DIETARY SUBSTITUTION OF MAIZE WITH KOLA POD HUSK MEAL ON GROWTH, HAEMATOLOGY AND SERUM PROFILE OF PIGS

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Abstract
The study aimed to assess the dietary effect of kola pod husk (KPH) meal on the growth, haematology and serum profile of weaner pigs. Forty weaner pigs of crossbreed and mixed sexes were randomly distributed to 5 dietary treatments in a completely randomized design of 8 pigs per treatment with a pig per replicate in a feeding trial that lasted for 84 days. Results showed a significantly ($p<0.05$) lower average weight gain (0.29-0.37 kg/pig/d) in pigs fed 20 - 40% KPH meal-based diets compared with the 0.46 kg/pig/d average weight gain in pigs fed 0 and 10% KPH meal-based diets. Feed conversion ratio (feed: gain ratio) was significantly ($p<0.001$) poorer (3.86-3.03) in pigs fed 20-40% KPH-based diets compared with those (2.45 and 2.46) on the control and 10% KPH meal. Total feed intake was similar ($p>0.05$) across the pigs fed the control and kola pod husk meal. Haematological parameters such as the red blood cell (RBC), mean corpuscular haemoglobin concentration (MCHC), haemoglobin concentration (HbC), lymphocytes, monocytes, and eosinophils of pigs were significantly ($p<0.05$) influenced at varying substitution levels of dietary KPH meal. Among the serum indices, only the blood urea and high-density lipoprotein were not significantly ($p>0.05$) influenced by dietary substitution of maize with KPH meal. Economics of production revealed a 10% KPH meal as the optimal and economic threshold of KPH meal substitution as an energy source for maize in pig diet. Thus, substitution of KPH meal for maize as an energy source in pigs' diet at a 10% level will help reduce the cost of pig production without posing any health challenges.

Keywords: cost, haematology, kola pod husk meal, pigs, serum profile

Introduction
The high cost of products such as meat, milk, eggs and other livestock products has been the bane of inadequate animal protein intake of the average citizen of the developing countries. Pathetically, an average Nigerian consumes less than 10 g of animal proteins per day (FAO, 2005), which is far below the minimum daily recommendation of 35 g (FAO, 2005). The resultant effect of this protein malnutrition is the high rate of infant morbidity and mortality (Agbede, 2004), low productivity, reduced growth and other challenges that impair healthy well-being. The identified cause of the low animal protein consumption is the high feed cost of intensively reared animals. Feed cost alone accounts for over 60% of the total production cost (Ogunsipe, 2017). Researchers have, in various studies, utilized agro waste such as cassava peel meal (Banjoko et al., 2008; Aderemi et al., 2012; Ogunsipe et al., 2015), palm kernel cake (Adesehinwa, 2007), Brewer's yeast (Sreeparvathy et al., 2012) among others, as alternative feed resources for the conventional feed ingredients in livestock diets to increase animal protein intake at an affordable cost. However, only a few results have

demonstrated the acceptability of these alternative energy resources as a partial substitute for maize in monogastric diets.

Another agro waste of importance is kola nut pod husk. Kola nut pod husk (KPH), a by-product resulting from removing the kola nut from the husk. World production of kola nut is estimated at 300,000 tons per annum, of which the average annual production in Nigeria is 162,500 tons of processed nuts, generating a yearly quantity of kola nut husk estimated at 90,000,000 kg (90,000 tons) (Brickell et al., 2002; FAO, 2002). Although this waste is seen as of no commercial and economic value in the livestock industry but presently, it is gaining attention because of its nutritive value. Nutritionally, kola nut pod husk contains appreciable levels of nutrients (crude protein 8-14.5%, crude fibre 7.5-17.3%) (Oluokun and Olalokun 1999; Adeyeye et al., 2021) and a substantial quantity of vitamins and minerals (Fabunmi et al., 2019). However, the nutritional value of kola nut pod husk is constrained by some anti-nutrients such as caffeine, theobromine, tannin, saponin, cardiac glycosides, among others (Benjamin et al., 2022). Thus, the present study aimed to assess the growth performance and haemato-biochemical profile of pigs fed a dietary substitution of maize with sun-dried kola pod husk meal.

Materials and Methods

Study site and chemical analysis

The study was conducted at the Aladenika Livestock Farm, located at KM 5, Ondo-Ore road. Freshly gathered kola nut pod husks were hosed down and sliced into tiny pieces. The sliced KPH were washed with clean water, sundried for 14 days with constant turning to ensure uniform drying. They were thereafter milled with a hammer mill to produce KPH meal. The KPH meal was analyzed for dry matter (DM) by oven drying method, ash content by muffle furnace, crude protein (CP) by Kjeldahl method, ether extract (EE) by Soxhlet fat analysis, crude fibre (CF) by acid and alkaline hydrolysis as described by AOAC (2002). The nitrogen free extract (NFE) was calculated by subtracting the values of other fractions from 100 (i.e NFE = 100-(DM + Ash + CP + EE + CF). Caffeine and theobromine determination were by Rade et al. (2008) and Bistol et al. (2002), respectively and gross energy (GE) was determined by the Gallenkamp Adiabatic Bomb Calorimeter (Model CBB-330-0104).

Feed preparation

Five experimental diets (Table 1) were formulated such that diet 1, which was the basal diet, contained 0% KPH meal while diets 2, 3, 4 and 5 had their maize substituted at 10, 20, 30 and 40% dietary KPH meal, respectively.

Table 1: *Gross composition of experimental diets.*

KPHM (%)	0	10	20	30	40
Diets	1	2	3	4	5
Ingredients (kg)					
Maize	54.50	49.05	43.60	38.15	32.70
Kola pod husk meal	-	5.45	10.90	16.35	21.80
Wheat offal	11.65	11.65	11.65	11.65	11.65
Soybean meal	13.10	13.10	13.10	13.10	13.10
Groundnut cake	8.30	8.30	8.30	8.30	8.30
Palm kernel cake	9.15	9.15	9.15	9.15	9.15
Bone meal	1.50	1.50	1.50	1.50	1.50
Oyster shell	0.50	0.50	0.50	0.50	0.50
Premix	0.50	0.50	0.50	0.50	0.50
Lysine	0.15	0.15	0.15	0.15	0.15
Methionine	0.15	0.15	0.15	0.15	0.15
Salt	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00
Nutrient composition of kola pod husk meal %					
Nutrients	**Kola nut pod husk meal**				
Dry matter	89.46				
Crude protein	8.21				
Crude fibre	21.02				
Ether extract	4.73				
Ash	8.55				
Nitrogen-free extract	46.95				
Gross energy (KJ/gDM)	1120.05				
Theobromine	3.15				
Caffeine	2.55				

Animal protocol and experimentation

The right to conduct the study was obtained from the Research Committee of the University, and the animals were certified clinically healthy for experimentation. Forty weaner pigs of crossbreed and mixed sexes were used for the feeding trial. The pigs were given a one-week adaptation period, during which they were fed a commercial grower diet and treated against parasitic infection by being given a 0.2 ml ivermectin injection intramuscularly. Thereafter, they were assigned to their respective diets in a completely randomized design of 5 treatments of 8 replicates per treatment, with one weaner pig per replicate. The pigs were fed at 5% body weight with water served ad libitum throughout the 84-day feeding trial.

Growth parameters

Data collected on daily feed intake were determined by subtracting the feed leftover from the feed given after removing every dirt. This is mathematically represented as:

$$\text{Feed Intake} = Feed\ Given - Leftover$$

Weight gain was calculated by subtracting the initial weight from the present weight as mathematically illustrated below:

$$\text{Weight gain} = Present\ weight - Initial\ weight$$

Feed conversion ratio was calculated using the following equation:

$$Feed\ conversion\ ratio = \frac{Feed\ intake}{Weight\ gain}$$

Blood evaluation

At the end of the feeding trial, 4 pigs per treatment, making a total of 20 pigs, were randomly sampled for blood collection. Blood samples were collected from the dorsal vein using a sterilized syringe and needle into two separate sterile bottles. Immediately after collection, the blood parameters were determined by Shenzhen Mind ray Auto Haematology Analyser, Model Bc-3200 (Shenzhen Mind ray Biochemical Electronics Co., Hamburg 20537, Germany) while the serum was analyzed for serum biochemical characteristics using commercial kits Reflectron® Plus 8C79 (Roche Diagnostic, GonbH Mahnheim, Germany).

Cost evaluation

The cost evaluation of pigs fed with KPH-based diets was determined using the following economic tools. The cost of feed was calculated based on the prevailing market price of the ingredients at the time of study.

$$\text{Feed cost ₦/kg} = \frac{\text{Total cost of feed compounded}}{\text{Total kg of the compounded feed}}$$

$$Cost\ of\ gain = Average\ weight\ gain\ x\ Feed\ cost\ \#/kg\ \text{gain}$$

$$\text{Feed cost ₦/kg gain} = \frac{\text{Feed cost }\#/\text{kg x Average feed intake}}{\text{Average weight gain}}$$

$$Saving\ cost\ \#/kg\ gain = \text{Feed cost ₦/kg gain of control diet} - Feed\ cost\ \#/kg\ \text{gain of the test diet}$$

$$\text{Cost reduction} = \frac{\text{Feed cost }\#/\text{kg of control diet} - \text{Feed cost }\#/\text{kg of test diet x Average feed intake}}{\text{Feed cost }\#/\text{kg of control diet}}\ x\ 100$$

$$\text{Relative cost benefit (RBC)} = \frac{\text{Feed cost }\#/\text{kg gain of control diet} - \text{Feed cost }\#/\text{kg gain of test diet}}{\text{Feed cost }\#/\text{kg gain of control diet}}\ x\ 100$$

Data collection and statistical analysis

Data collected on daily feed intake, weekly weight gain, feed: weight gain ratio, and blood and serum parameters were analyzed by one-way analysis of variance (ANOVA) using SPSS 2006. Where significant differences exist, the means were separated using Duncan's new Multiple Range Test (Duncan, 1955).

Results

Chemical composition

The results of the chemical profile of KPH meal used in the study, as presented in Table 1, show a crude protein of 8.21%, crude fat 4.73, crude fibre 21.02%, gross energy 1120.05 KJ/g, theobromine 3.15 % and caffeine 2.55 %

Performance indices

The weight gain as shown in Table 2 reveals a significant ($p<0.002$; 0.001) decrease in final live weight and average weight gain in pigs fed 20 - 40% KPH-based diets compared with those on the control and 10% KPH-based diets while feed: gain ratio decreased ($p<0.001$) progressively in pigs fed above 20% KPH-based diets compared with those on 0 and 10% KPH-based diets. However, total feed intake (93.84-96.21 kg/pig) and average feed intake (1.12-1.14 kg/pig/day) were similar ($p>0.05$) in pigs fed the control and KPH-based diets.

Table 2: *Performance and cost implications of pigs on varying levels of cocoa bean shell meal*

KPH meal (%)	0	10	20	30	40	SEM	Sig
Diets	1	2	3	4	5		
Performance characteristics							
Initial weight (kg)	9.36	9.39	9.43	9.28	9.41	0.28	0.37
Final live weight (kg/pig)	48.08^{a}	47.74^{a}	41.93^{ab}	35.08^{b}	33.62^{c}	3.41	0.002
Total weight gain (kg/pig)	38.72^{a}	38.35^{a}	32.57^{b}	25.82^{c}	24.21^{c}	2.18	0.001
Average weight gain (kg/pig/d)	0.46^{a}	0.46^{a}	0.37^{b}	0.31^{bc}	0.29^{c}	0.04	0.001
Total feed intake (kg/pig)	96.21	94.91	95.02	94.43	93.84	2.19	0.53
Average feed intake (kg/pig/d)	1.14	1.13	1.13	1.12	1.12	0.02	0.54
Feed: Gain	2.46^{a}	2.45^{a}	3.04^{b}	3.61^{c}	3.86^{d}	0.43	0.001
Cost analysis							
Feed cost ₦/kg	101.34^{a}	98.26^{b}	94.71^{c}	91.17^{d}	87.62^{e}	6.02	0.003
Cost of gain ₦	115.53	111.02	107.02	102.11	118.44	9.12	0.31
Feed cost ₦/kg gain	251.15^{c}	241.38^{c}	289.25^{b}	329.39^{a}	338.39^{a}	45.79	0.003
Saving cost ₦/kg gain	-	9.77	-38.10	-78.24	-87.24		
Cost reduction (%)	-	3.43	7.39	11.24	15.16		
Relative cost benefit (%)	-	3.89	-15.17	-31.15	-34.74		

^{abcde}Means with different superscripts along the same row are significantly different at $P<0.05$

Economic implications show a progressive decrease ($p<0.003$) in the feed cost ₦/kg as evidenced in the 3.43-15.16% cost reduction when KPH meal was substituted for maize in the diet. Although the cost of gain was similar ($p>0.05$) but the feed cost ₦/kg gain, which is a measure of the economic efficiency of feed utilization, was significantly ($p<0.003$) least (₦241.38) at 10% KPH meal substitution. This suggests the optimal and economic level of using KPH meal as an energy substitute for maize in the pig diet. The negative values obtained in the saving cost ₦/kg gain and relative cost benefit (%) of pigs fed 20-40% KPH-based diets implied additional cost incurred to raise a kg of pig weight.

Haematological indices

The erythrocyte sedimentation rate (ESR), PCV, MCH, MCV, neutrophils and basophils were not significant ($p>0.05$) in pigs fed the control and test diets. The RBC counts, MCHC, Hb, and lymphocytes were similar ($p>0.05$) in pigs fed 0 (control) and 10% KPH-based diets but significantly ($p<0.05$) decreased at 20-40% KPH-based diets. White blood cell counts, monocytes and eosinophils significantly ($p<0.002$-0.04) increased in pigs fed 20-40% of KPH meal (Table 3).

Table 3: *Haematological indices of pigs on varying levels of cocoa bean shell meal*

CBS (%)	0	10	20	30	40	SEM	Sig
Diets	1	2	3	4	5		
Haematological indices							
ESR (mm/hr)	3.90	4.03	4.47	4.13	4.00	0.19	0.34
PCV (%)	37.83	38.33	38.63	38.67	39.43	0.89	0.79
RBC (x 10^6 mm^3)	7.33^a	7.40^a	7.13^b	6.93^{bc}	6.80^c	0.13	0.04
MCH (pg)	18.37	17.97	18.97	18.57	18.48	0.18	0.37
MCHC (%)	35.61^a	34.70^{ab}	33.32^b	33.28^b	31.88^c	2.23	0.04
MCV (fl)	51.61	51.80	54.19	55.80	57.98	2.08	0.42
HbC (g/dl)	13.47^a	13.30^a	12.87^b	12.87^b	12.57^c	0.17	0.02
WBC (x 10^3/l)	7.80^c	8.67^c	12.20^b	13.33^{ab}	14.50^a	1.42	0.03
Differential count (%)							
Neu	39.63	40.66	40.73	40.60	40.43	0.70	0.41
Lym	52.70^a	51.10^{ab}	49.90^b	50.00^b	49.97^b	0.66	0.03
Mon	4.17^b	4.47^{ab}	4.57^{ab}	4.73^a	4.67^a	0.12	0.04
Eos	2.93^d	3.07^d	4.17^b	4.00^c	4.28^a	0.13	0.002
Bas	0.57	0.70	0.63	0.67	0.65	0.05	0.26

[abcd] Means with different superscripts along the same row are significantly different at $P<0.05$

Serum indices

Table 4 reveals that only the blood urea and high-density lipoprotein (HDL) of the experimental pigs were not significantly ($p>0.05$) influenced by dietary substitution of maize with KPH meal. The total serum protein (TSP), albumin and globulin concentrations decreased significantly ($p<0.05$) while the cholesterol, low density lipoprotein (LDL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) increased significantly ($p<0.05$) in pigs fed the test diets compared with those fed the control diet.

Table 4: Serum indices of pigs fed varying levels of cocoa bean shell meal

CBS (%)	0	10	20	30	40	SEM	Sig
Diets	1	2	3	4	5		
Serum indices							
Total serum protein (g/dl)	7.43^{a}	6.99^{b}	6.92^{b}	6.89^{b}	6.84^{b}	0.13	0.04
Albumin (g/dl)	4.11^{a}	3.80^{b}	3.89^{b}	3.84^{b}	3.85^{b}	0.06	0.02
Globulin (g/dl)	3.32^{a}	3.19^{ab}	3.03^{b}	3.05^{b}	2.99^{b}	0.10	0.03
Blood urea (mg/dl)	4.95	4.85	4.75	4.83	4.78	0.28	0.19
Cholesterol (mg/dl)	3.54^{c}	3.70^{bc}	3.89^{b}	4.00^{ab}	4.37^{a}	0.11	0.01
HDL (mmol/l)	1.13	1.00	1.00	1.10	1.00	0.04	0.98
LDL (mmol/l)	1.44^{d}	1.99^{c}	2.22^{b}	2.35^{ab}	2.48^{a}	0.07	0.001
AST (u/l)	9.17^{c}	10.43^{b}	11.04^{ab}	11.32^{ab}	12.09^{a}	0.32	0.004
ALT (u/l)	7.46^{c}	7.60^{bc}	7.93^{b}	8.50^{a}	8.61^{a}	0.32	0.02
ALP (u/l)	15.18^{d}	15.77^{c}	16.03^{c}	17.27^{b}	18.69^{a}	0.16	0.001
LDH (u/l)	10.83^{c}	11.07^{c}	12.54^{bc}	13.39^{b}	14.91^{a}	0.56	0.01

^{abcd}Means with different superscripts along the same row are significantly different at $P<0.05$

Discussion

The chemical compositions regarding CP (8.21%) and CF (21.02%) in this study were similar to the values reported by Oluokun and Olalokun (1999) and Adeyeye et al. (2021). Theobromine and caffeine contents of dried KPH meal (3.15 and 2.55 g/kg) used in this study were lower than the 5.22 g/kg and 3.51 g/kg kola pod husk meal (Adeyeye et al. 2021) but higher than the contents reported by Okoli et al. (2012) and Fabunmi et al. (2019). The differences might be due to the variety from which the pod was obtained or other environmental factors (Sánchez et al., 2023).

A decrease in weight gain of pigs observed at higher substitution with KPH meal in this study is agreeable with rabbits fed sun-dried kola pod husk meal (Eburu et al., 2020; Adeyeye et al., 2021), broilers and cockerels fed cocoa husk meal (Olubamiwa et al., 2002; Teguia et al., 2004). The decrease in weight gain at higher substitution levels could be attributed to destruction of the intestinal lining, possibly occasioned by theobromine, which could result in poor nutrient digestibility and absorbability (Muhammed et al., 2000). Growth performance of livestock has been reported to be negatively affected at higher substitution of kola pod husk meal (Emiola et al., 2006; Fabunmi et al., 2019) or dietary concentrations of theobromine (Muhammed et al., 2000). Anti-nutritional factors such as theobromine and caffeine have been reported to interfere with nutrient digestion and utilization, with consequent decrease in growth rates (Jayeola et al., 2001; Fabunmi et al., 2019). Also, the low catabolic process of theobromine to either 7-methylxanthine or 3-methylxanthine, then to xanthine and subsequent oxidation to uric acid in the liver (Dash and Gummadi, 2006) might result in poor nutrient utilization and subsequent poor growth rate (Adamafio, 2013). Higher intakes of theobromine and caffeine, and other anti-nutritional factors, have been reported to negatively affect animal performance by blocking the metabolic pathways and reducing nutrient bioavailability (Oloruntola et al., 2018). Similar weight of pigs fed up to

10% KPH-based diets in this study is in accordance with rabbits fed up to 10% kola pod husk meal (Eburu et al., 2020) and 10% cocoa husk meal (Olubamiwa et al., 2002).

The similar feed intake in the present study indicates and suggests the suitability of the test ingredient (kola pod husk meal) as a suitable substitute for maize in pig nutrition. This study is in agreement with previous reports on rabbits fed sun-dried kola pod husk (Eburu et al., 2020; Adeyeye et al., 2021) and broilers fed castor kernel cake (Akande and Odunsi, 2012) but apposite with broilers that received cocoa husk meal (Teguia et al., 2004). The similar consumption of KPH meal and maize indicates the palatability of KPH meal by pigs. Levels of feed intake could be attributed to factors such as texture, taste and odour. Hence, the chocolate odour, crispy and coarse-fine texture, and colour of KPH meal could have been responsible for its acceptability by pigs.

The similar feed conversion ratio in pigs fed 0 and 10% KPH meal is in tandem with snails fed the same level of KPH meal substitution (Owosibo et al., 2008). The non-significant difference in the feed conversion ratio (feed: gain ratio) up to 10% in this study, KPH meal could suggest the comparable nutrient density in KPH meal and maize, and beyond which the nutrient utilization could be impaired, leading to depressed weight gain. The reduction in feed cost ₦/kg gain, better saving cost and relative cost benefit in pigs fed at 10% dietary KPH meal as energy substitute for maize agree with birds fed cocoa husk meal (Olubamiwa et al., 2002; Teguia et al., 2004) and rabbits fed KPH meal (Ayinde et al., 2010). Results of the cost analysis that blend with those of the feed conversion ratio suggest that the threshold level of KPH meal in pig diet is 10%, beyond which there would be a loss.

The normal haematological range for healthy pigs are: PCV (32.0-50.0%), RBC (5.0-8.0 x 10^6), MCH (17.0-21.0pg), MCHC (30.0-36.0g/dl), MCV (50.0-68.0fl), HbC (10.0-16.0 (g/dl), WBC (11-22 x 10^3/l), neutrophils (28.0-51.0%), lymphocytes (39.0-62.0%), monocytes (2.0-10.0%), eosinophils (0.50-11.0%) and basophils (0.0-2.0%) as reported by RAR (2009) Etim et al. (2013) and Coronado (2014). The significantly lower RBC, MCH, HbC and lymphocytes of pigs fed dietary substitution with KPH meal might be due to erythropoietin deficiency, haemolysis or bone marrow failure (Gernsten, 2009; Bunn, 2011), or possibly poor protein utilization (Bunn, 2011) or the residual effect of theobromine and caffeine (Oloruntola et al., 2018; Adeyeye et al., 2021) while the higher WBC, monocytes and eosnophils could not be attributed to infection or tissue damage disease (Valencia, 2012) as the values obtained fall within the normal range for healthy pigs (Etim et al., 2014). Higher WBC but within the normal range for healthy pigs, as reported in this study, suggest the potential of KPH meal to produce immunologic activity in the animal system. Interestingly, the non-significant effect of KPH meal on some haemato-biochemical profiles confirms the safety of KPH meal up to a 10% substitution level to support the normal haematopoietic activity in the pigs.

The significant reduction in the TSP, albumin and globulin levels could be attributed to the effect of fibrous KPH meal by exerting protective action, thus decreasing protein availability and utilization by pigs or poor digestibility of the protein in kola pod husk meal (Bunn, 2011). However, there may be no cause for hypoalbuminaemia or hypoglobulinaemia as the values obtained were within the recommended range for healthy pigs (Rodostits et al., 2000). The significant increase in the cholesterol of pigs fed dietary KPH meal could be due to an increase in lipoproteins. The soluble effects of dietary fibre have been reported to influence cholesterol and LDL concentrations (Fasuyi et al., 2013). The cholesterol level obtained in this study suggests that the KPH meal might not contain high saponins that could bind to serum lipids (Matawalli et al., 2004). Increased ALP in pigs fed dietary substitution with KPH-based diets could be due to the congestion and obstruction of the biliary tract, as reported by Flecknell (2000) or possibly as a result of high fibre (Helen and Asagba, 2015).

The significant difference in AST, ALT, ALP and LDH in the pigs could be attributed to dietary treatments or anti-nutrient (Helen and Asagba, 2015). Irrespective of the differences in the serum metabolites in this study, the values fall within the range reported for healthy pigs (Adesehinwa, 2007; Adesehinwa et al., 2008; Coronado, 2014; Etim et al., 2014).

Conclusion

Results from this study further attest to the minimal use of kola pod husk meal in non-ruminant diets. Results showed that 10% was the optimal biological and economic level of KPH meal as an energy substitute for maize in the pig diet. Pig farmers in this part of the world, where KPH meal abounds and is presently not being competed for, are encouraged to minimally utilize KPH meal as an energy substitute for maize in the pig concentrate diet.

Recommendations

- Pig farmers are advised to substitute maize with kola pod husk meal as an energy source in the pig diet up to a 10% level.
- To improve the nutritive value of KPH meal in pig diet, there may be a need to carry out some processing techniques that will help detoxify the anti-nutrients, particularly theobromine and caffeine contents and add more value to the nutritional contents of the product

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