IN VITRO GAS PRODUCTION AND NUTRIENT UTILISATION INDICES AS AFFECTED BY SWEET ORANGE *(Citrus sinensis*) PEEL POWDER

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Abstract

This study investigated the effect of orange peel powder (OPP) supplementation on in vitro gas production and nutrient utilisation indices. The experimental design was completely randomized, and the dietary treatments were four experimental diets containing different levels of OPP (0, 10, 20, and 30 mg/g DM). The treatments were incubated for 48 hours. The results revealed that OPP supplementation had no significant ($p > 0.05$) effect on total gas production (25.33-29.00 mL/200mg DM), pH (6.65-6.85), and total volatile fatty acids (65.67-66.93 mL) concentration. Similarly, in vitro dry matter digestibility, short-chain fatty acids, metabolizable energy, partitioning factor, and microbial yield of diets were not affected by OPP supplementation. However, methane production (14.00-10.00 mL) and ammonia nitrogen (34.87-23.54 mg/dL) concentration decreased significantly ($p < 0.05$) with the addition of OPP in the diet, while the organic matter digestibility (64.50-74.00) increased. The study indicated that OPP at 30 mg/g could be used as a phytogenic additive to manipulate rumen fermentation.

Keywords: Orange peel, phytochemicals, additive, microbial fermentation, methane,

Description of Problem

Ruminant feed digestion is largely driven by microbial fermentation, producing volatile fatty acids (VFAs), ammonia-nitrogen, carbon dioxide, and methane. While VFAs supply the main energy to the animal, methane and excess ammonia production represent nutrient losses and contribute to greenhouse gas emissions, with methane being a major concern (1). In the past, synthetic antibiotics were used to enhance nutrient utilisation and reduce emissions, but their ban in 2006 due to health concerns has intensified the search for safe alternatives, with phytogenic additives emerging as promising options. Among the wide range of phytogenic resources, citrus by-products represent an underexploited but valuable option. Orange peel, a major by-product of juice industry, is abundantly available; even at street-side orange selling points and often discarded, causing environmental concerns. Chemically, it is rich in flavonoids, essential oils, tannins, and other phenolic compounds known for antimicrobial, antioxidant, and modulatory effects on rumen fermentation (2). Recent findings show that citrus phytochemicals improve digestibility and reduce fermentation lag phase (3). However, despite its abundance, the phytogenic potential of orange peel powder in ruminant feeding is still underexplored. This study, therefore, evaluates the effect of orange peel powder inclusion levels on *in vitro* gas production and nutrient utilisation indices, with emphasis on its potential as a sustainable feed additive in ruminant nutrition.

Materials and methods

Experimental site and preparation of orange peel powder

The experiment was conducted at the Laboratory of Animal Nutrition Department, Federal University of Agriculture, Abeokuta. Ogun State, Nigeria. Orange peel was collected from orange sellers around the Odeda local government area, Abeokuta, Ogun state. The peels were cleaned by removing the extraneous materials and were air-dried under a shed for 14 days. The dried peels were later ground with an electrical grinder and made to pass through a 1mm sieve to obtain the powder needed for the study. The orange peel powder (OPP) was then added to the experimental diet (*Megathrysus maximus* and concentrate in ratio 6:4) at varying inclusion levels of 0, 10, 20, and 30 mg/g DM as follows:

Treatment I: Diet + 0 mg/g DM OPP
Treatment 2: Diet + 10 mg/g DM OPP
Treatment 3: Diet + 20 mg/g DM OPP
Treatment 4: Diet + 30 mg/g DM OPP

In vitro gas production procedure and measurement of fermentation parameters

The *in vitro* gas production procedure of (4) was followed to incubate 200 mg of each treatment for 48 hours at 39±1°C, after which the gas production and methane (5) were determined. Each treatment was replicated ten times. The pH, ammonia nitrogen, and total volatile fatty acids concentration of the incubation fluid were determined (6, 7). The dry matter degradation (DMD) was calculated while metabolizable energy (ME), organic matter digestibility (OMD), and short-chain fatty acids (SCFAs) were estimated using gas volume at a 24 h incubation period as an index. The partitioning factor (PF) and microbial yield (MBY) of the sample incubated were also estimated according to (8)

$$DMD\ \% = \frac{[DMs - (DMr - DMb)]}{DMs} \times 100$$

$$ME\ (MJ/kg) = 2.20 + 0.136GV + 0.057CP + 0.0029(CF)^2 \quad (4)$$

$$OMD\ \% = 14.88 + 0.889GV + 0.45CP + 0.651A \quad (4)$$

$$SCFAs\ (\mu mol/g\ DM) = 0.0239GV - 0.0601 \quad (9)$$

PF = $PF = \frac{IVOMD}{GV\ (24h)}$

MBY (mg) = OMD + GV x 2.25

Where DMs = dry matter content of the substrate, DMr = dry matter content of the residue and DMb = dry matter content of the blank, GV = gas volume, CP = crude protein, CF = crude fibre, and A = ash.

Chemical and statistical analyses

The phytochemical compositions of orange peel, tannins, saponins, flavonoids, phenol, catechin and alkaloids were also determined (10, 11). Data collected were analysed in one-way analysis of variance in a completely randomised design using (12). The experimental model is as follows:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

where, Y_{ij} = observation, μ =population mean, T_i = treatments, ε_{ij} = residual error.

Results and discussion

The phytochemical analysis revealed that OPP contained tannin, phenol, flavonoids, catechin, alkaloids and saponin as shown in Table 1. These secondary metabolites have been reported to have many biological and therapeutic properties (13). Similar contents have been detected in orange peel by (14). Orange peel caused a significant reduction in methane and ammonia nitrogen concentration (Table 2). Secondary metabolites such as tannins and flavonoids in OPP might be responsible for reduced methane and ammonia nitrogen concentration of the diet. (15) reported enhanced rumen ecology and decreased methane production in diets supplemented with Rambutan peel powder. Tannins have beneficial effects by forming protein–tannin complex, decreasing the availability of feed protein for ruminal degradation and hence reducing ammonia nitrogen release (10).

Measurement of IVDMD and IVOMD has been widely used to assess the nutritional quality of feeds, due to its high correlation with *in vivo* digestibility (16). Supplementation of varying levels of orange peel powder had no significant effect on IVDMD, SCFAs, ME, partitioning factor and microbial yield but significantly improved IVOMD (Table 3). This suggests that levels of orange peel powder supplementation had no adverse effect on the majority of the microbial population involved in the fermentation process. The ability of several ruminal microorganisms to tolerate high concentrations of condensed tannin has been identified (17). Partitioning factor and microbial yield are valuable indicators of nutrient use efficiency in ruminants. Orange peel powder supplementation had no significant ($p > 0.05$) effect on these parameters. This indicates that the conversion of nutrients to microbial protein, which is crucial for animal growth and health, was not impaired.

Table 1: Phytochemical composition of orange peel powder

Parameters	Orange peel
Tannins (mg/100g)	735.386
Phenol (mg/100g)	173.150
Flavonoids (mg/100g)	860.986
Catechin (mg/100g)	217.260
Alkaloids %	6.73
Saponin %	4.09

Table 2: In vitro gas production and fermentation parameters of diets containing orange peel powder

Parameters	Inclusion levels of orange peel powder				SEM	p-value
	0 mg/g	10 mg/g	20 mg/g	30 mg/g		
Gas production						
Total GV (mL)	29.00	25.33	26.00	26.66	0.76	0.277
Methane (mL)	14.00^{a}	10.66^{b}	10.33^{b}	10.00^{b}	0.89	<0.001
%Methane	48.39^{a}	42.08^{b}	39.73^{b}	37.51^{b}	2.25	<0.001
Fermentation parameters						
pH	6.73	6.65	6.85	6.80	0.73	0.081
TVFAs (mM)	66.93	55.67	64.40	64.20	2.02	0.238
NH_3-N (mg/dL)	34.87^{a}	31.47^{a}	24.02^{b}	23.54^{b}	0.93	0.049

^{abc}Means on the same row having different superscripts are statistically different (P < 0.05), GV-gas volume, SEM-standard error of means, TVFAs-total volatile fatty acids, NH_3-N-ammonia nitrogen

Table 3: Effects of orange peel powder on nutrient utilisation indices

Parameters	Inclusion of orange peel powder				SEM	p-value
	0 mg/g	10 mg/g	20 mg/g	30 mg/g		
IVDMD (%)	62.10	60.62	59.43	60.01	0.68	0.606
IVOMD (%)	64.50^{b}	72.67^{a}	74.00^{a}	73.17^{a}	2.18	0.038
SCFA (µmol/DM)	0.63	0.59	0.56	0.58	0.03	0.606
ME (MJ/kg)	6.76	6.53	6.36	6.44	0.10	0.610
PF (mg/mL)	3.26	3.31	3.33	3.27	0.06	0.972
MBY (cfu/g)	153.22	148.38	144.95	147.05	2.43	0.73

^{abc}Means on the same row having different superscripts are statistically different (P < 0.05), IVDMD- in vitro dry matter digestibility, IVOMD-in vitro organic matter digestibility, SCFA-short chain fatty acids, ME-metabolizable energy, PF-partitioning factor, MBY- microbial yield

Conclusion and Application

The current study concluded that orange peel powder could be supplemented up to 30 mg/g of diet to improve the *in vitro* rumen fermentation and decrease methane production without adversely affecting gas production and nutrient utilisation indices.

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