

Effects of *Petiveria alliacea* (guinea hen weed) leaf extract on fermentation parameters, nutrient digestibility and faecal worm egg count in growing West African dwarf goats

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Abstract

Medicinal plants are used in animal feeding as natural antimicrobial to improve nutrient utilisation and health status of animals. This study assessed the effects of varying concentration of *Petiveria alliacea* leaf extract (PLE) on fermentation parameters, nutrient digestibility, nitrogen utilisation and faecal worm egg count of growing West African dwarf goats. Twenty-four (24) growing West African dwarf bucks with average body weight of 8.50 ± 0.55 kg were divided into four treatment groups with six bucks each in a completely randomized design. Each treatment group were administered varying concentrations (0, 2, 4 and 6 %) of PLE at 5 ml per animal and day and fed wilted *Panicum maximum* as basal feed and concentrate supplement. Results showed that oral administration of varying concentrations of PLE did not significantly ($p > 0.05$) influence ammonia-N, pH and total volatile fatty acids concentration of the rumen fluid of the experimental goats. Dry matter intake, weight gain and dry matter digestibility were also not affected ($p > 0.05$) by administration of PLE. Crude protein, NDF and ADF digestibility were higher ($p < 0.05$) in goats on 0 and 2 % concentrations and lower in those administered 4 and 6 % concentrations. Urinary N and total N excretion values were lowest in goats administered 6 % concentration of PLE. Nitrogen retained (% of N intake) was highest ($p < 0.05$) in goats administered 4 and 6 % concentration of PLE. Highest reduction (55.6 %) in faecal worm egg count was obtained at 6 % concentration of PLE. *Petiveria alliacea* leaf extract could be administered to West African dwarf goats at 4 % concentration for increased nitrogen retention and at 6 % concentration as anthelmintic.

Keywords: Anthelmintic, feed additive, livestock, medicinal plants, secondary compounds.

1 Introduction

Medicinal plants have received attention in recent years as feed additive in livestock feeding to replace antibiotics, which usage has been restricted or outrightly banned in some countries. Due to this, alternatives have been sought to synthetic drugs mainly from natural sources, especially from plants. Additives from plants are considered to be safe and cheaper. According to Atata *et al.* (2003), plants with medicinal property have made traditional medicine cheaper than orthodox medicine. Some natural products such as probiotics, dicarboxylic acids, plant extracts and exogenous

enzymes have been identified as alternatives to antibiotics feed additives (Joany & Morgavi, 2007). Pattayanak *et al.* (2010) reported that herbal preparations have been identified as valuable alternatives to antibiotics and are currently being adopted in many parts of the world for treating diseases. Medicinal plants have been found to contain secondary compounds also called secondary metabolites or phytochemicals such as tannins, saponins, glycosides and essential oils which can be used to improve digestibility, nutrient absorption, and health status by elimination of pathogens in the gut and overall performance of the animal. Phytochemicals are natural growth promoters incorporated into animal feed to enhance productivity (Gadde *et al.*, 2017). According to Abdurazak *et al.* (2000) the use of browse plants contain-

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ing secondary compounds as feed supplement for ruminants to improve their performance is gaining wide acceptance in many parts of the tropics. Phenolic compounds have been reported to exert strong anthelmintic activity (Feireira *et al.*, 2013). Tannins may have beneficial effects which include anthelmintic property, direct reduction of abdominal and intestinal infections and provision of by-pass protein for absorption in the intestine (Njidda & Ikhimiyoia, 2010).

Petiveria alliacea is a very important plant in traditional Latin America herbal medicine where it is used as an anti-rheumatic and anti-inflammatory plant, to treat fever headache, diabetes, malaria, arthritis, skin allergies, and cancer and to induce abortion (Perez-Leal *et al.*, 2006). According to Lopes-Martins *et al.* (2002) *P. alliacea* is a plant with sudorific, anti-venera, sedative, anthelmintic, emmenagogue, anaesthetic and depurative properties. It contains some phytochemical compounds like flavonoids, terpenoids and benzenoids which are commonly identified in the plant (Silva *et al.*, 2018) and it is rich in Sulphur-containing compounds that possess a broad-spectrum of *in vitro* microbial activity against pathogenic fungi and bacteria at low concentrations (Guedes *et al.*, 2009). An essential oil called Petiverin has also been reported to be found in the leaf and stem of *P. alliacea* (Kubec & Musa 2001; Luz *et al.*, 2016). Extract and meal of *P. alliacea* leaf and root have been investigated in diets of laying hens and broiler chickens (Sobayo *et al.*, 2017; Sobayo *et al.*, 2018; Oyeleke *et al.*, 2021a; Oyeleke *et al.*, 2021b) and on rumen fermentation and methane production *in vitro* (Adebayo *et al.*, 2021). There is a dearth of information on the effects of *P. alliacea* on nutrient utilisation and health of ruminants. Hence, this study seeks to investigate the effects of oral administration of *P. alliacea* leaf extract (PLE) on rumen fermentation parameters, dry matter intake, nutrient digestibility, nitrogen retention, and faecal worm egg count in West African dwarf goats.

2 Materials and methods

2.1 Study area

The study was carried out at the Small Ruminant Unit, Directorate of University farms, Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State, Nigeria. The University is located in the derived savannah vegetation zone of South West, Nigeria and lies on latitude 7°13'28" N and longitude 3°25'2" E with elevation of 127 m and altitude of 75 m above sea level (Google Earth, 2018). The area has a mean annual rainfall of 1,037 mm, a mean ambient temperature of about 34.7 °C and an average yearly humidity of 83 % (Metrological Station, Water Resources Management and Agro-meteorology Department, FUNAAB).

2.2 Collection and processing of *Petiveria alliacea* (guinea hen weed)

Collection and processing of *P. alliacea* was as reported by Adebayo *et al.* (2021); *P. alliacea* was harvested in a nearby village around the University. The leaves were plucked at the petiole and air dried to a constant moisture level. The dry leaves were milled to pass through a 1 mm sieve. Extraction was by a modification of the method described by Imaga & Bamigbetan (2013). About 100 ml of hot water was added to 0, 2, 4, and 6 g of the leaf powder in separate jars to obtain 0, 2, 4 and 6 % concentrations of the extract. The solution was allowed to stand for 20 minutes and thereafter sieved with Whatmann NO.1 filter paper to obtain a clear solution. The resulting different concentrations were stored in the refrigerator pending usage. The preparation was done weekly for daily use. The secondary compounds in *P. alliacea* as reported by Adebayo *et al.* (2021) are presented in table 1.

Table 1: Secondary compounds (%) in *Petiveria alliacea* at varying concentration.

Parameters	Concentration of <i>P. alliacea</i> leaf extract		
	2 g/100 mL	4 g/100 mL	6 g/100 mL
Tannin	0.0075	0.0143	0.0199
Phenol	0.0030	0.0032	0.0034
Oxalate	0.0027	0.0028	0.0030
Phytate	0.0017	0.0016	0.0016
Trypsin inhibitor	0.0101	0.0139	0.0175
Flavonoid	0.0187	0.0294	0.0414
Cyanogenic glycoside	0.000015	0.000022	0.000034
Alkaloid	0.12	0.20	0.26
Saponin	1.15	1.91	2.08

Source: Adebayo *et al.* (2021)

2.3 Experimental animals, management, and diets

Twenty-four West African dwarf bucks with an average weight of 8.50 ± 0.55 kg were purchased from villages around the University and kept in quarantine section for two weeks to monitor their health. They were maintained on wilted *Panicum maximum* and concentrate, and clean water was supplied *ad libitum*. Thereafter, they were divided into four treatment groups on weight equalisation basis and housed in individual pens. The study was divided into 2 weeks of adaptation, 10 weeks of feeding trial and 2 weeks of nutrient digestibility study. The weight of animals was taken on the first day of the experiment and fortnightly thereafter. The animals were fed 60 % wilted *P. maximum* as the basal diet and 40 % concentrate supplement at 4 % of their body

weight. The *P. maximum* was cut daily from an established pasture (which was cut back to 15 cm above ground level) after 8 weeks of re-growth and allowed to wilt for 24 hours before being fed to the animals. The concentrate diet was formulated to contain approximately 14 % crude protein with the following ingredients: Wheat offal-30 %, maize bran-27 %, palm kernel cake-20 %, rice bran -20 %, bone meal -2 % and salt-1 %. The feeds were offered in separate feeders and the animals were fed twice daily. Five millilitres (5 ml) of the varying concentrations (0, 2, 4 and 6 %) of *P. alliacea* leaf extract (PLE) were administered orally via syringe every morning to the four treatment groups respectively.

2.4 Apparent nutrient digestibility and nitrogen utilisation

This study was carried out at the end of the 70 days feeding trial for a 14-day period. All animals were transferred to a well disinfected individual metabolic cage. Known quantity of feed was offered and water was provided *ad libitum*. The first seven days was for adjustment and adaptation of the animals to the metabolic cage and the last 7 days was for collection of samples. Daily feed intake was calculated by deducting feed refusal from feed offered. Daily faecal output for each animal was collected and weighed fresh and 10 % of each day collection for each animal was dried in the oven at 60 °C to constant weight. At the end of the study, faecal samples from each animal were milled, pooled and stored pending analysis. Urine sample from each animal was also measured and 10 % aliquot was retained daily, bulked for each goat and stored in the freezer at -4 °C for subsequent analysis. Urine samples were collected in bottles containing 2 ml of 10 % H₂SO₄ solution to prevent ammonia-N loss and maintained pH below 3.0 (Chen & Gomez, 1992). Urine samples were analysed for nitrogen content and consequently nitrogen intake, nitrogen output and nitrogen retention (g day⁻¹) were calculated.

$$\text{digestibility (\%)} = \frac{\text{nutrient intake} - \text{faecal nutrient}}{\text{nutrient intake}} \times 100$$

$$\text{nitrogen absorbed} = \text{nitrogen intake} - \text{faecal nitrogen}$$

$$\text{nitrogen retained} = \text{nitrogen intake} - (\text{faecal nitrogen} + \text{urinary nitrogen})$$

2.5 Rumen fluid analysis

Rumen fluid was collected on the last day of nutrient digestibility study to determine total volatile fatty acid production and ammonia nitrogen concentration. Approximately 30 ml of rumen fluid was collected from each goat 6 hours after morning feeding and immediately after collection pH

was measured using a portable pH meter (HANNA instruments HI 98153) and thereafter the fluid was freed from coarse particles by filtration through four-layers of cheese cloth. One half of the fluid was acidified with few drops of concentrated H₂SO₄ and stored frozen at -20 °C for the determination of ammonia nitrogen concentration using steam distillation procedures (Ogubai & Sereke, 1997). The second half was stored frozen at -20 °C for the determination of total volatile fatty acid (TVFA) concentration using Markham apparatus as described by Barnett & Reid (1956). This was carried out by adding 2 ml of rumen fluid together with 1 ml 10 % potassium oxalate buffer and 1 ml oxalic acid injected into the Markham apparatus, where a distillate of 100 ml was collected. This was then titrated against a standard 0.01N NaOH with 2 drops of phenolphthalein as indicator. Concentration of TVFA was then calculated using the following equation:

$$\text{TVFA (mM)} = \frac{(\text{NaOH volume} \times \text{NaOH normality} \times 1000)}{\text{rumen inoculum volume}}$$

2.6 Faecal worm egg count determination

Faecal samples of about 4 g were collected directly from the rectum of each goat at the beginning of the experiment and at two-week intervals for identification of helminth eggs using floatation techniques (Ameen *et al.*, 2010). Three grams (3 g) of each faecal sample was ground and mixed with 42 mL of water. A saturated solution was poured into the mixture of faeces and water to float the eggs following the modified McMaster method (Miller *et al.*, 1998). Sample obtained from this was collected and put into both compartments of McMaster counting chamber/slide and then viewed under the microscope. The number of eggs obtained within each viewed area was multiplied by 100 to get the actual number of egg per gram.

2.7 Chemical analyses

Oven dried feed and faecal samples were analysed for their proximate composition according to AOAC (2000). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin were determined according to Van Soest *et al.* (1991).

2.8 Statistical analysis

Data obtained were subjected to one-way analysis of variance in a completely randomized design using version 9.1 of SAS software (SAS 2003) with the following model:

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

where Y_{ij} = observed variation, μ = population mean, T_i = effect of varying doses of leaf extract and e_{ij} = error term. Means were separated using Duncan's procedure of the same software.

3 Results

Table 2 shows the chemical composition of concentrate diet and *P. maximum*. The dry matter and crude protein content of the concentrate diet (93.12 % and 14.33 %) were higher than 74.35 % and 7.44 % obtained for *P. maximum*. However, neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) values of the concentrate diet (50.67 %, 25.33 % and 16.67 %) were lower than that of *P. maximum* (60.00 %, 43.67 % and 38.67 %).

Table 2: Chemical composition (%) of concentrate supplement and Panicum maximum.

Parameters	Concentrate supplement	Panicum maximum
Dry matter	93.1	74.4
Crude protein	14.3	7.4
Crude fibre	11.4	34.3
Ether extract	3.7	7.7
Ash	10.5	10.0
Nitrogen free extract	60.1	40.5
Neutral detergent fibre	50.7	60.0
Acid Detergent fibre	25.3	43.7
Acid Detergent lignin	16.7	38.7

Presented in table 3 are the fermentation parameters, dry matter intake, weight gain and apparent nutrient digestibility values of the experimental animals. Significant differences ($p < 0.05$) were observed in crude protein, ash, NDF and ADF digestibility. Crude protein, NDF and ADF digestibility were higher in goats receiving no extract and those on 2 % concentration of PLE while lower values were observed on goats receiving 4 and 6 % concentration of PLE. Higher ash digestibility values were recorded for goats administered varying concentrations of PLE and lower values were obtained for goats receiving no extract.

Table 4 shows the nitrogen balance and faecal worm egg count of West African dwarf goats administered varying concentration of PLE. All the parameters measured were significantly ($p < 0.05$) influenced by PLE administration except nitrogen intake and faecal nitrogen. Urinary nitrogen and total nitrogen excreted were lowest in goats administered 6 % concentration of PLE. However, nitrogen retained (g day^{-1}) was lower in goats receiving no PLE

and higher in those administered 4 and 6 % concentration of PLE. Faecal worm egg count reduction was highest (55.56 %) in goats administered 6 % concentration of PLE and lowest (16.67 %) in goats receiving no PLE.

4 Discussion

The dry matter composition of the concentrate supplement (93.1 %) and *Panicum maximum* (74.4 %) is an indication of high nutrient density. The CP content of concentrate supplement (14.3 %) was higher than that of *P. maximum* (7.4 %). The concentrate supplement was formulated to meet the CP requirement of growing goats as recommended by (NRC, 1981). The CP of *P. maximum* could only meet the maintenance requirement of the animals. According to Pugh (2020), 7 % CP is required for maintenance of mature healthy animals while higher level of dietary CP is required for growth, gestation and lactation. Concentrate feed are usually formulated for ruminants to meet up with the inadequacy of the basal roughage diet. Fibre fractions are sources of energy for ruminants and they are also necessary for proper rumen function. The neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) values obtained for *P. maximum* and concentrate supplement were low to moderate for ruminants. These values were indicative of higher digestibility of the diet. Isah *et al.* (2015) attributed higher digestibility of a diet to low fibre concentrations. Sha Du *et al.* (2016) also reported a negative correlation between dry matter digestibility and fibre fractions of feed, that is, the higher the fibre fractions the lower the dry matter digestibility and vice versa. According to Okunade *et al.* (2014) fibre plays an important role in voluntary intake and digestibility. The NDF of *P. maximum* and concentrate supplement was below $650 \text{ g kg}^{-1} \text{ DM}$ (65 %) threshold level at which cell wall inhibits feed intake, digestibility and animal performance (Meissner *et al.*, 1991).

The decrease in crude protein, NDF and ADF digestibility in goats administered 4 and 6 % concentration of *P. allicea* leaf extract (PLE) may be attributed to secondary compounds in PLE which were able to exert their effect at those levels. This result confirms the report of an *in vitro* study by Adebayo *et al.* (2021). The higher digestibility obtained at 0 and 2 % concentration may suggest none availability or lower content of secondary metabolites in the diets of the animals. *P. allicea* leaf extract at 4 and 6 % concentration was able to inhibit microbial activity hence reduced CP, NDF and ADF digestibility. Antinutritional factors form a more complex structure which may decrease protein digestibility (Butts *et al.*, 2012; Lowery *et al.*, 2012). Gurbuz *et al.* (2006) and McSweeney *et al.* (2001) reported that the effects

Table 3: Fermentation parameters, dry matter intake, weight gain and apparent nutrient digestibility of growing West African dwarf goats administered *Petiveria alliacea* leaf extract.

Parameters	Concentrations of <i>Petiveria alliacea</i> leaf extract (PLE)				SEM	P value
	0 %	2 %	4 %	6 %		
<i>Fermentation parameters</i>						
pH	6.2	6.1	6.3	6.3	0.07	0.38
Ammonia-N (mg dl ⁻¹)	26.4	30.6	29.5	23.8	1.99	0.75
Total volatile fatty acids (mM)	44.1	49.7	45.6	46.3	1.48	0.84
<i>Dry matter intake (g day⁻¹)</i>						
<i>Panicum</i>	238.9	226.1	206.6	196.2	7.52	0.09
Concentrate	155.4	148.8	166.6	151.1	5.09	0.15
Total intake	394.3	374.9	373.2	347.3	11.31	0.11
<i>Weight gain (kg)</i>						
Initial weight	9.0	8.6	9.0	8.5	0.16	0.56
Final weight	11.9	11.5	12.0	11.5	0.07	0.55
Total weight gain	2.9	2.9	3.0	3.0	0.01	0.27
Daily weight gain (g)	41.4	42.0	42.1	42.1	0.12	0.05
<i>Digestibility (%)</i>						
Dry matter	79.2	78.0	77.0	78.7	0.66	0.82
Crude protein	72.3 ^a	68.1 ^a	63.4 ^b	61.6 ^b	3.03	0.04
Ether extract	58.9	61.7	61.5	59.4	1.29	0.87
Ash	60.8 ^b	69.8 ^a	68.9 ^a	69.5 ^a	2.55	0.04
Neutral detergent fibre	70.1 ^a	66.1 ^a	58.3 ^b	61.5 ^b	3.55	0.03
Acid detergent fibre	48.5 ^a	51.7 ^a	44.0 ^b	39.5 ^b	3.39	0.03

^{ab} Means on the same row with different superscripts are significantly different ($p < 0.05$)

Table 4: Nitrogen balance and faecal worm egg count of growing West African dwarf goats administered *Petiveria alliacea* leaf extract (PLE).

Parameters	Concentrations of <i>P. alliacea</i> leaf extract				SEM	P value
	0 %	2 %	4 %	6 %		
<i>Nitrogen utilisation</i>						
N-intake (g day ⁻¹)	10.9	11.1	11.1	9.5	0.42	0.99
Faecal-N (g day ⁻¹)	1.4	1.4	1.3	1.3	0.08	0.86
Urinary-N (g day ⁻¹)	5.0 ^a	3.7 ^b	3.5 ^b	2.4 ^c	0.42	0.01
Total N-excreted (g day ⁻¹)	6.4 ^a	5.1 ^b	4.8 ^b	3.7 ^c	0.42	0.04
N absorbed (g day ⁻¹)	9.5 ^a	9.7 ^a	9.8 ^a	8.2 ^b	0.32	0.04
N retained (g day ⁻¹)	4.5 ^b	6.0 ^a	6.3 ^a	5.8 ^a	0.48	0.04
N retained (% of N intake)	40.9 ^c	52.3 ^b	56.9 ^a	60.1 ^a	3.96	0.03
<i>Faecal egg count</i>						
Initial faecal egg count (eggs g ⁻¹)	200. ^b	133.0 ^c	166.7 ^b	300.0 ^a	49.94	0.004
Final faecal egg count (eggs g ⁻¹)	166.7 ^a	100.0 ^b	100.0 ^b	133.3 ^b	22.89	0.04
Reduction in faecal egg count (%)	16.7 ^d	25.0 ^c	40.0 ^b	55.6 ^a	9.53	0.001

^{abcd} Means on the same row with different superscripts are significantly different ($p < 0.05$)

of tannin are associated with their ability to combine with dietary proteins, cell wall polymers such as cellulose, hemicellulose and pectin as well as minerals thus either retarding or preventing their microbial digestion. According to Jemiseye *et al.* (2019) tannin render protein unavailable for digestion

in the rumen due to formation of complexes thereby decreasing rumen ammonia concentrations and inhibiting fermentation of structural carbohydrates. Decreased feed digestibility had been reported with phytochemical additives (Aderao *et al.*, 2018; Joch *et al.*, 2019). Reduced CP digestibility may

suggest that there will be an increase in available protein at the lower gut for enzymatic digestion and absorption. Protein digested and absorbed in the lower gut as amino acids is utilized by the animals for productive purposes (growth, milk production and so on).

The reduction observed in urinary-N, total N excreted and N-absorbed in goats administered 6% PLE can be attributed to secondary compounds in PLE, which were able to reduce degradation of protein to ammonia-N by microbes in the rumen thus its availability in the lower tract for productive purposes. Reduced urinary-N excretion suggests reduced ruminal N degradability of the diet possibly due to protective action of secondary compounds on protein in the rumen. Plants containing tannin and saponin have been reported to reduce ammonia production in the rumen (Patra & Saxena, 2010; Bodas *et al.*, 2012) and invariably urinary-N. The trend observed in this study complement the findings of Mahgoub *et al.* (2008) that animals fed higher levels of tannins tend to produce higher levels of protein in the faeces and lower nitrogen levels in the urine. Positive N balance was obtained for all the animals in the treatment groups. An indication of adequate nutrient levels in the diets to meet the protein requirement of the animals. Nitrogen retention is the proportion of nitrogen utilized by animals from the total nitrogen intake for body processes (Saka *et al.*, 2020). Reduced N excretion and greater N retention recorded at 4 and 6% concentrations indicate superior protein metabolism, availability and utilisation by the goats. Alves *et al.* (2014) asserted that greater N retention may indicate better utilisation of dietary N and possibly higher muscle deposition and weight gain. The weight gain of animals at 4 and 6% concentration was numerically higher than at other concentrations. This implies that administration of PLE at 4 and 6% concentration may improve weight gain in animals.

The highest reduction obtained in faecal worm egg count at 6% concentration could suggest that the concentration of secondary compounds at the level was more sufficient in eliminating the worms than other concentrations. Anthelmintic activity of root and stem extract of *P. alliacea* has been reported (Lopez-Martins *et al.*, 2002; Flota-Burgos *et al.* 2017; Rosado-Aguilar *et al.*, 2020). These authors also attributed the anthelmintic activity to the presence of secondary compounds in the plant. Tannins have been reported to have effect on internal parasites (Butter *et al.*, 2000; Khan & Diaz-Hernandez, 2000) and provide by-pass-protein for absorption in the intestine (Njidda & Ikhimioya, 2010). Anthelmintic activity of phenols have also been reported (Feireira *et al.*, 2013, Udoha *et al.*, 2015). The results obtained from the study indicates that PLE could be used as anthelmintic for goats at 6% concentration. This will reduce

the cost of production as it will eliminate the purchase of anthelmintics by poor farmers in developing countries and also solve the problem of resistance of worms to some conventional anthelmintics. The overall effect will be increased productivity and high economic gain to farmers. Diseases caused by helminths in small ruminants in the tropics and subtropics have been reported to result in poor growth (Mini, 2012), lower productivity (Ombasa *et al.*, 2012), mortality (Mohammed *et al.*, 2013) and high economic losses (Alemu *et al.*, 2014). *P. alliacea* is within the reach of farmers as the shrub is commonly found in most farms and bushes in South-West Nigeria. Camargo (2007) reported that *P. alliacea* is a wild and perennial shrub that grows in Africa and tropical America.

5 Conclusions

Extract of *P. alliacea* leaves improved nitrogen retention in goats at 4% concentration but with highest reduction in faecal worm egg count at 6% concentration. This implies that for increased nitrogen retention and invariably weight gain the extract can be administered to goats at 4% concentration but as anthelmintic it can be administered at 6% concentration. Further investigation is recommended to determine its effect on blood profile, carcass/meat quality and immune response of the animals.

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Conflict of interest

The authors declare that they have no conflict of interest.

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