti-nutritional factors. This study investigated the effect of extrusion cooking variables on in vitro dry matter digestibility (IVDMD) of sorghum extrudate.The study followed a (3×2×2) factorial arrangement in a completely randomized design, each with three replicates, to determine the effect of feed moisture content, barrel temperature, and screw speed on IVDMD of sorghum extrudate using a co-rotating twin screw extruder. According to preliminary trials, the feed moisture content was between 40 to 50% (wet basis), the barrel temperaturewas 70 and 90 °C, and the screw speed was 280 and 300 rpm.The IVDMD of sorghum extrudate increased as feed moisture content and screw speed decreased. Among the tested range of extrusion cooking variables in this study, a combination of low feed moisture content (40%), high screw speed (300 rpm) and high barrel temperature (90 °C) resulted in high IVDMD. The study demonstrated that feed moisture content and screw speed are important extrusion cooking variables that can significantly influence the IVDMD of sorghum extrudates.

***Keywords**: Anti-nutritional factors,barrel temperature,high-tannin sorghum, screw speed,twin screw extruder*

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I. INTRODUCTION

Inrecent years, the demand for energy cereal sources for humans and animals has rapidly increased due to the rising human population and high demand for biofuel production in developed countries (Khanal et al., 2022). Thus, efforts to improve the utilisation of some underutilised, high-value, and drought-resilient crops for food, feed, and industrial use have increased (Rashwan et al., 2021;Selle et al., 2010). Sorghum(*Sorghum bicolor* (L.) Moench) is the fifth most important food and feed cereal crop globally, after maize, wheat, rice, and barley (Assefa et al., 2020).Sorghum does well in relatively hot and dry climates; amidst the accelerating global climate change (Orr et al., 2016).Sorghum has a wide range of uses. The grain is utilisedin brewing and making traditional bread from fermented dough and other food products such as *ugali*, *sadza*, and *uji*. Sorghum is also a source of livestock feed (Orr et al., 2016), and it is included in poultry diets as a constituent or sometimes the only energy source (Selle et al., 2010).The nutritional composition of sorghum grain is similar to that of maize grain, with a crude protein of 9.0% vs 8.5% and metabolizable energy of 3250 kcal/kg vs 3330 kcal/kg, respectively (Leeson & Summers, 2005). However, the nutrient digestibility of sorghum grain is lower in comparison with maize grain due to endogenous anti-nutritional factors (tannins, kafirins, and phytate content (Sohail et al., 2019).

Extrusion cooking is an advanced technology which uses a high-temperature, relatively short-time mechanism to combine moisture, pressure, temperature, and shearing impact to plasticise and cook starchy and/or proteinaceous food material resulting in molecular transformation and chemical reactions (Navale et al., 2015). The unique advantage of extrusion cooking over the other heat processing methods is that the food/feed material undergoes intense mechanical shear, thus breaking the covalent bonds in biopolymers hence intense structural disruption and mixing (Singh et al., 2007). Extrusion cooking changes the physical, chemical and nutritional properties of food/feed constituents. Some desirable effects of extrusion cooking on the extrudate product include the inactivation of anti-nutritional factors (trypsin inhibitors, haemagglutinins, tannins, and phytates), starch gelatinisation, formation of soluble dietary fibre and reduction of lipid oxidation (Singh et al., 2007). Although some undesirable effects, such as Maillard reactions between protein and sugars and loss of heat-labile vitamins,may occur, the desirable effects outweigh the undesirable effects.The quality of the extruded product depends on several factors, especially those that affect the temperature of the food/feed mass within the extruder barrel. As reviewed by Camire (2011), such factors include; feed composition (moisture), barrel temperature, screw speed, extruder model, feed particle size, feed rate, screw configuration, die properties, specific mechanical energy, and mass (product) temperature.Extrusion cooking has become very popular in food processing to produce breakfast cereals, ready-to-eat snack foods, and other textured foods. The technology has been recognised as the main method in the processing of food and feed, and it is attracting more attention and research (Rashwan et al., 2021; Alam et al., 2016; Riaz et al., 2009)

Many studies have been conducted on the effect of extrusion cooking variables on the nutritional quality of sorghum extrudates. However, most of these studies focussed on the physicochemical properties of the extrudates, such as expansion ratio, bulk density, and water absorption capacity, rather than their nutritional quality. Therefore, this studyinvestigatedthe effects of extrusion cooking variables on *in vitro* dry matter digestibility, nutritional composition, mineral bioavailability and anti-nutritional factors of sorghum extrudate.

II. MATERIALS AND METHODS

Study site

The study was undertaken at Egerton University, Department of Dairy and Food Science and Technology (extrusion cooking), and Animal Science laboratories (laboratory analysis). The University is located in Njoro Sub-County, Nakuru County, at 0° 23' S, 35° 55' N. The altitude of the area is 2,238 m above sea level. The temperature of the area averages 21^{o}C, and annual rainfall ranges between 900 to 1,020 mm (Egerton University Meteorological Station, 2019).

Materials

One indigenous variety of sorghum grain containing 4.83% tannin content (tannic acid equivalent) was sourced from Solai ward in Rongai sub-county, Nakuru County.The sorghum grain was cleaned to remove glumes before milling using a hammer mill with a 2 mm sieve (9FC-22A, Shandong Gongyou Group Limited, China). The flour was analysed in the lab before and after extrusion cooking.

Proximate Analysis and Cell Wall Constituents

Moisture, ash, crude fibre and ether extractwere determined by the Association of the Official Analytical Chemists (AOAC, 2006) methods 934.01, 942.05, 962.09 and 920.39, respectively. Protein content (N X 6.25) was determined by the AOAC Kjeldahl method (984.13).The Van Soest method was used to determine acid detergent fibre (ADF), acid detergent lignin (ADL) and neutral detergent fibre (NDF) (Van Soest et al., 1991).

Determination of Calcium and Phosphorus

Calcium was determined using the titrimetric method using calcium carbonate as the standard solution, asSiong (1989) described.Phosphorus was determined using the ascorbic acid method (Nielsen et al., 2017).

Determination of Total Phenolics and Tannin Content

The total tannins were evaluated following the procedure of Makkar and Makkar (2003). Tannins weredetermined by binding with polyvinylpolypyrrolidone (PVPP). The first step involved the measurement of the total phenolics. In the second step, tannins were precipitated together with the PVPP. The results were subtracted from the total phenols to determine the tannin as a percentage of tannic acid equivalent in dry matter.

Determination of Phytate Content

Phytate contentwas determined according to Wheeler and Ferrel (1971). Phytates were extracted with 50 ml of 3% trichloroacetic acid, precipitated as the ferric salt, and then Fe was estimated. Phytate was calculated assuming a constant molecular ratio of 4 Fe: 6 P in the precipitate.

Extrusion Cooking of Sorghum Flour

The flour was extruded in the Department of Dairy and Food Science and Technology using a co-rotating twin-screw extruder (PSHJ-20, Jiangsu Xinda Tech Limited, China).The study followed a (3×2×2) factorial arrangement in a completely randomized design, each with three replicates, to determine the effect of feed moisture content, barrel temperature, and screw speed on IVDMD of sorghum extrudate. According to preliminary trials, the feed moisture content was between 40 to 50% (wet basis), the barrel temperature was 70 and 90 °C, and the screw speed was 280 and 300 rpm. The experimental design comprising 12 treatments is summarised in Table 1.

Table 1. Factorial (3 x 2 x 2) experimental design used inextrusion cooking of sorghum

Factors	Levels
Feed moisture content (%)	40, 45, 50
Barrel temperature (°C)	70, 90
Screw speed (rpm)	280, 300

The feed moisture content was achieved by adding a calculated amount of distilled water before the extrusion cooking process. The first and second barrel temperature regimes were 40, 50, 60, 70°C and 60, 70, 80, 90°C, respectively. Extrudates were collected after the process stabilised and cooled at room temperature (21±4°C) and then dried in a hot air oven at 60°C for 24 h before grinding to pass through a 2 mm sieveand sealed in plastic bags for analysis of IVDMD.

In vitro Dry Matter Digestibility (IVDMD)

A two-phase *in vitro*dry matter digestibility methodwas used with modifications to simulate the digestive process of chicken (Boisen and Fernandez (1997). The treatments were the12 extrudates from the extrusion cooking process; each replicated three times. The effect of feed moisture content, barrel temperature, and screw speed on IVDMD of sorghum extrudate meal was determined using the following statistical model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_K + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \varepsilon_{ijk} \qquad i=1,2,3;\ j=1,2;\ k=1,2$$

where Y_{ijk} is observations made on the response variables; μ is the overall mean; α_i, β_j and γ_k is the effect of i^{th}, j^{th}, and k^{th}main factors in the experiment; $\alpha\beta_{ij} + \alpha\gamma_{ik} + \alpha\beta\gamma_{ijk}$ is the effect of the interaction of factors in the experiment; $\mathcal{E}_{ijk}$ is random error effect associated with the experiment.

Phase one (simulating the stomach of a chicken)

A ground feed sample (0.4 g) was weighed into a 250 ml digestion tube, followed by the addition of simulated stomach fluid. The fluid was composed of 1,550 U/mL pepsin (Sigma-Aldrich P6887, St. Louis, MO) to simulate the natural action of pepsin in the stomach fluid of chicken, postulated by Sturkie (1976). The stomach buffer solution contained 16.9 mmol/L of NaCl, 9.6 mmol/L of KCl, and ten mmol/L of HCl to simulate the natural ionic concentration of stomach fluid from chicken (Sturkie, 1976). The pH was adjusted to 2.0 at 39°C by adding 200 mmol/L of HCl. Exactly 2ml Chloramphenicol C-0378; Sigma-Aldrich, St. Louis, MO, USA (0.5g/100ml ethanol) was added in each conical flask to inhibit bacterial growth. The tubes were sealed using rubber stoppers and kept at 39°C in a water bath with continuous stirring until after 2 hours.

Phase two: (simulating intestines of a chicken)

The mixture from phase one was mixed with 80 ml of phosphate buffer (0.2M, pH 6.8) and 20 ml of 0.6M NaOH. The pH was adjusted to 6.8 using 1M HCl or 1M NaOH to achieve a stable environment for the activity of intestinal enzymes. Exactly 10.6 ml of artificial pancreatin P-1625 Sigma-Aldrich, St. Louis, MO, USA, containing 100 mg/1 litre buffer, was added to the mixture and incubated at 39°C with continuous stirring for 4 hours. The residues were put in 15 mL centrifuge tubes and centrifuged at 1250 x g for 10 min at a temperature of 5°C. The supernatant was taken carefully, rinsed with distilled water, and washed twice with 20 ml of 95% ethanol and 20 ml of 99.5% acetone. Drying was done in the oven for 12 hours at 70°C, and the weight of the residue was recorded.

The IVDMD was computed as described by Boisen and Fernandez (1997):

$$DM\ digestibility = \left(\frac{DM_{In} - DM_{RS}}{DM_{In}}\right) \times 100$$

where DM_{In} and DM_{RS} are the initial and residual DM, respectively.

Statistical analysis

The results were subjected to a three-way analysis of variance using the general linear model (GLM) of the SAS system, version 9.4(2013). Means were separated using the Least Significance Difference (LSD) test at a significance level of 0.05.

III. RESULTS

Table 2. Mean of squares of analysis of variance (ANOVA) of*in vitro* dry matter digestibility of sorghum extrudate

Source of variation	IVDMD (%)
FMC[a]	279.615***
SS[b]	565.085***
BT[c]	61.026[ns]
FMC×SS	223.249***
FMC×BT	187.19***
SS×BT	0.001[ns]
FMC×SS×BT	73.488*

*Significant at P < 0.05; ***Significant at P <0.001; [ns]not significant at P<0.05;[a]feed moisture content (%); [b]screw speed (rpm), [c]barrel temperature (°C).

Table 3. Effect of extrusion cooking variables on *in vitro* dry matter digestibility of sorghum extrudate

Extrusion cooking variable			IVDMD(%)[4]
FMC[1]	RPM[2]	BT[3]	
40	280	70	47.51±3.71[abc]
	280	90	47.74±3.65[abc]
	300	70	44.24±5.05[dc]
	300	90	50.98±7.76[a]
45	280	70	49.85±4.75[ab]
	280	90	46.52±7.98[bdc]
	300	70	42.09±1.63[de]
	300	90	34.50±3.13[f]
50	280	70	45.06±2.82[dc]
	280	90	43.63±3.21[dc]
	300	70	42.35±3.03[de]
	300	90	38.71±3.00[e]

[1]Feed moisture content (%); [2]Screw speed (rpm); [3]Barrel temperature (°C); [4] Values are means of 9 replications ± standard deviation. Means followed by the same letter in the same column are not significantly differentby the Least Significance Difference test at P < 0.05.

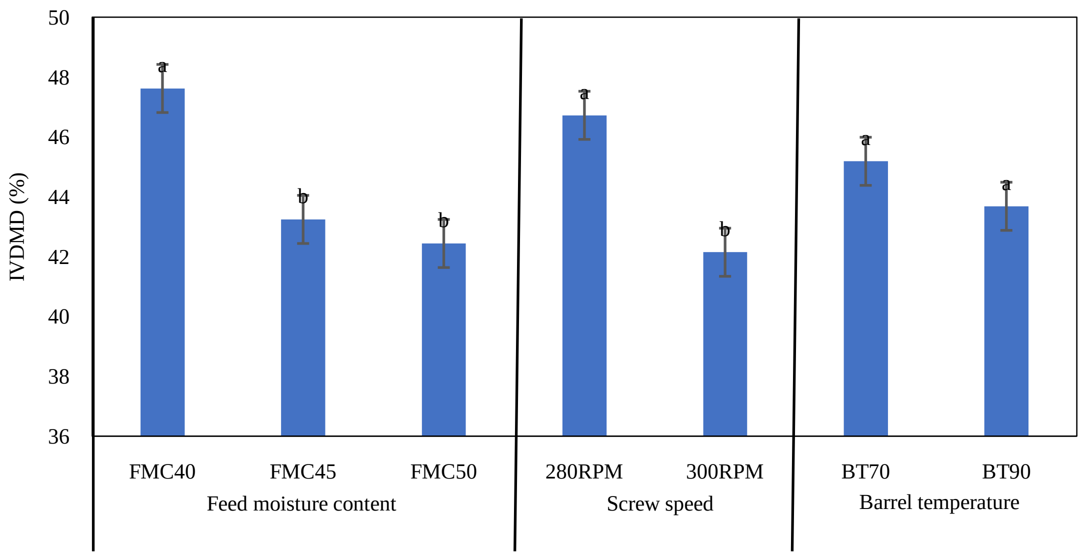

Figure 1. Means of *in vitro* dry matter digestibility of sorghum grain influenced by three extrusion cooking variables (feed moisture content, screw speed, and barrel temperature)

Each bar represents the mean ± standard error. Bars with the same letter within the same process variable are not significantly different at P < 0.05 by the Least Significance Difference test.

Table 4. Effect of extrusion cooking onthe chemical compositionof Sorghum

Parameter	Raw sorghum (g/100g)	Extruded sorghum (g/100g)	t-value	p-value
Moisture Content	8.62±0.14	5.68±0.08	17.77	<.0001
Ash	1.79±0.04	1.75±0.06	0.52	0.6331
Crude Protein	8.72±0.40	8.89±0.36	-0.31	0.7726
Crude Fibre	3.86±0.27	2.76±0.24	3.06	0.0375
Ether Extract	2.60±0.19	0.90±0.03	8.71	0.0111
Acid Detergent Fibre	9.46±0.26	4.72±0.19	14.94	0.0001
Acid Detergent Lignin	1.96± 0.00	0.42±0.12	12.69	0.0061
Neutral Detergent Fibre	30.19±1.57	13.43±0.75	9.61	0.0007
Calcium[1]	10.85±0.15	15.15±0.55	-7.61	0.0016
Phosphorus[1]	128.81± 0.50	177.62± 1.04	-42.44	<.0001
TEPH	6.82±0.01	5.62±0.03	39.85	<.0001
TET	4.83±0.03	4.23±0.06	9.43	0.0007
Phytates[1]	455±13.07	375.6±0.60	6.07	0.0258

TEPH=Total Extractable Phenolics (Tannic Acid equivalent); TET=Total Extractable Tannins (Tannic Acid equivalent): [1]= values expressed in mg/100g; values are expressed as mean ± standard error

IV. DISCUSSION

The*in vitro* dry matter digestibility of sorghum extrudate was significantly affected by feed moisture content and screw speed (Table 2). This is because feed moisture content influences the gelatinisation of starch, protein denaturation, barrel lubrication and the final quality of the extruded product. On the other hand, screw speed regulates the heat flow from the mechanical energy input to the food/feed material, shearing action, and the residence time of the feed/food material in the barrel. It is well-documented that these variables significantly influence the properties of the extruded product (Alam et al., 2016; Navale et al., 2015).The *in vitro* dry matter digestibility values of sorghum extrudate as affected by theextrusion process variables were summarised in Table 3. The IVDMD values ranged from 34.50 to 50.98%, depending on the extrusion cooking variables used. The highest IVDMD was attained at a feed moisture content, screw speed, and barrel temperature of 40%, 300 rpm, and 90^{o}C, respectively.

An increase in feed moisture content from 40 to 50%negativelyinfluencedthe IVDMD of sorghum extrudate (Figure 1). This was because high feed moisture content promotes the formation of protein aggregates due to the cross-linking of disulphide bonds between starch and protein (Dalbhagat et al., 2019), which lowers their accessibility for enzymatic hydrolysis. Moreover, high moisture content increases the lubricating effect in the extruder barrel, reducing the friction among the feed/food material, screw and barrel (Dalbhagat et al., 2019). It also decreases the viscosity and shortens the residence time of feed/food material, reducing the shearing effects on feed/food material (Jafari et al., 2017). Kim et al. (2006) observed a similar trend upon extrusion cooking of pastry wheat flour and attributed itto resistant starch formation.

As screw speed increased from 280 to 300rpm, there was a decrease in IVDMD of sorghum extrudate (Figure 1). It is probable that, at high screw speed, the increased degree of starch gelatinisationresulted in resistant starch formation(Sajilata et al., 2006).Similarly, at high screw speed, a higher degree of protein denaturationresulted in the formation of protein aggregates resistant to enzymatic hydrolysis, leading to lower IVDMD values (Singh et al., 2014). Similar findings were documented byGuha et al. (1997), who reported a decreased*in vitro* starch digestibility of rice flour when screw speed increased from 200 to 400 rpm.

The effect of extrusion cooking on the chemical composition of sorghumwassummarised in Table 4. Extrusion cooking significantly decreasedmoisture content, crude fibre, ether extract, acid detergent fibre, acid detergent lignin and neutral detergent fibre. Extrusion cooking reduced the moisture content of the sorghum extrudate,probably due to pressure and temperature differences, as the sorghum extrudate exited from high pressure and temperature to a low pressure and temperature zone resulting in expansion which promoted easy evaporation of moisture.Moisture content influences the shelf life of the extrudate product. The decrease in moisture content upon extrusion cooking was within the range observed by Byaruhanga et al. (2014), who researched on properties of sorghum extrudates in Uganda.Extrusion cooking significantly reducedcrude fibre, acid detergent fibre, acid detergent lignin, and neutral detergent fibre. This is probably due to a reduction in the molecular weight of pectin and hemicellulose molecules, which increased soluble dietary fibre. During the process, theβ1,4 linkages were significantly broken. This agrees withthe findings of a study on the properties of sorghum extrudate (Byaruhanga et al., 2014) and the extrusion cooking of oats (Zhang et al., 2009).The ether extracts were reduced significantly upon extrusion cooking. This was expected since, during extrusion cooking, some oil may bind starch and proteins, which makes them less extractable with nonpolar solvents (Gulati et al., 2020). According to the researchers, the binding of lipids to starch is not undesirable because it increases the oxidative stability of the lipids.The ether extract of 0.9% after extrusion was within the range reported byByaruhanga et al. (2014).

The bioavailability of minerals (Ca and P) increased significantly upon extrusion cooking. This was expected after the reduction in tannins and phytates,which bind them, thus reducing their bioavailability.Gulati et al. (2018) reported a 30% increase in P dialyzability upon extrusion cooking of beans.

The totalextractable phenolics and total extractable tannins were reduced significantly upon extrusion cooking (Table 4). Their decrease was probably due tothe destruction of polyphenols by the high barrel temperatures and moisture content which promoted decarboxylation, thus increasing the polymerisation of phenols and tannins.This was similar to reports of other studies investigating the effect of extrusion cooking on the total phenols, tannin content and antioxidant activity of sorghum grains (Morais Cardoso et al., 2015; Dlamini et al., 2007).Extrusion cooking significantly reduced phytates. The underlying mechanism is probably the thermal degradation of phytate, causing hydrolysation of inositol hexaphosphate to lower molecular weight forms. This agrees with the work of Gulati et al. (2018), who reported a 46% reduction in phytates upon the extrusion cooking of Great Northern beans.

Thus, extrusion cooking of sorghum flour under low feed moisture content (40%), high screw speed (300 rpm), and high barrel temperature (90^oC) conditions may produce an extruded product with high *in vitro* dry matter digestibility.

V. CONCLUSIONS

The study demonstrated that feed moisture content and screw speed are important extrusion cooking variables that can significantly influence the IVDMD of sorghum extrudates. These findings have practical applications for improving the utilisation of sorghum grain as an energy source cereal for both human and livestock diets for food and nutrition security.However, further studies are necessary to evaluate the other extrusion cooking variables, such as die diameter, and feed rate on IVDMD of sorghum extrudates.

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CONFLICT OF INTEREST

The authors declare no conflict of interest for this article.

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