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Microbial population, identification and *in vitro* studies of West African Dwarf goats fed diets with or without urea treated corncob

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Target Audience*: Ruminant Animal Scientists; Nutritionists; and Feed millers*

Abstract

Corncob is an agricultural by-product emanating from harvested maize which is considered as waste after maize seeds/grains are removed either in fresh or dry form. Effort can be made to fortify and utilise it as feed for ruminant animals using some substances. Thus, a study was conducted to evaluate microbial population, identification and in vitro studies of West African Dwarf (WAD) goats fed diets with or without urea treated corncob. Sixteen WAD goats of four animals/treatment were randomly allotted to four dietary treatments comprising of T1 (diet without urea treated corncob); T2 (diet with corncob treated with 1.5% urea); T3 (diet with corncob treated with 2% urea); and T4 (diet with corncob treated with 2.5% urea). The diets were fed for 12 weeks after which rumen liquor was collected from the animals for evaluations and in vitro studies. Data obtained were analysed using one-way ANOVA at 5% probability level. Results obtained revealed that bacteria population was highest ($p<0.05$) and lowest with values of 1.60 and 1.02×10^4cfu/ml, respectively for goats fed T3 and T2. Bacteria isolates common to all the treatments included Streptococcus faecalis and Eschericia coli; while Penicillin notatum was for fungi. TI had the highest significant ($p<0.05$) values for in vitro parameters like gas production at 33rd, 42nd and 45th hours of incubation; dry matter digestibility (41.67%) and CO_2 (15.00ml/200mg DM). It could be concluded from the study that inclusion of urea treated corncob generally improved microbial population, reduced value for CO_2, whereas T1 had highest dry matter digestibility.

Key Words: *West African Dwarf goats; Urea; Corncob; Microbial population; Microbial identification; In vitro study*

Description of Problem

There is high yield in maize production in Nigeria which is usually accompanied with huge quantity of corncob either after consumption in fresh form or shelling of grains in dry form. There are more than 21 million tonnes of plant by-products produced annually, among them is the corn crop residues which include; green corn, corn stover, corn stalk and corncobs (1). The corncob can be disposed by dropping it off on the floor; piling up in a place; and dumping in the river which can cause environmental and water pollution. It can

also be disposed by burning which will also cause air pollution by releasing some harmful gases to the air. Several uses of corncob include but not limited to carbon adsorbent (2); bacteria cellulose (3); carbon fuel cell (4); and Acetone-Butanol (5). Despite these applications of corncob in the industry, its usage is limited in livestock production due to high fibre content and low crude protein. Goat farming in developing countries rely on conventional feedstuffs whose costs are high and not readily available due to their competition with human

Corncobs have poor nutritive value which can be improved by different methods of treatment and supplementation. Effort to improve the quality of corncob as a feed for ruminant can be done by physical treatment, chemical treatment, biological treatment or combination of these treatments (6). Physical treatment can be done by combining chemical treatment such as ammoniation and biological treatment which is fermentation using a microbial cellulolytic starter. Urea is an inorganic compound that contains 46.7% of nitrogen. Urea treatment has, however, emerged as the system of choice used in the tropical farm level as it is best adapted to the condition of subsistence farming (7). The best way to improve the use of corncob for ruminant animals is to overcome the barrier to rumen microbial fermentation of lignocelluloses. Thus, the study was conducted to evaluate the effects of urea treated corncob inclusion in the diets of West African Dwarf goats on the microbial population, identification and in vitro studies.

Materials and Methods

Experimental site

The field work was conducted at the Small Ruminant Unit of the Directorate of University Farms (DUFARMS) Federal University of Agriculture Abeokuta (FUNAAB), Ogun State, Nigeria. Microbial population and identification were done at Microbiology Laboratory, College of Veterinary Medicine; while *in vitro* studies and feed analyses were done at the Department of Pasture and Range Management Laboratory, College of Animal Science and Livestock Production, FUNAAB. The area falls within Latitude 7°10N and Longitude 3°E in Odeda Local Government Area of Abeokuta, Ogun state, Nigeria (8). The area has a tropical climate characterized with an annual rainfall of about 1,037mm; minimum and maximum temperature of 20.66°C and 35.48°C, respectively. The vegetation lies in between the tropical rainforest and derived savannah

Preparation of Urea treated Corncob

Dry corncobs were obtained from DUFARMS after shelling of maize cobs, while feed-grade urea was purchased at a reputable feed mill in Abeokuta. The corncobs were sundried until a constant weight was obtained. Foreign materials present in the corncobs were handpicked, thereafter, the corncobs were taken to the feedmill for crushing. The crushed corncob was sieved using 5mm sieve. Known quantities (1.5%, 2.0%, and 2.5% of the sieved corncob) of the urea were dissolved in known volume (33.33cl/kg corncob) of water. Thereafter, the sieved corncob was mixed thoroughly with the respective urea solution. The mixed corncob was packed inside separate thick black polythene bags, compressed to remove air and tightened thoroughly. The bags were put inside air-tight plastic containers with weights put on the bags to further expel air and kept in a cool, dry place for 72hours, after which the bags were opened. The fermented corncob was spread on a clean platform on the floor for air-drying until a constant weight was

obtained. The air-dried fermented corncob was then mixed with other feed ingredients to make respective experimental diet.

The flow chart for the fermentation is presented in Figure 1.

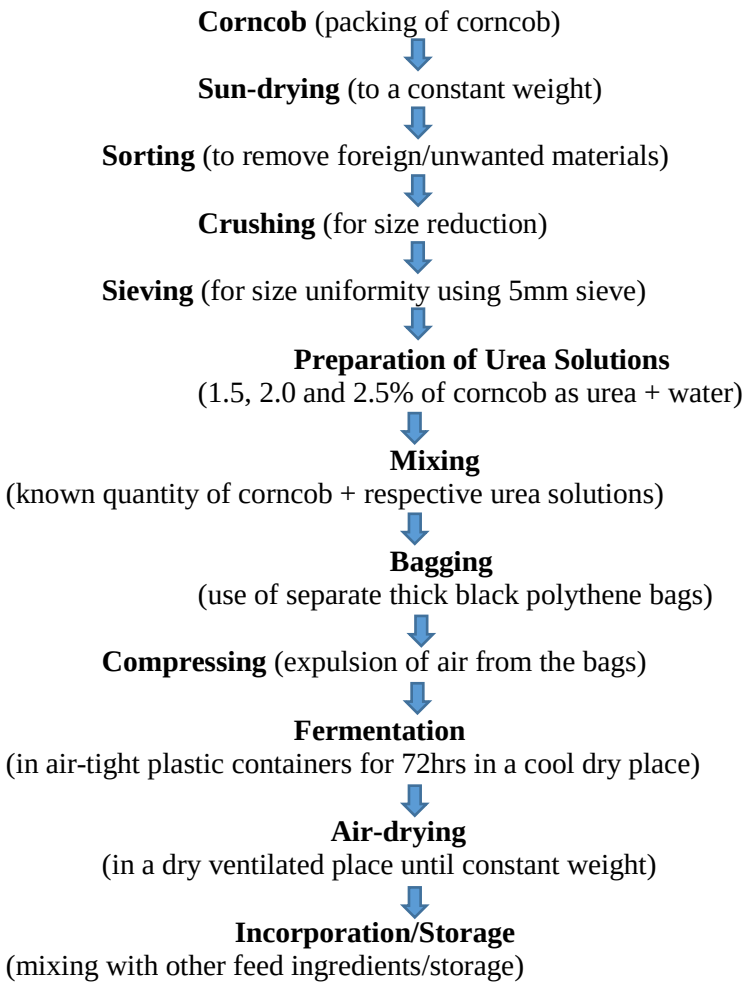

Figure 1: Flowchart showing steps involved in the preparation of urea treated corncob

Experimental Animals and Management

Sixteen (16) growing and physically healthy West African Dwarf bucks which were not up to a year were purchased from a reputable livestock market in Iwo, Osun State. Prior to the arrival of the goats, the experimental pens covered with aluminium roofing sheets with raised slatted floor were thoroughly swept, washed and disinfected. Upon the arrival of the animals, they were administered glucose (as an anti-stress) and prophylactic. PPR vaccine was also given in addition to other medications upon their arrival. The goats were observed for 4weeks for diseases/infections during that time. Concentrate and dry cassava peels were fed, while water was made available without restriction throughout the study period.

The goats were tagged and randomly allotted to 4 experimental diets (Table 1) with 4 goats per treatment. The goats were fed at 5% of their body weight per day. The goats were housed and fed individually during the study. The animals were fed experimental concentrate diets and wilted Guinea grass chopped to 5cm throughout the study which lasted for 12 weeks. Concentrate was fed in the morning (8:00am) while the grass was fed in the afternoon (1:00pm). Before feeding the animals each day, leftover/feed refused per animal is packed and weighed for both concentrate and grass fed.

Rumen Liquor Collection

Rumen liquor was collected at the end of the feeding trial (i.e. 12th week) from all the animals used using ½ inch suction tube before feeding in the morning. The tube was rinsed thoroughly for about 3 times using running distilled water before it was used for another animal. The liquor collected from each goat was dispensed in a clean sample bottle and kept in a pre-warmed flask (39°C) and taken to the laboratory as soon as the last animal was aspirated for necessary evaluations.

Table 1: Composition (%) of experimental concentrate diets

Ingredients	T1	T2	T3	T4
Maize	10.00	10.00	10.00	10.00
Corn bran	20.00	20.00	20.00	20.00
Wheat offal	30.00	00.00	00.00	00.00
Corn cob	0.00	30.00	30.00	30.00
Palm kernel cake	30.00	30.00	30.00	30.00
Bone	3.50	3.50	3.50	3.50
Limestone	3.00	3.00	3.00	3.00
Salt (NaCl)	3.00	3.00	3.00	3.00
*Grower's premix	0.50	0.50	0.50	0.50
Total	100	100	100	100

*****Contains vitamin A (IU) 10,000.000; vitamin D2 (IU) 2,000,000; vitamin E (IU)20,000; vitamin K (mg) 2250; riboflavin (mg) 5000; pyridoxine (mg) 275; biotin (mg) 50; pantothenic acid (mg) 7500; vitamin B1 (mg) 175; vitamin B12 (mg) 15.0; niacin (mg) 27,500; folic acid (mg) 7500; choline chloride (mg) 400; antioxidant (mg) 125; Fe (g) 20.0; Zn (g) 50.0; Mn (g) 80.0; Cu (g) 5.0g; I (g) 12.0; Co (mg) 200; Se (mg) 200

T1 is concentrate diet without corncob;
T2 is concentrate diet with corncob treated with 1.5% urea
T3 is concentrate diet with corncob treated with 2.0% urea
T4 is concentrate diet with corncob treated with 2.5% urea

Laboratory Analyses

The liquor collected was taken to Microbiology Laboratory for microbial count and identification determinations, while *in vitro* studies was carried out at Pasture and Range Management Laboratory, Federal University of Agriculture Abeokuta.

***1. Microbial Counts and Identification*:** Total bacteria and fungi counts were done according to Harrigan and Mc Cance (9). Pure culture of bacteria and fungi were determined according to the method of Frumholtz *et al*. (10).

2. In vitro Studies: This was determined following the procedure of Menke and Steingass (11). A sensitive scale was used to measure 200mg each of milled experimental

concentrate diets in four (4) replicates and then placed into 100ml graduated syringes. Separate rumen fluid (inoculum) was collected inside a pre-warmed flask (39°C) before feeding in the morning from a donor West African Dwarf goat using same procedure. The inoculum was strained through 2 layers of cheese cloth and mixed with Sodium an Ammonium Bicarbonate buffer (35g $NaHCO_3$ + 4g NH_4HCO_3 per litre) at a ratio of 1:2 (v/v) to prevent lowering of the pH of the rumen fluid which could result in decreased microbial activities. Thirty (30) milliliters of the buffered inoculum were added to each syringe containing the milled feed samples. The syringes were positioned vertically in a water bath and kept at 39°C. A blank syringe containing 30ml of the buffered inoculum was included as a control. All the syringes were gently shaken after the commencement of incubation at regular intervals of 3hours for 48hours. Gas released was read directly on the graduated syringes at those intervals.

Data obtained from *in vitro* gas production was fitted to the non-linear equation of Larbi *et al.* (12):

V (ml/0.2 g DM)= $GV(I-e^{-ct})$

Where *V* is the potential gas production, GV is the volume of gas and *ct* is the fractional rate of gas production.

Organic matter digestibility (OMD) was estimated as:

OMD = 14.88 + 0.889GV + 0.45CP + 0.651Ash (11).

Short-chain fatty acids (SCFA) was estimated as:

SCFA = 0.0239GV – 0.0601 (13).

Metabolizable energy (ME) was calculated as:

ME = 2.20 + 0.136GV + 0.057CP + $0.029CP^2$ (11).

Total gas volume (GV) was expressed as ml/0.2 g DM; OMD, CP and Ash as %; ME as MJ/kg DM; and SCFA as µmol/g DM.

Methane gas determination

The volume of methane gas produced by each dietary treatment was determined by dispensing 4ml of 10N sodium hydroxide into each incubated sample at the end of 48hr of incubation period. Sodium hydroxide was added to absorb Carbon IV Oxide produced during the process of fermentation and the remaining volume of gas was recorded as methane according to the method of Fievez *et al.* (14).

Chemical analysis

Samples of the experimental concentrate diets and grass fed were analysed for proximate composition according to the method of A.O.A.C. (15), while fibre fractions were determined according to procedure of Van Soest *et al.* (16).

Statistical analysis

Data obtained at the end of the study were subjected to one way analysis of variance (ANOVA) in a completely randomized design using SAS (17). Significant level was taken at 5% probability, while significant means were separated using Duncan's Multiple Range Test (18) of same statistical package.

Statistical model

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

Where:

Y_{ij} = Yield or output

μ = Population mean

T_i = Effects of treatment

Σ_{ij} = Residual error

Results and Discussion

Proximate and fibre fraction composition of experimental concentrate diets and Guinea grass

The proximate and fibre fraction compositions of concentrate diets and Guinea grass fed to the animals during the study is presented in Table 2.

Table 2: Proximate and fibre fraction compositions of experimental concentrate diets and Guinea grass

Parameters (%)	T1	T2	T3	T4	G. grass
Dry matter	93.00	91.00	92.00	91.00	88.10
Crude protein	11.11	6.90	8.45	10.07	9.40
Ether Extract	12.33	12.00	12.67	13.00	2.20
Crude Fibre	4.57	7.73	4.87	8.77	35.60
Ash	4.93	4.65	7.44	5.02	10.70
Nitrogen free extract	67.06	68.72	66.57	63.14	42.10
Organic Matter	95.07	95.35	95.13	94.98	89.30
Fibre Fractions					
Neutral Detergent Fibre	58.67	58.66	60.00	59.33	60.41
Acid Detergent Fibre	34.67	36.67	34.67	32.00	37.84
Acid Detergent Lignin	9.33	8.67	9.33	10.67	20.71
Hemicellulose	24.00	22.00	25.33	27.33	25.57
Cellulose	25.33	28.00	25.33	21.33	17.13
*ME (MJ/kg DM)	13.22	13.24	13.23	13.19	12.77

*Calculated using De Boever *et al.* (19) equation **ME** is Metabolisable Energy
T1 is concentrate diets without corncob; **G** is Guinea grass
T2 is concentrate with corncob treated with 1.5% urea,
T3 is concentrate with corncob treated with 2.0% urea
T4 is concentrate with corncob treated with 2.5% urea and.

Microbial population and identification of rumen liquor of West African Dwarf goats fed diets with or without urea treated corncob

The microbial population and identification of rumen liquor of West African Dwarf goats fed diets containing corncob treated with urea is presented in Table 3. The experimental concentrate diets had significant ($p<0.05$) influence on the bacteria population, whereas it had no effect ($p>0.05$) on fungi population. The count for bacteria population had a range of 1.02 to 1.60×10^4cfu/ml. The highest value was recorded for goats fed T3, while goats fed T2 had the lowest value for bacteria population. The range of 0.40 to $0.60\text{x}10^4$cfu/ml was recorded for fungi count, where goats fed diets containing urea treated corncob had higher ($p>0.05$) values numerically for fungi population compared to those without urea treated corncob with the least value for same parameter. This indicated that inclusion of urea treated corncob in the diets of the goats improved both bacteria and fungi populations by providing the required energy and protein for their growth. As the population of bacteria and fungi increase in the rumen, crude protein and fibre degradations will improve at reasonable rate and invariably microbial protein available for the animals will also increase. This is indicative of a normal rumen environment. Oni *et al.* (20) reported that population density of microbes could be attributed to availability of nutrient in the rumen which triggers microbial proliferation. Rumen bacteria population is dependent on ammonia concentration and pH of rumen fluid, and both factors are dependent on type of diet(s) fed to animals. Rumen bacteria is the principal agent for fermenting plant cell

wall carbohydrates hence they constitute the largest proportion of microorganisms in the rumen in relation to fungi.

The bacteria species isolated include: *Lactobacillus spp., Streptococcus faecalis, Pseudomonas spp., Proteus spp.* and *Eschericia coli*. According to Mould *et al.* (21) the main rumen bacteria species are *Fibrobacter succinogens, Ruminococcus amylophilus, Prevotella ruminicola, Butyrivibrio fibrosolvens, Ruminococcus spp., Selenomonas ruminantum, Streptococcus bovis, Eubacterium ruminatum, Lactobacillus spp. and Megasphaera elsdenii*. For this study, only *Streptococcus spp.* and *Lactobacillus spp.* were similar to those identified by the author. *Ruminococcus flavafaciens, Ruminococcus albus, Bacteroides succinogens* and *Butyrivibrio fibrosolvens* were reported by Preston and Leng (22) as the most common rumen bacteria for fibre degradation. This is contrary to this study where none of the microbes was present. This may be attributed to the technique used for identification. There have been reports of limitations in the use of culture-based technique to evaluate bacteria populations as it substantially underestimates the diversity of microorganism within the rumen (23).

Rumen fungi have been shown to digest cellulose and xylans which shows that they may play a role in helping the ruminant animals to digest plant materials (22). Rumen anaerobic fungi actively colonize plant cell walls and account for up to 8 to 12% of the microbial biomass in rumen (24). However, the fungi identified for this study were; *Aspergillus spp (Aspergillus flavus* and *Aspergillus fumigatus), Penicillium notatum* and *Fusarium oxysporum*.

Table 3: Microbial population and identification of rumen liquor of West African Dwarf goats fed diets containing corncob treated with urea

Parameters	T1	T2	T3	T4	SEM
Bacteria					
TBC × 10^4 cfu/ml	1.27	1.02	1.60	1.15	0.09
Lactobacillus spp.	+	−	+	+	
Streptococcus faecalis	+	+	+	+	
Pseudomonas spp.	+	−	+	+	
Eschericia coli	+	+	+	+	
Fungi					
TFC × 10^4 cfu/ml	0.40	0.52	0.60	0.60	0.53
Penicillin notatum	+	+	+	+	
Fusarium oxysporum	+	−	−	+	
Aspergillus flavus	−	+	+	+	
Aspergillus fumigatus	+	+	+	−	

[ab] Means on the same row having different superscripts are significantly different
T1 is concentrate diets without corncob
T2 is concentrate with corncob treated with 1.5% urea
T3 is concentrate with corncob treated with 2.0% urea
T4 is concentrate with corncob treated with 2.5% urea
SEM is Standard Error of Mean
TBC is Total Bacteria Count **TFC** is Total Fungi Count **cfu** is colony forming unit

Table 4: *In vitro* gas production (ml/200mg DM) of diets with or without urea treated corncob for 48hours of incubation

Hours of Incubation	Dietary Treatments				SEM
	T1	T2	T3	T4	
3	0.00	0.00	0.00	0.00	0.00
6	0.50	0.50	0.00	0.50	0.20
9	4.50	3.00	1.00	3.50	0.73
12	6.50	4.00	2.00	5.00	0.90
15	9.50	6.00	3.50	8.00	1.01
18	12.00	9.50	7.50	10.50	0.84
21	14.00	12.50	9.50	13.50	0.84
24	18.00	16.00	13.50	16.50	1.09
27	19.50	17.00	14.00	17.00	1.05
30	21.50	18.00	15.00	18.50	1.26
33	24.50[a]	20.00[ab]	15.00[b]	19.50[ab]	1.43
36	25.00	20.50	16.00	20.00	1.49
39	26.00	21.00	17.00	21.00	1.47
42	27.50[a]	22.00b[a]	17.50[b]	22.00[ab]	1.45
45	29.00[a]	22.00[ab]	18.50[b]	22.00[ab]	1.49
48	29.50	22.50	18.50	23.00	1.57

[ab] Means on the same row having different superscripts are significantly different ($p<0.05$)
T1 is concentrate diets without corncob
T2 is concentrate with corncob treated with 1.5% urea
T3 is concentrate with corncob treated with 2.0% urea
T4 is concentrate with corncob treated with 2.5% urea
SEM is Standard Error of Mean

In vitro gas production parameters, post-incubation parameters and gas production kinetics

The *in vitro* gas production technique is useful for determining the feed value of diets for farm animals. Digestibility can be estimated from *in vitro* gas production, which has the advantage of not only being less expensive but less time consuming too (25; 14). The *in vitro* gas production (ml/200mg DM) of concentrate diets with or without urea treated corncob fed to West African Dwarf goats for 48hours of incubation is presented in Table 4. Result obtained for *in vitro* gas production was significantly ($p<0.05$) influenced at 33rd, 42nd and 45th hours of incubation. Diet without urea treated corncob had the highest gas production of 24.50, 27.50 and 29.00ml/200mg DM at those periods, whereas diet containing 2.0% urea treated corncob had the least values of 15.00, 17.50 and 18.50ml/200mg DM.

In vitro post-incubation parameters and gas production kinetics of concentrate diets with or without urea treated corncob fed to West African Dwarf goats is presented in Table 5. The results indicated that Dry Matter Digestibility (DMD) and Carbon IV Oxide (CO_2) were significantly ($p<0.05$) influenced. Values obtained for DMD and CO_2 were highest (41.67% and 15.00ml/200mg DM, respectively) for concentrate diet without urea treated corncob.

The neutral detergent fibre (NDF) contents of the diets were high and this can cause increase in gas production, which is in agreement with the report that there exist correlations between volume of gas, moles

of volatile fatty acids produced and the mass of fibre digested in NDF samples (26). Highest value for *in vitro* DMD recorded for diet without urea treated corncob could possibly be due to high level of Crude Protein of the diet. The provision of protein may enhance the activity of the rumen microorganisms and improve digestibility of feed stuffs (27). Methane and CO_2 gases are energy losses to ruminants and are among the main greenhouse gases that contribute to global warming (28; 29).

Table 5: *In vitro* post-incubation parameters and gas production kinetics of diets with or without urea treated corncob

	Dietary Treatments				
Parameters	**T1**	**T2**	**T3**	**T4**	**SEM**
Post-Incubation					
OMD (%)	45.00	42.45	38.90	42.75	1.05
DMD (%)	41.67[a]	30.00[b]	28.30[b]	31.67[b]	1.91
ME (MJ/kg DM)	9.70	9.05	9.14	9.91	0.25
SCFA (µmol)	0.37	0.32	0.26	0.33	0.03
CO_2 (ml/200mg DM)	15.00[a]	9.00[b]	5.50[b]	8.00[b]	1.19
Methane (ml/200mg DM)	14.50	13.50	13.00	15.00	0.66
Gas Production Kinetics					
b (ml/200mg DM)	36.50	34.22	39.90	39.83	3.27
c (ml/hour)	0.03	0.03	0.02	0.04	0.00
Lag time (hour)	5.16	4.96	5.79	4.75	0.37

[abc] Means on the same row having different superscripts are significantly different ($p<0.05$)

T1 is concentrate diets without corncob, **T2** is concentrate with corncob treated with 1.5% urea, **T3** is concentrate with corncob treated with 2.0% urea, **T4** is concentrate with corncob treated with 2.5% urea and **SEM** is Standard Error of Mean

OMD is *Organic matter digestibility;* **DMD** is *Dry matter digestibility*
ME is *Metabolisable energy* **SCFA** is *Short chain fatty acid*
CO_2 is *Carbon IV Oxide*
b is *Volume of gas produced in time (t)* **c** is *Fractional rate of gas production*

Conclusion and Applications

The following conclusions were drawn from the results of the study:

1. Inclusion of corncob treated with urea at 2.0% had the highest bacteria population;
2. Bacteria isolates common to all the treatments were *Streptococcus faecalis* and *Eschericia coli*; while *Penicillin notatum* was for fungi;
3. Diet without urea treated corncob had the highest values for *in vitro* parameters like gas production at 33rd, 42nd and 45th hours of incubation; Dry Matter Digestibility (41.67%) and CO_2 (15.00ml/200mg DM).

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