Prospects of extruded grain legumes for meat-type chickens: Effect on endogenous enzyme activity, starch utilization, growth and organ development

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Abstract

Extrusion cooking, a high-temperature short-time processing technique holds promise for lowering enzyme inhibitor activities and improving nutrient bioavailability in grain legumes, thus improving their feeding value. The effect of extrusion cooking of two grain legumes; pigeon pea (*Cajanus cajan*) and bambara groundnut (*Vigna subterranean*) at varied temperatures on growth performance, endogenous enzyme activity and organ development of 35d-old meat-type chickens was investigated. Pigeon pea and bambara groundnut were milled and extruded at two temperatures (100°C and 140°C) from which six diets were formulated containing 30g/100g diet of non-extruded (control), 100°C or 140°C extrudates of bambara groundnut or pigeon pea. Two hundred and fifty-two, one day-old, meat-type chicks were allotted to the six diets in six replicates, seven birds each. Weight gain and feed intake (FI) were monitored weekly and feed conversion ratio (FCR) was estimated. Relative weight (g) of pancreas, proventriculus, gizzard and liver as well as duodenal, jejunal and ileal lengths and weights were measured. Activities of pancreatic amylase, proteases; chymotrypsin and trypsin, and brush border disaccharidases; sucrase and maltase were also assayed in the pancreas, ileal and jejunal digesta, and ileal and jejunal tissues respectively. Data was analyzed using ANOVA ($p < 0.05$) and pre-planned contrasts, elucidating the effect of extrusion (raw vs extruded; 100°C and 140°C) and temperatures (100°C vs 140°C) on each grain legume investigated. Weight gain of chickens on both bambara groundnut and pigeon pea diets were not affected by extrusion temperatures or extrusion throughout the study period except during 8-14d in the pigeon pea groups. However, FI significantly reduced and FCR improved with extrusion cooking and extrusion temperatures ($P < 0.05$) for meat-type chickens on the pigeon pea diets. The FI also reduced and FCR improved with extrusion cooking and extrusion temperatures for meat-type chickens on the bambara groundnut diets between 8-21d and 29-35d of age (FI: 604.83 ± 0.71 g vs 530.58 ± 6.47 g and 532.20 ± 15.36 g respectively; FCR: 3.89 ± 0.15 vs 3.25 ± 0.17 and 3.27 ± 0.21 , respectively). Relative organ weights, weights and lengths of intestinal segments as well as pancreatic amylase and protease activities were not influenced by extrusion cooking or extrusion temperatures.

Keywords: high-temperature short-time extrusion cooking; grain legumes; alternative energy-protein ingredients; broiler chicken

Prospection de légumineuses à grains extrudées pour les poulets de type viande : effet sur la croissance, le développement des organes, l'activité enzymatique endogène et l'utilisation de l'amidon chez les poulets de type viande

Résumé

La cuisson par extrusion, une technique de traitement de courte durée à haute température, est prometteuse pour réduire les activités des inhibiteurs enzymatiques et améliorer la

biodisponibilité des nutriments dans les légumineuses à grains, améliorant ainsi leur valeur nutritive. L'effet de la cuisson par extrusion de deux légumineuses à grains ; *Cajanus cajan* (pois cajan) et *Vigna subterranean* (arachide bambara) à des températures variées sur les performances de croissance, l'activité enzymatique endogène et le développement des organes de poulets de type viande âgés de 35 jours ont été étudiés. Le pois cajan et le pois cajan ont été broyés et extrudés à deux températures (100oC et 140oC) à partir desquels six régimes ont été formulés contenant 30g/100g de régime alimentaire non extrudé (témoin), 100oC ou 140oC d'extrudés de pois cajan ou de pois cajan. Deux cent cinquante-deux poussins de type viande d'un jour ont été répartis dans les six régimes en six répétitions, sept oiseaux chacun. Le gain de poids et la prise alimentaire (PA) ont été surveillés chaque semaine et le ratio de conversion alimentaire (RCA) a été estimé. Le poids relatif du pancréas, du proventricule, du gésier et du foie (g) ainsi que les longueurs et poids duodénaux, jéjunaux et iléaux ont été mesurés. Activités de l'amylase pancréatique, des protéases ; la chymotrypsine et la trypsine, et les disaccharidases de bordure en brosse; la sucrase et la maltase ont également été dosées dans le pancréas, le digesta iléal et jéjunal et les tissus iléaux et jéjunaux respectivement. Les données ont été analysées à l'aide d'ANOVA ($p < 0,05$) et de contrastes pré-planifiés, pour élucider l'effet de l'extrusion (brut vs extrudé ; 100 oC et 140 oC) et des températures (100 oC contre 140oC) sur chaque légumineuse à grains étudiée. Le gain de poids des poulets recevant à la fois des régimes à base d'arachide et de pois cajan n'a pas été affecté par les températures d'extrusion ou l'extrusion tout au long de la période d'étude, sauf pendant 8 à 14 jours dans les groupes de pois cajan. Cependant, le PA a diminué de manière significative ($P < 0,05$) et le RCA s'est amélioré avec la cuisson par extrusion et les températures d'extrusion pour les poulets de type viande sur les régimes de pois cajan. Le PA a également diminué et le RCA s'est amélioré avec la cuisson par extrusion et les températures d'extrusion pour les poulets de type viande sur les régimes à base d'arachide bambara entre 8-21 jours et 29-35 jours d'âge (PA : $604,83 \pm 0,71$ g vs $530,58 \pm 6,47$ g et $532,20 \pm 15,36$ g respectivement ; RCA : $3,89 \pm 0,15$ vs $3,25 \pm 0,17$ et $3,27 \pm 0,21$ respectivement). Les poids relatifs des organes, les poids et les longueurs des segments intestinaux ainsi que les activités de l'amylase et de la protéase pancréatiques n'étaient pas influencés par la cuisson par extrusion ou les températures d'extrusion.

Mots-clés : Cuisson par extrusion de courte durée à haute température ; légumineuses à grains; ingrédients protéinés énergétiques alternatifs; poulet à griller

Introduction

The high cost of conventional energy and protein ingredients used in poultry feeding has continued to negatively impact the growth of the poultry industry in Nigeria. Fluctuations in the supply and cost of maize and soyabean meal (Figure 1) are attributed to increased competition for maize and soyabean by the food, feed and biofuel industries, climate change induced weather change, occurrence of new crop pests and diseases, scarcity of resources (such as fertile land, water for irrigation and fertilizers), and, political and socioeconomic constraints. Lesser known

grain legumes such as kidney beans (*Phaseolus vulgaris* L.), field bean (*Vicia faba* L.), African yam bean (*Sphenostylis stenocarpa*), pea (*Pisum sativum* L.), lupin (*Lupinus albus* L.) and chickpea (*Cicer arietinum* L.), have been considered as alternative protein-energy ingredients for poultry (Arija *et al.*, 2006; Jozefiak *et al.*, 2006; Brenes *et al.*, 2008; Nalle *et al.*, 2011; Son and Ravindran, 2012; Raji *et al.*, 2016; Abdulla *et al.*, 2017) due to their superior protein value in comparison to maize, as well as similarity in gross energy when compared to maize and soyabean meal (Heuze *et al.*, 2016). However, the

partial resistance of legume starches to amylase digestion (Velasco *et al.*, 1997) and the presence of antinutritive bioactives (such as protease inhibitors, lectins, tannins, phytate and haemagglutinins) in grain legumes (FAO, 1995; Capos-Vegas *et al.*, 2016) stall their use as feed ingredients

for poultry. However, heat treatment of grain legumes by traditional and industrial methods are reported to irreversibly deactivates heat-labile antinutritive bioactives improving nutrient digestibility and bioavailability to varying degrees.(Alonso *et al.*, 1998, 2000a)

Figure 1. Trend of soyabean meal and maize prices 2016 -2021 (Personal communications)

High-temperature short-time (HTST) extrusion cooking, which is an industrial technique that subjects moist food/feed ingredients to a combination of high temperature, pressure and mechanical shear over a short time period, has been widely adopted in the food and feed industry due to its practicality, high productivity, efficiency, and nutrient retention. The ability of HTST cooking to influence nutrient bioavailability is pronounced in the protein and starch components of ingredients. Nutritional implications for HTST extrusion cooking of grain legumes has been investigated *in vitro* and *in vivo* and attributed to modified physicochemical properties of extrudates, thermal degradation of sugars and amino acids, and gelatinization of starch during the HTST extrusion cooking process. Other implications for HTST extrusion cooking of

grain legumes include significant lowering of feed rancidity and microbial contamination. Conversely, severe extrusion conditions have been implicated in impaired nutrient bioavailability owing to formation of late Maillard reaction products such as melanoides. Maillard's reaction is an integral part of feed processing arising from the binding of the amino group of reactive amino acids such as lysine, and the carbonyl group of reducing sugars such as glucose and lactose.(Singh *et al* 2007; Mavromichallis, 2015). Earlier reports of *in vitro* protein digestibility of *Sphenostylis stenocarpa* (African yam bean), *Cajanus cajan* (pigeon pea) and *Vigna subterranean* (bambara groundnut) showed a 22.16-30.8% improvement in protein digestibility when 100°C and 140°C extrudates of *Sphenostylis stenocarpa* and *Cajanus cajan* were

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assessed without any effect of extrusion temperature observed in extrudates of *Vigna subterranean*, while *in vitro* starch digestibility was improved in 140°C extrudates of *Cajanus cajan* and *Vigna subterranean* compared to 100°C extrudates, with no effect of varied extrusion temperatures in *Sphenostylis stenocarpa*. Hence this study seeks to validate these findings *in vivo* by investigating the effect of varied extrusion temperatures on the growth performance, organ development, endogenous enzyme activity and starch utilization in meat-type chickens fed *Cajanus cajan* (pigeon pea) and *Vigna subterranean* (bambara groundnut)-based diets.

Materials and methods

Test product, extrusion and diets

Cajanus cajan and *Vigna subterranean*

were purchased from a grain market in Ibadan, cleaned of debris and finely ground through a 0.5mm screen in the hammer mill. Thereafter, the milled grains were conditioned to 20% moisture and fed manually through a feeding unit at 33.6kg/h into a single-screw extruder of compression ratio, 3:1; screw length-to-diameter (L/D) ratio, 6:1 and barrel length, 317.50mm. The extruder operated at screw speed of 305 rpm through an 8mm die hole at varied extrusion temperatures of 100°C or 140°C. The unextruded and extruded grain legumes were assayed for nutritional composition (Table 1) and subsequently incorporated at 30% of a starter-grower broiler diet (Table 2) formulated to provide required nutrients to support growth and performance (NRC, 1994).

Table 1 : Determined composition of unextruded and extruded bambara groundnut (*Vigna subterranean*) and pigeon pea (*Cajanus cajan*)[†]

		Crude protein (%)	Crude fibre (%)	Ether extract (%)	Nitrogen-free extract (%)	Ash (%)	AMEn [‡] (kcal/kg)
Bambara groundnut (<i>Vigna subterranean</i>)	BBR	20.50 ± 0.01	4.03 ± 0.05	11.45 ± 1.28	51.42 ± 1.27	2.90 ± 0.00	2838.66
		20.27 ± 0.01	6.13 ± 0.46	5.15 ± 0.65	55.87 ± 0.87	3.63 ± 0.08	2492.14
	BB100	19.91 ± 0.01	6.03 ± 0.06	0.77 ± 0.18	60.36 ± 0.16	3.63 ± 0.03	2494.65
		21.20 ± 0.02	7.72 ± 0.25	8.50 ± 3.10	48.59 ± 2.74	4.88 ± 0.03	2172.62
	PPR	20.07 ± 0.00	8.02 ± 0.02	3.37 ± 0.16	55.58 ± 0.61	4.50 ± 0.20	2164.31
		18.66 ± 0.01	9.35 ± 0.13	2.03 ± 0.81	55.60 ± 0.69	5.02 ± 0.12	1888.19
Pigeon pea (<i>Cajanus cajan</i>)	PP100						
	PP140						

Values are presented as means of triplicate determinations ± standard deviation

BBR - unextruded bambara groundnut (*Vigna subterranean*); BB100 - Bambara groundnut (*Vigna subterranean*) extruded at 100°C; BB140 - bambara groundnut (*Vigna subterranean*) extruded at 140°C; PPR - unextruded pigeon pea (*Cajanus cajan*); PP100 - pigeon pea (*Cajanus cajan*) extruded at 100°C; PP140 - pigeon pea (*Cajanus cajan*) extruded at 140°C

[‡]Calculated value; European Table of Energy Values for Poultry Feedstuff (WPSA, 1986)

[†]Adeleye *et al.* (2020)

Table 2 : Ingredients and calculated nutrient composition of experimental diets (g/kg as fed) containing unextruded and extruded bambara groundnut and pigeon pea

Ingredients	bambara groundnut			pigeon pea		
	BBR	BB100	BB140	PPR	PP100	PP140
Maize	320	320	320	340	340	340
Soyabean meal (44% CP)	275	275	275	255	255	255
Bambara groundnut - unextruded	300	-	-	-	-	-
100°C bambara groundnut extrudate	-	300	-	-	-	-
140°C bambara groundnut extrudate	-	-	300	-	-	-
Pigeon pea - unextruded	-	-	-	300	-	-
100°C pigeon pea extrudate	-	-	-	-	300	-
140°C pigeon pea extrudate	-	-	-	-	-	300
Fish meal (72% CP)	30	30	30	30	30	30
Soy oil	42	42	42	42	42	42
Limestone	10	10	10	10	10	10
Dicalcium phosphate	15	15	15	15	15	15
Vitamin-mineral premix	2.5	2.5	2.5	2.5	2.5	2.5
Lysine- HCl	1	1	1	1	1	1
DL methionine	2	2	2	2	2	2
Sodium chloride	2.5	2.5	2.5	2.5	2.5	2.5
Calculated nutrients						
Metabolizable energy (ME, kcal/kg)	3054	2950	2950	2874	2872	2790
Crude protein, %	23.3	23.2	23.1	22.8	22.4	22.0
Crude fibre, %	3.9	4.5	4.5	4.9	5.0	5.4
Calcium, %	1.1	1.1	1.1	1.1	1.1	1.1
Methionine, %	0.6	0.6	0.6	0.6	0.6	0.6
Lysine, %	1.4	1.4	1.4	1.5	1.5	1.5
Phosphorus, %	0.6	0.6	0.6	0.7	0.7	0.7

¹Optimix poultry chick nourisher, Animal Care, Nigeria. Provided the following per kg/diet: vitamin A, 20,000 IU; vitamin D3, 4,000 IU; vitamin E, 20 mg; vitamin K3, 4 mg; vitamin B1, 3 mg; vitamin B2, 10 mg; niacin 16 mg; calpan 16 mg; vitamin B6, 6 mg; vitamin B12, 0.03 mg; choline chloride, 200 mg; folic acid, 2 mg; biotin, 0.1 mg; manganese, 120 mg; iron, 40 mg; zinc, 100 mg; copper, 10 mg; iodine, 2 mg; cobalt, 0.4 mg; selenium, 0.4 mg; antioxidant, 250 mg

²Calculated analysis based on nutritional values of feed ingredients (NRC, 1994; Feedipedia)

³BBR - unextruded bambara groundnut (*Vigna subterranean*); BB100 - bambara groundnut (*Vigna subterranean*) extruded at 100°C; BB140 - bambara groundnut (*Vigna subterranean*) extruded at 140°C; PPR - unextruded pigeon pea (*Cajanus cajan*); PP100 - pigeon pea (*Cajanus cajan*) extruded at 100°C; PP140 - pigeon pea (*Cajanus cajan*) extruded at 140°C

Experimental site, housing and animals

This study was conducted at the Poultry Unit, Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria and lasted for 35 days. A total of 252 day-old chicks were purchased from a reputable hatchery in Ibadan metropolis. Chicks were weighed on arrival and transferred to a deep

litter house and brooded on wood shavings for seven days.

Temperature was maintained at 32°C and a 24L:0D, lighting programme was observed during the brooding period, and thereafter ambient temperature of 24-29°C and natural illumination (14L:10D) was observed for the rest of the study. At the end of the

brooding period, the chicks were weighed and randomly allocated to one of 36 groups (7 chicks per group) based on weight equalization using the Experimental Animal Allotment Program (Kim and Lindemann, 2007). Each group was housed in a unit with dimensions; 148cm × 96cm × 116cm within a battery cage system and randomly allotted to one of the experimental diets in six replicates. Diets were provided in mash form with titanium dioxide added at 0.5% of the complete diet as an indigestible marker. Diets and water were offered *ad libitum*.

Sampling and data collection

Each chick was weighed on day 14, 21, 28 and 35 of the study. Feed consumption was measured per unit at the same time and feed conversion ratio was calculated weekly. At 21d of age, two birds per replicate were euthanized by exsanguination, the pancreas, jejunum and ileum were biopsied and digesta harvested from the distal jejunum and ileum. Biopsies were homogenized in saline water on ice for about 5 minutes and subsequently centrifuged at 4000 ×g for 10 minutes to remove larger cell debris with only small losses of enzyme activity. The supernatant was transferred to a 2mL tube and stored at -20°C for estimation of the activity of amylase and disaccharidases; sucrase and maltase. Digesta was centrifuged at 12000 ×g for 5 minutes and the supernatant transferred to a 2mL tube and stored at -20°C for estimation of the activity of serine proteases; trypsin and chymotrypsin. From day 32-34 of the study, samples of excreta were collected daily and subsequently pooled on replicate basis. At 35d of age, another two chickens were randomly selected per replicate and euthanized by exsanguination. Small intestinal sections; duodenum, jejunum and ileum were harvested, measured using a tape measure and then weighed, and the relative length (cm/100g BW) and weight (g/100g BW) were estimated. The pancreas, liver,

proventriculus and gizzard were also harvested, cleaned of adhering tissues, weighed, and relative organ weights were computed. Digesta was also collected from the distal jejunum and ileum, and approximately 5cm of the jejunum and ileum was collected, flushed with distilled water and fixed in 10% formalin for 48 hours. Thereafter, the jejunal and ileal sections were dehydrated, embedded in paraffin, sliced into 5 µm thick sections and fixed on slides. Samples of digesta and excreta were dried in a forced air oven at 55°C prior to chemical analyses.

Chemical analyses

Amylase activity was estimated by the reaction of crude enzyme extracts of the pancreas with QCA amylase liquid which contains the chromogenic substrate 5-ethylidene-G- ρ -nitrophenol bound to a maltose oligosaccharide (Química Clínica Aplicada S.A), and disaccharidase; sucrase and maltase activity estimated by the method of Dahiqvist (1984) based on the reaction between crude enzyme extracts of the jejunal and ileal tissues, disaccharide standards solutions and a glucose/peroxidase reagent (Química Clínica Aplicada S.A). Activity of the serine proteases; trypsin and chymotrypsin in crude extracts of the digesta were determined by their reaction with the chromogenic substrates N-benzoyl-DL-arginine-4-nitroanilide hydrochloride (BAPNA, B-4875, Sigma-Aldrich) and N-benzoyl-L-tyrosine- ρ -nitroanilide (BTPNA, B-6760, Sigma-Aldrich), respectively. Jejunal and ileal digesta, and excreta samples were assayed for moisture (AOAC, 1995) and total starch (Vasantan, 2001), and residual starch was calculated. The jejunal and ileal tissue slides were stained with haematoxylin and eosin and subsequently examined under a digital light microscope (VJ-2005-DN Model; Bio-Microscope) fitted with mitotic images plus 2.0 digital microscopy software and measurements of the villus height, crypt

depth and thickness of the *muscularis* were done using the TS VIEW CX Image software (File version 6.2.4.3).

Statistical analysis

Data obtained were subjected to a one-way analysis of variance and pre-planned contrasts elucidating the effect of extrusion (raw vs extruded; 100°C and 140°C) and temperatures (100°C vs 140°C) on each grain legume investigated. The JASP statistical software (version 0.14.1) (JASP, 2020) was used, and significance was based on a 5% probability level.

Results and discussion

Bambara groundnut and pigeon pea are sources of energy and crude protein (~ 37-45% starch and 18-28% crude protein) (Oshodi *et al.*, 1993; Adeleye *et al.*, 2020b). However, like other grain legumes, their use in poultry diets is impaired by their inherent antinutritional factors resulting in poor utilization of these grains by consuming animals (Singh *et al.*, 2007). *In vitro* evaluations have established that the heat treatment associated with extrusion cooking has the ability to achieve deactivation of these inherent antinutrients as well as improve the digestibility of their starch and protein (Adeleye *et al.*, 2020a,b). The determined composition and estimated apparent metabolizable energy corrected for nitrogen (AMEn) of extruded and unextruded bambara groundnut (*Vigna subterranean*) and pigeon pea (*Cajanus cajan*) are shown in Table 1, and a detailed discourse of the effect of extrusion cooking and extrusion temperatures on their nutritional profile is provided in an earlier publication (Adeleye *et al.*, 2020b). Earlier research support the feeding of heat treated bambara groundnut and pigeon pea up to 30 and 27% respectively in the diets of meat-type chickens without detrimental effects (Ani and Okeke, 2011; Bello *et al.*, 2005), informing the inclusion of 100 and 140°C

extrudates of bambara groundnut and pigeon pea at 30% of the diet in the current study. The performance of meat-type chickens fed the experimental diets from 8-35d is summarized in Table 3. There was no significant effect of extrusion cooking of bambara groundnut at different extrusion cooking temperatures (PP100 vs PP140) on body weight gain (BWG) and feed conversion ratio (FCR), however, extrusion cooking (unextruded vs extruded) significantly reduced body weight gain ($p = 0.05$) of meat-type chickens on 15-21d. A reduction in feed intake as a result of extrusion cooking (unextruded vs extruded) was also observed at 8-14d, 15-21d and 29-35d, while FCR was improved during the same period. Feed intake declined significantly and FCR improved with extrusion cooking of pigeon pea (unextruded vs extruded) at higher temperature (PP100 vs PP140) throughout the study, while extrusion cooking of pigeon pea (unextruded vs extruded) at different temperatures (PP100 vs PP140) significantly improved BWG at 15-21d. Extrusion of grain legumes seems to have different effects on growth performance of meat-type chickens and may be attributed to differences in extrusion characteristics, extruder properties, physicochemical properties of different grain legumes in digesta (Hejdysz *et al.*, 2018) or null effect of the extrusion process on heat stable antinutritional bioactives such as phytate (Rutkowski *et al.*, 2016). For example, similar to our observations in the current study feeding of extruded faba beans at 300g/kg diet depressed feed intake and improved FCR without any significant effect on body weight gain (Hejdysz *et al.*, 2019). However, extrusion cooking of narrow-leaved lupin seeds did not influence feed intake, body weight gain, or FCR of meat-type chickens at 0-14d and 15-35d (Hejdysz *et al.*, 2018), while extrusion of peas did not influence feed intake, BWG, or FCR of meat-type

Table 3: Performance of meat-type chickens fed unextruded or extruded bambara groundnut and pigeon pea-based diets from 8-35d

Treatments	8-14 d			15-21 d			22-28 d			29-35 d		
	BWG (g)	FI	FCR (g)	BWG (g)	FI	FCR (g)	BWG (g)	FI	FCR (g)	BWG (g)	FI	FCR
BBR	87.04 ± 4.06	179.65 ± 0.34	2.17 ± 0.11	130.88 ± 5.04	291.29 ± 0.63	2.30 ± 0.09	149.57 ± 6.26	402.31 ± 16.92	2.85 ± 0.19	151.05 ± 8.20	604.83 ± 0.71	3.89 ± 0.15
BD100	81.72 ± 3.53	141.60 ± 0.27	1.87 ± 0.09	123.33 ± 4.08	243.25 ± 3.27	2.06 ± 0.08	159.79 ± 6.99	378.43 ± 9.16	2.54 ± 0.13	137.16 ± 6.05	530.58 ± 6.47	3.25 ± 0.17
BD140	85.15 ± 3.44	135.01 ± 0.25	1.71 ± 0.08	112.83 ± 4.68	220.0 ± 0.22	2.07 ± 0.08	153.62 ± 5.74	377.31 ± 4.49	2.53 ± 0.96	157.61 ± 9.88	532.20 ± 15.36	3.27 ± 0.21
<i>p</i> -contrast values												
Unextruded vs extruded	NS	0.00	0.02	0.05	0.00	0.05	NS	NS	NS	NS	0.00	0.01
BB100 vs BB140	NS	0.00	NS	NS	0.00	NS	NS	NS	NS	NS	NS	NS
PPR	90.74 ± 2.67	171.87 ± 0.24	1.92 ± 0.05	97.57 ± 7.01	283.42 ± 0.25	2.98 ± 0.13	129.37 ± 7.74	445.25 ± 0.34	3.38 ± 0.15	144.6 ± 6.09	604.24 ± 0.30	3.88 ± 0.14
PP100	79.88 ± 2.99	142.57 ± 0.26	1.91 ± 0.09	105.32 ± 5.37	233.0 ± 2.39	2.48 ± 0.14	128.74 ± 8.49	366.5 ± 0.22	2.90 ± 0.17	151.85 ± 7.60	506.39 ± 0.30	3.42 ± 0.18
PP140	85.56 ± 3.01	128.65 ± 0.25	1.61 ± 0.08	128.97 ± 4.61	213.34 ± 0.22	1.77 ± 0.09	127.38 ± 6.66	365.01 ± 0.32	2.64 ± 0.08	169.10 ± 13.82	493.84 ± 0.24	2.52 ± 0.17
<i>p</i> -contrast values												
Unextruded vs extruded	0.03	0.00	0.04	0.01	0.00	0.00	NS	0.00	0.00	NS	0.00	0.00
PP100 vs PP140	NS	0.00	0.01	0.00	0.00	0.00	NS	0.00	0.16	NS	0.00	0.00

¹BBR - unextruded bambara groundnut (*Vigna subterranean*); BB100 - bambara groundnut (*Vigna subterranean*) extruded at 100 °C; BB140 - bambara groundnut (*Vigna subterranean*) extruded at 140 °C; PPR - unextruded pigeon pea (*Cajanus cajan*); PP100 - pigeon pea (*Cajanus cajan*) extruded at 100 °C; PP140 - pigeon pea (*Cajanus cajan*) extruded at 140 °C

²BWG – Body weight gain (g/period); FI – Feed intake (g/period); FCR – Feed conversion ratio

chickens when fed at 350-356g/kg of diet in the grower (11-28d) and finisher (29-42d) phases (Diaz *et al.*, 2006). The effects of extrusion and temperature on the relative weight and length of intestinal sections and relative organ weights of meat-type chickens fed the experimental diets are summarized in Figures 2 and 3. No changes in relative weight and length of intestinal segments; duodenum, jejunum, and ileum, as well as organs; proventriculus, gizzard, pancreas and liver, were observed in meat-type chickens when unextruded and extruded test diets compared. To the contrary, Arija *et al.* (2006) reported a decrease in the weight of digestive accessory organs; pancreas and liver, and the jejunum in response to extrusion cooking of kidney beans incorporated in the diet of meat-type chickens at 300g/kg of the total diet. The effects of extrusion and extrusion temperatures on endogenous enzyme activity; pancreatic amylase, trypsin and chymotrypsin activity of meat-type chickens fed the experimental diets are reported in Table 4. No effect of extrusion cooking (unextruded *vs* extruded) or

extrusion temperatures was observed on the activity of pancreatic amylase as well as trypsin and chymotrypsin activity in jejunal and ileal digesta of meat-type chickens fed unextruded or extruded bambara groundnut and pigeon pea-based diets. The activity of jejunal and ileal disaccharidases was also unaffected by extrusion cooking (unextruded *vs* extruded). However, a significant effect of extrusion temperatures was observed on jejunal maltase activity in meat-type chickens on the extruded bambara groundnut diets (BB100 *vs* BB140) and ileal sucrase in meat-type chickens on the extruded pigeon pea diets (PP100 *vs* PP140). Compensatory activities of the pancreas of chickens in response to upregulated cholecystokinin production in the presence of protease inhibitors results in upregulation of pancreatic enzyme secretions and consequently pancreatic hypertrophy and hyperplasia (Clarke and Wiseman, 2005; Hill, 2003). However in this study no differences were observed in the activity of pancreatic amylase and proteases in meat-type chickens fed the unextruded *vs* extruded grain legumes in their diets.

Figure 2. Effect of extrusion and extrusion temperatures on: A. Relative intestinal weight of meat-type chickens fed a bambara groundnut-based diet. B. Relative intestinal weight of meat-type chickens fed pigeon pea-based diets C. Relative intestinal length of meat-type chickens fed a bambara groundnut-based diet. D. Relative intestinal length of meat-type chickens fed pigeon pea-based diets and from 8-35d.

Figure 3. Effect of extrusion and extrusion temperatures on: A. Relative organ weights of meat-type chickens fed a bambara groundnut-based diet. B. Relative organ weights of meat-type chickens fed pigeon pea-based diets

Table 4 : Effect of extrusion and extrusion temperatures on pancreatic amylase, intestinal proteases and disaccharidases of meat-type chicken fed unextruded or extruded bambara groundnut and pigeon pea-based diets from 8-12d

Treatments	Jejunal proteases				Ileal proteases			Jejunal disaccharidases			Ileal disaccharidases		
	Pancreatic amylase ($\times 10^3$ U/g tissue)	Trypsin (U/g digesta)	Chymotrypsin (U/g digesta)	Trypsin (U/g digesta)	Chymotrypsin (U/g digesta)	Trypsin (U/g digesta)	Chymotrypsin (U/g digesta)	maltase ($\times 10^2$ U/g protein)	sucrase ($\times 10^2$ U/g protein)	maltase ($\times 10^2$ U/g protein)	sucrase ($\times 10^2$ U/g protein)	maltase ($\times 10^2$ U/g protein)	sucrase ($\times 10^2$ U/g protein)
BBR	19.65 $\pm$ 32.78	19.12 $\pm$ 10.49	3.80 $\pm$ 2.11	7.44 $\pm$ 7.40	11.87 $\pm$ 19.45	1.72 $\pm$ 1.28	3.34 $\pm$ 5.35	1.72 $\pm$ 1.28	3.34 $\pm$ 5.35	0.54 $\pm$ 0.32	0.15 $\pm$ 0.05	0.15 $\pm$ 0.05	0.15 $\pm$ 0.05
BB100	18.32 $\pm$ 16.97	17.19 $\pm$ 9.38	7.57 $\pm$ 3.15	20.07 $\pm$ 6.89	2.07 $\pm$ 0.42	1.72 $\pm$ 1.08	3.40 $\pm$ 3.18	1.72 $\pm$ 1.08	3.40 $\pm$ 3.18	0.45 $\pm$ 0.17	0.18 $\pm$ 0.10	0.18 $\pm$ 0.10	0.18 $\pm$ 0.10
BB140	23.04 $\pm$ 23.33	15.21 $\pm$ 8.84	8.87 $\pm$ 8.22	26.18 $\pm$ 1.32	1.79 $\pm$ 0.90	3.86 $\pm$ 2.15	6.44 $\pm$ 7.77	3.86 $\pm$ 2.15	6.44 $\pm$ 7.77	0.64 $\pm$ 0.37	0.15 $\pm$ 0.09	0.15 $\pm$ 0.09	0.15 $\pm$ 0.09
<i>p</i> -contrast values													
Unextruded	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
vs extruded													
BB100	NS	NS	NS	NS	NS	NS	NS	0.003	NS	NS	NS	NS	NS
BB140													
PPR	18.08 $\pm$ 13.36	16.96 $\pm$ 10.79	13.82 $\pm$ 4.96	15.63 $\pm$ 21.71	10.38 $\pm$ 6.53	2.02 $\pm$ 1.38	3.52 $\pm$ 2.86	2.02 $\pm$ 1.38	3.52 $\pm$ 2.86	0.46 $\pm$ 0.33	0.11 $\pm$ 0.09	0.11 $\pm$ 0.09	0.11 $\pm$ 0.09
PP100	14.61 $\pm$ 8.15	35.50 $\pm$ 22.05	18.35 $\pm$ 8.70	26.48 $\pm$ 8.41	14.15 $\pm$ 14.25	2.93 $\pm$ 2.29	6.89 $\pm$ 6.08	2.93 $\pm$ 2.29	6.89 $\pm$ 6.08	0.65 $\pm$ 0.19	0.10 $\pm$ 0.08	0.10 $\pm$ 0.08	0.10 $\pm$ 0.08
PP140	27.31 $\pm$ 43.84	20.03 $\pm$ 17.27	11.89 $\pm$ 6.58	19.41 $\pm$ 9.75	7.46 $\pm$ 10.42	2.57 $\pm$ 1.68	4.10 $\pm$ 4.66	2.57 $\pm$ 1.68	4.10 $\pm$ 4.66	0.51 $\pm$ 0.32	0.15 $\pm$ 0.09	0.15 $\pm$ 0.09	0.15 $\pm$ 0.09
<i>p</i> contrast values -													
Unextruded	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
vs extruded													
PP100	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
PP140													

¹BBR - unextruded bambara groundnut (*Vigna subterranean*); BB100 - bambara groundnut (*Vigna subterranean*) extruded at 100 °C; BB140 - bambara groundnut (*Vigna subterranean*) extruded at 140 °C; PPR - unextruded pigeon pea (*Cajanus cajan*); PP100 - pigeon pea (*Cajanus cajan*) extruded at 100 °C; PP140 - pigeon pea (*Cajanus cajan*) extruded at 140 °C

The effects of extrusion and extrusion temperatures on residual starch in the jejunal and ileal digesta and excreta of meat-type chickens fed the experimental diets are illustrated in Figure 4. No effect of extrusion cooking or extrusion temperatures was observed on residual starch content of the jejunal and ileal digesta, and excreta of meat-type chickens fed the bambara groundnut based diets. However a significant effect of extrusion temperature was observed on the residual starch content of the ileal digesta and excreta of chickens fed the pigeon pea diets, with significantly higher residual starch recorded in chickens on the PP100 diets compared to the PP140 diets, and significantly lower residual starch recorded in chickens on the PP100 diets compared to the PP140 diets. Extrusion cooking of ingredients containing starch is accompanied by significant starch gelatinization, melting and fragmentation. The disruption of the granular structure of starch achieved during the extrusion cooking process increases substrate availability to enzymes and starch digestibility (Abd ElKhalek and Janassens, 2010). In the current study, the measure of residual starch in the jejunal and ileal digesta, and excreta was used to evaluate the extent of starch digestion in response to extrusion of the grain legumes. In contrast to our findings in this study, inclusion of 140°C extrudates of peas at 250g/kg of the diet of meat-type chickens improved apparent ileal digestibility of starch without any improvement in the apparent metabolizable energy of the diets (Nalle *et al.*, 2011). Similar studies investigating faba beans extrudates at 300g/kg of the diet of meat-type chickens also improved apparent ileal digestibility of starch as well as nitrogen-corrected apparent metabolizable energy of the diets (Hejdysz *et al.*, 2019). The absence of an improvement in starch digestibility of

extruded grain legumes compared to the unextruded forms as observed in the current study could be attributed to the formation of amylose-lipid complexes or maillard reaction products which are able to impair starch digestibility (Bhatnagar and Hanna, 1994; Pellegrini *et al.*, 2020).

The effects of extrusion and extrusion temperatures on the small intestinal microstructure of meat-type chickens fed the experimental diets are summarised in Table 5. The crypt depth and thickness of the *muscularis* in the jejunum and ileum of meat-type chickens fed the pigeon pea diets increased significantly with extrusion cooking and under higher extrusion temperatures, while the villus height: crypt depth ratio decreased under similar conditions. While no effect of extrusion cooking (unextruded *vs* extruded) was observed in the histological structure of the jejunum of meat-type chickens fed the bambara groundnut diets, a decrease in the crypt depth and thickness of the *muscularis*, and increase in villus height: crypt depth in response to extrusion of bambara groundnut was observed in the ileum of meat-type chickens. Extrusion at a higher temperature also significantly decreased thickness of the *muscularis* of the jejunum and increased villus height and villus height: crypt depth in the ileum of meat-type chickens fed the bambara diet. The villus height, crypt depth and thickness of the *muscularis* are often used as indices of intestinal health. While an increase in villus height interprets as an increase in surface area which supports a higher capacity for absorption of digested nutrients, an increase in crypt depth is suggestive of an increase in tissue turnover which is required for villi renewal (Ma and Guo, 2008). Previous studies have shown seemingly inconsistent reports on the effect of extrusion cooking on the small intestinal microstructure. While inclusion of extruded coloured flower-pea at 150-300g/kg of meat-type chicken diets had no

Figure 4. Effect of extrusion and extrusion temperatures on residual starch of the jejunal and ileal digesta and excreta of meat-type chickens fed A. Bambara groundnut-based diets from 8-35d, or B. Pigeon pea-based diets from 8-35d

Table 5 : Effect of extrusion and extrusion temperatures on the small intestinal microstructure of meat-type chickens fed unextruded or extruded Bambara groundnut and pigeon pea-based diets from 8-35d

Treatments	Jejunum							Ileum						
	Villus height (µm)	Crypt depth (µm)	thickness of the muscularis(µm)	Villus height: crypt depth	Villus height (µm)	Crypt depth (µm)	thickness of the muscularis (µm)	Villus height: crypt depth	Villus height (µm)	Crypt depth (µm)	thickness of the muscularis (µm)	Villus height: crypt depth	Villus height (µm)	Crypt depth (µm)
BBR	1526.41 ± 143.10	216.22 ± 24.62	285.35 ± 60.84	7.36 ± 0.48	1113.50 ± 243.35	252.14 ± 20.21	293.37 ± 4.62	4.46 ± 1.12						
BB100	1805.11 ± 258.16	233.57 ± 29.70	352.19 ± 26.66	7.78 ± 1.06	1072.89 ± 146.88	146.22 ± 22.51	167.27 ± 21.60	7.43 ± 1.25						
BB140	1706.37 ± 136.51	258.59 ± 37.91	276.95 ± 54.78	6.74 ± 1.32	1646.51 ± 319.28	124.51 ± 43.96	143.90 ± 22.59	14.58 ± 5.47						
<i>p</i> -contrast values														
Unextruded vs extruded	NS	NS	NS	NS	NS	< 0.001	< 0.001	< 0.001						
BB100 vs BB140	NS	NS	0.005	NS	0.001	NS	NS	< 0.001						
PPR	1075.85 ± 324.69	107.52 ± 22.14	126.04 ± 20.87	10.39 ± 3.61	1447.03 ± 155.70	159.99 ± 17.81	190.54 ± 18.66	9.18 ± 1.73						
PP100	849.67 ± 62.90	118.04 ± 32.78	181.94 ± 9.94	7.48 ± 1.34	1672.02 ± 219.58	279.87 ± 51.29	257.68 ± 7.42	6.26 ± 1.96						
PP140	1813.07 ± 96.50	244.33 ± 53.16	186.32 ± 30.22	7.65 ± 1.37	1354.68 ± 348.87	179.02 ± 24.19	383.44 ± 53.39	7.74 ± 2.47						
<i>p</i> -contrast values														
Unextruded vs extruded	0.03	< 0.001	0.011	0.011	NS	< 0.001	< 0.001	NS						
PP100 vs PP140	< 0.001	< 0.001	< 0.001	< 0.001	NS	< 0.001	< 0.001	< 0.001						

¹BBR - unextruded Bambara groundnut (*Vigna subterranean*); BB100 - Bambara groundnut (*Vigna subterranean*) extruded at 100 °C; BB140 - Bambara groundnut (*Vigna subterranean*) extruded at 140°C; PPR - unextruded Pigeon pea (*Cajanus cajan*); PP100 - Pigeon pea (*Cajanus cajan*) extruded at 100°C; PP140 - Pigeon pea (*Cajanus cajan*) extruded at 140°C

effect on jejunal microstructure, inclusion of extruded full-fat soyabean at 40-120g/kg diet significantly decreases villus height and crypt depth (Foltyn *et al.*, 2013).

Conclusion

The extrusion of bambara groundnut (*Vigna subterranean*) and pigeon pea (*Cajanus cajan*) at either 100°C or 140°C had varied effects in meat-type chickens. Extrusion cooking of the grain legumes at 100°C or 140°C decreased feed intake throughout the study without negatively impacting on body weight gain at the end of the 35d period, whilst improving feed conversion. Extrusion cooking and extrusion temperatures also had a null effect on development of the gastrointestinal tract; gizzard and small intestine, digestive accessory organs; pancreas and liver, as well as enzyme secretory functions of the pancreas and starch utilization. However the small intestinal microstructure was influenced by extrusion cooking and extrusion temperature, especially in meat-type chickens fed the extruded pigeon pea diet.

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