Effect of unconventional feeds on production cost, growth performance and expression of quantitative genes in growing pigs

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ABSTRACT

This study examined the effects of unconventional feed on performance characteristics and candidate gene expression in growing pigs of 3-5months of age. A Total of forty-five (45) growing pigs were randomly allotted into five treatments consisted of whole maize (T1), brewer's dried grain (T2), cassava peel meal (T3), plantain peel meal (T4), and corn husk meal (T5), at 35% inclusion rate. Performance data were collected for 12weeks, while duodenum and liver samples were collected after the experiment for gene expression analysis. Data were analyzed in a completely randomized design. Diets significantly (p<0.05) affected growth performance with T2 showing the highest average of daily weight gain at 0.21±0.02; while T5 had the least value. Feed efficiency was highest for T1 and T2 (0.10±0.02) and lowest was T5 (0.07±0.17). Cost-benefit analysis were significantly different (p<0.05) with T4 showing the highest gross margin. Insulin-like growth factor1 and leptin gene were differentially expressed in tissues, while no significant differences (p>0.05) existed for growth hormone gene and ryanodine receptor1 gene. The results showed commendable gross margin, however, cognizance should be taken in the quantity of brewer's dried grain fed to pigs since it increased the expression of ryanodine receptor 1 gene that leads to porcine malignant hypertermia and pale soft exudates pork.
Keywords: Gene expression, Growth Performance, Gross margin, Unconventional feeds, Pigs

INTRODUCTION

The cost of feed has been the biggest variable in pig farming and most systems of livestock farming. Feed efficiency has been the most determinant factor in examining the profit in pork production enterprise and this can be calculated using feed gain ratio (Onteru *et al.*, 2013). Nonconventional feed resources are feeds that are not been used in the preparation of livestock rations and as a result, are not contained in commercial diets produced by feed production companies, and they include but not limited to; spent brewers grain, cassava peel, plantain peels, corn

husk, and sugar cane bargasse (Amata, 2014). The inclusion of non-conventional feed resources in livestock rations with suitable and complete feed technology that can utilize the feed sources with maximum efficiency will assist in reducing production cost and controlling agro-waste products in our locality. The cost of conventional feed ingredients such as maize, wheat, cassava, and sweet potato flour has forced most pig farmers to feed pigs with mainly agro-industrial by-products (Amafule *et al.*, 2009; Nnadi *et al.*, 2010). These farmers use the waste without taking into consideration its effect on production cost, nutrient utilization and pork quality.

Cassava peel meal has been posited to record a better feed cost/kg of weight gain for growing pigs compared to maize-based diet (Adesehinwa *et al.*, 2008; Ekwe *et al.*, 2011). Conversely, an increase in dietary energy content in the diet of growing pigs has also been identified to improve fat accumulation in pigs, which could be attributable to the direct deposition of fat in the adipocyte (Yang *et al.*, 2012). The fat deposition in turn affects the lean meat percentage which is a major consideration for pork quality and profit margin in marketing situation.

Genes associated with feed consumption and its related parameters such as weight gain and fat deposition have been reported by Ciobanu *et al.* (2004); Faria *et al.* (2006); Biziene *et al.* (2011) and Onteru *et al.* (2013). The associations between growth hormone polymorphism and economic traits in pigs have been identified as the candidate in the investigation of phenotypic variance among polygenic characters in the pigs (Faria *et al.*, 2006).

Reports have shown that nutritional factors can relate to some regulatory networks such as tissue-specific developmental and hormonal factors as well as dietary energy in the control of gene expression (Park *et al.*, 2012). Different fat types have been reported to have no significant effect on chemical composition and meat quality as well as growth performance in growing-finishing pigs. Dietary fat types have also been shown to affect fatty-acid composition and insulin signaling-related gene expression in pigs (Park *et al.*, 2012). Other studies indicated that energy requirements of pigs are affected by differences in age, sex, genotype, physiological state, environment and feeding strategy (Kil *et al.*, 2013).

The chief goal of genomics experiments in pigs are centered on genes regulating economic traits, and some candidate genes associated with these traits such as leptin gene, Insulin-Like Growth Factor1, Growth Hormone gene, Rynaodine Receptor1 gene, myostatin gene, and melanocortin etc. These genes are sometimes expressed as a result of hormonal concentration and fat tissue deposition (Kolodziej *et al.*, 2009).

Previous studies on the use of unconventional feed ingredients as whole or partial replacement for maize and other grains have focused on weight gains and feed conversion ratio without interrogating quantitative genes that are associated with growth and pork quality in pigs. Hence, the major candidate genes in pigs needed to be studied for researchers and pork producers to understand the relationship that exists between how genes are expressed in response to change in feed efficiency, feed conversion ratio and average daily gain as they directly impacts intramuscular fat and back fat thickness (Yin *et al.*, 2009; Park *et al.*, 2012).

This study was therefore designed to evaluate the cost of production, growth performance, andexpression of candidate genes in metabolic tissues of growing pigs.

MATERIALS AND METHODS

The procedures for this experiment was considered and approved by the Board of studies; Department of Animal Science and Faculty of Agriculture, Delta State University, Asaba Campus

Experimental Site

The experiment was conducted at the Teaching and Research farm of the Department of Animal Science, Delta State University, Asaba Campus, Asaba. Asaba is located in Southern

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Nigeria. The annual rainfall in Asaba ranges from 1800 mm to 3000 mm while its temperature ranges from 27.50°C-30°C. The area experiences two seasons in a year, the dry and rainy seasons. The rainy season starts in April and ends in October while the dry season commences from November and terminates in March (NMET, 2010). The rainfall and sunshine in this area are evenly distributed.

Experimental Design, Animals and Management

A total of forty-five (45) growing commercial pigs of Duroc and Large white crosses within ages 3-5 months with body weight range of 8-14 kg were used for this study. Feed and water were provided *ad libitum* throughout the duration of the experiment. Maize, fresh cassava peels, plantain peels and corn husk were sourced locally from farmers, sundried and milled, while brewers 'spent grain was collected from a brewery and sun-dried. The five treatments consisted of whole maize (T1), brewer's dried grain (T2), cassava peel meal (T3), plantain peel meal (T4), and corn husk meal (T5), at 35% (Table 1). The 45 growing pigs were randomly allotted to five dietary treatments at nine (9) animals per treat-

ment. The experiment lasted for 12 weeks (84 days). Proximate compositions of test ingredients were obtained using method of AOAC (2010).

Data Collection and Handling

The data collected were daily feed intake and body weight weekly. Feed intake and body weight were measured with the aid of weighing scale to the nearest gram. At the end of this exercise, three animals were randomly selected from each treatment for gene expression. The quantity of the feed provided daily and the residue of the previous day's feed was weighed to determine the daily feed intake. To monitor the growth pattern of the animals in response to the experimental treatments, the animals' weight was taken once a week before the morning feeding. Weight gain was calculated as the final live weight minus the initial live weight. Average daily weight gain (ADG) was obtained by adding together the weight of pigs in the replicate, subtracting it from the previous week's value and dividing the result by the number of days in a week. Feed intake was determined by subtracting the quantity leftover from the quantity offered. Average daily feed intake (ADFI) was then computed thus: daily feed intake was divided by the number of pigs in

Table 1. Ingredients of experimental diets[*]

Ingredients	T1	T2	T3	T4	T5
Whole maize	35	0	0	0	0
BDG	0	35	0	0	0
CPM	0	0	35	0	0
PPM	0	0	0	35	0
CHM	0	0	0	0	35
PKC	25	25	25	25	25
Wheat offal	10	21	8	10	9
Fish meal (72%)	2	1	3	2	3
Bone meal	4	4	4	4	4
Salt	0.5	0.5	0.5	0.5	0.5
Premix	0.5	0.5	0.5	0.5	0.5
Soya bean meal	23	13	26	23	23
Calculated Composition					
Crude Protein%	20.34	20.47	19.86	20.72	19.29
NFE%	50.68	42.00	50.31	46.61	44.32
Crude Fibre%	7.72	13.81	9.79	10.19	16.99
Crude Fat%	8.35	9.02	7.65	8.00	8.02
Crude Ash%	4.14	5.45	4.06	5.71	4.30

CPM- cassava peal meal, PPM- plantain peel meal, BDG- brewers dried grain, CHM- corn husk meal, PKC- palm kernel cake.
[*]g/kg percentage inclusion and calculated composition.

the replicate. Feed conversion ratio (FCR) was calculated as feed intake that was divided by weight gain while feed efficiency was calculated as the inverse of feed conversion ratio.

Feed Cost/Gain

The market cost of the feed ingredients at the time of the experiment was used to compute the total cost of 100 kg of each of the five experimental diets. The cost per kg of each diet was calculated by multiplying the percentage composition of the feedstuffs with the price per kg and summing all. The total feed intake multiplied by cost per kg feed gave the feed cost. Feed cost per kg weight gain was calculated as FCR x cost per kg of diet.

Sample Harvest

Three animals from each treatment group were euthanized after 12 weeks of feeding trial. The liver and duodenum samples were collected from three animals from each treatment and stored in RNALater to examine expression of genes influencing growth and pork quality in pigs. The genes analyzed were the growth hormone gene (GH) receptor region, porcine ryanodine receptor 1 (RYR1), insulin-like growth factor-1 (IGF-1), and leptin Gene.

RNA Concentration, Reverse Transcription and Real-time Polymerase chain reaction

RNA extraction, Reverse Transcriptionand Real-time Polymerase chain reaction were done according to Sorhue et al. (2021).The mRNA expression level of Leptin gene, Growth Hormone Gene (GH), Porcine Ryanodine Receptor 1 (RYR1), Insulin-Like Growth Factor1 (IGF-1), were measured using a 7500 Real-Time PCR System, gene expression was calculated using the Delta-Delta CT method as reported bySorhue et al. (2021).

Primers Used

Table 2 shows the genes, sequences (forward and reverse), product length and gene bank accession numbers of the primers used for the study.

Statistical Analysis

All data collected were subjected to analysis of variance (ANOVA) in a completely randomized design using the R-Statistical package. Prism 7.0 software (Graphpad Software Inc, San Diego, California) was used in generating the figure. Comparisons between expression patterns among tissues were analyzed with paired t-test using the R Statistical Package.

RESULTS AND DISCUSSION

Table 3 shows the proximate composition of the test ingredients. The percentage crude protein was highest for BDG (21.19%) compared to the least value of 3.96% for CHM. Crude fat was highest for BDG (4.94%) while the lowest value of 1.07% was recorded for CPM. The crude fiber content of test ingredients was highest for CHM (28.75%) and lowest for WM (2.27%). Total ash content was highest for BDG (6.96%) and lowest for WM (2.22%) while the results for Nitrogen free extract revealed that BDG recorded the least, with value of 36.94% and CPM recording the highest (73.03%). The proximate composition of whole maize in this study compares favorably with previous reports (David et al., 2016).The crude protein content of BDG recorded in this study is less than 22.5%; 22.45% and 22.49 from previous studies (Amaefule et al., 2006a; Amaefule et al., 2006b; Amaefule et al., 2009). The crude fat was lower than 6.25%, 6.15% and 6.25% as reported by Amaefule et al. (2006a); Amaefule et al. (2006b); Amaefule et al. (2009). respectively. The crude fiber was also lower than 21%, 16.25% and 21% reported earlier (Amaefule et al., 2006a; Amaefule et al., 2006b; Amaefule et al., 2009). The total ash percentage of 6.96% was higher than 4.70%, 4.30%, and 4.70% according to reports of Amaefule et al. (2006a); Amaefule et al., (2006b; Amaefule et al., 2009). However, the NFE content of 36.94% reported in this study was higher than 35.1%, 35.06 and 35.06% reported by Amaefule et al. (2006a); Amaefule et al. (2006b); Amaefule et al. (2009). The CP content of 2.54% for CPM in this study is similar to 2.54% (Abu et al., 2015),

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Table 2. Used primers

Genes		Sequence(5'->3') Forward and Reverse Primers	Product length	Gene bank Accession no
GH	FP	GATGCGGGAGCTGGAGGATGGC	193	NM213869.1
	RP	GCTCTCCACGAAGCGGCGAC		
IGF1	FP	GATGCGGGAGCTGGAGGATGGC	152	NM213883.2
	RP	GCTCTCCACGAAGCGGCGAC		
GAPDH	FP	CGGGACATCAAGGAGAAGC	273	DQ845173
	RP	ACAGCACCGTGTTGGCGTAGAG		
Leptin	FP	TGTTCGCTGTGGCATCCGTGGT	150	NM213840.1
	RP	CACGGCGGCATTTATCACCGGCT		
RYR1	FP	CGCAGCAACCGGGACAGCCT	296	001001534.1
	RP	CGCTGACCCCGGTGCCCATT		

GH-Growth Hormone Gene, IGF1-Insulin-like Growth Factor1, GAPDH- House keeping gene, Leptin- Leptin gene, RYR1- Ryanodine Receptor1 gene, FP-Forward primer, RP-Reverse primer .

Table 3. Proximate composition of test ingredients

Parameter	WM	BDG	CPM	PPM	CHM
	--- % ---				
Moisture	11.88	12.85	12.17	12.46	9.69
Crude Protein	9.56	21.19	2.54	10.10	3.96
Crude Fat	3.06	4.98	1.07	2.06	2.11
Crude Fiber	2.27	17.08	8.18	9.32	28.75
Total Ash	2.22	6.96	3.01	6.69	2.66
NFE	71.01	36.94	73.03	59.37	52.83

WM-whole maize, BDG-brewer's dried grain, CPM-cassava peel meal, PPM-plantain peel meal, CHM-corn husk meal.

but less than 11.09% and 5.19% reported by Ajagbe *et al.* (2020); Ekpo *et al.* (2015). The crude fat content of 1.07% recorded for CPM in this study is higher than 0.70% reported by Abu *et al.*,(2015) and less than 3.25% and 5.02% reported by Ajagbe *et al.* (2020; Ekpo *et al.* (2015). The crude fiber content of 8.18% for CPM is lower than 9.03%, 10.62% and 19.72% in line with previous reports (Abu *et al.*, 2015; Ekpo *et al.*, 2015; Ajagbe *et al.*, 2020;). The total ash of 3.01% is similar to 3.17% reported by Abu *et al.* (2015) but lower than 5.77% and 7.26% reported by Ajagbe *et al.* (2020); Ekpo *et al.* (2015). The NFE content of 73.03% for CPM

in this study is similar to 74.83% reported by Abu *et al.* (2015), and far higher than 52.63%, and 62.81% recorded by Ajagbe *et al.* (2020); Ekpo *et al.*, 2015). The CP value of 10.10% for PPM is similar to 9.86% reported by Oluwatosin and Solomon (2017), but higher than 6.69% and 2.93% reported for ripe plantain peels and unripe plantain peels respectively (Adamu *et al.*, 2017). The value of 2.06% for crude fat recorded for PPM is lower than 7.74% and 6% reported by Oluwatosin and Solomon (2017); Adamu *et al.* (2017) respectively, but was however higher than 1.70% reported by Adamu *et al.* (2017).The crude fiber value of 9.23% for PPM is similar to

9% reported by Shadrach *et al.* (2020) but less than 18.75% and 11.25% reported for soaked ripe plantain peels (Oluwatosin and Solomon, 2017; Shadrach *et al.*, 2020). However, value of 9.32% for crude fibre of PPM, was higher than 3.71% and 2.53% reported by Adamu *et al.* (2017). Total ash value of 6.69% is less than 14.77%, 10.35% and 13.88% reported by Oluwatosin and Solomon (2017); Shadrach *et al.* (2020) but higher than 6.30% and 1% reported by Adamu *et al.* (2017). The NFE value of 59.37% obtained for PPM in the present study is higher than 40.98%, 42.94% and 45.53, 28.60% and 54.01% reported by Oluwatosin and Solomon (2017); Adamu *et al.* (2017); Shadrach *et al.* (2020). The crude Ash of 2.66% is less than 4.83%, the crude fat of 2.11% is less than 17.20%, the crude protein value of 3.97% obtained for CHM in this study is lower than 7%, the crude fiber of 28.75% is higher than 3.45% while the NFE values of 52.83% recorded for CHM is lesser than 60.37% according to previous reports (Madika *et al.*, 2017).

Table 4 shows the performance evaluation of growing pigs fed different unconventional feed sources. The result from the present study showed that the initial weight of the experimental animals were similar hence the values did not differ significantly (P>0.05) from each other, while significant differences (p<0.05) were observed for average final weight with the control treatment having the highest weight while treatment fed corn husk based diet recorded the least value. Highly significant differences (p<0.01)

were recorded for average total weight gain with T2 gaining more weight than T1 (control) while T5 gained the least weight. This can be attributable to the varying nutrient composition of the experimental diets among the treatment (Meyer and Brendemuhl, 2013). The differences observed in this study have also been reported for growing-finishing pigs (Park *et al.*, 2012) when different dietary fat types were fed to pigs. Earlier reports (Sullivan *et al.*, 2005; Stein *et al.*, 2009) showed that energy sources from different grains can also compete favorably with maize as obtained with brewers dried grain in comparison with maize in this study. The lowest weight gain on daily basis was recorded by treatment five (corn husk meal); this is expected due to the high fibre content and low palatability that possibly resulted in reduced feed intake (Amata, 2014; Selene *et al.*, 2018). The low body weight recorded by treatment 5 was evident on the low feed intake compared to the other treatments, with only the plantain peel (T2) sitting slightly closer to it. Report of Ekwe *et al.* (2011),suggests that feed intake was higher in dried cassava peel because the animals tend to consume more feed compared to maize based diet in other to meet their metabolizable energy requirements which is not in agreement with the feed intake findings of this current study, as the animals consumed more of whole maize which contains more energy as compared to the unconventional and low dietary energy feeds used. The feed conversion ratio (FCR) among treatment groups were significantly different (p<0.05), with control treatment hav-

Table 4. Performance evaluation of growing pigs fed unconventional dietary feed sources

Parameters	Treatments				
	T1	T2	T3	T4	T5
IWT	8.88 ± 0.44^{ns}	8.01 ± 0.09^{ns}	9.45 ± 1.01^{ns}	8.50 ± 0.68^{ns}	7.92 ± 0.33^{ns}
FWT	26.06 ± 0.77^a	25.48 ± 1.65^a	23.72 ± 1.63^{ab}	20.50 ± 0.70^{bc}	17.07 ± 1.05^c
TWT	17.18 ± 0.33^a	17.42 ± 1.66^a	14.27 ± 1.52^{ab}	11.99 ± 0.46^{bc}	9.13 ± 0.96^c
DFI	2.03 ± 0.00^a	2.03 ± 0.00^a	1.92 ± 0.02^b	1.72 ± 0.02^c	1.57 ± 0.01^d
ADG	0.20 ± 0.01^a	0.21 ± 0.02^a	0.17 ± 0.02^{ab}	0.14 ± 0.01^{bc}	0.11 ± 0.01^c
FCR	9.91 ± 0.19^a	9.93 ± 0.87^a	11.5 ± 21.04^a	12.0 ± 40.42^{ab}	14.69 ± 2.48^b
FE	0.10 ± 0.02^a	0.10 ± 0.11^a	0.09 ± 0.19^{ab}	0.08 ± 0.03^{ab}	0.07 ± 0.17^{bc}

Values are means ±SE
Means within row with different superscript are significantly different (p< 0.05)
IWT: initial weight, FWT: final weight, TWT: total weight gain, DFI: average daily feed intake, ADG: average daily gain, FCR: feed conversion ratio, FE: feed efficiency.

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Table 5. Cost-benefit analysis of growing pigs fed unconventional feed sources

Parameters	Treatments				
	T1	T2	T3	T4	T5
Cost/kg Feed (₦)	124.64±0.18[a]	89.64±0.26[b]	89.64±0.24[b]	94.89±0.16[c]	89.64±0.20[b]
Total feed consumed (kg)	57.03±0.88[a]	48.02±0.58[bd]	53.76±0.58[ad]	56.70±0.58[a]	43.82±0.58[bc]
Cost of feed consumed (₦)	7208.21±0.58[a]	4304.51±1.15[b]	4819.05±0.57[c]	5380.20±0.58[d]	3927.02±0.58[e]
Cost/kg gain(₦)	411.73±0.58[a]	359.66±0.58[b]	344.22±0.58[c]	314.09±1.00[d]	439.04±0.58[e]
Cost diff./kg wt gain (₦)	0.00±0.00[a]	52.07±0.58[b]	67.51±0.58[c]	97.64±0.58[d]	-27.31±0.58[e]
Gross Margin (₦)	188.27±0.27[a]	240.34±0.58[b]	255.78±0.13[c]	285.91±0.21[d]	160.96±0.58[e]

Means within row with different superscript are significantly different (p< 0.05)
Cost/kg feed = cost per kg of experimental diet; Total feed consumed = Total feed consumed per pig; Cost of feed consumed = cost/kg feed x total feed consumed pig; Cost of feed/kg weight gain = cost/kg feed x feed conversion ratio; Cost differential/kg weight = cost/weight gain in treatment 1 – cost/kg weight/treatment; Gross margin = price/kg pork – total cost of producing 1 kg pork. ₦ = Naira. 1₦ =$365, ₦600/kg pork.

ing the highest and closely followed by T2. The findings recorded for feed conversion ratio in the present study contradicts the report of Joven *et al.* (2014) which stated that feed conversion ratio was not affected by diets types when replacing barley with higher levels of olive cake in the feed of finishing pigs. However, the study agrees with previous reports that different dietary fat sources affects average daily gain and feed conversion ratio (Spurlock *et al.*, 2000). Other reports shows no significant differences (p<0.05) when different mixtures of palm kernel meal and cassava based diets were fed to growing pigs in terms of feed conversion ratio and bodyweight gain but was not the same for feed intake (Purlock *et al.*, 2000), which is in agreement with this study; that dietary feed types significantly affectedfeed intake, while the report for FCR and body weight gain by (Fatufe *et al.*, 2007) does not agree with this findings. Average daily feed intake was lowest for T5 and was highest for T1 and T2 with highly significant differences (p<0.01), which is not in agreement with earlier reports that low dietary protein and energy diet did not significantly affect feed intake and average body weight gain of growing-finishing pigs (Hong *et al.*, 2016). Feed intake were not significantly affected by dietary treatment in broiler chickens fed cassava peel based diet (Adesehinwa *et al.*, 2008), which is also not in agreement with the present study, as feed intake was significantly different across the dietary treatments and could be attributed to differences in the species of livestock used. More so, reports that average daily energy intake increased linearly with dietary energy concentrations, which in turn

affects average daily weight gain (Amafule *et al.*, 2009) agrees with the reports in the present study. Animals that consume diets containing higher energy contents tend to gain more weight in our study. The faster growth rate as a result of higher dietary energy intake has been reported to increase fat deposition in Langtang pigs which is definitely caused through direct deposition of fat in the adipocyte (Amafule *et al.*, 2009).

The result of feed efficiency in the present study is in line with the reports of (John *et al.*, 2015) that feed efficiency is expressed on dietary energy intakes; treatments with higher energy content tend to record higher feed efficiency which is feed consumed to growth achieved in this present study. Higher inclusion of peels from plantain and banana has been reported to negatively affect feed efficiency as reported by Abel *et al.* (2015) which was also observed in the present study with plantain peel based diet, which is higher than corn husk meal for feed efficiency values. The low feed intake of plantain peel based diet can be attributable to the presence of some anti-nutritional factors present in the ingredients as well as low palatability of the feed when compared to maize and brewer's dried grain. The least performance in terms of average final weight, average total weight gain, average daily weight gain and feed efficiency was recorded for corn husk based diet which could be attributable to the poor feed intake of experimental animals receiving the diets. While the corn-husk based diet performed poorly, it could help in reducing the feeding cost for producers as well as reducing wastage in farms thereby encouraging

sustainable agriculture (Noblet and Goff, 2001; Bindelle *et al.*, 2008;Selene *et al.*, 2018).

Table 5 shows that the cost/kg of feed were significantly different (P<0.05) across treatments with T1 recording the highest value, while T2, T3, and T5 recorded the least with same value of 89.64. Total feed consumed were significantly different (P<0.05) between treatments with T1 recording the highest feed intake, while the least feed intake value was recorded for T5. Cost of feed consumed was significantly different across treatments (P<0.05), with T1 commanding the highest feed cost of 7208.21 as against 3927.02 for T5. However, cost of feed/ kg of weight gain was highest for T5, the lowest cost/kg weight gain was recorded for T4 and the values were significantly different across treatments (P<0.05). The cost difference between T1 and the other treatments were also significantly different (P<0.05) with T4 giving the best performance of 97.64 while T5 showing the lowest with a negative value of -27.31. The gross margin obtained showed that T4 is more profitable than T1 and other treatment groups while T5 recorded the least gross margin and the differences between the treatments were significantly different (P<0.05). The cost per kg of feed in this study is higher than reports when graded levels of cassava based diet were fed to pigs (Ekpo *et al.*, 2015) but were lower in other reports when cassava plant meals were used in pig feeding (Adeyemi and Akinfala, 2019). The cost differentials reported in this study were however lower than the reports of ((Ekpo *et al.*, 2015) while the gross margin in this study is greater than reports from Ekpo *et al.* (2015) while the cost per kg weight gain is lesser than values reported by Adeyemi and Akinfala (2019) but comparable to Ekpo *et al.* (2015). Alternative ingredients to produce high fiber diets is a way of reducing feeding cost while obtaining appreciable weight gain in pig production.This study agrees with reports from Adeyemi and Akinfala (2019) that there was notable decrease in the cost of production when cassava plant meal completely replaced maize in the diets of growing pigs as evident in this study with CPM having a gross mar-

gin of N255.78 compared to maize of N188.27. With exception of CHM, the other treatments (PPM, CPM and BDG) generally outperformed WM in terms of profit margin. The comparative advantage in of T3 and T4 in terms of feed efficiency in relation to T1 vis-a-vis cost of production agrees with reports from (Lammers *et al.*, 2007)that a lower cost diet with a poorer feed efficiency may have a more competitive feeding cost per Kilogram of weight gain. Since T1 was significantly higher than T2, T3, T4 in terms of cost of feed consumed and cost per kilogram of weight gain.

Differential Expression of candidate genes in the liver and duodenum of growing pigs

Figure 1 and Figure 2 shows the relative expression of the genes in the liver and duodenum respectively. Ryanodine receptor 1 and leptin were more expressed in T2 and T3 in both the liver and duodenum.All the genes studied were upregulated in both tissues. Figure3 shows the expression of the Growth Hormone gene in the tissues of growing pigs.The fold change revealed that the growth hormone gene is more abundant in the duodenum of pigs receiving T3 in a ratioof 1.04:1.00, 0.50, 0.19, and 0.05 as compared with T1, T4, T2 and T5. Growth hormone genewas expressed 20.8folds in T3 compared to T5, while T1 was expressed 20folds compared to T5. The result of fold change indicates relative expression of GH in the liver of growing pigsin the following order, 1.00:0.60:0.57:0.32:0.23 for T1:T3:T5:T4:T2. However, there were no significant differences (p>0.05) in the expression patterns of GH gene among treatments in the liver, duodenum as well as comparison between the two tissues. Reports of Biziene *et al.* (2011) showed that average daily gain is negatively correlated with serum growth hormone levels, the result of T1 in the present study agrees with the reports (Tanga *et al.*, 2005; Wang *et al.*, 2014), that the growth hormone gene promotes somatic growth in vertebrates by boosting average daily gain. More so, the result obtained for T3 contradicts earlier reports (Tanga *et al.*, 2005; Wang *et al.*, 2014), as the animals receiving T3 did not out
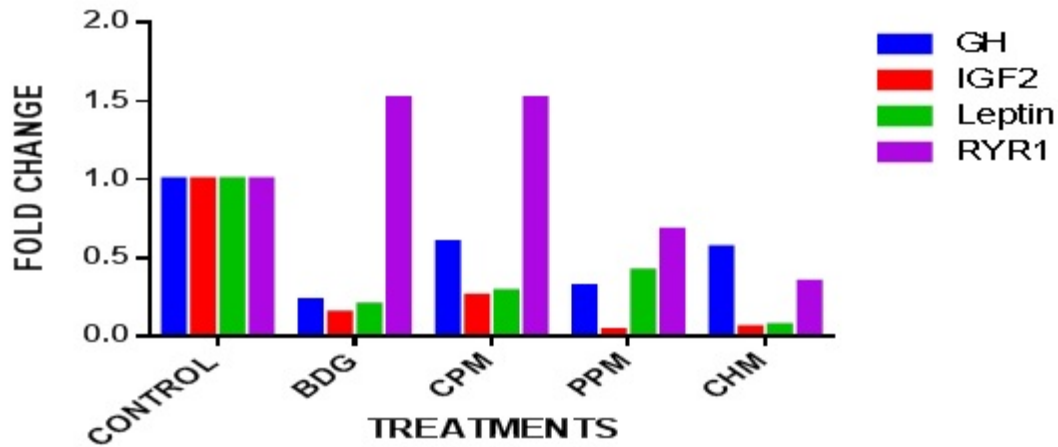
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Figure1. Gene expression in the liver of growing pigs fed different unconventional feeds. Control-whole maize, BDG-brewer's dried grain-, CPM-cassava peel meal, PPM-plantain peel meal, CHM- corn husk meal
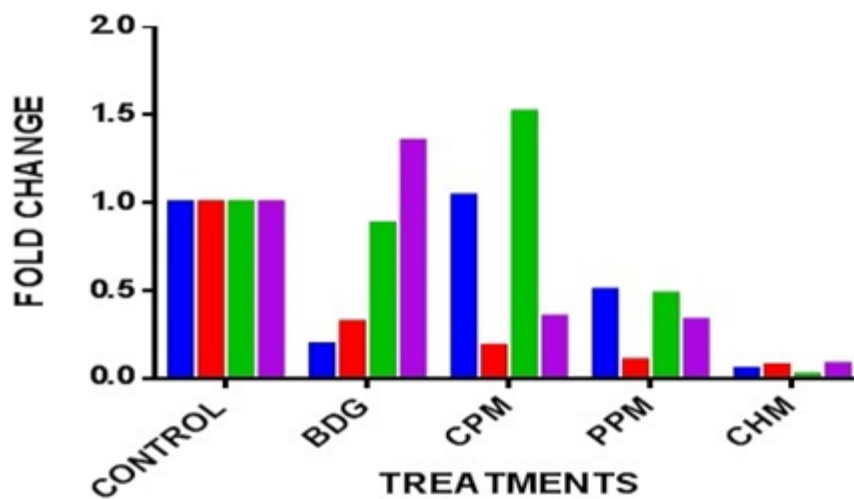

Figure 2. Gene expression in the Duodenum of growing pigs fed different unconventional feed. Control-whole maize, BDG-brewer's dried grain-, CPM-cassava peel meal, PPM-plantain peel meal, CHM- corn husk meal.

perform animals from treatments with low expression of the growth hormone gene as it relates to body weight gain. GH is a peptide hormone, and the insertion of the gene could improve weight gain, and other economic traits in the treatment with low body weight gain since earlier findings indicates that insertions of GH into growing pigs increased growth rate (Ju *et al.*, 2015).The growth hormone gene regulates growth, development and various metabolic activities in vertebrate animals which imply it affects all aspect of animal production including the physiological, morphological, metabolic, behavioral, reproductive, and immunological as

well as promoting skeletal system growth, and facilitates amino acid incorporation during protein synthesis (Wang *et al.*, 2014). While GH improved growth and FCR as reported in this study, over expression of the growth hormone gene may also lead to pathological challenges (Hausmana and Barba, 2008; Yang *et al.*, 2019). Figure 4 shows the expression of Insulin-Like Growth factor1 in tissues of growing pigs. The expression of the IGF1 gene in the duodenum was not significantly different among the treatment groups. The result of fold change shows that IGF1 gene is more expressed in the control treatment (T1) compared to the other dietary
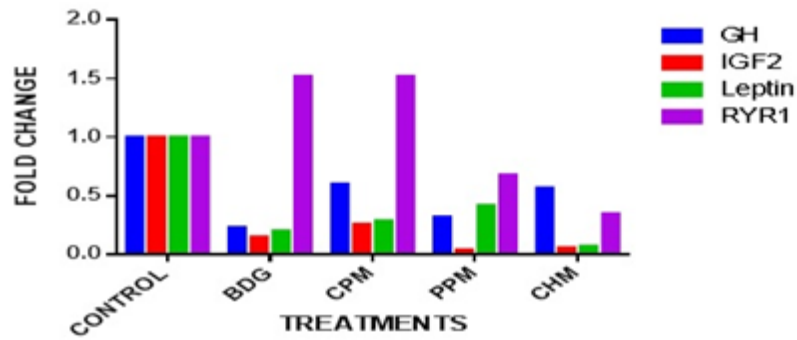
Figure1. Gene expression in the liver of growing pigs fed different unconventional feeds. Control-whole maize, BDG-brewer's dried grain-, CPM-cassava peel meal, PPM-plantain peel meal, CHM-corn husk meal.
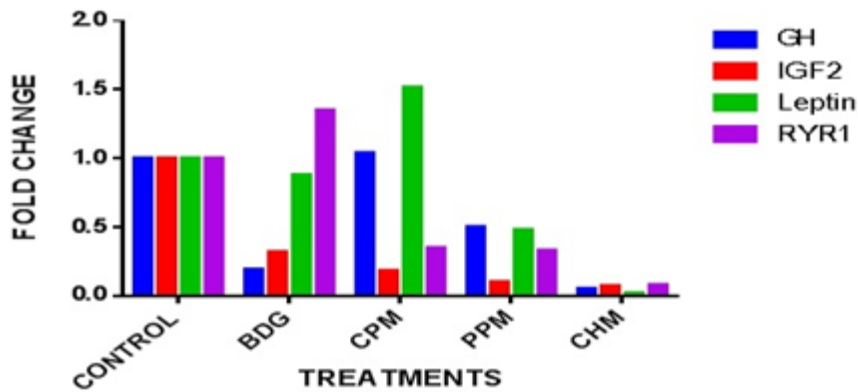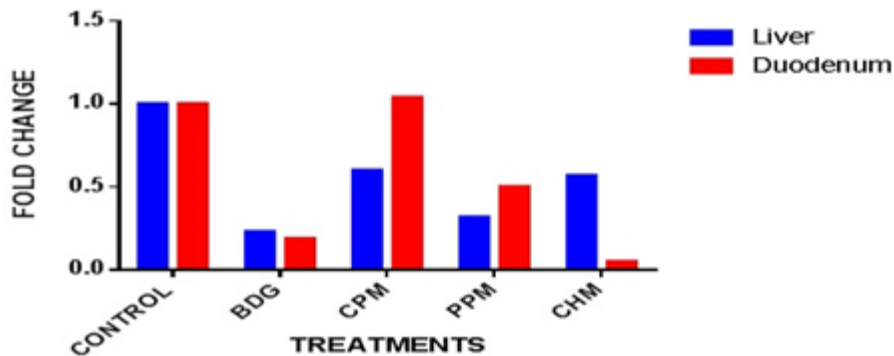

Figure 2. Gene expression in the Duodenum of growing pigs fed different unconventional feed. Control-whole maize, BDG-brewer's dried grain-, CPM-cassava peel meal, PPM-plantain peel meal, CHM- corn husk meal.

Figure 3. Growth Hormone Gene expression in the liver of growing pigs fed different unconventional feeds. Control-whole maize, BDG-brewer's dried grain-, CPM-cassava peel meal, PPM-plantain peel meal, CHM- corn husk meal

groups, with treatment five recording the least value. However, significant differences ($p < 0.05$) were observed in the expression patterns of IGF1 gene in the liver of growing pigs, as it was in the duodenum, while the least abundance of

the gene was recorded for T4 as opposed to T5 in the duodenum. Expression patterns in the duodenum and liver showed that there is highly significant difference ($p < 0.01$) in the expressions patterns of IGF2 in T1, T2 and T5 while in T3 and

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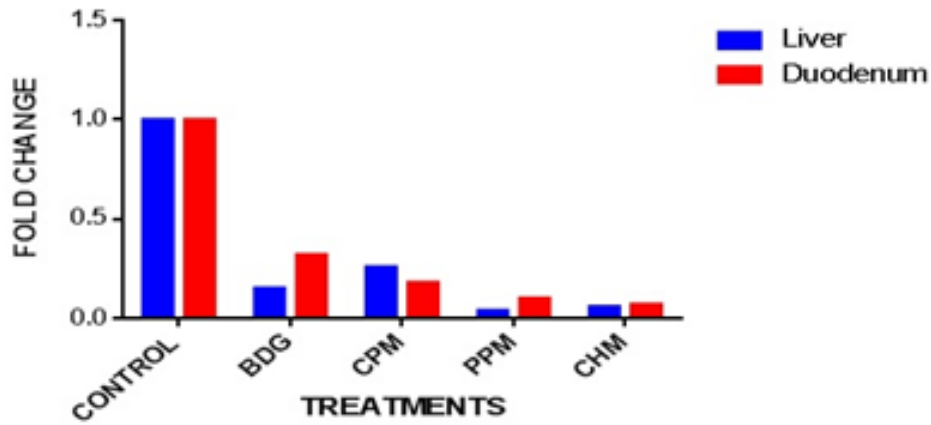*J. Indonesian Trop. Anim. Agric. 47(4):301-317, December 2022*

Figure 4. Insulin-Like Growth factor1 Gene expression in the liver of growing pigs fed different unconventional feeds. Control-whole maize, BDG-brewer's dried grain-, CPM-cassava peel meal, PPM-plantain peel meal, CHM- corn husk meal.
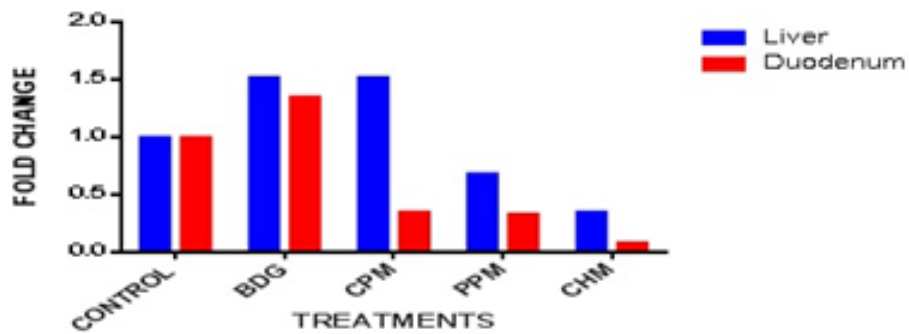

Figure 5. Ryanodine Receptor 1 Gene expression in the liver of growing pigs fed different unconventional feeds. Control-whole maize, BDG-brewer's dried grain-, CPM-cassava peel meal, PPM-plantain peel meal, CHM- corn husk meal.
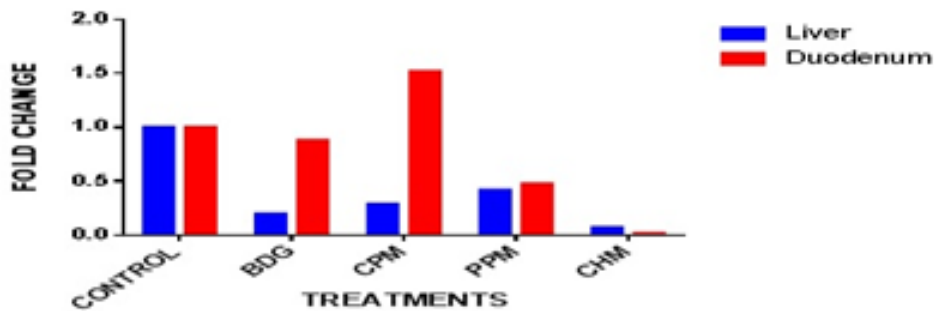

Figure 6. Leptin Gene expression in the liver of growing pigs fed different unconventional feeds. Control-whole maize, BDG-brewer's dried grain-, CPM-cassava peel meal, PPM-plantain peel meal, CHM- corn husk meal.

T4, the expression patterns of the IGF1 gene was shown to be non-significant ($p > 0.05$) between the liver and the duodenum. However, the mean expression values were inconsistent among the treatments as the mean values were not higher for a particular tissue across treatments. The expression analysis of the 1GF1 in the liver and duodenum in the present study is in tandem with earlier report of Wang *et al.* (2014) that IGF1 gene has complex biological functions and plays

an important role in animal growth. The expression of this gene favours body weight gain in the treatment groups studied. T1, T2 and T3 had the highest abundance of the target gene and were also best performers in terms of ADG and FCR, which corresponds with reports of Schmoelzl *et al.* (1996) and Biziene *et al.* (2011). This implies that insertion of the IGF1 gene in pigs may result in improved weight gain and other economically important traits. Figure5 below shows the expression of Ryanodine receptor1 gene in tissues of growing pigs. The expression patterns of RYR1 gene in the duodenum of growing pigs were not significantly different ($p>0.05$) among treatment groups. The RYR1 gene is more abundant in pigs fed BDG based diet as compared to maize, which is different from the other genes so reported in this present study. The same trend was followed by the expression pattern in the liver except for T3 where the abundance level increased compared to the duodenum. The expression pattern of RYR1 gene was consistently higher in the liver for all treatment groups compared to the duodenum, except for treatment two (T2), were expression in the duodenum was slightly higher than that of the liver. The differences observed were not significant ($p>0.05$) across treatments with mean difference ranging from -0.33 for T2 to 1.95 for T5. RYRI was expressed in 17 folds in T2 compared to T5, 1.35 folds in T2 compared to T1, and 3.8f olds in T2 compared to T3 and T4. The implication of this expression pattern is that while BDG tend to improve weight gain and feed efficiency in growing pigs, it triggers the abundance of RYR1 gene which is a candidate gene for pale soft exudates pork. The skeletal muscle isoform which is the RYR1 has been shown to be expressed predominantly in skeletal muscle; expression was also detected in the oesophagus and brain (Fujii *et al.*, 1991). It has also been observed that a single point mutation in the porcine gene for the skeletal muscle ryanodine receptor was found to be correlated with muscle hyperthermia in five major breeds of lean, heavily muscled swine (Jovanovic *et al.*, 2005). In porcine muscle hyperthermia, these studies identified the skeletal

muscle sarcoplasmic reticulum Ca2+ release channel gene as the site of the defect. The gene functions in the release of calcium from sarcosplasmicrecticulum to the cytoplasm and thereby plays a key role in triggering muscle contraction following depolinization of T-tubes. It also mediates the release of Ca^{2+} from intracellular stores in neurons, and may thereby promote prolonged Ca2+ signalling in the brain. RYR1 is required for normal embryonic development of muscle fibres and skeletal muscles. The RYR1 gene was reported to be the only factor causing porcine stress syndrome as revealed by expression studies (Martens, 1997). Previous reports posits that growth rate, fed conversion, and the ratio between meat and fat is often associated with porcine stress syndrome, which is a higher incidence of muscles diseases, and a reduced meat quality as pale soft exudates in pork (Paiao *et al.*, 2013). It has been suggested that one possible way of improving pork quality is by breeding out the porcine RYR1 mutation for the elimination of a major porcine stress syndrome, and pale soft exudates (Sera *et al.*, 2011; Georgescu *et al.*, 2014); this report conforms to the result of this present study, whereby the RYR1 gene is more abundant in treatments associated with high feed intake and higher body weight gain which may lead to simple accumulation of fat in the adipose tissues. It may lead to porcine stress syndrome and therefore produce pale soft exudates pork that will be poor in lean meat. Figure 6 shows the expression of leptin gene expression in the liver and duodenum of growing pigs. The relative expression patterns in fold change is shown with treatment 3 (cassava peel diet) showing a higher abundance of leptin gene in the duodenum, with fold change of 1, 0.20, 0.29, 0.42, and 0.07 while 1, 0.88, 1.52, 0.48 and 0.02 was recorded in the liver. While significant differences ($p<0.05$) were obtained in the expression patterns in the liver, nonsignificant difference ($p>0.05$) were recorded in the duodenum, and same trend ($p>0.05$) were followed when the two tissues were compared. Leptin was also more abundant in the liver than other tissues in earlier reports (Gaige *et al.*, 2003). This present study is also in tandem with

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reports by Gaige *et al.* (2003) and Barba *et al.* (2001), that the leptin gene reduces feed intake because animals with low abundance of leptin in the tissues studied tend to get the lowest feed intake as evident in pigs fed diet containing corn husk meal in T5, though the trend was not consistent between tissues. Treatment five recorded the lowest amount of leptin as well as the lowest amount of feed intake but it also recorded the least weight gain and final body weight gain. Treatment five was also observed to have recorded the best feed conversion ratio which could be used as a justification to the regulation of feed intake for optimum results. Although some initial report (Lopez *et al.*, 2007) stated that the leptin was first identified as the deficient product found in obese mouse; this implies that treatment animals with low abundance of leptin are prone to fat accumulation. The leptin gene (Brennan and Mantzros, 2006) is associated with dietary energy regulation and decreases carbohydrate absorption, but treatments two and treatments one with appreciable amount of leptin expressed in both the liver and duodenum tend to outperform treatment four and treatment five with the lowest abundance of the leptin gene in terms of average daily gain and daily feed intake. Leptin is not directly intended for reduced feed intake that will eventually lead to reduced fat, but rather to stimulate physiological adaptation to hunger (Ahima *et al.*, 1996). Although pigs fed diet containing fibre and low digestible energy reduced feed intake and body weight in this study, weaning stress could also reduce feed intake and growth rate (Xiong *et al.*, 2019). However, having gone through the same weaning conditions, the pigs therefore responded differently to the different treatment diets used in this study.

CONCLUSION

This study showed that pigs respond differently to unconventional dietary feed sources as expected, since they tend to contain varying nutrient composition. While corn husk meal impaired feed intake, growth and feed efficiency, it also impaired the expression of RYR1 gene

which is known to cause pale soft exudates pork. Plantain peel meal and cassava peel meal can be used in saving feeding cost for farmers due to the high gross margin recorded.

CONFLICT OF INTEREST

All the authors involved in this research work declared that they have no competing interest from conceptualization to the final writing of the manuscript.

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