IN VITRO FEED DIGESTIBILITY USING FIVE BACTERIA AND THEIR CRUDE ENZYMES OBTAINED FROM COW RUMEN

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INTRODUCTION

- Supply of meat is inadequate in developing Countries (FAO, 2017).
- Poor animal productivity resulting from poor digestibility of the principal constituents of livestock feeds is a factor (Mussatto and Teixeira, 2010).
- Cow's gut is a complex system packed with a consortium of microorganisms responsible for animal nutrition uptake and overall health (Myer *et al*., 2015).
- Bacteria are the predominant rumen microbes that enzymatically convert plant biomas into utilizable fermentation end- products (Zhou *et al*., 2009).
- Enzyme technology broadly involves production, isolation, purification and applications (Penner, 2014).
- Amylase, cellulase, pectinase, lipase and protease are of utmost importance in livestock feed digestion. Thus, this study focused on bacterial production and application of these crude enzymes.

MATERIALS AND METHODS

Sample collection

Cultures of five already identified rumen bacteria were obtained from the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria and their respective specific hydrolysis were produced while the conventional cow feed concentrate was commercially sourced.

Analyses of feed

- Proximate composition (AOAC, 2012)
- In-vitro digestibility test via gas production technique (Menke and Steingas, 1998) with slight modification by Akinfemi *et al*., (2009) as shown in plate 1.
- Feed digestibility characteristics (Getachew *et al*., 2002).

Extrapolations

- ME (MJ/Kg DM) = 2.20 + (0.136 x GV) + (0.057 x CP) + (0.0029 x CF)
- OMD (%) = 14.88 + (0.889 x GV) + (0.057 x CP) + (0.0029 x CF)
- SCFAs (mmol) = (0.0239 x GV) – 0.0601
- Where; ME: metabolizable energy, OMD: organic matter digestibility,

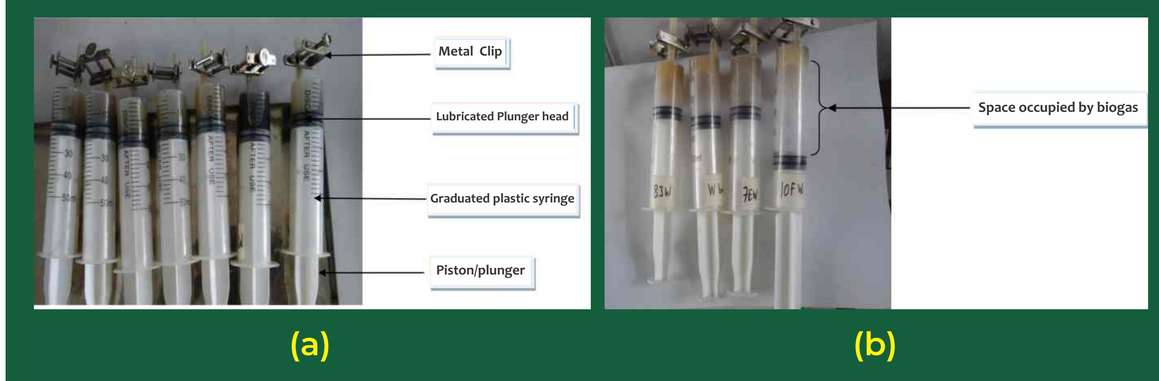

Plate 1: In vitro gas production at (a) 0 h incubation (b) 24 h incubation

RESULTS

Enzyme production and screening:
Five (5) autochthonous bacteria resident in the rumen of cow were isolated and identified as *Klebsiella edwardsii*, *Photobacterium damselae*, *Pseudomonas aeruginosa*, *Stenotrophomonas matophilia*, *Burkhoderia cepacia* to be best in specific activity of crude amylase, cellulase, pectinase, protease and lipase respectively.

Digestibility (rate of disappearance) of Feed:
- Combined whole cells and crude amylase produced detectable volume of biogas (2 ml/0.5 g DM) at 3 h of incubation (Table 1) while whole cells of *K. edwardsii* produced highest volume (36 ml) at termination of 24 h (Figure 1).
- *K. edwardsii* had the highest digestibility of 96.0 %, W*P.a* had the least (74%) while others had 92-94% (Figure 2).

Proximate Composition and Digestibility Characteristics of Feed:
The feed sample was high in CHO content (62.32 %) making it a good bio-degradable substrate for rumen bacteria with an estimated ME of 8.02 MJ/Kg, while OMD and SCFAs were 47.81 % and 0.80 mmol respectively.

Table 1: In vitro feed digestibility using whole cells and their crude enzymes singly and in combination

	Mean Gas production (ml) less blank at 3 h intervals over 24 h incubation period								
Isolate code	0 h	3 h	6 h	9 h	12 h	15 h	18 h	21 h	24 h
W*K.e*	0	0	1	1	2	9	17	28	36
W*P.d*	0	0	1	1	2	4	5	6	7
W*P.a*	0	1	1	1	1	1	1	1	1
W*S.m*	0	1	1	1	1	1	2	6	10
W*B.c*	0	0	0	2	3	4	4	4	4
W-bulk	0	2	2	3	11	14	15	15	16
E*K.e*	0	2	2	2	2	2	2	4	8
E*P.d*	0	0	1	1	1	1	2	4	6
E*P.a*	0	1	1	1	1	1	1	1	1
E*S.m*	0	0	0	0	0	0	0	0	0
E*B.c*	0	0	1	1	1	1	2	4	4
E-bulk	0	1	1	1	1	4	13	16	18

Key: W*K.e,* = whole cells of *K. edwardsii*, W*P.d* = whole cells of *P. damselae*, W*P.a* = whole cells *P. aeruginosa*, W*S.m* = whole cells of *S. matophilia*, W*B.c* = whole cells of *B. cepacia,* W-bulk = combined whole bacterial cells, E*K.e* = crude amylase, E*P.d* = crude cellulase, E*P.a* = crude pectinase, E*S.m* = crude protease, E*B.c* = crude lipase, E-bulk = combined crude enzymes.

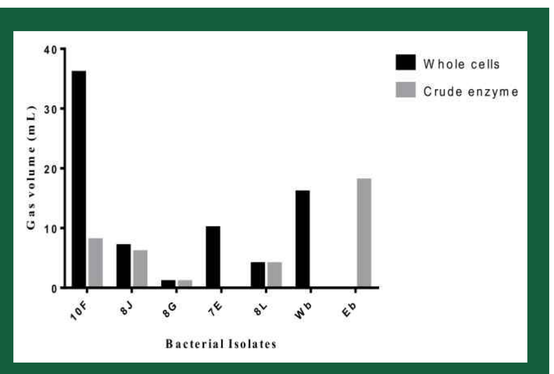

Figure 1: *In vitro* gas production at 24 h incubation

Key: 10F: *K.edwardsii*, 8J: *P damselae*, 8G: *Pseudomonas aeruginosa*, 7E: S. *matophilia*, 8L: *B. cepacia,* Wb: combined whole bacterial cells, Eb: combined crude enzymes

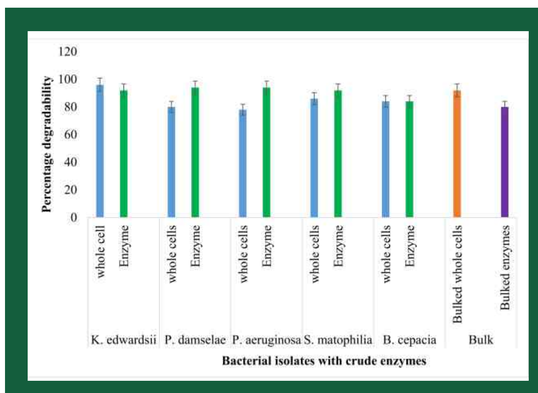

Figure 2: Percentage digestibility of conventional cow feed

Table 2: Proximate composition and digestibility characteristics of conventional cow feed

Parameter	Mean composition
% MC	6.46
% A	7.40
% CF	10.37
% EE	8.07
% CP	15.75
%NFE	51.95
%CHO	62.32
ME (MJ/Kg DM)	8.02
OMD (%)	47.81
SCFAs (mmol)	0.80

Key: MC = moisture content, A = ash, CF = crude fibre, EE = ether extract, CP = crude protein, NFE = nitrogen-free extract, CHO = carbohydrate, ME = metabolizable energy, OMD = organic matter digestibility, SCFAs = short chain fatty acid.

CONCLUSION

- *Klebsiella edwardsii* culture had the outstanding potential to spontaneously degrade the starch component of livestock feed into soluble and utilizable forms in addition to its ability to produce a methane-rich biogas whose flammability was tested.
- Synergy of combined whole bacterial cells were found more effective for partial feed degradation to enhance prompt nutrient uptake and utilization by farm animals so as to increase the animal protein supply for human consumption.

RECOMMENDATION

Detailed molecular studies on isolate conventionally identified as *Klebsiella edwardsii* in this study is hereby recommended as it showed the potential to produce many hydrolases which enhanced its exceptional ability to digest the feed *in vitro.*

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