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Evaluation of the Effective Cowpea [*Vigna unguiculata* (L.) Walp] Sprouting or Roasting for Pig Feeding

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ABSTRACT

Background: The study investigated the effective roasting or sprouting of cowpeas (*Vigna unguiculata*) based on effects on *in vitro* digestibility (IVDMD), supported by measurement of key chemical components and trypsin inhibitor activity (TIA).

Methods: The *in vitro* dry matter digestibility (IVDMD) of raw and all processed cowpeas were evaluated using standard, 3-step (gastric-ileal-colon) simulation of porcine digestion, modified for micro (0.5 g) sample digestion. Standard methods were employed to track processing effects on Ash, CP, Fat, ADF, NDF and trypsin inhibitor activity (TIA) at key processing points.

Result: Sprouting for 2 and 3 days significantly decreased ($p<0.05$) gastric-ileal IVDMD while increasing ($p<0.05$) colon IVDMD. Total (steps 1-3) IVDMD increased ($p<0.05$) in 2-day (0.911) and 4-day (0.902) sprouts. The 20-minute cowpea roasting to 105°C terminal grain temperature resulted in high ($p<0.05$) step 3 and total IVDMD coefficients. The lowest ($p>0.05$) total IVDMD was recorded in 15-minute (0.883) (95°C terminal grain temperature) roasts. In both experiments 1 and 2, quantitative changes in IVDMD were consistent with the changes in the chemical components (ADF, NDF, fat, CP, minerals) and trypsin inhibitor activity. In conclusion, the compartmental and total IVDMD and quantitative change in chemical components and TIA indicated 4 days sprouting and 20-minute (105°C) roasting were respectively most effective for cowpea processing.

Key words: Antinutrtional factors, Digestibility, Plant protein.

INTRODUCTION

The cowpea is a highly agroecologically adaptable leguminous crop with whole grain protein content in the range 16% to 31% and also rich in starch, minerals and functional compounds, including the B group vitamins (Boukar *et al*., 2011).

Subject to varietal differences, the globulins are the dominant protein, with low levels of albumins, glutelins and prolamins (Gupta, 2020; Gonçalves *et al.,* 2016). Details of the structure of the main storage protein (vicilin) were previously described by Kimura *et al.* (2008); Oliveira *et al.* (2021).

As a dietary complement to cereals, typical of the legumes, cowpea protein is rich in lysine and tryptophan, though relative to animal protein, it is deficient in the sulphur containing amino acids (methionine, cysteine) (Frota *et al.,* 2017).

For monogastric stock feed, cowpea protein value may be limited in digestibility and gut absorption of amino acids, due to antinutritional factors (Frota *et al*., 2017). Among key cowpea antinutrients are protease inhibitors and lectins (Boukar *et al*., 2015). Trypsin inhibitors are considered the most important of the protease inhibitors (Kochhar *et al.,* 1988). Processing techniques to mitigate antinutrient effects include soaking, autoclaving, pelleting, dry roasting, dehulling, germination/ sprouting or fermentation (Udensi *et al*., 2007). Thermal processing is traditionally preferred, given the protease inhibitors and lectins are particularly known to be heat labile (Boukar *et al*., 2015). Thermal processing also improves starch digestion through gelatinization (Boukar *et al*., 2015). Among bioprocessing methods, germination and sprouting are advantageous in being non-energy consumptive and are known to substantially improve the nutritive value of legume seeds through stored nutrient mobilization coupled to biosynthesis and antinutrient detoxifying germination metabolism (Boukar *et al.,* 2015). Roasting and sprouting time are one of the things to be considered during processing of legumes to eliminate trypsin inhibitor activity.

The aim of the study was to evaluate the effects of sprouting period and roasting thermal intensity on porcine *in vitro* dry matter digestibility of cowpea (*Vigna unguiculata*) of either sprouting or roasting cowpeas. This study involved two experiments designed to separately evaluate the effective sprouting or roasting of cowpeas [*Vigna unguiculata* (L.) Walp.] for feeding to growing pigs. In both experiments, the evaluation adopted standard *in vitro* porcine digestion, modified for rapid, micro (5 g) sample

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dry matter digestibility measurement, supported by spot analyses for trypsin inhibitor activity and key chemical components.

MATERIALS AND METHODS

Cowpea grain sampling and pre-processing: Cowpeas [*Vigna unguiculata* (L.) Walp.] (Southern cowpea) were sourced from the local market (Thusano grain Products, Makhdo, Limpopo Province, South Africa). Standard procedures were followed to obtain representative analytical samples of cowpeas from the bulk supply. Damaged grains (cracked and weevil-bored) and all debris were manually removed.

Cowpea sampling and pre-processing

Sprouted cowpea

Screened and cleaned cowpeas were sterilized by soaking for 30 minutes in 20% sodium hypochlorite solution. Sterile grains were soaked overnight (approximately 12 hours) in tap water within sterilized soaking drums, after which the grains were subjected to 4-day open-air sprouting at ambient conditions during April 2019 (mean and diurnal temperature range ± 30°C and 24°C-35°C, respectively). Samples were collected for raw, 2-days sprout and 4-days sprouts. Sampled sprouts were rinsed in distilled water and the growth/germinant was terminated by oven-drying to a constant weight in a 105°C, forced air oven, after which they were dry-cooled and stored in a desiccator. Samples were then milled to pass through a 1 mm sieve and stored to be used for *in vitro* digestion.

Roasted cowpea

Screened and cleaned cowpeas were roasted in 20 kg batches within a cylindrical (Length = 1.5 m; Diameter = 0.50 m) manually rotating, cast-iron, gas heated drum. Pre-roasting, the rotating empty roasting drum was heated to a maximum initial constant maximal interior temperature of 150°C, after which cowpeas were subjected to 10, 20 and 30-minute roasting, which culminated in respective internal drum temperatures at 55°C, 105°C and 130°C. The highest or terminal roasting temperature was the point at which cowpeas turned golden brown, to avoid over-roasting. Sampling was done at 55°C, 105°C and 130°C (10, 20 and 30-minute) then forced air oven drying, after which they were dry-cooled and stored in a desiccator. Samples were then milled to pass through a 1 mm sieve and stored to be used for *in vitro* digestion.

In vitro digestion

Sprouted (experiment 1) or roasted (experiment 2) cowpea samples were subjected to 3-step *in vitro* porcine digestion (Boisen and Fernández, 1997), with modifications for micro-sample (0.5 g) dry matter digestibility evaluation (Fushai *et al.* 2019). Samples milled to pass through a 1 mm sieve were oven-dried to a constant weight in a 105°C, forced air oven, after which they were dry-cooled and stored in a desiccator. Approximately 0.5 g samples were weighed into similarly dried Ankom® F57 filter bags which had been pre-rinsed in pure acetone (Acetone for HPLC, ≥99.8% (Sigma-Aldrich® product) 34850). Empty and the sample filter bags were pre-weighed and suspended in digestive media within 250 ml glass digestion bottles immersed in a shaking water bath (CNW Model, WBS 450-B) set at 39°C.

Stepwise simulation of pig digestion was conducted concurrently for experiments 1 and 2 as follows; as follows:

Step one (gastric digestion)

To simulate gastric digestion, sprouted and roasted cowpeas were subjected to 2-hour incubation in phosphate buffer solution in which pH was adjusted to 2.0 using 1 M HCl/M NaOH solutions. To each of the jars were added 87.5 ml phosphate buffer (Phosphate buffer for microbiology, APHA, pH 7.2 (Sigma-Aldrich) 17202) (0.1 M, pH 6.0) and hydrochloric acid 35 ml HCl (0.2 M) solutions (Hydrochloric acid puriss. p.a., ACS reagent, reag. ISO, reag. Ph. Eur., fuming, ≥37%, APHA ≤10 (Sigma-Aldrich) 30721) and the pH adjusted to 2.0 using HCl or sodium hydroxide (NaOH) (Sodium hydroxide BioXtra, ≥98% (acidimetric), pellets (anhydrous) (Sigma-Aldrich) S8045) solution. The jars were then placed on CNW Model, WBS 450-B, 39°C Water Bath Thermostatic Vibration. A 3.5 ml aliquot of a freshly prepared pepsin solution containing 10 mg/ml pepsin (Pepsin from porcine gastric mucosa powder, ≥250 units/mg solid (Sigma) P7000) was then added to the mixture. To prevent bacterial growth, 1.7 ml of a chloramphenicol solution (0.5 g Chloramphenicol ≥98% (HPLC) (Sigma) C0378, per 100 m thanol) were added to the digestion medium and subsequently digested for 2 h using the stop watch timer.

Step two (Small intestine digestion)

After the pepsin digestion, to simulate the environment of the small intestine, pH within jars was adjusted to 6.8 by adding 35 ml of sodium phosphate buffer solution (0.2 M, pH 6.8) and 17.5 ml of NaOH (0.6 M, pH 13.8). A 3.5 ml aliquot of freshly prepared pancreatin solution containing 50 mg pancreatin (Pancreatin from porcine pancreas powder, suitable for cell culture, 4 × USP specifications (Sigma) P3292) was then added to each jar and digestion was continued for another 5 h.

Step three (Large intestine digestion)

To simulate colon digestion, the medium in each jar was completely discarded and replaced with218.75 ml of freshly prepared phosphate buffer (0.1 M, pH 4.8). Sample residues from gastic+intestinal digestion were further digested with 1.75 ml Viscozyme (Viscozyme® L cellulolytic enzyme mixture (Sigma) V2010) for 24 h in a freshly prepared buffer with pH of 4.8 and incubated in a shaking incubator for 18 h at 39°C.

Chemical analyses

Raw cowpeas and the two and 4-day sprouts (experiment 1) and for 10, 20 and 30 roasts (experiment 2) were analysed

for key nutrients, fibre components and protease inhibitor activity (Table 1). Oven-drying two grams' samples determined dry matter at 105°C for 48 hours (AOAC, 2000 method 976.050); Ash by heating two-gram samples at 550°C overnight in an electric furnace (AOAC, 2000 method 923.03); Nitrogen using the micro-Kjeldahl method (AOAC, 2000 method 976.05). Ether extract (EE) by Soxlet extraction (AOAC 2000 method 920.39); Neutral (NDF) and acid (ADF) detergent fibre, according to (Van Soest and Mason, 1991).

To determine minerals, samples were subjected to acid digestion, followed by the determination of calcium by atomic absorption spectrophotometry (Brand GBC, Mod. Avanta PM) (AOAC, 2000; method 968.08) and phosphorous by colourimetry (Clesceri *et al*., 1989, method 4500-P).

Trypsin inhibitor activity (TIA) was assayed according to the American Oil Chemists' Society procedures (AOCS; 1998; method Ba 12-75). One-gram samples were incubated in 50 µl with 20 µl of commercial bovine trypsin (1 mg mL^{-1}) at 37°C for 15 min. Thereafter, a 40 µl of stock solution of 10 mg mL^{-1} in (Dimethyl Sulfoxide) BApNA was added to the solution and the mixture was further incubated at 37°C for 30 minutes. The reaction was terminated by adding 500 µl of 10% glacial acetic acid and the absorbance was measured at 410 nm against a reference to correct for the absorbance from the yellow pigment of the crude extract. Trypsin inhibitor activity was estimated by the difference between the activity recorded with and without inhibitors (Nair *et al*., 2013).

Statistical analysis

The estimated of IVDMD coefficients were analysed separately for the sprouted (experiment 1) or roasted (experiment 2) cowpeas. For each experiment, parameters were subjected to One-Way ANOVA using the GLM of MINITAB software (Version 17.0) using the model.

$$T_{ij} = \mu + T_i + \varepsilon_{ij}$$

Where,

Y_{ij}= Observation (IVDMD) parameter value on the i^{th} processing level.

µ= Overall mean.

T_i= i^{th} processing level.

εij = Random error.

Tukey's test was used to compare means where significant differences occurred.

RESULTS AND DISCUSSION

In vitro dry matter digestibility of sprouted cowpeas

The IVDMD coefficients of raw (control) and sprouted cowpeas, the measured key-spot chemical components and the inhibitor activity auxiliary variables are presented in Table 1. Quantitative effects on the main auxiliary explanatory variables (TIA, crude protein, acid and neutral detergent fibre) are plotted in Table 2. Sprouting influenced the compartmental and total digestion ($p<0.001$). The steps 1-2 (gastric-ileal) IVDMD dropped ($p<0.05$) in the two and three-day sprouts, the sprouts which had highest ($p<0.05$) step three IVDMD. The total (steps 1-3) IVDMD coefficient increased ($p<0.05$) in 2-4 day sprouts ($p<0.05$).

Sprouting affected the compartmental IVDMD in unpredictable fashion, with the high total digestibility observed in 2-4-day sprouts (Table 2). The pattern of total IVDMD digestibility was largely quantitatively consistent with confounding effects of the increasing fibre content and reduction in TIA (Table 2). In previous studies, soaking and sprouting similarly altered the compositional quality of cowpea sprouts (Devi *et al*., 2015). The positive trend in CP in cowpea sprouts (Table 2) was quantitatively similar to the trend reported in 2-5 day sprouts (Malomo *et al*., 2013), though without effect on the chemical score, essential amino acid index, biological value and requirement index (Malomo *et al*., 2013).

Table 1: Trypsin inhibitor activity and chemical components of raw, sprouted or roasted cowpeas.

Parameters	Raw cowpeas	Experiment 1		Experiment 2		
		1 Sprouting period (days)		2 Extent of roasting		
				Duration (minutes)		
		2	4	10	20	30
				Endpoint grain temperature (°C)		
				55	105	130
Trypsin inhibitor activity (Units mg^{-1} DM)	5.63	8.7	4.63	5.25	2.26	1.67
Chemical components (% DM)						
Crude protein	25.9	28.2	29.1	25.7	26	26
Ether extract	1.6	1.2	0.9	1.12	1.31	1.2
NDF	37.3	23.6	36.7	19	24	25.4
ADF	12.5	12.9	18.7	12.1	13.4	10.1
Ca	0.07	0.09	0.10	0.09	0.09	0.09
P	0.4	0.53	0.55	0.51	0.51	0.52

[1]Sprouting; 12-hour pre-soaking in water in water, four-day open-air sprouting at ambient conditions.

Abdelatief (2011) reported higher chymotrypsin inhibitor (15.02 TIU/mg protein) in unprocessed cowpeas compared to those previously (Sumathi and Pattabiraman, 1976) reported for cowpeas (7.2 TIU/mg protein) and for and soybeans (6.6 TIU/mg protein). The reasons for higher levels of these enzyme inhibitors in the experimental local cowpea seeds could be attributed to adverse environmental conditions as well as varietal differences (Abdelatief, 2011). In different cowpea cultivars, Devi *et al.* (2015) reported 29-56% decrease in TIA with soaking. Khattab and Arntfield (2009) reported similar TIA reduction in soaked cowpeas. Though protease inhibitor activity tends to decrease as germination proceeds (Kayembe and van Rensburg, 2013), in the present study, the lowest level TIA was obtained after day 4 sprouting. In sprouts, initial TIA (day 2) was recorded before gradual depletion (Table 2). Antinutritional factors may be endogenous, partially as byproducts during the processing of proteins (Gilani *et al*., 2011) in legume seeds. Sprouting increases, the permeability of cell membrane, increasing the amount of antinutrient leaching (Ali *et al*., 2022). On a residual DM basis, similar to the nutrient, such change in TIA could merely reflect depletion of readily soluble and, or metabolized organic matter, in as much as soluble inhibitors might also not be recovered in solid digesta.

Crude protein and embryonic structural carbohydrate constituents also increased in 4-day sprouts (Nonogaki *et al*., 2010). Similar to the present study, sprouting cowpeas for 4 days increased CP (Uppal and Bains, 2012), in addition to crude fibre (Uppal and Bains, 2012; Devi *et al*., 2015), calcium (Devi *et al*., 2015). In previous studies, IVDMD also depended on the sprouting period Recharla *et al.* (2019). Soaking (8-10 hours) and sprouting (1-3 hours) increased cowpea *in vitro* protein digestibility from 6 to 17% (Uppal and Bains, 2012). However, in the present study, given small indigestible residue sample recovery from the *in vitro* digestion, computation of DM digestibility and nutrient and anti-nutrient content expression on DM basis should be carefully interpreted.

In vitro dry matter digestibility of roasted cowpeas

The IVDMD coefficients of raw (control) and roasted cowpeas are presented in Table 1. Roasting influenced the partial step 3 and the total IVDMD ($p<0.05$), with no effect on steps 1-2 IVDMD ($p>0.05$). The roasting increased ($p<0.05$) both step 3 and the total IVDMD to peak in the 20-minute (105°C) roasts ($p<0.05$). The lowest ($p<0.05$) total IVDMD was recorded in 15-minute (95°C) roasts ($p<0.05$). Quantitative effects on the main auxiliary explanatory variables (TIA, crude protein, acid and neutral detergent fibre) are plotted in Table 3.

Roasting influenced the partial step 3 and the total IVDMD, without effect on steps 1-2 IVDMD. The roasting increased both step 3 and the total IVDMD to peak in the 20-minute (105°C) roasts ($p<0.05$), which was considered most effective processing. Heat inactivated cowpea enzyme inhibitors (Khatoon and Prakash, 2005). Udensi *et al.* (2007) reported heat lability phytic acid. Trypsin inhibitor thermal stability persisted to 80°C, after which activity decreased to detectable low levels at 100°C (Kansal *et al*., 2008), which is consistent with the apparent deactivation in the present study (Table 3). Trypsin inhibitor thermal stability is attributed to the rigid, compact protein structure which is stabilized by several disulfide linkages (de la Sierra *et al*., 1999).

In legumes, perhaps of equal importance to macromolecular depolymerization facilitated nutrient release is antinutrient detoxification to expose biomolecules to plant endogenous and animal enzymatic degradation. The main proteinaceous anti-nutritional factors in cowpeas are trypsin inhibitors and lectins (Mihailovi *et al*., 2005). The molecular architecture Kimura *et al.* (2008); Oliveira *et al.* (2012) of these key proteins is relevant to optimal seed processing. The trypsin inhibition assay is the standard test for the efficacy of thermal, hydrothermal and methods used to process grain legumes for food (Miki *et al.,* 2009). Nutritionally, TIA is classified (Miki *et al.,* 2009) into very low (2-4 TIU mg^{-1} DM), low (4-7 TIU mg^{-1} DM), medium (7-10 TIU mg^{-1} DM) and high (10-13 TIU mg^{-1} DM). A 2 TIU level is considered the minimal threshold enzyme-inhibiting TIA (Miki *et al*., 2009).

Roasting affects DM digestibility by gelatinizing resistant starch (Uppal and Bains, 2012), protein denaturation and breaking of cross-linkages (Khatoon and Prakash, 2005) and through complex mallard oxidation reactions involving carbohydrates, lipids and nitrogenous compounds (Khatoon and Prakash, 2005), processes which when excessive, may negatively affect digestibility.

Table 2: Effects of cowpea sprouting period on *in vitro* dry matter digestibility.

In vitro digestion	N		Sprouted cowpeas				SEM	Significance
			2 Sprouting period (days)					
		Raw cowpeas	1	2	3	4		
			Digestibility					
Step 1-2	14	0.822^{ab}	0.832^{a}	0.781^{b}	0.772^{b}	0.803^{ab}	0.00486	***
Step 3	14	0.061^{b}	0.074^{b}	0.133^{a}	0.132^{a}	0.101^{ab}	0.00605	***
Total	14	0.880^{b}	0.903^{ab}	0.911^{a}	0.901^{ab}	0.902^{a}	0.00336	***

^{ab}means within a row with different letter superscripts are significantly different at ($p<0.05$); SEM- Standard error of the mean; *** -Significant at $p<0.001$.

Table 3: Effects of the extent of roasting cowpeas on *in vitro* dry matter digestibility.

In vitro digestion	N	Raw cowpeas	Roasted cowpeas				Significance	
			2 Extent of roasting					
			Duration (minutes)					
		10	15	20	30	SEM		
			Terminal grain temperature (°C)					
			55	95	105	130		
			DM digestibility					
Step 1-2	14	0.824	0.822	0.820	0.811	0.821	0.00586	ns
Step 3	14	0.061^{b}	0.104^{b}	0.063^{b}	0.172^{a}	0.112^{ab}	0.00734	***
Total	14	0.882^{cd}	0.921^{bc}	0.883^{d}	0.983^{a}	0.932^{b}	0.00449	***

^{abcd} means within a row with different letter superscripts are significantly different at ($p<0.05$); SEM- Standard error of the mean; *** -Significant at $p<0.001$.

Overall, the major legume antinutrients are heat labile (Khatoon and Prakash, 2005).

Roasting increased the lipid and protein content of cereal seeds such as millet (Sade, 2009), maize (Oboh *et al*., 2010) and sesame (Makinde and Akinoso, 2014), an effect attributed to the destruction of cell structure which enables efficient release of the oil reserve (Cuevas-Rodriguez *et al*., 2004). In the current study, roasting had insignificant quantitative effect on cowpea lipid content. The quantitative effect of roasting was less pronounced on fibre components, in contrast to greater reduction of the TIA (Table 3).

CONCLUSION

The study concluded that cowpea sprouting and roasting are potent tool to improve DM digestibility, only if optimally calibrated to maximize the nutritional benefit.

Further studies are recommended which include chemically specific residual digesta and solute analyses, to track the nutritional significance of the observed alterations in gut compartmental and the total IVDMD, with validatory *in vivo* studies.

Conflict of interest

There were no conflicts of interest among the authors.

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