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Dietary supplementation of *Kigelia africana* fruits to rabbit bucks fed cottonseed meal on digesta pH and digesta microbial population

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Abstract

Medicinal plants are abundant in phytochemicals that has significant effects on digestion. This study was carried out to evaluate the effects of dietary supplementation of Kigelia africana fruits (KAF) on digestibility, digesta pH and intestinal microbial populations on rabbit bucks fed cottonseed meals. Sixty rabbit bucks were divided into five groups with twelve replicates in a completely randomized design. Each treatment group had: (T1) 100% SBM - CSM -KAF (T2) 100% CSM- KAF, (T3) 100% CSM + KAF powder, (T4) 100% CSM + KAF extract, (T5) 100% CSM + KAF powder + KAF extract (blend mixtures) Diets were formulated to meet the nutrient requirements for Rabbit bucks and all other management practices were strictly observed. During 84 days feeding trial, feed intake and feacal samples were determined. After, bucks were slaughtered and intestinal digesta samples were collected to assess microbial populations in the stomach and caecum. Microbial populations were determined by 10-fold serial dilution and total coliform count (TCC), Total anaerobic plate count (TAPC), total anaerobic plate count (TAnPC) and fungi coliform counts were determined. Result indicated that digestible crude protein was significantly (P<0.05) lower in bucks without supplementation and those supplemented with KAF powder but significantly (P<0.05) higher in bucks supplemented with extract and blend mixtures. Acidity of digesta significantly (P<0.05) increased in bucks fed CSM diets with or without supplementation. Supplementation of KAF to CSM diets significantly (P<0.05) suppressed intestinal microbial populations but significantly (P<0.05) higher in non-supplemented. It was concluded that KAF supplementation to CSM improved digestibility but reduced microbial populations in both stomach and caecum.

Keywords: *Rabbits, Cottonseed-meal, Kigelia africana fruits. Digesta, Stomach and Caecum*

Introduction

The medicinal plants are of high therapeutic value which have recently gained popular interest since the prohibition on the usage of antibiotics by the European Union in 2006 due to anticipated adverse effect, toxicity and high cost. (Oluwafemi et al., 2020). According to Musa et al. (2020) there are over 200,000 species of medicinal plant species where about 800 plant species have been used by different communities for curing different diseases and they contain several minerals, vitamins, protein and essential fatty acids. *Kigelia africana* is one of such identified medicinal plants. *Kigelia. africana* (Lam) Benth, belongs to the family bignoniaceae and genus of flowering plants. It is commonly referred as sausage or cucumber tree. The plants are found to contain alkaloids, flavonoids, phenols, saponins, tannins, oxalate, terpenoids, steroids etc. Alagbe, et al. (2020) reported ability to function as an antimicrobial, anti-inflammatory, antiviral, antioxidant anti-ulcer, anthelminthic and anti-implantation. *Kigelia africana* is a tropical African plant widely grown and distributed in southern, central and West Africa. In Nigeria, the native name is called 'Pandoro' (Yoruba) 'uturbun' (Ibo), (Aiyeloja and Bello, 2006) and 'Hantsargiwa' (Hausa) (Blench, 2003). Grace et al., (2002), reported antibacterial activity of the fruit *K. africana* in rats, and that fruits enhance the modulation of beneficial intestinal microbes by controlling potential pathogens, improved nutrient digestion and absorption and enzyme activity to enhance better weight gain and feed conversion efficiency due to the presence of several bioactive chemicals. In view of its potential, this study focuses on evaluation of supplementation of KAF to CSM diets to address deleterious effects of gossypol in cottonseed cake.

Materials and Methods

Experimental site

The experiment was conducted at the Rabbit Research and Production Unit of the Teaching and Research Farm, Osun State University, College of Agricultural Sciences and Management Ejigbo, campus, Ejigbo Osun State Nigeria. The university is located at coordinates; $7^{0}45^{1}42^{1}$N $4^{0}36^{1}04^{1}$ E of latitude and longitude in a forest zone of Nigeria.

Preparation of CSC, Powder and Extract of *Kigelia africana* fruits

Cottonseed meal

Cottonseed cakes (CSC) were obtained from cotton milling industry in Nigeria. The materials were sundried and milled to obtain cottonseed meal (CSM). The moisture content was determined by drying until constant weight in hot-air oven and packed in tight polytene sacks for proximate/ chemical and photochemical analysis.

The matured ripe fruits of *K. africana* were collected based on ethno-pharmacological information from the Forestry at Teaching and Research Farm Ejigbo, Osun State University. The botanical identification of the plant and fruits was done at the Forestry Research Institute of Nigeria, Ibadan where a voucher specimen number- FHI/08257 was recorded for ease of identification. Fruits thoroughly washed with distilled water to remove soil and other bound particles, air dried separately until a constant weight obtained and was divided into two parts. First was pulverized to using grinding machine to powder. Second was aqueous extraction performed according to the method of Gwatido et al. (2018) with minor modification in the laboratory. Blend mixtures were performed by dissolving 10g of ground sample (Kigelia africana fruits) mixed with water together (1:1) dissolved in 1000 ml water, stirred continuously and kept in the refrigerator for 48 hours. The extract was filtered using Whatman filter paper No.1 to obtain filtrate (KAF extract). Processed KAF (powder, extract and blend mixtures) were later stored in well labeled airtight containers and kept for further analysis. Proximate compositions of test materials and experiment diets were determined by using Official Method of Analysis Chemistry (AOAC, 2000). Phytochemical evaluation of tannins, alkaloids, saponins, flavonoids, phenols, oxalate, glycosides, steroids and terpenoids were estimated using methods described by Atamgba, et al. (2015) and Odebiyi, et al. (1978). All data generated were subjected to ANOVA and significant means separated by Duncan's multiple range test of the same statistical package. KAF powder was added to the experimental diets while extract and blend-mixtures were added to experimental diet containing powder and addition of the extract to the diet was done daily for group in the blend mixture KAF samples; powder, extract and blend-mixtures were analyzed for proximate composition according to AOAC, (2000)

Table 1: Chemical composition of test materials and gossypol levels

Composition	SBM (%)	CSC (%)	KAF (%)	SEM
Moisture	8.12[b]	5.37[c]	9.80[a]	0.54
Crude Protein	42.50[a]	37.45[b]	16.31[c]	0.58
Crude Fibre	7.98[c]	11.60[b]	21.02[a]	0.51
Ether Extract	6.10[c]	8.15[b]	9.28[a]	1.65
Ash	4.80[c]	5.54[b]	7.57[a]	0.14
NFE	28.50[c]	31.89[b]	36.20[a]	0.83
Calcium	2.82[a]	2.45[b]	2.14[c]	0.21
Phosphorus	5.50[b]	12.70[a]	3.36[c]	0.45
Methionine	6.80	5.50	-	-
Threonine	28.90	20.50	-	-
Lysine	17.80	14.50	-	-
Leucine	34.50	21.30	-	-
Isoleucine	20.10	16.50	-	-
Arginine	24.10	23.69	-	-
Gossypol ug/ml	0.57		-	

SBM = soybean meal, CSC = cottonseed meal, KAF= *Kigelia africana* fruits, NFE = Nitrogen Free extract

Table 2: Phytochemical analysis of Kigelia africana fruits. (1000g/mL)

Test/Extraction Samples	Quantitative Powder	Qualitative	Qualitative Extract	Qualitative	Quantitative Blend-mixtures	Qualitative
Alkaloids	-	-	-	+	-	-
Terpenoids	-	+	2.34	-	-	-
Flavonoids	50.62[c]	++	55.42[b]	++	59.70[a]	++
Phenolic compounds	108.06[c]	+++	115.01[a]	+++	113.08[b]	++
Tannins	126.14[a]	+++	86.65[c]	++	121.09[b]	++
Saponins	22.56[a]	++	18.37[c]	+	20.81[b]	+++
Reducing sugars	76.89[c]	++	80.74[b]	+++	84.23[a]	+++
SEM	6.22		4.19		5.89	

+ slightly present, ++ highly present, +++ densely present, - not present

Animals, Management and Diet composition

Sixty (60) rabbit bucks of crossbred (mixed population) aged between 7-8 weeks old were balanced for weight, caged individually and randomly allocated to five dietary treatments containing CSM with or without KAF supplementation in a completely randomized design (CRD). Animals were adjusted to adaptation period of two weeks after which they were subjected to 84 days of feeding trial. Twelve (12) bucks were allotted to each treatment, and each rabbit serves as replicates. Table 3 shows the composition of experimental diets. The followings are the treatments. Treatment (T1) had 100% SBM without supplementation. Treatment (T2) had CSM without KAF supplementation. Treatment (T3) had CSM supplemented with KAF powder 100g/100kg feed. Treatment (T4) supplemented with extract of KAF at 100mg/bodyweight/ animal. Treatment five (T5) was blend-mixture supplement KAF of powder and extract.

Table 3: Gross composition of experimental diets and calculated nutrients

Ingredients (%)	T1	T2	T3	T4	T5
Maize	43.24	43.24	43.24	43.24	43.24
Soybean meal	20. 76	-	-	-	-
Cottonseed cake	-	20.76	20.76	20.76	20.76
Rice husk	21.00	21.00	21.00	21.00	21.00
Brewers dried grain	10.00	10.00	10.00	10.00	10.00
Fish meal 72%)	2.00	2.00	2.00	2.00	2.00
Oyster shell	2.00	2.00	2.00	2.00	2.00
Bone meal	0.25	0.25	0.25	0.25	0.25
Premix	0.25	0.25	0.25	0.25	0.25
Salt	0.20	0.20	0.20	0.20	0.20
Lysine	0.10	0.10	0.10	0.10	0.10
Methionine	0.20	0.20	0.20	0.20	0.20
Total	100	100	100	100	100
Calculated Nutrients					
Crude protein (%)	16.34	15.72	15.72	15.72	15.72
Crude Fibre (%)	10.22	11.57	11.57	11.57	11.57
ME (Kcal/kg)	2582	2436	2436	2436	2436
Lysine	0.86	0.64	0.64	0.64	0.64
Methionine	0.27	0.25	0.25	0.25	0.25

*Premix composition (per kg of diet): vitamin A, 12,500 IU; vitamin D3,2500 IU; vitamin E, 50.00k3, 2.50mg; vitamin B1, 3.00mg; vitamin B2, 6.00mg; vitamin B6. 6.00mg; niacin, 40mg; calcium pantothenate, 10mg; biotin, 0.08mg; vitamin B12,0.26mg; folic acid, 1.00mg; chlorine chloride, 300mg; manganese, 100mg; iron, 50mg; zinc, 45mg; copper, 2.00mg; meal. BDG- Brewer's dried grain, ME- Metabolizable Energy, Control T_1 (100% SBM + 0% CSC), T_2 (100% CSC + 0% KAF), T_3 (100% CSC +100% KAF powder), T4 (100% CSC +100% KAF extract), T_5 (100% CSC +T_3 +T_4)

Data collection

Microbial populations determination

A 10-fold serial dilution method, in which 1% peptone solution was mixed with stomach and caeca samples collected from 3 rabbits per treatment for microbial analysis. The mixture was poured on Mac Conkey agar plates and lactobacilli medium III agar plates to determine the colony forming unit (cfu) in each gram of caeca sample by means of pour plate method. E. coli was cultured on Eosin Methylene Blue (EMB) agar at 37℃

for 48 hours. Lactobacilli were enumerated on Rogosa Sharpe agar at 37°C for 72 hours. Colonies with metallic green sheen colours were considered as E. coli while white colours signify colonies of lactobacilli. All records were computed according to the methods outlined by Suriya, et al. (2014). Laboratory procedures: Proximate analysis of experimental diets was determined by standard methods as described by AOAC (1990).

Results

Table 4 represents the digesta microbial population of rabbits fed cottonseed cake-based diet supplemented with or without *Kigelia africana* fruits. All the parameters examined were significantly (P<0.05) affected. There was response to diet in Total Coliform Count (TCC) in the stomach of rabbit fed the experimental diets while a linear response was observed in the caecum. Significantly higher (P<0.05) TCC was recorded in rabbits fed control diets (T1) 18.00 cfuM^{-2} and CSC-based diet (T2) respectively) while the least value was recorded in rabbit group fed CSC-diet with *Kigelia. africana* powder (T3). 10.00 cfu/M^{-1} The TCC of the caecum decreased (P<0.05) in (T1-14.80cfu/M^{-1}, T2-14.00cfu/M^{-1}, T3-10.00 cfu/M^{-1} and T4-9.00cfu/$M^{-1,}$ T5-8.00cfu cf/M^{-1}respectively. A linear decrease along T1 to T3 was observed for the Total Aerobic Plate Count (TAPC) in the stomach (102×10^4cfug^{-1}, 93×10^4cfug^{-1}, 74×10^4cfug^{-1}) whereas it then increased for T4 (85×10^4cfug^{-1}), T5 (89×10^4 cfug^{-1}), while that of caecum was a quadratic response. Higher (P<0.05) TAPC value (97.67 $\times10^4$cfu$g^{-1)}$ was observed in the control group while the lowest value (67.00 $\times10^4$cfug^{-1}) was recorded in the group fed CSC-diet with *Kigelia africana* fruit powder.

Total Anaerobic Plate Count (TAnPC) value showed significantly higher (P<0.05) value for T2 both in the stomach and caecum, while lower value was observed in T3 at both sites too. The Fungi count had significant elevated values for T2 both in the stomach and caecum while the least value was recorded in T4 for both sites.

Table 4. Digesta microbial population of rabbit bucks fed cottonseed cake supplemented with *Kigelia africana* fruits

Parameters	Site	T1	T2	T3	T4	T5	SEM
TCC (cfuM^{-2})	Stomach	18.00^a	19.00^a	10.00^c	13.00^b	14.00^b	0.83
	Caecum	14.80^a	14.00^a	10.00^b	9.00^b	8.00^b	0.61
TAPC (10^4cfug^{-1})	Stomach	102.00^a	93.00^b	74.00^d	85.00^c	89.00^{bc}	3.17
	Caecum	97.60^a	87.00^b	76.00^c	67.00^d	72.00^{bc}	2.70
TAnPC(cfug^{-1})	Stomach	27.00^d	65.00^a	31.00^c	42.00^b	39.00^{bc}	2.77
	Caecum	37.40^b	72.00^a	24.00^c	26.60^c	24.00^c	3.54
Fungi Count(10^2cfuM^{-1})	Stomach	6.00^{ab}	7.00^a	5.00^b	4.00^c	4.60^{bc}	0.46
	Caecum	8.40^{ab}	9.00^a	7.00^b	4.00^c	5.68^{bc}	0.57

^{abcd} Means with different superscript along the same row are significantly different (P<0.05). SEM-Standard Error of Mean, T1- Control (SBM-based diet), T2- Cotton seed cake without *Kigelia africana*, T3- Cotton seed cake with *Kigelia africana* powder, T4- Cotton seed cake with *Kigelia africana* extract, T5- Cottonseed cake with *Kigelia africana* powder and extract TCC-Total Coliform Count, TAPC- Total Aerobic Plate count (x 10^4cfug^{-1}), TAnPC-Total Anaerobic Plate count

Discussion

Rabbits are pseudo - ruminants which occupy ecological niche similar to ruminants because of gut microbial fermentation. The higher these microbial populations, the faster the rate of digestion. KAF is a natural substance of antioxidant properties that could be used to control the pathogenic microbial population that are normal inhabitant in the intestinal tract of the rabbits. The decrease in bacterial populations may led to resultant decrease in microbial population. TCC was decreased in the stomach and caecum sites of bucks fed T3 however, KAF powder supplementation reduced TCC better in stomach than caecum. TAPC was suppressed in both stomach and caecum of rabbit bucks fed CSC supplemented with KAF. More suppressive capacity of KAF powder reflected in T3. A linear decrease observed in fungi count imply the potential of KAF to reduce the fungi count, this reduction could be attributed to KAF supplementation. Gruptal et al., (2019) observed no significance difference of anti-bacterial and anti-fungi effect of aqueous extract of KAF. Skrivanova et al., (2010) observed no significant decreased in number of TAPC and TAnPC but a significant decrease in TCC but consistent with Hyun, et al., (2018) and Shittu, et al., (2020) reported that phytogenic feed additives can reduce the activity of intestinal bacteria by competitive exclusion due to the presence of phenols, alternation of bacteria cells and preventing the development of virulence structures in pathogenic microorganisms. The test material has also proven its ability to depopulate beneficial bacteria and could not maintain dysbiosis. This result is in consonance with Abu-Hafsa et al., (2016) who also observed decrease in fermentative activity in hindgut and reduction in bacterial count of some microbial communities.

Conclusion

In conclusion, feeding CSC diet supplemented with KAF to rabbits reduces the number of coliforms in both the stomach and caecum, in addition, the reduction in the total number of anaerobic bacteria was more pronounced in the caecum than stomach. The nature of this effect is not confined to one group of microbes but appears to affect a few different groups of bacteria both pathogenic and beneficial microorganisms.

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