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Utilisation of Bovine Rumen Filtrate for Biodegradation of Sweet Orange Peel (*Citrus sinensis*) and Effect on Rabbit Performance

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Abstract

A feeding trial was conducted with four to six-week old rabbits (n=30) to determine their growth and nutrient digestibility on diets in order to evaluate the maize replacement value of sweet orange peel biodegraded with rumen filtrate. Rumen filtrate was obtained from the rumen content and liquor of four randomly selected slaughtered cattle, to which water was added in ratio 1:1, and filtrate manually squeezed out. Freshly collected sweet orange peel was divided to five batches of 5 kg each. Rumen filtrate was added to and mixed with one of 5 kg each in ratio 1:5, 2:5, 3:5, 4:5 and 5:5 and biodegraded for 24 hrs, sundried and milled. Each of replaced 50% maize in the control diet (T1) of rabbits to obtain diets T2, T3, T4, T5 and T6, respectively. Thirty mixed breed exotic rabbit of both sexes were randomly allocated to six diets of five rabbits each. Rabbits were fed ad libitum and provided adequate drinking water in a 77-day feeding trial. Growth indices and coefficient of digestibility of nutrients were determined. Experimental diets had no significant effect ($p>0.05$) on final live body weight, feed intake, body weight gain, feed conversion and protein intake, but protein efficiency ratio differed significantly ($p<0.05$) with higher value of 2.10 in T5. Diets had varied significant effect ($p<0.05$) on the coefficients of digestibility of nutrients and improved crude protein digestibility. Bovine rumen filtrate can be used to treat sweet orange peel in a ratio of 4 litre rumen filtrate : 5kg for its biodegradation to improve its feed value, and as a replacement for dietary maize at 50% level in growing rabbit diet.

Keywords: *Rumen filtrate, biodegradation, peel, rabbit*

Introduction

Food is a necessity in human life as it helps in body growth and sustenance of diverse life activities. However, the world is facing food insecurity which is a threat to human survival most especially in the developing countries. The shortage in animal food production with increase in population growth brings about the current food crisis in many developing countries. In Nigeria, over 70% of the citizens are involved in agriculture and mainly at subsistence level (Babamaaji and Ekwe, 2023). In spite of its contribution to the nation's economy, the sector confronts a myriad of problems which affect productivity, prominent among which are climate change, land degradation which reduces cultivable land mass, inadequate local production of feed ingredients, post-harvest losses and high production cost. These have adverse effect on agricultural productivity, causing increased food importation due to population increase and decreased food sufficiency. Nigeria's population is estimated at 223.8 million with a growth rate of 2.41% (Worldometer, 2024), and dietary protein deficiency is a major problem partly, because the demand for protein of animal origin is greater than supply. The recent average animal protein intake in Nigeria put at 6 – 8 g/day (Have et al., 2020) is dismal. The global average daily total protein intake based on a reference intake value of 0.83 g/kg of body weight is 64 g, and in advanced economies more than 60% of protein intake comes from animal sources whereas, in Africa, India and other food deficient countries it ranges from 20 – 25% of total protein (Capper et al., 2013). Animal production in Nigeria remains underexploited, thus, a dire need for an appreciable step-up in livestock production to mitigate the critical shortage of animal protein using animals like the domestic rabbit which when compared with other livestock is characterized by early sexual maturity, high prolificacy, relatively short gestation period, short generation interval, high productive potential, rapid growth, in addition to its ability to utilize forages and fibrous plant materials and agricultural wastes. Rabbit is thus regarded as an animal at the nexus of food production and bioscience research for sustainable development in developing countries (Sikiru et al., 2020). Sweet orange peels are abundant citrus wastes in most part of Nigeria because of the heavy consumption of the fruit. It is high in metabolisable energy content, and similar to maize in crude protein (Jobo and Oluremi, 2023) and can be an alternative feed ingredient to maize in rabbit nutrition because of its relatively high crude fibre.

The study determined the feed value of sweet orange peels biodegraded with rumen filtrate from bovine species in grower rabbit performance

Materials And Methods

The feeding trial was carried out in the Rabbitary unit at the Teaching and Research Farm, Federal University of Agriculture, Makurdi, Nigeria located in latitude 60^0 - 80^0 N and Longitude 6^0 - 10^0 E. Makurdi has an annual precipitation ranging between 508 mm to 1016 mm, for a six to eight-month period, minimum temperature of 22.80 ^oC and maximum temperature of 40.03 ^oC and, relative humidity between 37.3% and 59.2% (Audu et al., 2022).

Fresh sweet orange peels (SOP) were collected from orange fruit retailers on the Campus and Wadata, in Makurdi metropolis and treated with rumen filtrate (RF). Fresh rumen content together with liquor was collected in covered plastics buckets from four randomly selected slaughtered cattle in a Government abattoir within the metropolis. The RF was analysed for its microbial content (Cheesbrough, 2005) in the University's Veterinary Microbiology Laboratory. Water was added to rumen content and liquor in the ratio of 1 L : 1 kg of rumen content, thoroughly stirred and squeezed manually to extract the rumen filtrate. The RF was then added to and mixed with SOP in the ratio of 1 L : 5 kg, 2 L : 5 kg, 3 L : 5 kg, 4 L : 5 kg and 5 L : 5 kg, to obtain biodegraded SOP 1,, SOP 2, SOP 3, SOP 4 and SOP 5,, respectively. Each mixture was poured in a feed sack, kept in a shade and allowed to ferment for 24 hours. Thereafter, biodegraded SOPs were spread thinly on a concrete floor and sundried until about 10% moisture content was attained within 24 – 48 hours. Samples of SOP 1,, SOP 2, SOP 3, SOP 4 and SOP 5 were milled and analysed for its proximate constituents (A.O.A.C., 2015) at the University's Animal Nutrition Laboratory and the metabolisable energy calculated (Pauzenga, 1985) as shown in Table 1.
Six experimental diets were formulated, using sweet orange peel (SOP) biodegraded with rumen filtrate (RF) in ratios 1 litre RF : 5 kg SOP (SOP 1), 2 litre RF : 5 kg SOP (SOP 2), 3 litre RF : 5 kg SOP (SOP 3), 4 litre RF : 5 kg SOP (SOP 4), and 5 litre RF : 5 kg SOP (SOP 5), as replacement for 50% maize in the control diet (T1), to obtain diets T2, T3, T4, T5 and T6, respectively (Table 2). The experimental animals were four to six-week old 30 mixed breed exotic rabbits of both sexes, housed in separate cages, and randomly assigned to the six diets at the rate of five rabbit per diet group. Each rabbit in a diet group served as a replicate, all rabbits were fed *ad-libitum* and drinking water supplied without restriction. The feeding trial lasted for 77 days. The experiment was a completely block randomized design. Medications given were antibiotics (Tridox) intramuscularly, coccidiostat (Amprolium) and Zantriviral were given orally using water as the administration route. Vitalyte was given as antistress.

Table 1: Proximate constituents of Biodegraded sweet orange peel (%DM)

Nutrients	SOP1	SOP2	SOP3	S0P4	SOP5
Dry matter	89.40	88.86	87.78	88.73	85.48
Crude protein (CP)	5.87	6.40	6.98	7.39	9.73
Crude fibre	9.97	10.03	10.48	11.75	12.20
Ether extract (EE)	1.84	2.23	2.10	2.30	2.11
Ash	9.32	10.38	10.21	10.10	10.40
Nitrogen free extract	73.00	70.42	70.23	68.45	65.56
ME[1] (kcal/kg)	2958.01	2917.56	2921.74	2890.29	2858.51

[1]ME = Metabolisable energy, SOP = sweet orange peel.

Table 2: Gross composition of experimental diets

Ingredients (%)	T1	T2	T3	T4	T5	T6
Maize	41.44	20.72	20.72	20.72	20.72	20.72
Sweet orange peel	0	20.72	20.72	20.72	20.72	20.72
Fixed ingredients[1]	58.56	58.56	58.56	58.56	58.56	58.56
Total	100	100	100	100	100	100

[1]Fixed ingredients (%); soybean 5.00, blood meal 0.50, rice bran 27.30, brewers dried grain 22.45, bone ash 2.81, vitamin/mineral premix 0.25, table salt 0.25.

The performance data collected were;

i. Initial body weight (IBW)
ii. Final body weight (FBW)
iii. Body weight gain (BWG) = $FBW - IBW \ / \ 96 \ days$
iv. Daily feed intake (DFI) = $\frac{Feed\ supplied - Unconsumed\ feed}{96\ days}$
v. Feed conversion ratio (FCR) = $\frac{DFI}{BWG}$
vi. Protein intake (PI) = DFI x % crude protein in feed
vii. Protein efficiency ratio (PER) = BWG / PI

At the end of the eleventh week of the feeding trial, three rabbits were selected per treatment having an average weight similar to treatments average weight, and used for nutrient digestibility evaluation. Experimental diets were withdrawn 18 hours prior to commencement of digestibility trial to allow for the elimination of the residual feed in the gut. Thereafter, 75% of their daily feed intake was supplied to each rabbit for four days and the corresponding faecal droppings collected and used to determine coefficient of digestibility of nutrients. Wet faecal collection was done daily using mosquito net tied under respective cages, weighed, oven dried at 105 ^{o}C for 24 hrs and weighed. The faecal droppings for each replicate was bulked and subjected to proximate analysis at the Nutrition Laboratory of the Department of Animal Nutrition in the Federal University of Agriculture Makurdi. Nutrient digestibility was computed as;
Nutrient digestibility = Nutrient consumed – Nutrient in faeces / Nutrient consumed x 100.

Data generated were subjected to the analysis of variance (ANOVA) using SPSS (2012), and the means of significantly ($P<0.05$) different indices separated with the least significant difference (LSD).

Results And Discussion

The microbial analysis of the rumen content showed that its homogenous mixture contained bacteria *E.coli* and *Klebseilla* spp. in the range of 2.4 x 10^6 – 2.8 x 10^6 cfu/g and *Aspergillus* spp. *a* fungal spp. in the range of 0.5 x 10^6 – 0.6 x 10^6 cfu/g. The ruminal ecosystem has a large diversity of microorganisms; bacteria, fungi and protozoa (Castillo-Gonzalez, 2014), working synergistically for the nutritional transformation of forage, the main feed of ruminant animals to produce metabolic energy for various physiological activities. The presence of these bacterial and fungal isolates in rumen filtrate shows that there are some ruminal microbes which can survive outside the ruminal ecosystem and can be useful to stimulate biodegradation process *in vitro* to improve the feed value of some crop residues and agricultural wastes such as sweet orange peels. The crude protein content of biodegraded sweet orange peel increased as the quantity of RF added to SOP increased from 1:5 to 5:5. The microbial load in the rumen filtrate was expected to increase the higher the quantity of rumen filtrate added to SOP. This can cause higher cell multiplication and more microbial cell mass thereby causing the increase in the crude protein content of SOP. The crude fibre level of the biodegraded SOP increased while, the nitrogen free extracts decreased as the ratio of the mixture of RF to SOP increased from 1:5 to 5:5, implying that crude fibre in the biodegraded SOP may contain higher indigestible fractions. The microbial isolates in the rumen filtrate *Klebseilla* spp., *E.coli* and *Aspergillus* spp. appeared unable to alter the structure of SOP by reducing its crude fibre content. Ash content increased as the RF : SOP ratio increased from 1:5 to 5:5, possibly because of the mineral content in the rumen mass due to mostly forage basal diet consumed by the cattle. The higher ether extract in the rumen filtrate treated SOP might be the consequence of the lipolytic activity of *E.coli*, one of the microbial isolates (Henne et al., 2020). There was a slight decrease in the metabolisable energy content of biodegraded sweet orange peel as the rumen filtrate added increased from 1:5 to 5:5. This may have been due to increased fibre and ash, and reduced digestible carbohydrate in the form of nitrogen free extract as the volume of rumen filtrate added to the sweet orange peel for biodegradation increased.

The experimental diets had no significant ($P>0.05$) effect on the FBW, daily BWG, DFI, FCR and daily PI, except PER which significantly ($P<0.05$) increased with higher amount of the rumen filtrate added to the sweet orange peel as shown on Table 3. Rabbits in diet T5, which contained 50% maize replacement with SOP treated with rumen filtrate in ratio 4:5 had a significantly ($P<0.05$) higher PER of 2.10. This result showed that as the quantity of rumen filtrate added to SOP for its biodegradation increased, PER of growing rabbits tended to increase significantly ($P<0.05$). This, most likely stimulated the relatively higher mean final body weight of 1461.40 g of the rabbits in T5 compared to the other dietary treatments.

Table 3: Effect of experimental diets on the performance of grower rabbits

Performance Indices	T1	T2	T3	T4	T5	T6	SEM
IBW (g)	418.33	425.83	425.83	425.83	425.83	376.66	14.78
FBW(g)	1317.00	1338.40	1318.50	1187.00	1461.40	1335.00	32.55
BWG(g)	11.80	12.70	11.76	9.61	12.99	12.46	0.44
DFI(g)	58.88	59.62	61.58	52.58	58.82	53.61	1.64
FCR	5.07	5.45	5.32	5.57	4.56	4.41	0.17
PI(g)	6.19	7.41	6.45	5.79	6.17	6.07	0.20
PER	1.89^{ab}	1.58^{b}	1.61^{b}	1.65^{b}	2.10^{a}	2.07^{a}	0.06

^{ab}Means with different superscripts on the same row are significantly different ($P<0.05$), SEM = standard error of mean, IBW=Initial body weight, FBW=Final body weight, BWG=Body weight gain, DFI=Daily feed intake, FCR=Feed conversion ratio, PI=Protein intake, PER=Protein efficiency ratio.

Table 4: Nutrients Digestibility of grower Rabbits fed diets containing sweet orange (*Citrus sinensis*) peel treated with bovine rumen filtrate

	Experimental Diets						
Parameters	T1	T2	T3	T4	T5	T6	SEM
Dry matter	79.83[a]	60.41[bc]	55.97[c]	72.77[ab]	71.78[abc]	65.97[abc]	4.92
Crude protein	79.12[a]	75.73[ab]	64.92[b]	81.29[a]	81.26[a]	74.41[ab]	3.97
Crude fibre	37.26 [a]	54.97 [a]	44.18 [a]	55.24 [a]	63.37 [a]	53.65 [a]	8.53
Ether extract	88.22[ab]	86.76[bc]	81.49[c]	93.69[a]	91.19[ab]	87.24[b]	1.76
Nitrogen free extract	79.48[a]	67.29[bc]	65.96[c]	76.87[abc]	78.75[ab]	68.48[abc]	3.58

[abc]Means within rows with different superscripts are significantly different (P<0.05), SEM- Standard error mean,
T1= Control Diet (Maize based)
T2= Diets containing 50% biodegraded sweet orange peel as a replacement for maize.
T3= Diets containing 50% biodegraded sweet orange peel as a replacement for maize.
T4= Diets containing 50% biodegraded sweet orange peel as a replacement for maize.
T5= Diets containing 50% biodegraded sweet orange peel as a replacement for maize.
T6= Diets containing 50% biodegraded sweet orange peel as a replacement for maize.

The non-significant (P>0.05) variation of the FBW, BWG, DFI. FCR and daily PI of rabbits irrespective of the dietary treatments, is an evidence of the nutritional potential of biodegraded SOP to serve as a replacement feed resource for maize which is an energy feed ingredient in growing rabbit nutrition. Biodegradation of SOP using bovine rumen filtrate enhanced the nutritive value of SOP for growing rabbit feeding. Thus biodegraded SOP obtained when bovine rumen filtrate is added to SOP in ratio of 1:4 to 5:5 can be used to replace 50% of maize in the diet of growing rabbits. This observation has elicited increased level of utilization of SOP as a replacement for dietary maize at 50% level in the diet of rabbit as against the 30% maize replacement value earlier reported (Oluremi et al., 2018).

The result of the coefficients of digestibility of nutrients by grower rabbits is presented in Table 4. Diets containing biodegraded sweet orange peel had significant (P<0.05) effect on dry matter, crude protein, ether extract, and nitrogen free extract while, the crude fibre did not vary significantly (P>0.05) across the treatment groups. Whereas, the values obtained varied from 55.97% to 79.83% dry matter, 64.92% to 81.29% crude protein, 37.26% to 63.37% crude fibre, 81.49% to 93.69% ether extract, 65.96% to 79.48% nitrogen free extract, none had a particular sequence of variation except crude protein digestibility. Crude protein digestibility range was lower than 78.63% to 84.64% reported by Oluremi et al. (2018) when grower rabbits were fed biodegraded sweet orange peel-based diets. The crude protein digestibility of 81.29% and 81.26% in T4 and T5, respectively were higher than 79.12% in T1 (control dietary group). This suggests a high efficiency in crude protein utilization by rabbits in dietary groups T4 and T5. The crude fibre, dry matter and ether extract values range of 37.26% to 63.37%, 55.97% to 79.83% and 81.49% to 93.69% were lower than 60.48% to 74.72%, 69.49% to 75.41% and 91.93% to 94.79%, respectively reported for grower rabbits fed biodegraded sweet orange peel-based diets (Oluremi et al., 2018). However, nitrogen free extract digestibility coefficient range of 65.96% to 79.48% obtained in this study was similar to digestibility values of 67.48% to 75.54% reported by these authors for growing rabbits fed biodegraded sweet orange peel-based diets. The significant effect (P<0.05) of the experimental diets on the digestibility of dry matter, ether extract and nitrogen free extract showed that the replacement of dietary maize with biodegraded sweet orange peel affected the nutrient quality of diets. While, dietary crude fibre is important for gut motility, digestibility of crude fibre is important in the overall nutrient utilization in rabbit because of the depressive effect it has on the utilization of other nutrients needed for growth and development. The result obtained in the present study did not show that dietary crude fibre caused any adverse effect on rabbit because the live weight of rabbits in T2, T3, T4, T5 and T6 were higher than the live weight of rabbits in T1 (control group).

The bovine rumen filtrate used contained three microbial isolates *Klebseilla* spp., *Escherchia Coli* and *Aspergillus* spp. which possibly improved the feed value of raw SOP treated in ratio of 1 litre RF : 5 kg SOP to 5 litre RF : 5 kg SOP during biodegradation. The FBW, BWG, FI, FCR and PI were similar irrespective of the experimental groups, except PER which increased significantly up to 2.10 when the volume of rumen filtrate added to the sweet orange peel for biodegradation was 4 litre RF : 5 kg SOP (T5). The growth response of rabbits fed biodegraded SOP based diet in ratio 4:5 at 50% maize replacement thus have a comparative advantage over the other treatments and can be used to replace 50% of maize in the diet of growing rabbits.

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