

**ORIGINAL RESEARCH ARTICLE****The effect of replacement of molasses with pineapple waste in multi-nutrient block on *in vitro* gas production and digestibility****\*Isah, O. A. and Kojeku, F. T***Federal University of Agriculture, Abeokuta, Nigeria**\*Correspondence author email: [bukolaisah13@gmail.com](mailto:bukolaisah13@gmail.com)***ABSTRACT**

*Pineapple waste can be a good replacement for molasses being easily sourced for; relatively cheaper among other nonconventional agro-industrial wastes and does not compete with man with man and other farm animals as source of feed. Thus, the study was carried out to evaluate the effect of replacement of molasses with pineapple waste in multi-nutrient block on *in vitro* gas production and digestibility. Four (4) different blocks in a completely randomized design (CRD) consisting of four (4) replacement levels of molasses with pineapple wastes (0%, 10%, 20%, 30%) were formulated thus: 0%P/30%M, 10%P/20%M, 20%P/10%M, 30%P/0%M respectively. Proximate composition of blocks was determined. 0.2g of feed block samples were incubated using rumen liquor from West African dwarf sheep as inoculum. Gas production at 3 hours intervals between 0 and 48 hours of incubation periods were recorded. Post incubation dry matter digestibility, organic matter digestibility, metabolizable energy, methane and short chain fatty acids were estimated at 24 hour period. Results showed that multi-nutrient blocks with 30% pineapple waste had highest ( $p < 0.05$ ) content of dry matter (49.60%), and ether extract (22.33%) but lowest ( $P < 0.05$ ) crude protein content. The gas production increased as incubation period progressed. Significant differences were observed ( $P < 0.05$ ) in gas production after 6 hours post incubation. Results showed that the *in vitro* methane production, metabolisable energy and organic matter digestibility reduced ( $P < 0.05$ ) at 20% and 30% levels of pineapple waste inclusion. Short chain fatty acid (SCFA) (0.12mmol), reduced at 30% level of inclusion. It is evident that replacement of molasses with pineapple wastes, as much as 20% might improve performance of the West African dwarf goats and reduce enteric methane gas production when feed block is offered.*

**Keywords:** Molasses, Pineapple waste, Nutrient digestibility West African dwarf sheep**INTRODUCTION**

Provision of adequate good quality feed to livestock to obtain an optimal performance is a major challenge all over the world. Ruminants are mostly fed on low quality roughages, which are poor in protein, energy, minerals and vitamin contents. The principles of improving the use of poor quality roughages by ruminants include supplementation with fermentable nitrogen and minerals which could by-pass the rumen and this could be in form of multi-nutrient blocks (Habib *et al.*, 1991). The pineapple solid waste from pineapple processing is estimated to be 40-50% from fresh fruit as pineapple peels and core (Buckle, 1989). Pineapple waste contains high concentration of biodegradable organic material and suspended solid (Correia *et al.*, 2004). Pineapple waste is basically composed of residual pulp, peel, and cores. Pineapple waste could be a good replacement for molasses as it could easily be sourced, cheaper and does not compete with man and other farm animals as source of feed. Information on the replacement value of molasses with pineapple waste in multi-nutrient feed block is scarce.

The study therefore aimed at evaluating the effect of replacement of molasses with pineapple waste at various levels of inclusion, on *in vitro* gas production and degradation parameters of multi-nutrient.

**MATERIALS AND METHODS****Experimental site, ingredient and preparation of multi-nutrient block**

This study was carried out at the analytical laboratory of the Department of Animal Nutrition, College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta. The location is situated in the rainforest zone of south-western Nigeria on Latitude 7°13', 49.66°N, Longitude 3°26'11.98"E and altitude 98m above sea level. The climate is humid with mean annual rainfall of 1003mm, annual mean temperature range of 31.9 to 34.8°C and humidity of 79.7 to 90.1% (Google earth, 2010). Four different multi-nutrient blocks (MNB) were made following the protocol of Molina-Alcaide *et al.* (2008) with little modification. Four replacement levels of molasses with pineapple wastes (0%, 10%, 20%, 30%) were formulated to

Table 1: Percentage composition of the experimental feed blocks (%)

| Ingredients                | 0%P/30%M | 10%P/20%M | 20%P/10%M | 30%P/0%M |
|----------------------------|----------|-----------|-----------|----------|
| Molasses                   | 30.00    | 20.00     | 10.00     | 0.00     |
| Pineapple waste            | 0.00     | 10.00     | 20.00     | 30.00    |
| Urea                       | 5.00     | 5.00      | 5.00      | 5.00     |
| Limestone powder           | 10.00    | 10.00     | 10.00     | 10.00    |
| <i>Chromolaena odorata</i> | 10.00    | 10.00     | 10.00     | 10.00    |
| PKC                        | 15.00    | 15.00     | 15.00     | 15.00    |
| Wheat offal                | 25.00    | 25.00     | 25.00     | 25.00    |
| Oyster shell               | 4.00     | 4.00      | 4.00      | 4.00     |
| *Premix                    | 0.50     | 0.50      | 0.50      | 0.50     |
| Common salt                | 0.50     | 0.50      | 0.50      | 0.50     |
| Total                      | 100.00   | 100.00    | 100.00    | 100.00   |

0%P/30%M = 0% of pineapple wastes with 30% of molasses

20%P/10%M = 20% of pineapple wastes with 10% of molasses

10%P/20%M = 10% of pineapple wastes with 20% of molasses.

30%P/0%M = 30% of pineapple wastes with 0% molasses.

\* Premix: Vitamin A (750000IU), Vitamin D3 (100000IU), Vitamin E (1800mg), Vitamin B1(500mg), Vitamin B2 (1000mg), Vitamin D-Pantothenic acid (3200mg), Vitamin B6 (180mg), Vitamin B12 (5mg), Vitamin C (5000mg), Vitamin K (700mg), Nicotinic acid (4000mg), Folic acid (50mg), Choline chloride (63000mg), Manganese (35000mg), Cu (1500mg), Cobalt (180mg), Iron (10000mg), Iodine (720mg), Zinc(1500mg)

contain 0% pineapple with 30% molasses (0%P/30%M), 10% pineapple with 20% molasses (10%P/20%M), 20% pineapple with 10% molasses (20%P/10%M) and 30% pineapple with 0% molasses (30%P/0%M). Pineapple waste sourced from FUMAN Plc, Apata Ibadan, Oyo state (Pulp and core alone) was used to replace sugarcane molasses sourced from Dangote sugar industry, Apapa Lagos at 0, 10, 20 and 30 % level of inclusion. Other ingredients used were urea, wheat offal, palm kernel cake, salt, and premix and limestone powder (Table 1). Known quantity of wet pineapple waste was properly crushed in a mortar and thoroughly mixed with wheat offal, premix and palm kernel cake in a bowl. Limestone powder was dissolved in water (400 mL/100g) in another separate container. Known quantity of urea and salt were also dissolved in another 400 mL of water after which molasses was added and thoroughly mixed. The resulting mixture (water, salt, urea and molasses) was added to the first mixture (Pineapple waste, wheat offal, palm kernel cake and premix) and stirred thoroughly. Limestone/water mixture was also added and mixed thoroughly. Another 200 mL of water was added to the mixture. Plastic molds were used in casting the multi-nutrient blocks. Each separate mixture was compressed into the block molds. The feed blocks were made on the floor under a roofed open sided building. The molds were removed and blocks were air-dried at room temperature for 3 – 4 days and stored until needed. About 50g each of multi-nutrient blocks was sampled pulverized and ground for proximate composition and fibre analysis.

### ***In vitro* gas production procedure**

Two hundred milligrams each of ground multi-nutrient block samples were weighed into 100mL calibrated syringes with pistons lubricated with vaseline. A buffered mineral solution was prepared consisting of  $\text{NaHCO}_3 + \text{Na}_2\text{HPO}_4 + \text{KCl} + \text{NaCl} + \text{MgSO}_4 \cdot 7\text{H}_2\text{O} + \text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  (1:4, v/v) and stirred at  $39 \pm 1^\circ\text{C}$  under continuous flushing with carbon dioxide. Rumen fluid was collected from three goats that were previously fed with concentrate (20 % maize, 20 % corn bran, 25 % wheat offal, 20 % Palm kernel cake, 10% ground nut cake, 4 % oyster shell, 0.5 % common salt, 0.25 % fish meal and 0.25 % grower premix). The liquor was collected into a pre-warmed thermos flask and was later filtered through three layers of cheesecloth with carbon dioxide flushing. About 30 mL of buffered rumen fluid was taken into syringes containing the samples. The syringes were placed in a water bath pre-heated at  $39^\circ\text{C}$ . The samples were shaken every 30 minutes. Gas production rates were recorded at 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39, 42, 45 and 48 hour of incubation and each syringe was gently swirled after reading.

Samples incubated for 24 hours were analysed to determine methane production such that 4mL of 1.0N NaOH (Sodium hydroxide) was introduced into the incubation syringe through the silicon tube fixed at the tip of 5mL syringe capacity that contained the 4mL NaOH. The clip was carefully unscrewed and reagent introduced. The content was agitated while syringe plunger began to shift position to occupy the vacuum created by the absorption of  $\text{CO}_2$ . The volume of methane was read on the calibration. After 48 hours of incubation, samples were centrifuged (Model 90 -1) at

Table 2: Proximate composition of the experimental feed blocks (%)

| Parameters    | 0%P/30%M            | 10%P/20%M           | 20%P/10%M           | 30%P/0%M           | SEM  |
|---------------|---------------------|---------------------|---------------------|--------------------|------|
| Dry matter    | 32.18 <sup>d</sup>  | 37.15 <sup>c</sup>  | 43.19 <sup>b</sup>  | 49.60 <sup>a</sup> | 2.01 |
| Crude protein | 23.57 <sup>a</sup>  | 21.93 <sup>ab</sup> | 19.83 <sup>bc</sup> | 19.10 <sup>c</sup> | 0.62 |
| Ether extract | 17.50 <sup>bc</sup> | 13.83 <sup>c</sup>  | 20.50 <sup>ab</sup> | 22.33 <sup>a</sup> | 1.12 |
| Ash           | 30.83               | 30.33               | 31.00               | 31.83              | 0.37 |
| Crude fibre   | 3.83                | 5.17                | 4.50                | 5.33               | 0.26 |
| NDF           | 36.67 <sup>c</sup>  | 47.33 <sup>b</sup>  | 52.00 <sup>b</sup>  | 53.33 <sup>a</sup> | 2.09 |
| ADF           | 30.67 <sup>b</sup>  | 32.67 <sup>b</sup>  | 33.33 <sup>b</sup>  | 38.67 <sup>a</sup> | 1.11 |
| ADL           | 7.33 <sup>ab</sup>  | 8.67 <sup>a</sup>   | 6.00 <sup>a</sup>   | 10.00 <sup>a</sup> | 0.56 |

<sup>abc</sup> means on the same row having different superscript were significantly (P<0.05) different.

0%P/30%M = 0% of pineapple wastes with 30% of molasses

10%P/20%M = 10% of pineapple wastes with 20% of molasses.

20%P/10%M = 20% of pineapple wastes with 10% of molasses

30%P/0%M = 30% of pineapple wastes with 0% molasses.

NDF = Neutral detergent fibre

ADF = Acid detergent fibre

ADL = Acid detergent lignin

the speed of 2300 rpm for 10 minutes. The residue was washed with warm water until the rinse water was plain. At the end of washing, samples were dried at 65°C and the weight of the oven-dried residues was recorded. The metabolisable energy (MJ/Kg DM), organic matter digestibility (OMD %), short chain fatty acid, dry matter digestibility were estimated from the volume of gas produced after 24 hour of incubation. Metabolizable energy (ME, MJ/kg DM) was calculated as  $ME = 2.20 + 0.136 \text{ GV} + 0.057 \text{ CP} + 0.0029 \text{ CF}$  (Menke and Steingass, 1988). Organic matter digestibility (OMD%) was assessed as  $OMD = 14.88 + 0.889 \text{ GV} + 0.45 \text{ CP} + 0.651 \text{ XA}$  (Menke *et al.*, 1979). Short chain fatty acids (SCFA) as  $SCFA = 0.0239 \text{ GV} + 0.0601 \text{ XA}$  (Getachew *et al.*, 1995). Where GV, CP, CF and XA are total gas volume, crude protein, crude fibre and ash respectively

of the incubated samples. *In vitro* dry matter digestibility (%) was calculated as weight of pre - incubated sample minus weight of post - incubated sample divided by weight of pre - incubated sample multiplied by 100. Feed block samples were also analysed for Proximate composition (AOAC, 1995), fibre fraction (Van Soest, 1991).

### Statistical analysis

Data were analyzed by the analysis of variance using the General Linear model procedures of SAS (1999). Treatment means were compared using Duncan Multiple range F-test (1955).

Table 3: *In vitro* gas production of multi-nutrient block (MNB) with pineapple wastes as replacement for molasses

| Incubation (hr) | 0%P/30%M            | 10%P/20%M          | 20%P/10%M           | 30%P/0%M           | SEM  |
|-----------------|---------------------|--------------------|---------------------|--------------------|------|
| 3.00            | 1.33                | 2.00               | 2.00                | 1.00               | 0.26 |
| 6.00            | 5.67                | 6.33               | 4.00                | 3.33               | 0.53 |
| 9.00            | 10.00 <sup>ab</sup> | 11.33 <sup>a</sup> | 7.33 <sup>b</sup>   | 6.67 <sup>b</sup>  | 0.74 |
| 12.00           | 10.67 <sup>ab</sup> | 13.67 <sup>a</sup> | 8.00 <sup>b</sup>   | 6.67 <sup>b</sup>  | 0.96 |
| 15.00           | 12.33 <sup>ab</sup> | 15.33 <sup>a</sup> | 9.67 <sup>bc</sup>  | 7.33 <sup>c</sup>  | 1.11 |
| 18.00           | 13.00 <sup>ab</sup> | 17.00 <sup>a</sup> | 10.00 <sup>ab</sup> | 7.67 <sup>b</sup>  | 1.37 |
| 21.00           | 14.00 <sup>ab</sup> | 19.00 <sup>a</sup> | 11.67 <sup>bc</sup> | 7.67 <sup>c</sup>  | 1.49 |
| 24.00           | 15.67 <sup>ab</sup> | 19.67 <sup>a</sup> | 12.33 <sup>ab</sup> | 7.67 <sup>b</sup>  | 1.62 |
| 27.00           | 16.00 <sup>ab</sup> | 20.67 <sup>a</sup> | 13.00 <sup>ab</sup> | 8.33 <sup>b</sup>  | 1.67 |
| 30.00           | 16.67 <sup>ab</sup> | 22.00 <sup>a</sup> | 13.67 <sup>ab</sup> | 9.00 <sup>b</sup>  | 1.80 |
| 33.00           | 17.33 <sup>ab</sup> | 22.33 <sup>a</sup> | 14.00 <sup>ab</sup> | 9.00 <sup>b</sup>  | 1.85 |
| 36.00           | 17.67 <sup>ab</sup> | 23.33 <sup>a</sup> | 15.00 <sup>ab</sup> | 9.33 <sup>b</sup>  | 1.89 |
| 39.00           | 18.00 <sup>ab</sup> | 24.67 <sup>a</sup> | 16.33 <sup>ab</sup> | 10.33 <sup>b</sup> | 1.87 |
| 42.00           | 18.33 <sup>ab</sup> | 24.67 <sup>a</sup> | 16.33 <sup>ab</sup> | 10.33 <sup>b</sup> | 1.95 |
| 45.00           | 19.67 <sup>ab</sup> | 25.33 <sup>a</sup> | 18.00 <sup>ab</sup> | 11.33 <sup>b</sup> | 2.03 |
| 48.00           | 19.33 <sup>ab</sup> | 26.00 <sup>a</sup> | 18.00 <sup>ab</sup> | 11.33 <sup>b</sup> | 2.06 |

<sup>ab</sup> means on the same row having different superscript were significantly (P<0.05) different.

0%P/30%M = 0% of pineapple wastes with 30% of molasses

10%P/20%M = 10% of pineapple wastes with 20% of molasses

20%P/10%M = 20% of pineapple wastes with 10% of molasses

30%P/0%M = 30% of pineapple wastes with 0% molasses

Table 4: *In vitro* degradation parameters of multi-nutrient block (MNB) with pineapple waste and molasses

| Parameters          | 0%P/30%M            | 10%P/20%M          | 20%P/10%M           | 30%P/0%M            | SEM  |
|---------------------|---------------------|--------------------|---------------------|---------------------|------|
| Methane(ml/200mgDM) | 14.00 <sup>a</sup>  | 15.00 <sup>a</sup> | 11.00 <sup>b</sup>  | 11.00 <sup>b</sup>  | 0.58 |
| IVDMD (%)           | 76.25 <sup>a</sup>  | 70.75 <sup>c</sup> | 73.00 <sup>bc</sup> | 75.50 <sup>ab</sup> | 0.74 |
| SC FA (mmol)        | 0.31 <sup>a</sup>   | 0.41 <sup>a</sup>  | 0.23 <sup>ab</sup>  | 0.12 <sup>b</sup>   | 0.04 |
| ME(MJ/kg)           | 5.68 <sup>ab</sup>  | 6.13 <sup>a</sup>  | 5.02 <sup>bc</sup>  | 4.34 <sup>c</sup>   | 0.24 |
| OMD (%)             | 59.49 <sup>ab</sup> | 61.98 <sup>a</sup> | 54.95 <sup>bc</sup> | 51.01 <sup>c</sup>  | 1.51 |

<sup>abc</sup> means on the same row having different superscript were significantly (P<0.05) different.

0%P/30%M = 0% of pineapple wastes with 30% of molasses

10%P/20%M = 10% of pineapple wastes with 20% of molasses

20%P/10%M = 20% of pineapple wastes with 10% of molasses

30%P/0%M = 30% of pineapple wastes with 0% molasses

IVDMD = *In vitro* dry matter degradability

SCFA = Short chain fatty acids

ME = Metabolisable energy

OMD = Organic matter digestibility

## RESULTS

### The proximate composition of multi-nutrient feed blocks

Proximate composition of multi-nutrient feed blocks is presented in Table 2. Significant differences were observed (P<0.05) in dry matter, crude protein and ether extract content of the feed blocks. The dry matter content (49.60%) was highest (P<0.05) in feed block containing 30%P/0%M while crude protein of the feed blocks containing 0%P/30%M had the highest value (23.57%) compared with other treatments. Crude fibre values ranged from 3.83% (0%P/30%M) to 5.33% (30%P/0%M). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) increased as the quantity of pineapple waste increased in the block. Ash values ranged from 30.33% (10%P/20%M) to 31.83% (30%P/0%M). Ether extract was significantly higher in the feed blocks containing 30%P/0%M (22.33%) and 20%P/10%M (20.50%) compared with other treatments.

### *In vitro* gas production (mL/200 mg DM) of multi-nutrient feed blocks

The *in vitro* gas production of multi-nutrient blocks (MNB) with pineapple wastes as replacement for molasses is presented in Table 3. The results showed that significant differences (P<0.05) were observed in gas production at all incubation hours except at 3 and 6 hours. The multi-nutrient blocks with 10%P/20%M had the highest value (13.67mL) while those containing 30%P/0%M recorded the lowest value (6.67mL) of gas production. The gas production at 24 hours post incubation period ranged between 19.67 mL (10%P/20%M) and 7.67 mL (30%P/0%M). The differences in gas production from 24 to 48 hours post incubation showed similar trend. The gas production at 3 and 6 hours post incubation ranged from 1.00 to 2.00mL and 3.33 to 6.33mL respectively.

### *In vitro* degradation parameters and methane production of multi-nutrient feed blocks

*In vitro* degradation parameters and methane production of multi-nutrient feed block is shown in Table 4. Significant (P<0.05) differences were observed in the short chain fatty acids, *in vitro* dry matter degradability (IVDMD), methane production, metabolisable energy and organic matter digestibility. The methane production had higher value from feed block containing 0%P/30%M (14.00mL/200g DM) and 10%P/20%M (15.00mL/200g DM) with lower value (11.00mL/200g DM) from feed block containing 20%P/10%M and 30%P/0%M. The lowest short chain fatty acid (0.12μ) was recorded from feed block containing 30%P/0%M. The IVDMD value for the feed blocks containing 0%P/30%M (76.25%) and those containing 30%P/0%M (75.50%) were higher than values of those obtained for 10%P/20%M (70.75%) and 20%P/10%M (73.00%). The multi-nutrient block containing 0%P/30%M and 10%P/20%M had the highest value (5.68 and 6.13MJ/kg respectively) of metabolizable energy, while those containing 30%P/0%M recorded the least value (4.34MJ/kg). Organic matter digestibility result follow similar trend.

## DISCUSSION

Proximate analysis is extensively employed for quick estimation of nutrient potentials of feedstuff (D' Mello, 1992). Dry matter content was highest in the feed block contained 30%P/0%M compared with other treatments, probably due to the high fibre and low moisture content of the pineapple waste (Correia *et al*, 2004). The relatively high crude protein (CP) contents of 23.57 – 19.10% in the feed blocks observed in the present study were well above the 8%-11% recommended by Gatenby (2002) for growing lamb, and minimum CP requirements of 7% was recommended for ruminants in the tropics (Minson, 1980). However, Waruiru (2004) reported lower CP of 6.38. The low crude fibre NDF,

ADF and ADL recorded in feed block containing 0%P/30%M (without pineapple waste inclusion) was in line with the report of Correia *et al.* (2004) that by-product of pineapple processing industry contains high fiber and soluble carbohydrates with low protein contents. Gas production is a reflection of all the nutrients fermented, soluble as well as insoluble; and fractions that were not fermentable do not contribute to gas production. The *in vitro* gas production system helps to better quantify nutrient utilization, and its accuracy in describing digestibility in animals has been validated in numerous experiments (Getachew *et al.*, 2004). The gas production in this study increased as the incubation hour increased from 3 to 48 hours. However, replacement of molasses with pineapple waste reduced gas production. Gas production is an indirect measure of substrate degradation, particularly carbohydrate fraction, and is a good predictor for the production of volatile fatty acid. Differences in gas production of feed blocks could be due to the proportion and nature of their fibre (Rubanza *et al.*, 2003). Indeed, the high fibre level of feed blocks with pineapple waste might be responsible for the reduced gas production.

Significant reduction in methane (Johnson and Johnson, 1995) can be achieved by manipulating animal diets. The *in vitro* gas method can be used to study the efficiency of feed utilization and to examine animal waste components that impact the environment in order to develop appropriate mitigation strategies (Getachew *et al.*, 2004). The significant variation observed in methane production in the present study showed a decrease in methane production with increasing quantity of pineapple waste in the feed block. The same trend was observed in the total gas production and short chain fatty acid estimates. This might be due to the high fibre content of the pineapple waste. *In vitro* methane production of multi – nutrient blocks made with different protein sources ranged between 13.00 to 16.00 mL/200mg DM. Inclusion of 20% and 30% pineapple waste reduced ( $P<0.05$ ) *in vitro* methane production. This is noteworthy because of the practical implication of the greenhouse gas on the environment. The *in vitro* method is used to predict dry matter intake (Blummel and Ørskov, 1993), digestibility (Khazaal *et al.*, 1993) and metabolisable energy (Babayemi and Bamikole, 2006) in ruminants. The IVDMD in feed blocks containing 0%P/30%M had the highest value, indicating that molasses had a relatively good binding effect and a seeming source of rapidly fermentable nutrients compared to pineapple waste (Khanum *et al.*, 2006).

Short chain fatty acid is a reflection of the energy availability in feedstuff. The observed value of short

chain fatty acid of feed block is consistent with the trend observed in gas production, which was significantly evident after 24 hours of incubation. The organic matter digestibility and metabolizable energy values of blocks are reflection of their fermentation ability (Salem *et al.*, 2007). Organic matter digestibility observed is closely related to gas production; the digestibility of measured organic matter was closely correlated with that predicted from gas production (Getachew *et al.*, 2004).

## CONCLUSION

Based on the result of this study, feed blocks with 0%, 10% and 20% pineapple waste inclusion had similar ( $P>0.05$ ) short chain fatty acid and *in vitro* gas production. This implied that replacement of molasses with pineapple waste up to 20% did not reduce short chain fatty acid and gas production. Furthermore, metabolisable energy, organic matter digestibility and methane production were not affected when pineapple waste was not more than 10% in the feed block. However, complete replacement of molasses with pineapple waste for total tract *in vivo* digestibility study is recommended to further investigate the lower tract digestibility potential of feed block without molasses inclusion.

## CONFLICT OF INTEREST

There is no conflict of interest in the conduct and publication of this research work.

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