IJAS Iranian Journal of Applied Animal Science

# Effects of Inoculation and Fermentation Time on *in vitro* Digestibility, Microbial Population and Rumen Fermentation Characteristics of Industrial Potato Waste

Research Article

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Received on: 8 Apr 2024
Revised on: 25 Sep 2024
Accepted on: 15 Nov 2024
Online Published on: Dec 2024

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Online version is available on: www.ijas.ir
doi.org/10.71798/ijas.2024.1194988

## ABSTRACT

Potato processing generates waste that is estimated to be around 12-20% of the original potato weight. The waste can further be processed or incorporated into animal feed formulations. However, there is limited information on potentials of industrial potato waste (IPW) as ruminant feedstuff. The study aimed to determine the effect of inoculation and fermentation time on *in vitro* organic matter digestibility (IVOMD), metabolizable energy (ME), rumen microbial population and ruminal fermentation characteristics. The experiment involved inoculation of IPW with zero inoculum (control), *Lactiplantibacillus plantarum* (MW296876), *Saccharomyces cerevisiae* (MW296931) and *Aspergillus oryzae* (MW297015). The experimental design was completely randomized design (CRD) with factorial arrangement (4 treatments×5 replications×4 fermentation time). After inoculation and fermentation, the substrates were subjected to anaerobic incubation, and gas volumes were recorded at 3, 6, 12, 24, 48 and 72 h. The results revealed that there was no significant (P>0.05) interaction between treatment and fermentation time on gas production and IVOMD. The rumen microbial population revealed that total bacteria, total methanogens, *Ruminococcus flavafaciens*, *Ruminococcus albus* and *Fibrobacter succinogens* had no significant (P>0.05) interaction between the effect of treatment and fermentation time. However, total protozoa, total fungi and *Butyrivibrio fibrisolvens* exhibited a significant (P<0.05) interaction. Although the methane content (7.11±1.49-8.07±0.32 mM) of *A. oryzae* did not change across the fermentation time, the values recorded were lowest (P<0.05) compared to 7.77 - 13.03 mM recorded for the other treatments. *A. oryzae* recorded highest (P<0.05) concentration (1299.40-2085.29 µg/100 mL) of C18:0 (stearic acid) across all the fermentation time. It was concluded that microbial inoculation of IPW affects net gas production, it improves biohydrogenation process and reduces methane production. Among the three inocula used, *A. oryzae* is recommended because it recorded highest content of stearic acid via biohydrogenation process, and reduced methane gas production.

KEY WORDS biohydrogenation, fermentation, inoculation, rumen metabolites.

## INTRODUCTION

Over decades, different approaches have been developed to evaluate digestibility of feedstuff in ruminant animals. *In vivo* digestibility, although it is the most reliable, is expensive and difficult to carry out, because it requires the use of a large number of animals and a large amount of experimental diets (Krizsan *et al.* 2012). Besides, *in vivo* trial requires about two weeks to acclimatise experimental animals to a metabolism cage and another seven days for the collection of faecal and urine samples (Buonaiuto *et al.* 2021; Cavallini *et al.* 2021).

Consequent to the limitations of *in vivo* trial, *in vitro* digestibility becomes an alternative method to evaluate organic matter digestibility in ruminants. The method is considered less expensive and simpler than *in vivo* and *in sacco* methods (Olowu and Firincioğlu, 2019). It was discovered that gas production from *in vitro* incubation of feedstuff has a strong correlation (r=0.98) to the digestibility of organic matter determined *in vivo* using sheep (Menke *et al*. 1979). *In vitro* gas production offers an easy and fast approach to determining metabolizable energy and organic matter digestibility of a substrate at just 24 h of incubation (Menke and Steingass, 1988). In addition, the technique provides an opportunity to further determine fermentation kinetics and bio-hydrogenation of fatty acids as well as an opportunity to analyse rumen metabolites and rumen microbes (Nur Atikah *et al*. 2018).

Although the *in vitro* gas production method is popular in the evaluation of ruminant feed because of its numerous advantages, there are several factors that affect the accuracy of the technique, these factors include the form of feed sample, composition of buffer, ratio of rumen fluid to buffer, liquid-gas-interface, stirring and prevailing atmospheric pressure (Okhonlaye *et al*. 2020).

In Malaysia, IPW is substantially produced by potato processing plants. The term IPW is synonymous to potato waste, potato processing waste, potato peels, potato by-product, potato residue and industrial potato peels (Hamed *et al*. 2011; Ncobela *et al*. 2017; Franco *et al*. 2021). In the present study, IPW refers to the by-products from potato processing plants.

Depending on the processing method and variety of potatoes, IPW can range between 12% to 20% of the original fresh weight (Sepelev and Galoburda, 2015). The higher composition of peels in the IPW virtually increases fibre content and decreases antinutritional factors (ANF) especially glycoalkaloid compounds. More so, the peels have a higher concentration of phenolic compounds than the tuber fraction (Muhammad *et al*. 2024).

Several studies were conducted on fermentation of IPW with microbial species such as *S. cerevisiae*, *A. niger*, *Streptococcus thermophilus*, and *Bacillus subtilis* via a solid-state fermentation. A short-term fermentation provides an opportunity to quickly improve the nutritional value and reduce antinutrient content of substrate within few days (Muhammad *et al*. 2023). Afterwards, the substrate could be dried to ensure longer shelf-life. From available literature, studies conducted on fermentation of IPW via solid-state fermentation were mostly related to pharmaceuticals, breweries, enzyme assays and proximate constituents (Waseem Ali *et al*. 2017). Hence, there are virtually limited works on fermentation of IPW related to ruminant nutrition.

Considering the challenges and trends of the ruminant production system in Malaysia (Abdullah *et al*. 2020), IPW will certainly increase the availability of concentrate diets to ruminants, especially to farmers that are in close proximity to potato processing plants (Hoshide *et al*. 2006). Another limitation of IPW is high moisture content (~87%). Hence, transportation of fresh IPW will increase cost of transportation vis a vis dry matter content. In spite of the limitations of IPW, its potentials to increase body growth of ruminant animals has been documented in previous works with sheep, goats, cattle, and buffaloes (Makkar, 2003; Hamed *et al*. 2011; Toplu *et al*. 2013).

The objective of this study is to determine the digestibility and rumen fermentation characteristics of IPW fermented with *L. plantarum* (MW296876), *S. cerevisiae* (MW296931) and *A. oryzae* (MW297015). The study also aims to demonstrate that IPW can be used as an additional feed options for ruminant animals.

## MATERIALS AND METHODS

### Preparation of isolates

*Lactiplantibacillus* spp. (B0027), *Saccharomyces* spp. (Y0001) and *Aspergillus* spp. (F0017) isolates were sourced from the Malaysian Agricultural Research and Development Institute (MARDI). The pure isolates were then grown on appropriate media: *Lactiplantibacillus* isolate was grown on de Man Rogosa and Sharpe (MRS) broth and agar, *Saccharomyces* isolate was grown on potato dextrose broth and agar (PDA), while *Aspergillus* isolate was grown on potato dextrose agar only. Molecular characterization was carried out by extracting DNA from each isolates. The genomic DNA was then subjected to polymerase chain reaction. Sequencing of the nucleotides from each isolate was carried out by Apical Scientific company. The nucleotide sequences were further edited and analysed using Bio-edit software (version 7.0). The sequences were then deposited in the basic local alignment search tool (BLAST) on the National Centre for Biotechnology Information (NCBI) database. Hence, a discrete accession number of MW296876, MW296931 and MW297015 was assigned to *L. plantarum*, *S. cerevisiae*, and *A. oryzae*, respectively.

In order to determine the optimum growth and colony forming units (cfu), a single colony from *L. plantarum* and *S. cerevisiae* was sub-cultured to 1 mL MRS and PDA broth, respectively. The cultures were then incubated for 24, 48 and 72 h at 30 °C. It was found that both *L. plantarum* and *S. cerevisiae* reached optimum growth at 48 h of incubation. Similarly, about 5 $mm^2$ of *A. oryzae* was sub-cultured to PDA and incubated at 30 °C for 24, 48, 72 and 96 h. It was observed that the optimum and full growth of *A. oryzae* was at 96 h.

**Preparation of substrate**

IPW is a by-product from food industry that process potato into finished products such as chips, French fries and flakes. It is composed of potato peels (skin), potato flesh, spoiled potatoes and low grade cuttings (broken/pieces). IPW contains appreciable quantity of macro nutrients as presented in Table 1.

**Table 1** Nutrient and energy content of industrial potato waste

| Nutrient | Content (g/kg) |
|---|---|
| Crude protein | 20.40 |
| Ether extract | 3.00 |
| Ash | 29.70 |
| Organic matter | 924.50 |
| Neutral detergent fibre | 515.10 |
| Acid detergent fibre | 157.90 |
| Acid detergent lignin | 59.80 |
| Total starch | 403.10 |
| Energy (MJ/kg DM) | 15.49 |

For the purpose of this study, a substantial quantity of IPW was collected from French Fries (Malaysia) Sdn, Bhd. The material was then oven dried at 65 °C, cooled at room temperature (~ 28 °C), milled, sieved to pass 1.0 mm and preserved in a cold room (4 °C) until required for proximate analysis and solid-state fermentation.

About 100 g dry matter (DM) of the sample (substrate) was placed in a 250 mL Erlenmeyer flask, the moisture content was then adjusted to 60% by adding 140 mL water, the flask was then covered with a thin layer of paraffin. Thus, a total of eighty samples were prepared and divided into four (4) treatment groups, each treatment contained five replicates.

The first treatment group (control) was not inoculated with any microbe, while the remaining three treatment groups were inoculated with live cells of *L. plantarum* (MW296876) at $1\times10^5$/g (Abdul Rahman *et al.* 2017), *S. cerevisiae* (MW296931) at $1\times10^5$/g (Abdul Rahman *et al.* 2017) and *A. oryzae* (MW297015) at about $cm^2$/50 g (Ramin *et al.* 2011). Then, five replicates from each treatment were subjected to fermentation time of 0, 24, 48 and 72 h, the incubation temperature was maintained at 35 °C. A flowchart of the experimental set-up is presented in Figure 1. Termination of fermentation was carried out by increasing the incubation temperature to 65 °C until a constant weight of substrate was observed. The inoculated substrates were then carefully removed from the Erlenmeyer flask and preserved in a refrigerator until required for *in vitro* gas production.

***In vitro* gas production**

Substrates from inoculated and fermented IPW were subjected to *in vitro* gas production using a method and procedure described by Menke and Steingass (1988). The *in vitro* gas production experiment was conducted in three runs on separate days, with three replicates per treatment × fermentation time. A total of sixty (60) syringes were used during the incubation, the syringes consisted 48 IPW substrate samples (4 treatments×3 replicates×4 fermentation time), 2 concentrate standard, 2 hay standard, 2 mixtures of concentrate and hay standard, and 6 blanks (buffered-rumen fluid without any feed sample). Feed standards (with standard gas production of 44.16 mL/24 h and 62.62 mL/24 h at 400 m above sea level) were sourced from University of Hohenheim, Stuttgart, Germany.

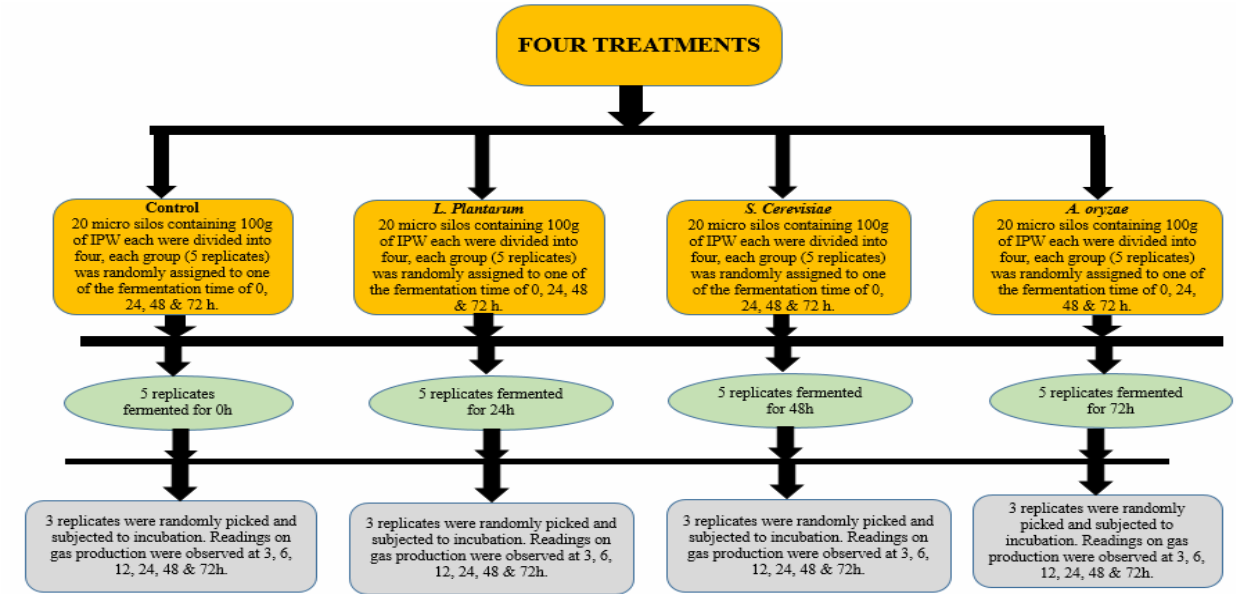


**Figure 1** A flowchart showing experimental set-up

The standards were used to correct gas production and make inference with samples' gas production. During the incubation, volume of gas production was recorded at 3, 6, 12, 24, 48 and 72 h. After the termination of incubation at 72 h, the pH of the rumen fluid was immediately measured using a pH meter (Mettler-Toledo). From the total 30 mL rumen fluid used per syringe, an aliquot of samples was collected for molecular determination of microbial population and rumen metabolites such as volatile fatty acids (VFA), ammonia-nitrogen, and long chain fatty acids. The aliquots were preserved at −20 °C until required for further analyses.

The volumes of gas produced were fitted into a rumen kinetics model as described by (Orskov and Mcdonald, 1979):

$$Y= a + b\ (1-e^{-ct})$$

Where:
Y: volume (mL) of gas production at time t (h).
a: volume (mL) of gas production from the immediately soluble fraction.
b: volume (mL) of gas production from the insoluble fraction.
c: gas production rate constant from the insoluble fraction (mL/h).
t: incubation time.

Net gas production (NGP) data were analysed for rumen kinetics using NEWAY Excel software. The *in vitro* organic matter digestibility (IVOMD) and metabolizable energy (ME) were calculated using prediction equations described by (Menke and Steingass, 1988):

$$IVOMD\ (\%)= 14.88 + 0.8893GP + 0.0448CP + 0.0651A,$$

Where:
GP: net gas production at 24 h (mL/200 mg) of incubation.
CP: percentage of crude protein content.
A: percentage of ash content of the substrate.

$$ME\ (MJ/kg\ DM)= -0.27 + 0.1546IVOMD - 0.0133A + 0.0169EE + 0.0009CP$$

Where:
IVOMD= *in vitro* organic matter digestibility.
A: percentage of ash content of the substrate.
EE: percentage of ether extract content of the substrate.
CP: percentage of crude protein content.

Similarly, the volume of methane produced was also calculated using a prediction model described by Owens and Goetsch (1988):

$CH_4$= 0.5 × acetate (mM) + 0.5 × butyrate (mM) − 0.25 × propionate (mM).

**Chemical assays**

Ammonia nitrogen ($NH_3$-N) in rumen fluid was determined according to the procedure described by (Soloranzo, 1969). Volatile fatty acids (VFAs) and fatty acids were extracted according to the procedure described by (Ebrahimi *et al.* 2017). Both VFA and long chain fatty acids were determined using gas-liquid chromatography (GC, Agilent 6890N). The dimensions of columns used for separation of long chain fatty acids and VFAs were 100 m × 0.25 mm ID × 0.2 µm (film thickness) and 15 m, 0.32 mm ID and 0.25 µm (film thickness), respectively.

**Microbial DNA extraction**

The extraction of microbial DNA from rumen fluid after *in vitro* gas production was carried out using FavorPrep™ Stool DNA Isolation Kit. The procedure for the extraction was according to the manufacturer's specifications and protocols. After isolation of the microbial DNA, the quality and yield of the DNA were measured using a spectrophotometer (NanoDrop™ 2000c spectrophotometer, Thermo Scientific™).

**Quantification of microbial population**

After termination of *in vitro* gas production which lasted for 72 h, a species-specific quantitative polymerase chain reaction (qPCR) was carried out using CFX96™ Real-Time PCR (Bio-Rad, USA). The qPCR was used to quantify total population of bacteria, protozoa, fungi and some important classes of cellulolytic bacteria such as *F. succinogens*, *B. fibrisolvens*, *R. albus*, *Methanobrevibacter ruminantium* and methanogenic archaea as shown in Table 5. The reaction of qPCR was carried out on 20 µL using ChamQ Universal SYBR® (qPCR Master Mix). The qPCR reaction was made up of 10 µL of SYBR, 0.4 µL of forward primer, 0.4 µL of reverse primer, 2 µL of template DNA, and 7.2 µL of deionised distilled water. The qPCR reactions were then placed in PCR strips (0.2 mL) with flat caps. The qPCR conditions applied to each well were according to the specifications of ChamQ Universal SYBR®. A melting curve analysis was carried out at the end of 40 amplification cycles to determine the specificity of amplification. The primers used for target microbial DNA are presented in Table 2.

Table 2 Primers used to target microbial DNA in qPCR reactions

| Target microbes | R/F | Primer sequence 5´ to 3´ | Amplicon size (bp) | Reference |
|---|---|---|---|---|
| Total bacteria | R | CCATTGTAGCACGTGTGTAGCC | 145 | (Koike and Kobayashi, 2001) |
| | F | CGGCAACGAGCGCAACCC | | |
| Total protozoa | R | GCTTTCGWTGGTAGTGTATT | 223 | (Sylvester *et al*. 2004) |
| | F | CTTGCCCTCYAATCGTWCT | | |
| Total fungi | R | CAAATTCACAAAGGGTAGGATGATT | 121 | (Lane, 1991) |
| | F | GAGGAAGTAAAAGTCGTAACAAGGTTTC | | |
| Total methanogens | R | CGGTCTTGCCCAGCTCTTATTC | 160 | (Zhou *et al*. 2009) |
| | F | CCGGAGATGGAACCTGAGAC | | |
| *Ruminococcus flavafaciens* | R | CCTTTAAGACAGGAGTTTACAA | 259 | (Koike and Kobayashi, 2001) |
| | F | TCTGGAAACGGATGGTA | | |
| *Fibrobacter succinogenes* | R | CGCCTGCCCCTGAACTATC | 122 | Lane (1991) |
| | F | GTTCGGAATTACTGGGCGTAAA | | |
| *Butyrivibrio fibrisolvens* | R | CCAACACCTAGTATTCATC | 417 | (Boeckaert *et al*. 2008) |
| | F | GYGAAGAAGTATTTCGGTAT | | |
| *Ruminococcus albus* | R | CCTCCTTGCGGTTAGAACA | 175 | (Koike and Kobayashi, 2001) |
| | F | CCCTAAAAGCAGTCTTAGTTCG | | |
| Methanobacteriales | R | TACCGTCGTCCACTCTT | 343 | (Yu *et al*. 2005) |
| | F | CGWAGGGAAGCTGTTAAGT | | |

### Statistical analysis

All observations were subjected to exploratory data analyses such as normality test (Shapiro–Wilk test) and homogeneity of variances (Levene's test). Hence, the basic assumptions of independence, normal distribution, homogeneity of variance and absence of outliers were met before employing the parametric test of two-way ANOVA. Similarly, microbial data were log10-transformed before the analysis. Means of treatments were computed and compared for differences by Duncan multiple range test. The differences between two means were considered to be statistically significant at 5% probability level ($P<0.05$). Results were presented as means ± standard error.

Data generated from *in vitro* gas production, fermentation characteristics and microbial population were subjected to two – way analysis of variance (ANOVA) using a general linear model (GLM) of Statistical Analysis System (SAS) software version 9.4 (SAS, 2011). The statistical model used was as follows:

$$Y_{ijr} = \mu + t_i + d_j + (td)_{ij} + \varepsilon_{ijr}$$

Where:

$Y_{ijr}$: dependant observation on effect of different levels of treatment and fermentation time.

$\mu$: overall mean of the observations.

$t_i$: effect of treatment levels.

$d_j$: effect of fermentation time.

$(td)_{ij}$= effect of interaction between treatment and fermentation time.

$\varepsilon_{ijr}$: random error.

However, microbial DNA extracted from treatments at zero fermentation time (0 h) were pooled together since no effect of the treatment was expected at zero hour.

## RESULTS AND DISCUSSION

The cumulative net gas production of IPW fermented with *L. plantarum* (MW296876), *S. cerevisiae* (MW296931) and *A. oryzae* (MW297015) was presented in Table 3 and Figures 2, 3, 4 and 5.

The result showed a significant ($P<0.05$) interaction between treatments and the time of fermentation. At 3 and 6 h incubation period, IPW inoculated with *L. plantarum* and fermented for 24 h recorded highest ($P<0.05$) net gas production compared to other treatment groups. However, at 12 h incubation, the net gas production of IPW fermented with *L. plantarum* for 24 h was similar ($P>0.05$) to *S. cerevisiae* and *A. oryzae*, but higher ($P<0.05$) than the control group. Similarly, at 24 h incubation period, the *L. plantarum* treatment recorded highest ($P<0.05$) net gas production than the control and other treatment groups which were similar ($P>0.05$).

*In vitro* gas production has a strong correlation to *in vivo* feed digestibility because gas production and metabolizable energy (ME) represent the fermentation of nutrients in the rumen. Previous studies reported that lactic acid bacteria and yeast can survive in the rumen, hence they alter the parameters of rumen fermentation, and rumen microflora (Elghandour *et al*. 2020b). Also, *in vitro* fermentation of feed materials result in the production of various types of rumen metabolites (Ibrahim *et al*. 2018).

**Table 3** *In vitro* net gas production (mL/200 mg DM) of industrial potato waste fermented with *Lactobacillus plantarum*, *Saccharomyces cerevisiae* and *Aspergillus oryzae*

| Incubation period (h) | Fermentation time (h) | Treatment | | | | P-value | |
|---|---|---|---|---|---|---|---|
| | | Control | *L. plantarum* | *S. cerevisiae* | *A. oryzae* | Treatment | Treatment*Time |
| 3 | 0 | 7.50±0.76 | 8.33±1.20$^{b}$ | 7.33±0.17 | 7.83±1.09 | 0.8666 | 0.275 |
| | 24 | 6.67±0.73$^{B}$ | 11.50±0.29$^{aA}$ | 6.33±0.73$^{B}$ | 7.67±0.88$^{B}$ | 0.0026 | 0.275 |
| | 48 | 7.50±0.29 | 8.17±0.88$^{b}$ | 7.83±1.01 | 7.50±1.44 | 0.9563 | 0.275 |
| | 72 | 7.83±1.48 | 10.33±0.33$^{a}$ | 7.00±0.29 | 8.83±1.09 | 0.1546 | 0.275 |
| | P-value | 0.8300 | 0.0438 | 0.4612 | 0.8431 | | |
| 6 | 0 | 18.67±1.00 | 20.02±1.93 | 17.50±0.17 | 20.67±1.53 | 0.3941 | 0.174 |
| | 24 | 15.67±1.00$^{B}$ | 21.34±0.73$^{A}$ | 16.17±0.50$^{B}$ | 17.17±1.32$^{B}$ | 0.0102 | 0.174 |
| | 48 | 16.67±1.0 | 17.17±0.76 | 17.17±0.76 | 14.34±2.49 | 0.4924 | 0.174 |
| | 72 | 17.84±1.74 | 18.67±0.58 | 15.84±0.44 | 16.67±0.58 | 0.2622 | 0.174 |
| | P-value | 0.3873 | 0.1355 | 0.1448 | 0.1263 | | |
| 12 | 0 | 29.67±1.26 | 31.84±2.84 | 27.50±0.17 | 33.50±2.46$^{a}$ | 0.2383 | 0.1203 |
| | 24 | 24.50±1.09$^{B}$ | 30.84±1.20$^{A}$ | 26.34±1.17$^{AB}$ | 26.84±1.86$^{abAB}$ | 0.0474 | 0.1203 |
| | 48 | 26.00±1.92 | 26.00±1.59 | 26.17±0.58 | 21.34±3.44$^{b}$ | 0.3661 | 0.1203 |
| | 72 | 27.84±1.88 | 26.84±0.88 | 24.50±0.60 | 26.95±1.87$^{ab}$ | 0.4433 | 0.1203 |
| | P-value | 0.1916 | 0.1250 | 0.0997 | 0.0422 | | |
| 24 | 0 | 42.92±1.30 | 46.26±2.35 | 42.59±0.50 | 47.59±3.28 | 0.3283 | 0.1446 |
| | 24 | 41.26±2.46$^{B}$ | 47.92±1.09$^{A}$ | 41.09±0.58$^{B}$ | 41.42±2.33$^{B}$ | 0.0428 | 0.1446 |
| | 48 | 41.92±1.74 | 42.09±1.53 | 42.59±2.57 | 35.59±3.40 | 0.2128 | 0.1446 |
| | 72 | 44.92±2.68 | 43.09±1.53 | 41.09±1.04 | 41.87±2.70 | 0.6189 | 0.1446 |
| | P-value | 0.6505 | 0.1252 | 0.7812 | 0.1128 | | |
| 48 | 0 | 68.93±5.20 | 64.09±1.33$^{c}$ | 67.26±1.00 | 71.09±4.94 | 0.6084 | 0.4266 |
| | 24 | 70.93±5.05 | 79.26±0.76$^{a}$ | 67.09±1.88 | 67.93±5.81 | 0.2007 | 0.4266 |
| | 48 | 68.43±2.95 | 71.26±1.50$^{b}$ | 70.26±6.66 | 57.43±9.88 | 0.4159 | 0.4266 |
| | 72 | 75.26±4.44 | 70.76±2.84$^{b}$ | 68.59±3.06 | 67.20±5.98 | 0.5885 | 0.4266 |
| | P-value | 0.7066 | 0.0024 | 0.9280 | 0.5636 | | |
| 72 | 0 | 61.93±5.64 | 57.59±1.33$^{c}$ | 60.43±1.45 | 65.59±4.48 | 0.5298 | 0.4590 |
| | 24 | 63.93±6.17 | 74.43±2.04$^{a}$ | 63.59±0.60 | 71.26±10.25 | 0.5325 | 0.4590 |
| | 48 | 62.59±2.89 | 66.76±1.76$^{b}$ | 65.09±6.33 | 53.43±7.65 | 0.3439 | 0.4590 |
| | 72 | 67.76±4.31 | 66.26±2.02$^{b}$ | 62.76±1.50 | 64.04±6.87 | 0.8406 | 0.4590 |
| | P-value | 0.8379 | 0.0014 | 0.7977 | 0.4569 | | |
| | pH after incubation | | | | | | |
| | 0 | 6.63±0.03 | 6.71±0.06 | 6.62±0.03 | 6.65±0.02 | 0.3655 | 0.7671 |
| | 24 | 6.58±0.01$^{B}$ | 6.59±0.01$^{B}$ | 6.58±0.02$^{B}$ | 6.68±0.04$^{A}$ | 0.0352 | 0.7671 |
| | 48 | 6.60±0.05 | 6.66±0.01 | 6.60±0.04 | 6.62±0.04 | 0.6541 | 0.7671 |
| | 72 | 6.50±0.13 | 6.62±0.03 | 6.61±0.05 | 6.64±0.03 | 0.5880 | 0.7671 |
| | P-value | 0.6367 | 0.1331 | 0.8743 | 0.6475 | | |
| Kinetic constant[1] | | | | | | | |
| | 0 | -1.36±1.04 | -2.95±0.63$^{c}$ | -1.71±0.42 | -1.96±1.19 | 0.6302 | 0.0664 |
| | 24 | -2.56±0.49$^{B}$ | 2.41±0.62$^{aA}$ | -1.92±0.82$^{B}$ | 0.68±0.75$^{A}$ | 0.0028 | 0.0664 |
| a | 48 | -1.4±0.68 | -0.57±1.27$^{bc}$ | -1.2±0.72 | -0.9±1.41 | 0.9553 | 0.0664 |
| | 72 | -1.92±0.79 | 1.91±0.44$^{ba}$ | -1.9±1.02 | -0.09±1.82 | 0.1288 | 0.0664 |
| | P-value | 0.6772 | 0.0052 | 0.8631 | 0.5769 | | |
| | 0 | 71.20±5.17 | 67.96±2.71$^{b}$ | 70.24±1.06 | 74.23±5.56 | 0.7510 | 0.7647 |
| | 24 | 78.22±7.26 | 79.47±1.20$^{a}$ | 74.21±2.89 | 93.66±26.03 | 0.7649 | 0.7647 |
| b | 48 | 72.75±4.03 | 77.39±2.80$^{a}$ | 75.50±7.99 | 62.51±10.60 | 0.4916 | 0.7647 |
| | 72 | 80.63±5.10 | 71.11±2.54$^{ab}$ | 74.67±4.17 | 72.60±9.55 | 0.6954 | 0.7647 |
| | P-value | 0.6032 | 0.0356 | 0.8651 | 0.5558 | | |
| | 0 | 69.84±5.93 | 65.01±2.45$^{c}$ | 68.53±1.39 | 72.27±5.05 | 0.6718 | 0.7197 |
| | 24 | 75.65±7.22 | 81.88±2.31$^{a}$ | 72.29±2.18 | 94.34±26.67 | 0.7001 | 0.7197 |
| (a+b) | 48 | 71.35±3.43 | 76.82±3.02$^{ab}$ | 74.40±8.09 | 61.60±10.57 | 0.4815 | 0.7197 |
| | 72 | 78.71±5.24 | 73.02±2.35$^{bc}$ | 72.78±3.24 | 72.51±9.51 | 0.8486 | 0.7197 |
| | P-value | 0.6811 | 0.0093 | 0.8254 | 0.5253 | | |
| | 0 | 0.05±0.006 | 0.07±0.01$^{a}$ | 0.05±0.002 | 0.06±0.001 | 0.2064 | 0.6031 |
| | 24 | 0.04±0.003 | 0.04±0.003$^{b}$ | 0.04±0.001 | 0.04±0.01 | 0.8933 | 0.6031 |
| c | 48 | 0.04±0.001 | 0.04±0.01$^{b}$ | 0.04±0.004 | 0.045±0.01 | 0.8587 | 0.6031 |
| | 72 | 0.04±0.004 | 0.04±0.001$^{b}$ | 0.04±0.0005 | 0.04±0.01 | 0.8476 | 0.6031 |
| | P-value | 0.4538 | 0.0199 | 0.2610 | 0.5900 | | |

$^{A, B:}$ the means within the same row with different letter, are significantly different (P<0.05).

$^{a, b:}$ the means within the same column with different letter, are significantly different (P<0.05).

[1] Kinetic constant a: volume (mL) of gas production from the immediately soluble fraction; b: volume (mL) of gas production from the insoluble fraction; c: gas production rate constant from the insoluble fraction (mL/h) and (a+b): potential gas production (mL/200 mg DM).

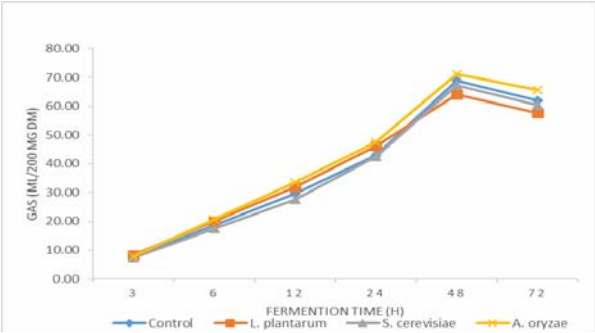


**Figure 2** Net gas production of IPW fermented at zero hour

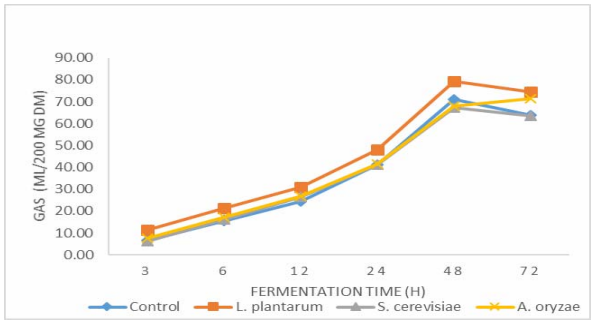


**Figure 3** Net gas production of IPW fermented for 24 hours

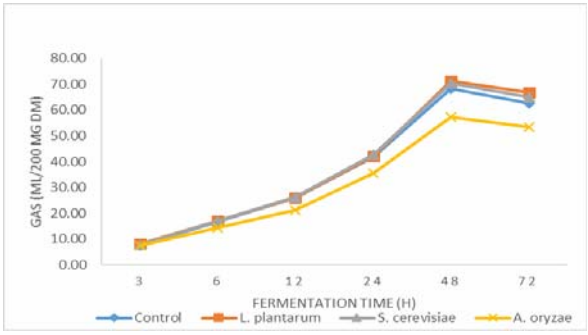


**Figure 4** Net gas production of IPW fermented for 48 hours

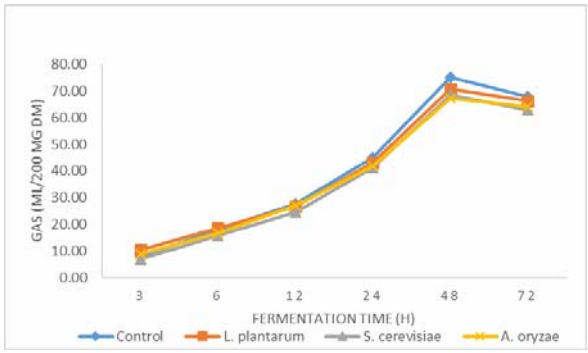


**Figure 5** Net gas production of IPW fermented for 72 hours

The present work observed that cumulative net gas production dropped after 48 h of incubation. The drop of gas production was related to the fact that our blanks recorded an average of 12.5 mL at 72 h of incubation, which was higher than the net gas production from each samples at the same time (72 h). Thus, when blank's gas volume (12.5 mL) was deducted from the gas production of each sample, the cumulative net gas production of the samples became lower or plateau at 72 h of incubation. Therefore, it was deduced that since IPW substrates contain high amount of starch, the rumen microorganisms would quickly ferment the samples, but the blanks that contained high amount of roughage from the previous animal's diet would certainly continue to produce gas slowly beyond 48 h. Therefore, the drop of gas production at 72 h of incubation was ascribed to the effect of previous diet in the rumen liquor.

This study observed that IPW fermented for 24 h with *L. plantarum* recorded highest net gas production (47.92 mL) at 24 h incubation period. Thereafter, the treatments had no effect on the net gas production. Notwithstanding, it was reported that the effects of nutrient proportion and nutrient sources are more pronounced in the first 12 h of incubation, but the extent of the effect varies according to the soluble carbohydrate (Baffa *et al.* 2023).

The finding of the present study confirmed a prior report that inoculation of the substrate did not affect cumulative net gas production (Babaeinasab *et al.* 2015). Similarly, gas production of IPW fermented at 48 and 72 h were similar (0.05). Across all the treatments, the volume of gas (41.09 – 47.92 mL) produced at 24 h ($GP_{24}$) incubation was lower compared to 47.10 – 55.40 mL reported for potato-wheat straw silage (Babaeinasab *et al.* 2015). From previous studies, factors that affect *in vitro* gas production include atmospheric pressure, anaerobic condition, composition of the culture medium, amount of microbial inoculum, nutrient composition of substrate, antinutrient factors and microbial biomass yield (MBY), which was reported to have a negative correlation to gas production (Contreras-Govea *et al.* 2011). However, it was reported that some inoculants resulted in gas production similar to uninoculated substrates (Muck *et al.* 2007; Contreras-Govea *et al.* 2011).

For the kinetic constants, only IPW inoculated with *L. plantarum* showed differences across the fermentation time. The *L. plantarum* treatment group fermented for 24 h recorded highest degradation of soluble fraction than the control, *S. cerevisiae*, and *A. oryzae* treatment groups. In general, the degradation constants 'b' (insoluble/potentially degradable fraction) and 'c' (rate of degradation of 'b') found in this study were comparable to those found in a corn-supplemented diet, but the 'c' disagrees with rate of degradation reported for postbiotic-supplemented diet (Ibrahim *et al.* 2018).

**Table 4** *In vitro* digestibility (%) and metabolizable energy (MJ/kg DM) of industrial potato waste fermented at different time (h) with *Lactobacillus plantarum*, *Saccharomyces cerevisiae* and *Aspergillus oryzae*

| Item[1] (%) | Fermentation Time (h) | Treatment | | | | P-value | |
|---|---|---|---|---|---|---|---|
| | | Control | *L. plantarum* | *S. cerevisiae* | *A. oryzae* | Treatment | Treatment*Time |
| IVOMD (%) | 0 | 53.35±1.19 | 56.22±2.08 | 52.93±0.45 | 57.33±2.92 | 0.3443 | 0.1491 |
| | 24 | 51.82±2.20 | 57.68±0.97 | 51.61±0.50 | 51.89±2.08 | 0.0750 | 0.1491 |
| | 48 | 52.43±1.56 | 52.50±1.36 | 52.92±2.27 | 46.68±3.03 | 0.2081 | 0.1491 |
| | 72 | 55.11±2.84 | 53.40±1.34 | 51.70±0.93 | 52.33±2.40 | 0.6148 | 0.1491 |
| | P-value | 0.6487 | 0.1240 | 0.8012 | 0.1139 | | |
| ME (MJ/kg DM) | 0 | 7.95±0.18 | 8.42±0.32 | 7.91±0.07 | 8.60±0.45 | 0.3198 | 0.1456 |
| | 24 | 7.71±0.33 | 8.64±0.15 | 7.71±0.08 | 7.76±0.32 | 0.0729 | 0.1456 |
| | 48 | 7.81±0.24 | 7.84±0.21 | 7.91±0.35 | 6.95±0.47 | 0.2200 | 0.1456 |
| | 72 | 8.21±0.37 | 7.97±0.21 | 7.70±0.14 | 7.82±0.36 | 0.6389 | 0.1456 |
| | P-value | 0.6552 | 0.1238 | 0.7778 | 0.1121 | | |

IVOMD: *in vitro* organic matter digestibility and ME: metabolizable energy.

Also, our 'b' contradicted degradation constant reported for potato-wheat straw silage (Babaeinasab *et al*. 2015). We also observed that our negative 'a' (immediately soluble fraction) conformed with values recorded by Ibrahim *et al*. (2018). The negative net gas production recorded in the current study was due to a delayed fermentation of soluble fraction due to time delay before rumen microbes colonize the substrate to start the fermentation process or it could be related to a time lag after the microbial degradation of soluble fraction ('a') before fermentation of potentially degradable fraction ('b') (Blümmel and Becker, 1997).

The IVOMD and ME of IPW fermented with zero inoculum (control), *L. plantarum, S. cerevisiae and A. oryzae* at different fermentation time revealed that there was no significant interaction between the effect of the microbes and the fermentation time (Table 4). The results of this work revealed that inoculation and fermentation of IPW did not influence both OMD and ME.

Although inoculation of ruminant feeds with *L. plantarum* has been reported to increase fermentation parameters (Direkvandi *et al*. 2021); the effect of inoculants on silages to improve fermentation characteristics such as gas production and organic matter digestibility was largely negative. However, it was found that some inoculants used on silages increase *in vitro* digestibility (Muck, 2010; Contreras-Govea *et al*. 2011). Even though simple sugars are known to influence *in vitro* digestibility because they get completely digested within six (6) hour incubation (Palmonari *et al*. 2021), the IPW used in the current study contains mainly starch and fibre with low simple sugar content. Also, our results on *in vitro* organic matter digestibility did not differ across the treatments despite the fact that complex carbohydrates such as starch are known to be broken-down by amylase produced by amylolytic and cellulolytic bacteria to yield simple sugars (Palmonari *et al*. 2021; Hua *et al*. 2022).

In the current study, the control (uninoculated and unfermented) did not show any significant difference in gas production and digestibility over IPW inoculated with *L. plantarum*, *S. cerevisiae* and *A. oryzae*. Hence, the finding of the present work did not agree with the previous report that uninoculated silage produced higher net gas production than the inoculated substrates (Muck *et al*. 2007). It should be noted that the absence of a significant increase in gas production and digestibility from inoculated IPW could be due to the presence of phenolic and glycoalkaloid contents that are known to affect gas production. Antinutritional factors such as tannin are toxic to rumen microbes, thus they suppress their fermentative function (Chalchissa *et al*. 2023).

Nevertheless, the presence of live inocula (probiotics) or non-viable microbial cells (para-probiotics) or their by-products (postbiotics) in the rumen have been reported to alter fermentation characteristics by increasing digestibility and fungal population, and also by reducing methane production (Elghandour *et al*. 2020a; Castillo-González *et al*. 2014; Candyrine *et al*. 2017; Ibrahim *et al*. 2018).

Results on rumen microbial population (Table 5) revealed that total bacteria, total methanogens, *R. flavafaciens*, *R. albus* and *F. succinogens* had no significant ($P>0.05$) interaction between the effect of treatment and fermentation time.

However, total protozoa, total fungi and *B. fibrisolvens* exhibited a significant ($P<0.05$) interaction. It was observed that fermentation time influenced population of protozoa in the control and *A. oryzae* treatment groups. But, the fungal population was affected by fermentation time in the control and *A. oryzae* treatment groups.

The current study recorded $Log_{10}$ 10.99 – 11.54 total bacteria per mL more than the previous works that used either high starch or conventional diets (Adebayo *et al*. 2018; Ibrahim *et al*. 2018; Norrapoke *et al*. 2018).

**Table 5** Rumen microbial population ($Log_{10}$ copy no/mL) of industrial potato waste fermented at different time (h) with *Lactiplantibacillus plantarum*, *Saccharomyces cerevisiae*, and *Aspergillus oryzae*

| Microorganism[1] | Fermentation period (h) | Treatment | | | | P-value | |
|---|---|---|---|---|---|---|---|
| | | Control | *L. plantarum* | *S. cerevisiae* | *A. oryzae* | Treatment | Treatment*Time |
| Total bacteria | 0 | 11.54±0.00 | 11.54±0.00 | 11.54±0.00 | 11.54±0.00 | NA | 0.8222 |
| | 24 | 11.13±0.02 | 11.16±0.13 | 11.10±0.21 | 11.25±0.09 | 0.9202 | 0.8222 |
| | 48 | 10.99±0.14 | 11.05±0.10 | 10.92±0.02 | 11.37±0.07 | 0.0807 | 0.8222 |
| | 72 | 11.03±0.10 | 11.29±0.38 | 11.09±0.15 | 11.13±0.13 | 0.8487 | 0.8222 |
| | p-value | 0.1132 | 0.4609 | 0.0932 | 0.0978 | | |
| Total protozoa | 0 | 6.67±0.12$^{b}$ | 6.67±0.12 | 6.67±0.12$^{b}$ | 6.67±0.12 | NA | 0.0021 |
| | 24 | 7.19±0.06$^{aA}$ | 6.64±0.03$^{C}$ | 6.99±0.02$^{aB}$ | 6.75±0.00$^{C}$ | 0.0012 | 0.0021 |
| | 48 | 6.54±0.13$^{bB}$ | 6.71±0.03$^{AB}$ | 6.5±0.01$^{bB}$ | 6.90±0.02$^{A}$ | 0.0427 | 0.0021 |
| | 72 | 6.82±0.02$^{b}$ | 6.76±0.05 | 6.97±0.06$^{a}$ | 6.79±0.12 | 0.2738 | 0.0021 |
| | p-value | 0.0280 | 0.6474 | 0.0147 | 0.3746 | | |
| Total fungi | 0 | 5.56±0.10$^{ab}$ | 5.56±0.10 | 5.56±0.10 | 5.56±0.10$^{c}$ | NA | 0.0287 |
| | 24 | 5.79±0.05$^{a}$ | 5.91±0.11 | 5.82±0.11 | 5.65±0.005$^{b}$ | 0.2749 | 0.0287 |
| | 48 | 5.45±0.005$^{bB}$ | 5.99±0.22$^{A}$ | 5.93±0.00$^{A}$ | 5.61±0.05$^{bAB}$ | 0.0271 | 0.0287 |
| | 72 | 5.42±0.05$^{bB}$ | 5.84±0.05$^{A}$ | 5.93±0.14$^{A}$ | 5.98±0.09$^{aA}$ | 0.0357 | 0.0287 |
| | p-value | 0.0397 | 0.2644 | 0.1583 | 0.0435 | | |
| Total methanogens | 0 | 7.66±0.12 | 7.66±0.12 | 7.66±0.12 | 7.66±0.12 | NA | 0.4929 |
| | 24 | 7.53±0.09 | 7.51±0.05 | 7.57±0.04 | 7.51±0.07 | 0.8847 | 0.4929 |
| | 48 | 7.37±0.07 | 7.48±0.07 | 7.38±0.05 | 7.51±0.05 | 0.3479 | 0.4929 |
| | 72 | 7.33±0.13 | 7.61±0.07 | 7.65±0.11 | 7.42±0.01 | 0.1718 | 0.4929 |
| | p-value | 0.2367 | 0.4192 | 0.2200 | 0.2629 | | |
| *B. fibrisolvens* | 0 | 0.64±0.10$^{ab}$ | 0.64±0.10 | 0.64±0.10 | 0.64±0.10$^{b}$ | NA | 0.0288 |
| | 24 | 0.86±0.05$^{a}$ | 0.98±0.11 | 0.88±0.10 | 0.72±0.01$^{b}$ | 0.2650 | 0.0288 |
| | 48 | 0.53±0.01$^{bB}$ | 1.05±0.21$^{A}$ | 0.99±0.00$^{A}$ | 0.69±0.05$^{bAB}$ | 0.0285 | 0.0288 |
| | 72 | 0.5±0.05$^{bB}$ | 0.91±0.05$^{A}$ | 1.00±0.14$^{A}$ | 1.04±0.09$^{aA}$ | 0.0370 | 0.0288 |
| | p-value | 0.0390 | 0.2634 | 0.1547 | 0.0434 | | |
| *R. flavafaciens* | 0 | 5.74±0.06 | 5.74±0.06 | 5.74±0.06 | 5.74±0.06$^{a}$ | NA | 0.1372 |
| | 24 | 5.53±0.00 | 5.72±0.06 | 5.66±0.13 | 5.53±0.05$^{b}$ | 0.3171 | 0.1372 |
| | 48 | 5.51±0.06 | 5.61±0.06 | 5.55±0.02 | 5.72±0.02$^{a}$ | 0.0855 | 0.1372 |
| | 72 | 5.54±0.06 | 5.81±0.03 | 5.70±0.12 | 5.54±0.01$^{b}$ | 0.1167 | 0.1372 |
| | p-value | 0.0871 | 0.2013 | 0.5600 | 0.0363 | | |
| *R. albus* | 0 | 9.37±0.07$^{a}$ | 9.37±0.07 | 9.37±0.07 | 9.37±0.07 | NA | 0.3048 |
| | 24 | 9.24±0.06$^{ab}$ | 9.19±0.06 | 9.17±0.01 | 9.34±0.01 | 0.1240 | 0.3048 |
| | 48 | 9.21±0.02$^{ab}$ | 9.22±0.01 | 9.11±0.22 | 9.27±0.03 | 0.7961 | 0.3048 |
| | 72 | 9.05±0.04$^{b}$ | 9.32±0.02 | 9.34±0.09 | 9.23±0.07 | 0.0800 | 0.3048 |
| | p-value | 0.0446 | 0.1282 | 0.4619 | 0.2918 | | |
| *F. succinogens* | 0 | 4.93±0.02 | 4.93±0.02 | 4.93±0.02 | 4.93±0.02 | NA | 0.0537 |
| | 24 | 4.70±0.11 | 4.89±0.03 | 4.92±0.02 | 4.94±0.03 | 0.1327 | 0.0537 |
| | 48 | 4.76±0.01 | 4.91±0.04 | 4.87±008 | 5.05±0.10 | 0.1469 | 0.0537 |
| | 72 | 4.62±0.08 | 4.97±0.02 | 5.01±0.11 | 4.75±0.12 | 0.0979 | 0.0537 |
| | p-value | 0.1111 | 0.3220 | 0.5936 | 0.2068 | | |
| *Methanobacteriales* | 0 | 2.74±0.04 | 2.74±0.05 | 2.74±0.06 | 2.74±0.07 | NA | 0.4563 |
| | 24 | 2.91±0.07 | 2.90±0.07 | 2.93±0.07 | 2.92±0.07 | 1.0000 | 0.4563 |
| | 48 | 2.79±0.03 | 2.91±0.19 | 3.34±0.56 | 2.82±0.00 | 0.5739 | 0.4563 |
| | 72 | 2.75±0.05 | 2.70±0.09 | 2.93±0.08 | 3.00±0.18 | 0.2919 | 0.4563 |
| | p-value | 0.1484 | 0.5642 | 0.5849 | 0.4658 | | |

[A, B]: The means within the same row with different letter, are significantly different (P<0.05).
[a, b]: The means within the same column with different letter, are significantly different (P<0.05).
NA: not analysed (NB: DNA templates from samples at zero hour across treatments were pooled together, hence an average of technical replicate was used).

But, the population of protozoa, fungi, methanogen and *B. fibrisolvens* varied compared with observations reported for sheep and goat fed commercial concentrate mixture (Candyrine *et al*. 2017). The differences in the microbial population observed in the present work and previous studies could be related to several factors which include the nature of substrates, type of animal that donate rumen fluid, nature of animal diet prior to rumen collection and type of inoculant.

It was also observed that the inoculation of IPW with *L. plantarum*, *S. cerevisiae* and *A. oryzae* increased the population of rumen fungi as previously reported

(Zhu *et al*. 2017). Therefore, this study deduced that the effect of fungi in stimulating growth of cellulolytic bacteria (Elghandour, *et al*. 2020a) is vice versa. It was further observed that inoculation of IPW with *A. oryzae* reduced the population of methanogenic archaea which produce most of the methane in the rumen by reducing $CO_2$ to CH4 in the presence of $H_2$ (Van de Pol *et al*. 2017).

The rumen ammonia nitrogen ($NH_3$-N), volatile fatty acids (VFA's) and total volatile fatty acids (TVFA's) had a significant ($P<0.05$) interaction between treatment effect (inoculation) and fermentation time (Table 6). But, there was no interaction ($P>0.05$) on the methane gas production. Also, among the treatment groups, only *S. cerevisiae* exhibited a linear decrease ($P<0.05$) of methane concentration across the fermentation time.

IPW inoculated with *L. plantarum* and *S. cerevisiae* increased $NH_3$-N. The increase was probably due to a rapid breakdown of proteins and other nitrogenous compounds. Nevertheless, the concentration of $NH_3$-N in all the treatment combinations was above the minimum level of 5 mg/dL (50 mg/L) required to support the growth of rumen microbes. However, the $NH_3$-N (56.0–76.12 mg/L) recorded in this work was lower compared to 11.3 – 12.0 mg/dL $NH_3$-N reported for potato-wheat straw silage incubated for 24 h (Babaeinasab *et al*. 2015), but comparable to $NH_3$-N concentration at 6 h post-feeding of West African goat fed either cassava peel and urea treated sweet potato peel based diets (Adebayo *et al*. 2018).

IPW inoculated with *L. plantarum* and *S. cerevisiae* produced more acetate than the control and *A. oryzae* treatment groups. However, inoculation of IPW did not alter the concentration of propionate and isovaleric acid, but the effect of inoculation was observed in isobutyrate content. Nevertheless, inoculation with *S. cerevisiae* seemed to consistently increase butyrate. On the overall effect, inoculation of IPW with *L. plantarum* and *S. cerevisiae* increased total volatile fatty acid (TVFA) more than the control and *A. oryzae* treatment groups.

The findings of this study confirmed the report of Muck *et al*. (2007) that inoculation increases VFA production. It was observed that the acetate content between the present work and the report of Babaeinasab *et al*. (2015) greatly varied. The difference could be related to the fact that, in this work, IPW was not mixed with any fodder. Hence, the study recorded low acetic acid content and highier contents of propionic and butyric acids. It was therefore deduced that IPW cannot be fed to ruminants alone without fodder as a basal diet (Franco *et al*. 2021; Raina *et al*. 2023). This is because high starch or concentrate diet produces more propionate and butyrate which could subsequently result in acidosis and milk fat depression (Gómez *et al*. 2016). Starch-rich diets cause alteration of rumen microbial ecology by favouring bacteria producing propionic acid over methanogens. Although a high acetate-propionate ratio is known to increase milk fat at the expense of milk yield, diets rich in starch in a good proportion have positive effects on milk yield and composition (Gómez *et al*. 2016). In view of the result on methane, there was no significant interaction between the treatment and fermentation time. Also, inoculation of IPW with *L. plantarum* and *S. cerevisiae* increased the production of methane more than the uninoculated substrate. It was observed that the inoculation of IPW with *S. cerevisiae* did not conform with a recent report that *S. cerevisiae* reduced methane production (Elghandour, *et al*. 2020b). A high concentration of methane observed in *L. plantarum* could be associated with the fact that *L. plantarum* treatment group produced higher content of acetate, propionate, and butyrate which were known to correlate with methane production (Lyons *et al*. 2018). Invariably, IPW inoculated with *A. oryzae* reduced methane production lower than the other treatments. Although the abundance of rumen fungi was reported to have a strong positive correlation to methane emission in dairy cattle (Lopez-Garcia *et al*. 2022). Our findings revealed that inoculation of IPW with *A. oryzae* did not produce rumen fungi above other treatments. It seemed that *A. oryzae* produced postbiotics that suppressed methane production, thus it might increase bioenergetics and animal productivity. Previous works have reported some beneficial effects of postbiotics and fermentation extract from *A. oryzae*. In particular, a recent study on the effect of *A. oryzae* postbiotic (AO postbiotic) on dairy cattle reported a reduced heat-induced inflammation and oxidative stress (Kaufman *et al*. 2021). Similarly, it was also reported that *A. oryzae* fermentation extract improved feed intake and lactation (Sallam *et al*. 2020).

The rumen fatty acid contents of IPW fermented with *L. plantarum*, *S. cerevisiae*, and *A. oryzae* were presented in Table 7. The palmitic and stearic acids recorded a significant ($P<0.05$) interaction between the treatments and fermentation time, while oleic and linoleic acids had no significant ($P>0.05$) interaction.

**Table 6** Rumen ammonia nitrogen (mg/L), methane gas production and volatile fatty acids (mM) of industrial potato waste fermented at different times with *Lactiplantibacillus plantarum*, *Saccharomyces cerevisiae*, and *Aspergillus oryzae*

| Metabolite | Time (h) | Treatments | | | | P-value | |
|---|---|---|---|---|---|---|---|
| | | Control | *L. plantarum* | *S. cerevisiae* | *A. oryzae* | Treatment | Treatment * Time |
| $NH_3$-N | 0 | 74.36±0.05$^{a}$ | 74.43±0.03$^{b}$ | 74.37±0.06$^{b}$ | 74.39±0.06$^{a}$ | 0.7706 | <.0001 |
| | 24 | 62.72±0.03$^{bC}$ | 66.57±0.04$^{cB}$ | 66.46±0.02$^{cB}$ | 67.70±0.03$^{bA}$ | <.0001 | <.0001 |
| | 48 | 56.00±0.02$^{dD}$ | 74.23±0.03$^{bB}$ | 74.96±0.04$^{aA}$ | 65.52±0.04$^{cC}$ | <.0001 | <.0001 |
| | 72 | 58.69±0.01$^{cD}$ | 76.12±0.25$^{aA}$ | 66.32±0.11$^{cB}$ | 64.49±0.02$^{dC}$ | <.0001 | <.0001 |
| | P-value | <.0001 | <.0001 | <.0001 | <.0001 | | |
| Acetate | 0 | 14.9±0.65$^{B}$ | 21.44±0.52$^{A}$ | 19.92±0.83$^{abA}$ | 13.53±1.26$^{B}$ | 0.0005 | 0.0002 |
| | 24 | 14.09±0.57$^{B}$ | 21.46±0.53$^{A}$ | 18.72±0.92$^{bAB}$ | 14.28±1.91$^{B}$ | 0.0043 | 0.0002 |
| | 48 | 16.06±3.19$^{AB}$ | 19.57±1.40$^{A}$ | 22.87±0.95$^{aA}$ | 9.78±0.27$^{B}$ | 0.005 | 0.0002 |
| | 72 | 19.87±1.02$^{A}$ | 20.40±0.34$^{A}$ | 14.80±0.60$^{cB}$ | 12.54±0.40$^{C}$ | <.0001 | |
| | P-value | 0.167 | 0.3506 | 0.001 | 0.1064 | | |
| Propionate | 0 | 11.55±0.42$^{AB}$ | 16.13±0.48$^{A}$ | 14.52±1.10$^{bAB}$ | 7.72±2.91$^{B}$ | 0.0251 | 0.0045 |
| | 24 | 11.56±0.32 | 15.75±0.45 | 15.79±0.18$^{ab}$ | 10.88±2.62 | 0.0551 | 0.0045 |
| | 48 | 12.59±2.31 | 14.41±1.64 | 18.83±0.99$^{a}$ | 12.48±0.02 | 0.0544 | 0.0045 |
| | 72 | 15.76±1.11 | 14.49±0.51 | 12.63±0.59$^{b}$ | 16.03±1.17 | 0.0954 | 0.0045 |
| | P-value | 0.1518 | 0.4787 | 0.0037 | 0.1045 | | |
| Isobutyrate | 0 | 1.14±0.08$^{B}$ | 1.53±0.04$^{A}$ | 1.49±0.07$^{bA}$ | 1.26±0.09$^{bAB}$ | 0.0123 | 0.0002 |
| | 24 | 0.97±0.02$^{B}$ | 1.53±0.14$^{A}$ | 1.44±0.03$^{bA}$ | 1.41±0.06$^{abA}$ | 0.0038 | 0.0002 |
| | 48 | 1.11±0.22$^{B}$ | 1.26±0.13$^{B}$ | 1.76±0.07$^{aA}$ | 1.65±0.10$^{bB}$ | 0.0375 | 0.0002 |
| | 72 | 1.32±0.07$^{B}$ | 1.34±0.03$^{B}$ | 1.18±0.01$^{cB}$ | 1.65±0.10$^{aA}$ | 0.0034 | 0.0002 |
| | P-value | 0.2947 | 0.2018 | 0.0003 | 0.017 | | |
| Butyrate | 0 | 7.95±0.28$^{C}$ | 11.32±0.02$^{A}$ | 10.87±0.95$^{bAB}$ | 8.54±0.54$^{bBC}$ | 0.0064 | 0.0003 |
| | 24 | 7.22±0.20$^{AB}$ | 11.63±1.35$^{A}$ | 10.84±0.06$^{bA}$ | 8.02±1.27$^{bB}$ | 0.0266 | 0.0003 |
| | 48 | 8.10±1.56$^{B}$ | 8.96±0.92$^{B}$ | 12.61±0.41$^{aA}$ | 8.08±0.07$^{abB}$ | 0.0254 | 0.0003 |
| | 72 | 9.52±0.47$^{B}$ | 9.38±0.25$^{B}$ | 8.46±0.19$^{cB}$ | 11.61±0.67$^{aA}$ | 0.0057 | 0.0003 |
| | P-value | 0.3315 | 0.118 | 0.0038 | 0.0303 | | |
| Isovaleric | 0 | 2.14±0.05$^{B}$ | 3.03±0.05$^{A}$ | 2.93±0.011$^{abA}$ | 2.89±0.22$^{A}$ | 0.0041 | <.0001 |
| | 24 | 3.04±0.71 | 2.96±0.35 | 2.96±0.11$^{ab}$ | 3.09±0.69 | 0.1125 | <.0001 |
| | 48 | 2.95±1.32 | 2.52±0.244 | 3.58±0.24$^{a}$ | 2.44±0.10 | 0.6453 | <.0001 |
| | 72 | 2.47±0.20$^{B}$ | 2.61±0.03$^{B}$ | 2.37±0.09$^{bB}$ | 3.37±0.23$^{A}$ | 0.0082 | <.0001 |
| | P-value | 0.8171 | 0.3254 | 0.0033 | 0.5166 | | |
| Methane | 0 | 8.53±0.36$^{BC}$ | 12.35±0.15$^{A}$ | 11.76±0.54$^{abAB}$ | 7.11±1.49$^{C}$ | 0.0049 | 0.6140 |
| | 24 | 7.77±0.30$^{BC}$ | 12.61±0.94$^{A}$ | 10.83±0.44$^{bAB}$ | 7.26±1.56$^{C}$ | 0.0117 | 0.6140 |
| | 48 | 8.93±1.79$^{BC}$ | 10.66±0.75$^{AB}$ | 13.03±0.43$^{aA}$ | 5.81±0.09$^{C}$ | 0.0055 | 0.6140 |
| | 72 | 10.75±0.47$^{A}$ | 11.27±0.17$^{A}$ | 8.47±0.30$^{cB}$ | 8.07±0.32$^{B}$ | 0.0003 | 0.6140 |
| | P-value | 0.2333 | 0.1636 | 0.0005 | 0.5595 | | |
| TVFA | 0 | 37.68±1.48$^{B}$ | 53.45±1.11$^{A}$ | 49.73±2.85$^{bA}$ | 33.95±3.85$^{B}$ | 0.0018 | 0.0014 |
| | 24 | 36.89±1.81$^{B}$ | 53.33±1.77$^{A}$ | 49.75±1.31$^{bAB}$ | 38.34±5.39$^{B}$ | 0.0115 | 0.0014 |
| | 48 | 40.81±8.40$^{AB}$ | 46.73±4.29$^{AB}$ | 59.65±2.65$^{aA}$ | 34.06±0.11$^{B}$ | 0.0318 | 0.0014 |
| | 72 | 48.95±2.87$^{A}$ | 48.22±0.48$^{AB}$ | 39.45±1.38$^{cB}$ | 45.20±2.44$^{AB}$ | 0.0379 | 0.0014 |
| | P-value | 0.3012 | 0.1795 | 0.0013 | 0.1603 | | |

$^{A, B}$: The means within the same row with different letter, are significantly different (P<0.05).
$^{a, b}$: The means within the same column with different letter, are significantly different (P<0.05).
TVFA: total volatile fatty acids.

**Table 7** Rumen fatty acid content (µg/100 mL) from *in vitro* gas production of industrial potato waste fermented at different times with *Lactiplantibacillus plantarum*, *Saccharomyces cerevisiae*, and *Aspergillus oryzae*

| Metabolite | Treatments | | | | | P-value | |
|---|---|---|---|---|---|---|---|
| | Time | Control | *L. plantarum* | *S. cerevisiae* | *A. oryzae* | Treatment | Treatment *Time |
| C12:0 (lauric) | 0 | 131.60±21.66 | 95.92±0.48 | 89.00±0.61 | 119.71±26.92 | 0.3293 | 0.1369 |
| | 24 | 91.83±7.10$^{B}$ | 96.67±0.47$^{B}$ | NS[2] | 116.41±2.09$^{A}$ | 0.0146 | 0.1369 |
| | 48 | 95.44±5.01 | 84.60±1.25 | NS | 177.28±52.26 | 0.1389 | 0.1369 |
| | 72 | 87.25±5.03 | 101.95±7.93 | NS | NS | 0.1926 | 0.1369 |
| | P-value | 0.1016 | 0.0794 | NV | 0.4169 | | |
| C14:0 (myristic) | 0 | 109.94±7.32$^{B}$ | 110.64±2.03$^{bB}$ | 143.06±23.19$^{B}$ | 232.56±4.07$^{A}$ | 0.0003 | 0.4715 |
| | 24 | 115.20±0.24$^{B}$ | 108.41±0.36$^{bB}$ | NS | 272.49±58.87$^{A}$ | 0.0236 | 0.4715 |
| | 48 | 125.44±8.01 | 134.84±10.18$^{a}$ | NS | 1013±787.53 | 0.3501 | 0.4715 |
| | 72 | 128.26±4.53$^{AB}$ | 93.25±0.25$^{bB}$ | NS | 149.40±17.61$^{A}$ | 0.0247 | 0.4715 |
| | P-value | 0.1739 | 0.0033 | Null | 0.4298 | | |
| C16:0 (palmitic) | 0 | 553.92±70 | 708.29±79.81 | 563.00±145.50 | 1396.54±645.09 | 0.3043 | 0.0748 |
| | 24 | 499.44±135.60$^{B}$ | 558.46±150.66$^{B}$ | 867.20±107.06$^{B}$ | 2624.29±218.55$^{A}$ | <.0001 | 0.0748 |
| | 48 | 622.02±157.35$^{B}$ | 522.13±173.24$^{B}$ | 785.47±125.91$^{B}$ | 2200.62±213.02$^{A}$ | 0.0004 | 0.0748 |
| | 72 | 508.52±141.19$^{CB}$ | 322.36±86.62$^{C}$ | 812.71±44.72$^{B}$ | 1761.64±163.51$^{A}$ | 0.0001 | 0.0748 |
| | P-value | 0.904 | 0.2829 | 0.3072 | 0.1764 | | |
| C18:0 (stearic) | 0 | 370.62±31.26$^{B}$ | 446.53±43.97$^{B}$ | 370.62±70.18$^{B}$ | 1299.40±77.34$^{bA}$ | <.0001 | 0.0045 |
| | 24 | 343.83±58.05$^{C}$ | 388.48±113.93$^{BC}$ | 651.93±80.50$^{B}$ | 2085.29±85.19$^{aA}$ | <.0001 | 0.0045 |
| | 48 | 406.34±110.55$^{B}$ | 392.95±116.10$^{B}$ | 553.69±96.70$^{B}$ | 1841.75±253.34$^{ab}$ $^{A}$ | 0.0005 | 0.0045 |
| | 72 | 415.27±7.73$^{BC}$ | 334.90±27.89$^{C}$ | 607.28±11.81$^{B}$ | 1174.37±122.37$^{bA}$ | <.0001 | 0.0045 |
| | P-value | 0.8508 | 0.8349 | 0.1001 | 0.0081 | | |
| C18:1n-9 cis/ΣMUFA (oleic) | 0 | 560.99±58.83 | 426.18±17.39 | 1343.77±94.08$^{a}$ | 1323.19±595.38 | 0.1248 | 0.1884 |
| | 24 | 444.44±1.29$^{C}$ | 404.44±133.26$^{C}$ | 1282.89±87.30$^{abB}$ | 2339.64±15.68$^{A}$ | <.0001 | 0.1884 |
| | 48 | 460.97±65.81 | 360.95±42.83 | 995.87±49.01$^{b}$ | 1275.51±625.28 | 0.2081 | 0.1884 |
| | 72 | 447.92±140.98$^{C}$ | 782.78±212.11$^{C}$ | 1252.45±13.05$^{abB}$ | 1965.64±56.54$^{A}$ | 0.0002 | 0.1884 |
| | P-value | 0.7283 | 0.1500 | 0.0315 | 0.3060 | | |
| C18:2n-6 cis/ΣPUFA (linoleic) | 0 | 59.67±8.93$^{a}$ | 46.11±3.58 | 47.79±4.27$^{a}$ | 436.85±388.24 | 0.4462 | 0.3607 |
| | 24 | 38.48±0.30$^{b}$ | 45.67±8.20 | 32.65±0.58$^{b}$ | 40.51±2.14 | 0.2632 | 0.3607 |
| | 48 | 38.67±0.64$^{b}$ | 111.67±76.76 | 27.38±0.65$^{b}$ | 36.69±2.29 | 0.4284 | 0.3607 |
| | 72 | 38.30±3.72$^{b}$ | 32.80±0.44 | 30.28±0.13$^{b}$ | 35.36±5.10 | 0.3761 | 0.3607 |
| | P-value | 0.0343 | 0.5048 | 0.0007 | 0.4191 | | |
| ΣSFA | 0 | 1166.07±127.89$^{B}$ | 1361.38±121.79$^{B}$ | 1165.68±195.13$^{B}$ | 3048.21±677.82$^{bA}$ | 0.0167 | 0.0200 |
| | 24 | 1050.29±186.15$^{B}$ | 1152.02±262.35$^{B}$ | 1519.13±179.82$^{B}$ | 5098.48±344.12$^{aA}$ | <.0001 | 0.0200 |
| | 48 | 1249.25±268.22$^{B}$ | 1134.51±280.42$^{B}$ | 1339.17±210.09$^{B}$ | 5232.65±845.42$^{aA}$ | 0.0007 | 0.0200 |
| | 72 | 1139.29±154.21$^{B}$ | 852.47±102.65$^{B}$ | 1419.99±54.87$^{B}$ | 3085.40±302.66$^{bA}$ | <.0001 | 0.0200 |
| | P-value | 0.9049 | 0.4371 | 0.5468 | 0.0451 | | |
| ΣUFA | 0 | 1225.74±135.06$^{B}$ | 1407.50±124.98$^{B}$ | 1213.46±199.10$^{B}$ | 3485.06±291.92$^{bA}$ | <.0001 | 0.0308 |
| | 24 | 1088.77±186.44$^{B}$ | 1197.70±257.99$^{B}$ | 1551.77±180.28$^{B}$ | 5139±346.25$^{aA}$ | <.0001 | 0.0308 |
| | 48 | 1287.92±267.59$^{B}$ | 1246.19±219.45$^{B}$ | 1366.54±210.73$^{B}$ | 5269.34±843.96$^{aA}$ | 0.0006 | 0.0308 |
| | 72 | 1177.60±157.69$^{B}$ | 885.27±103.06$^{B}$ | 1450.27±54.89$^{B}$ | 3120.76±301.50$^{bA}$ | <.0001 | 0.0308 |
| | P-value | 0.9011 | 0.324 | 0.586 | 0.0325 | | |

$^{A, B}$: The means within the same row with different letter, are significantly different (P<0.05).
$^{a, b}$: The means within the same column with different letter, are significantly different (P<0.05).
SFA: saturated fatty acid; UFA: unsaturated fatty acid; MUFA: mono saturated fatty acid and PUFA: poly unsaturated fatty acids.
NS: no synthesis and NV: no value.

Similarly, the sum of saturated fatty acids and the total unsaturated fatty acids had a significant (P<0.05) interaction. In view of the observations on rumen biohydrogenation of IPW inoculated with *L. plantarum, S. cerevisiae,* and *A. oryzae*; only stearic acid showed a significant (P<0.05) interaction between treatment and fermentation time. The current study observed that inoculated IPW altered rumen biohydrogenation. From previous literature, fatty acids found in potato are C12:0 (lauric acid), C14:0 (myristic acid), C18:0 (stearic acid), C18:1n-9 (oleic acid), C18:2n-6 (linoleic acid), C18:3n-3 (linolenic acid), and others of negligible quantities such as C18:1n-9 (trans-oleic acid), C20:3n-6 (eicosatrienoic acid), C20:5n-3 (eicosapentaenoic acid) and C22:6n-3 (docosahexaenoic acid) (Cotrufo and Lunsetter, 1964; Ramadan and Oraby, 2016). Nevertheless, the current study observed some inconsistent traces of C:21:0 (heneicosanoic acid), C20:1 (cis-11-eicosonate), C22:0 (behenic acid) and C20:3n-6 (cis-8, 11, 14-eicosatrienoic). No lauric acid was found in the blank samples used during the *in vitro* gas production, it was also observed that there was no synthesis of lauric acid and myristic acid in *S. cerevisiae* treatment group from fermentation time of 24, 48, and 72 h. It seemed that IPW fermented with *S. cerevisiae* favoured the synthesis of long-chain fatty acids from lauric acid and myristic acid. Lauric acid has been shown to improve fermentation characteris-

tics, and influence a significant reduction of protozoal population and methane production (Faciola and Broderick, 2014; Zhou *et al*. 2018). However, in the current study, the presence of lauric acid neither altered the population of protozoa and methanogens nor did it reduce the concentration of methane. Therefore, the variation could be related to the concentration of lauric acid supplementation used in the previous works (Hristov *et al*. 2011; Faciola and Broderick, 2014) compared to negligible lauric acid content in the rumen samples of the present work. Thus, the lauric acid content (87.25-131.60 µg/100 mL) in the current study was not enough to cause any defaunation as a result of the bursting of protozoa when they could not transform accumulated fatty acids in their bodies (Ibrahim *et al*. 2021). The limited number of fatty acids recorded in this study across the treatments was due to the fact that the substrate used in the current study was not a balanced diet or a feedstuff with a wider profile of fatty acids.

Reduction of polyunsaturated fatty acid (PUFA) [C18:2n-6 cis (linoleic)] to monounsaturated fatty acid (MUFA) [C18:1n-9 cis (oleic)] and finally to C18:0 (stearic) was an indication that linoleic acid was highly subjected to biohydrogenation, since stearic acid is the end product of 18-carbon unsaturated fatty acid (UFA) (McKain *et al*. 2010; Ibrahim *et al*. 2021). From the results of biohydrogenation, IPW inoculated with *A. oryzae* recorded the highest content of both saturated (3048.21-5232.76 µg/100 mL) and unsaturated fatty acids (3120.76 - 5269.34 µg/100 mL) than the sum of saturated and unsaturated fatty acids recorded in the control (1050.29-1249.25 and 1088.77-1287.92 µg/100 mL), *L. plantarum* (852.47-1361.38 and 885.27-1407.50 µg/100 mL) and *S. cerevisiae* (1165.68-1519.13 and 1213.46-1551.77 µg/100 mL) treatment groups. Consequently, the concentration of fatty acids in *A. oryzae* treatment group could be related to *B. fibrisolvens* recorded in *A. oryzae*. Even though *B. fibrisolvens* is a butyrate-producing bacterium, it is also associated with high biohydrogenation activity (Candyrine *et al*. 2017).

## CONCLUSION

The *in vitro* digestibility of IPW inoculated with *L. plantarum* (MW296876), *S. cerevisiae* (MW296931) and *A. oryzae* (MW297015) revealed that inoculation does not increase net gas production beyond 24 h incubation period, and it does not increase organic matter digestibility, and metabolizable energy. However, inoculated IPW increases the population of different classes of rumen microbiota higher than the uninoculated substrate. Among the three inocula, *L. plantarum* (MW296876) and *S. cerevisiae* (MW296931) tend to increase rumen ammonia-nitrogen and total volatile fatty acids. Specifically, *S. cerevisiae* (MW296931) consistently increased butyrate. Substrate inoculated with *A. oryzae* (MW297015) depressed methane production, and recorded the highest concentration of fatty acids. Consequent to the above, IPW appears as a potential feedstuff for ruminants, especially if it were inoculated with *A. oryzae* (MW297015) and subjected to a short-term fermentation for at least two days. Future study on long term effects of IPW on animal health and feasibility study on large scale processing of IPW to ruminant feed are recommended.

## ACKNOWLEDGEMENT

The authors wish to acknowledge the technical assistance and laboratory facilities of the Department of Animal Science, University Putra Malaysia.

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