IN VITRO ASSESSMENT OF GRAIN OF PARADISE ADDITIVE AS ANTIMCROBIAL GROWTH PROMOTER: EFFECT IN RUMINANT DIET

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ABSTRACT
This research was carried out to assess the replacement of conventional antibiotics (tetracycline) with a spice such as Grain of Paradise (Aframomum melegueta) as a growth promoter in ruminant production. The results of proximate, fibre and phytochemical analyses of the spice revealed the presence of appreciable quantities of tannins, saponins, phenols, oxalates, alkaloids etc. The in vitro studies using inoculum from cattle showed that at 24th, 30th and 33rd hour of incubation, similar but lowest gas production was observed in substrate containing 2% tetracycline (17.00, 21.33 and 22.67 mL/200mg) and 2% spice (18.00, 21.67 and 23.00 mL/200mg) respectively, than other treatments. Percentage methane/total gas produced by 1% spice additive (18.89 mL/200mg) is similar (p>0.05) to 2% spice additive (19.44 mL/200mg), but lower (p<0,05) than in 2% tetracycline additive (35.36 mL/200mg). Ammonia nitrogen produced by substrate containing 3% spice additive had lowest value (13.61mL/0.80 mg/dL). Inclusion of spice at various levels has no effect on total Volatile Fatty Acids (VFA) and individual VFA production. In conclusion, spice such as Grain of Paradise (Aframomum melegueta), can therefore be used as a growth promoter feed additive to replace tetracycline at 2% inclusion and to reduce the effect of enteric methane production by ruminant animals. This might solve the problem of residual effects of antimicrobials in meat produced by ruminant animals for human consumption.
Key words: Spice, Antimicrobial alternatives, Ruminant Production

INTRODUCTION

The use of antibiotics as growth promoters in animal feeds is a widely applied practice in livestock industry because antibiotics is found to improve overall health of livestock, increase animal body weight gain and feed efficiency (Yajing Ban and Le Luo Guan, 2021). However, misuse of antibiotics by the livestock industry has resulted in an increase in antimicrobial resistance which has been recognized as a big challenge for human and animal health (TResearch, 2020). Data from thirteen (13) to twenty-seven (27) countries in Africa indicate that 279 to 3558 tonnes of antimicrobials were used in animal production from 2015 to 2019, despite the EU ban on antimicrobials in 2006 (Castanon, 2007). Tetracycline and polypeptides constitute the largest proportion of antimicrobials used (Wang, *et al* 2015). There is real demand among animal producers for alternative feed additives and consumers for more natural, safe products in the human food supply chain. There is paucity of information on the use of *Aframomum melegueta* (Grain of paradise- 'Oburo' in Yoruba, 'Urioma' in Delta Ibo) in animal production and health. This research therefore aims at investigating replacement of conventional antibiotics (such as Tetracycline) with *Aframomum melegueta* as growth promoter in ruminant diet.

MATERIALS AND METHODS

The research was carried out in the Department of Animal Nutrition Laboratory, Federal University of Agriculture, Abeokuta located in the rain forest vegetation zone of south-western Nigeria on latitude 7°13'57.55N, longitude 3°26'12.65E and altitude 76N above sea level (Google Earth, 2021), between the period of May and December, 2023. *Aframomum melegueta* seed was purchased locally, *Panicum maximum* grass was harvested, both were air- dried, milled and stored in air- tight containers pending analyses. Concentrate ration was formulated containing Maize (20%), Cassava peels (15%), Wheat offal (30%), Soy bean meal (10%), Palm kernel cake (20%), Bone meal (3%), Salt (1.5%), and Premix (0.5%). Total mixed ration (TMR) for the experiment was formulated containing 60% grass, 40% concentrate. *Aframomum melegueta* was incorporated as additive into the TMR at graded levels (0, 1, 2, 3, 4 and 5%) to form the various dietary treatments, 2% of Tetracycline powder as additive

was used as the control treatment. Proximate (AOAC, 2000) and fibre (Van Soest,1990) compositions of concentrate ration and *P. maximum*, including anti-nutritional factor components of the spice were analyzed. *In vitro* gas production, greenhouse gas (methane) production, ammonia nitrogen, individual volatile fatty acid (VFA) and total VFA were determined (Babayemi *et al.,* 2004 and Fievez *et al.,* 2005).

Results

In Table 1, results of phytochemical analysis of the selected spice revealed that there are appreciable quantities of tannins, saponins, phenols, oxalates, alkaloids etc., present in the spice. *In vitro* gas studies (Table 2) showed that at the 12th hour of incubation, inclusion of spice at various levels increased ($p<0.05$) the volume of gas produced which ranged from 12.67 to 14.00 mL/200mg, while similar ($p>0,05$) gas volume was recorded for substrate with 2% tetracycline (8.67 mL/200mg) and 0% (no spice, no tetracycline) inclusion (8.50 mL/200mg). However, at 24th, 30th and 33rd hour of incubation, similar but lowest gas production was observed in substrate containing 2% tetracycline (17.00, 21.33 and 22.67 mL/200mg) and 2% spice (18.00, 21.67 and 23.00 mL/200mg) respectively. Substrate with 2% spice additive produced lowest ($p<0.05$) methane gas (5.00 mL/200mg), similar ($p>0.05$) to 1% spice additive (5.67 mL/200mg), but 2% tetracycline additive produced the highest methane gas volume (10.67 mL/200mg). Percentage methane/total gas produced by 1% spice additive (18.89 mL/200mg) was similar ($p<0.05$) to 2% spice additive (19.44 mL/200mg), but lower ($p<0,05$) than 2% tetracycline additive (35.36 mL/200mg). In Table 3, Ammonia nitrogen (NH_4N) produced by substrate containing 3% spice additive had lowest value (13.61mL/0.80 mg/dL), while substrate with 2% tetracycline additive produced highest ($p<0.05$) volume (1.50 mL/25.52 mg/dL). Differences in the various VFAs produced were not significant.

Table 1: Chemical Components of Concentrate, Panicum grass and Spice (*Aframomum melegueta*) used in the study

Proximate Composition (%DM)	***Aframomum melegueta***	**Concentrate**	**Panicum grass**
Crude protein	8.42	11.49	7.70
Ether extract	8.84	6.30	4.69
Ash	4.07	7.18	12.33
Crude fibre	14.80	10.06	19.16
Fibre fractions (%DM)			
Neutral Detergent fibre	39.35	40.28	70.55
Acid Detergent fibre	28.50	16.72	42.72
Acid Detergent lignin	8.25	7.10	11.15
Phytogenic Contents			
Tannins (mgTAE/100g)	1.03	ND	ND
Phenol (mgGAE/g)	50.31	ND	ND
Oxalate (mg/100g)	11,76	ND	ND
Phytate (mg/100g)	3.77	ND	ND
C. Glycosides(mg/100g)	0.64	ND	ND
Flavonoids (%)	6.60	ND	ND
Alkaloids (%)	4.48	ND	ND
Saponins (mg/100g)	2.03	ND	ND

ND: Not determined

Table 2: Effects of *Aframomum melegueta* (Grain of Paradise) on *in vitro* gas production, CO_2 and methane production (mL/200mg of substrate)

Incubation hour (hr)	Inclusion levels of *Aframomum melegueta* (%)						Tetracycline 2	SEM	P value
	0	1	2	3	4	5			
3	1.00	1.33	2.00	1.67	1.33	1.67	1.33	0.170	0.934
6	2.00^{b}	5.67^{a}	5.00^{ab}	5.33^{a}	5.00^{ab}	6.00^{a}	5.00^{ab}	0.372	0.017
9	5.50^{b}	10.00^{a}	9.33^{ab}	9.00^{ab}	9.00^{ab}	10.00^{a}	6.33^{ab}	0.506	0.142
12	8.50^{c}	13.33^{a}	13.33^{a}	12.67^{b}	13.00^{ab}	14.00^{a}	8.67^{c}	0.580	0.029
15	10.50	16.00	11.33	15.67	15.00	16.67	10.67	0.792	0.248
18	13.50	18.33	13.33	18.33	18.00	19.33	13.33	0.847	0.321
21	16.00	20.33	16.33	21.00	20.33	22.00	15.00	0.885	0.354
24	18.50^{b}	22.33^{ab}	18.00^{b}	23.67^{ab}	23.67^{ab}	25.00^{a}	17.00^{b}	0.910	0.019
27	22.00	24.67	20.67	27.00	26.00	27.33	19.00	0.977	0.287
30	25.00^{ab}	27.00^{ab}	21.67^{b}	29.67^{a}	28.33^{a}	28.67^{a}	21.33^{bc}	0.944	0.018
33	28.50^{a}	29.00^{a}	23.00^{b}	30.00^{a}	29.00^{a}	29.33^{a}	22.67^{b}	0.834	0.013
36	30.00	30.00	24.00	30.00	30.00	30.00	24.00	0.774	0.119
39	30.00	30.00	25.00	30.00	30.00	30.00	26.33	0.622	0.242
42	30.00	30.00	26.00	30.00	30.00	35.20	26.67	0.528	0.422
45	30.00	30.00	26.33	30.00	30.00	30.00	29.33	0.463	0.498
48	30.00	30.00	26.67	30.00	30.00	30.00	30.00	0.481	0.466
CH_4	7.50^{ab}	5.67^{b}	5.00^{b}	6.33^{ab}	6.33^{ab}	6.00^{ab}	10.67^{a}	0.534	0.022
% CH_4	25.00^{ab}	18.89^{b}	19.44^{b}	21.11^{ab}	21.11^{ab}	20.00^{ab}	35.56^{a}	1.765	0.030

TI = Test ingredient, SEM = Standard error of mean

Table 3: Effects of *Aframomum melegueta* on *in vitro* post incubation parameters (200mg of substrate)

Parameters	Inclusion of *Aframomum melegueta* (%)						Tetracycline 2	SEM	P value
	0	1	2	3	4	5			
NH_3N (mL)	0.85^{d}	1. 15^{b}	1.10^{bc}	0.80^{d}	0.95b^{cd}	0.90^{cd}	1.50^{a}	0.064	0.001
NH_3N(mg/dL)	14.46^{d}	19.56^{b}	18.71^{bc}	13.61^{d}	16.16b^{cd}	15.31^{cd}	25.52^{a}	1.094	0.001
TVFA	5.59	5.46	5.79	5.51	560	5.27	6.02	0.106	0.602
Acetate	3.73	3.64	3.84	3.67	3.73	3.52	4.01	0.071	0.602
Propionate	0.37	0.36	0.39	0.37	0.37	0.35	0.40	0.007	0.602
Butyrate	0.25	0.24	0.26	0.24	0.25	0.23	0.27	0.005	0.602
Total	**4.35**	**4.25**	**4.50**	**4.28**	**4.35**	**4.10**	**4.68**	**0.082**	**0.602**

TI = Test ingredient, SEM = Standard error of mean

DISCUSSION

Chemical composition of the concentrate, grass, and spice assayed in the present study showed that the crude protein content of the concentrate (11.40%) and Panicum grass (7.70%) is enough to meet rumen microbial requirements. The CP (8.42%), Tannins (1.03 mg/100g), Saponins (2.03 mg/100g), and Flavonoids (6.60%) contents of spice reported in this study are comparable to the results of other researchers. Nwachoko *et al*, (2015) reported 13.3% CP and 1.05% Tannins for grain of paradise ('Oburo' in Yoruba, 'Urioma' seed in Delta State, Nigeria), while Alligator pepper (containing 13.01% CP, 5.74 mgGAE/g Flavonoid, 1.01 mgTAE/100g Tannins, 51.60% Alkaloids, 35.52 mgGAE/g Phenols) and Black pepper (containing 10.97% CP, 6.43 mg GAE/g Flavonoid, 1.66 mgTAE/100g Tannins, 15.73% Alkaloids, 41.73 mg GAE/g Phenols) respectively were reported (Okunade et al., 2019). Uduenevwo *et al*., (2022) reported (3.14%CP), (4.04% Flavonoids), (0.17% Tannins) and (0.38% Saponins) for Ethiopian pepper. The amount of gas produced by the substrate with different additives could be used to predict a ranking of DMI intake (Blummel *et al*., 2005). According to the *in vitro* results observed in this study, it could be predicted that ruminant animals fed substrate with 1 or 2% spice additive might have low DMI because of the low volume of *in vitro* gas production (IVGP) observed. However, it is important to note that either spice or tetracycline inclusion did not affect VFA production, while the benefit of methane gas (a greenhouse gas) reduction, is of great importance to preserve our climate.

CONCLUSION AND RECOMMENDATION

Spices such as Grain of Paradise (*Aframomum melegueta*), can be used as growth promoter to replace Tetracycline at 2% inclusion and to reduce the effect of enteric methane production by ruminants. This might solve the problem of residual effects of Antimicrobials in meat produced by ruminants for human consumption. It is recommended that further research be carried out using live animals to confirm the response of animals to the replacement of this spice (Grain of Paradise- *Aframomum melegueta*) as a growth promoter feed additive (1 – 2% inclusion) with tetracycline.

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