



Tropentag 2014, Prague, Czech Republic
September 17-19, 2014

Conference on International Research on Food Security, Natural Resource
Management and Rural Development
organised by the Czech University of Life Sciences Prague

Early Stage Identification of Rumen Microorganisms from Selected Nigerian Breeds of Cattle

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INTRODUCTION

Cattle are ungulates (Grubb, 2005) with a complex stomach system (Kudo *et al.*, 1995) modified for rumination; they are raised as livestock for meat production, dairy animals for milk production and as draft animals. Their by-products also serve as valuable raw materials for manufacturing industries; horns and hoofs in ceramics, bones in livestock feed mills, hide and skin for leather production while carcasses and dung could be stabilized into organic manure and biogas production. (Parawira, 2004).

Nigerian Breeds of Cattle: About twelve breeds (Plate 1) are reported for Nigeria but extensive studies on origin, adaptation and management have been conducted on Kuri, N'dama, Muturu, Gudali and Red bororo/Fulani (DAGRIS, 2007).

The rumen (Plate 1) is the first and the largest compartment where continuous anaerobic fermentation takes place by a complex consortium of microorganisms (Kudo *et al.*, 1995) thus, characterized as the World's largest fermentation process (Weimer, 1992).

This study aimed at enumerating, identifying and determining the percentage occurrence of the total heterophilic bacteria and fungi resident in the rumen of cattle with respect to different breeds raised in Nigeria.

The kinetics of fermentative activities in the rumen are a result of feed in-take, as a large part of the feed is composed of lignocellulosic crop-residues, this study assessed the micro-flora associated with rumen of selected cattle breeds commonly raised in Nigeria.

MATERIALS AND METHODS

The four breeds identified for this work were both male and female animals of Bunaji (white Fulani), Keteku (Futumi), Bokolo (N'dama) and Djeli (Sokoto Gudali) according to the description of DAGRIS, (2007).

A kitchen-spoonful of rumen content was aseptically scooped immediately after opening the rumen, into pre-labeled sterile empty bottles from 4 different portions of each rumen from skinned animals across the identified breeds. In this way, four (4) animals were examined over a period of 12 weeks.

All glassware were sterilised at 160°C for two hours in the hot-air oven, culture media were autoclaved at a temperature of 121°C under a pressure of 15 psi (100Kpa, 1.05kg/cm³, 15lb/sq inch) for 20 minutes according to the method of Chamberland modified by Jacquelyn (1993), and the bench surface was disinfected with 70% ethyl alcohol, an incineration to red hot was employed for sterilizing inoculating loops and scalpels.

Modified methods prescribed by Cowan and Steel (1985) and Buchman and Gibbons (2000) were carefully adopted for culturing the total heterophilic bacteria (THB) and total heterophilic fungi (THF). One ml each of 100-fold serially diluted suspension was plated out with nutrient agar (NA) and incubated at 37°C for 36-48 hrs to estimate the bacterial colonies formed.

The procedure for the THF, according to Borneman *et al.*, (1992), was similar to that of THB used except that malt extract (ME) agar (containing antibiotics) was employed as the culture medium, also, the plates were incubated uninvertedly at 30°C for 5-7 days (Okore, 2004).

Colonial characteristics, cell morphology, spore formation and biochemical reactions (Cheesbrough, 2006) led to the identification of bacteria and fungi that are autochthonous to cattle rumen.

Analysis of variance with means separated by the method of Least Significant Differences of the level of 5% ($p < 0.05$) as described by SAS (1999).

RESULTS AND DISCUSSION

Rumen pH and Total Viable Microbial Counts

The pH measurement ranged between 5.65 for female Keteku (Kf) and 6.90 for male Bunaji (Bm) (Table 1). No significant difference ($p > 0.05$) was observed between male Djeli (Dm) and female Djeli (Df), male Keteku (Km) and male Bokolo (Bkm) exhibited the same pH-value (6.60). However, the standard error based on sex of the selected animals revealed a significant difference ($p < 0.05$) in pH between male (6.71) and female (6.10) cattle.

Majority of rumen bacteria are also described as obligate anaerobes, having an optimal pH range of 6.0-6.9 at an optimum temperature of 39°C. The bacteria can tolerate a considerably higher level of organic acids without affecting adversely their metabolism.

The pH conditions of the sampled rumens could be said to be in agreement with the optimal range (6.0-6.9) described by Kamra, (2005), but partially differed from the old report of Johnson, (1963), which recorded optimal rumen pH of 6.7-7.0. Slightly acidic rumen pH (5.90 and 5.65) exhibited by both Bf and Kf respectively, could have resulted from the feed ingredients they were fed with, as feed rich in soluble sugars are capable of lowering the rumen pH when they are finally broken down (Yen and Brune, 2007; Orpin, 1977b).

Total Heterophilic Bacteria (THB)

As shown in Table 1, Dm and Df recorded both the highest (1.125×10^{11}) and the lowest (9.1×10^8) populations of THB respectively with a significant dissimilarity ($p < 0.05$) existing between them (Table 1). Bm, Bf, Km, Kf, Bkm and Bkf recorded THB in the range of 2.25×10^{10} - 5.3×10^{10} . Although, Bf (2.25×10^{10}), Bkf (2.25×10^{10}) and Df (9.1×10^8) were significantly indifferent ($p > 0.05$). Significant difference ($p < 0.05$), however, existed between male (5.8125×10^{10}) and female (2.4728×10^{10}) cattle selected for this study, as revealed by the standard error based on sex. The bacterial load obtained in this work agrees with the result obtained by Kamra, (2005) in which the efficiency of ruminants to utilize a wide variety of feeds (Stanton and Canale, 1980) was based on a highly diversified rumen microbial ecosystem consisting of bacteria (10^{10} - 10^{11} cells/ml). All the microorganisms isolated at the early stage from the selected breeds of cattle in this study differ from those identified by

Czerkawski (1986) as reported by Yokoyama (1993). However, variations in microbial load have been extensively dealt with according to the reports of Orscov, (1993a)

Total Heterophilic Fungi (THF)

The fungal load ranged between 1.5×10^3 (value enumerated for Bm, Bkm and Dm) and 6.0×10^5 (count recorded for Bf) with no growth observed for Bkf (Table 1), thus, the counts obtained in this study are in agreement with the fungal load obtained by Kamra, (2005). However, standard error based on sex revealed a significant difference ($p < 0.05$) male (2.4875×10^4) and female (2.26376×10^5) cattle selected for this study, implying that cows generally, are more loaded with fungi than bulls.

Obligate anaerobic fungi found in the rumen have an active and positive role to play in fibre degradation (Paul *et al.*, 2003). Founty and Joblin, (1990) had also reported that fungi have additional advantage of better penetration of the lignocellulosic feeds over the cellulose-degrading bacteria due to the presence of different enzymes like *protease* and *esterase* in addition to *cellulase* and *hemicellulase*.

Table 1: Rumen pH and Total Viable Counts for both Heterophilic Bacteria and Fungi

Breed of Animal	THB	THF	pH Value
Bm	$5.1E10^c \pm 2.0E9$	$1.5E3^c \pm 5.0E2$	$6.90^a \pm 0.1$
Bf	$2.25E10^f \pm 5.0E8$	$6.0E5^a \pm 1.0E5$	$5.90^e \pm 0.1$
Fm	$3.4E10^e \pm 1.5E9$	$9.5E4^c \pm 5.0E3$	$6.60^c \pm 0.1$
Ff	$5.3E10^b \pm 1.0E9$	$3.0E5^b \pm 2.9E5$	$5.65^f \pm 0.05$
Bklm	$3.6E10^d \pm 1.5E9$	$1.5E3^c \pm 5.0E2$	$6.60^c \pm 0.1$
Bklf	$2.3E10^f \pm 1.5E9$	NIL ^c	$6.10^d \pm 0.1$
Dm	$1.1E11^a \pm 1.5E9$	$1.5E3^c \pm 5.0E2$	$6.75^b \pm 0.05$
Df	$9.1E8^f \pm 1.0E7$	$5.5E3^c \pm 5.0E2$	$6.75^b \pm 0.15$

Values are means \pm Standard deviation of duplicate readings. Values on the same column with different superscripts are significantly different ($p < 0.05$).

THB: Total Heterophilic Bacteria, THF: Total Heterophilic Fungi, pH: hydrogen ion concentration, Bm: male Bunaji, Bf: female Bunaji, Fm: male Futumi, Ff: female Futumi, Bklm: male Bokolo, Bklf: female Bokolo, Dm: male Djeli, Df: female Djeli, E8: $\times 10^8$, E10: $\times 10^{10}$, E11: $\times 10^{11}$.

Okareh *et al.*, (2012) attributed population variation between bacteria and fungi to the competitive and unfavourable conditions created around fungi. Such include; variation in pH resulting from C/N ratio (Yen and Brune, 2007), nutrient availability (Eze and Agbo, 2010), the toxic effect of phytochemicals in feeds (Akin and Rigsby, 1987) and the parasitic effect of *Cladosporium herbarium* on other fungi (Prior, 1966).

Fungi found in rumen are involved in the early part of degradation (hydrolysis and acidogenesis) by means of extra-cellular digestion of the substrates, depending on the nature of substrate (Atlas, 1984). Flint *et al.* (2008) reported that the removal of fungi from bovine stomachs had significantly reduced effect on the amount of fibrous feed breakdown within rumen. Ruminal obligate anaerobic fungi make up only 5-10% of microbes but are absent on diets poor in fibre.

Despite their low numbers, the fungi still occupy an important niche in the rumen because they hydrolyze some ester linkages between lignin and hemi-cellulose or cellulose, and help break down digesta particles (Paul *et al.*, 2003). The partial digestion of microbial carcasses releases high quality protein to cattle and allows them to thrive on grasses and other vegetation. The efficiency of utilization of 'feed energy' is the amounts lost as heat (45%), faeces (40%), and urine together with combustible gases (10%). Whereas, the energy converted to products (tissue accretion) is as low as 5% (Ferrell, 1993).

Isolates

Species of *Proteus*, *Klebsiella* and *Citrobacter* are peculiar to cow dung (Vinnerås *et al.*, (2008), however, *Klebsiella edwardsii* was isolated from rumen of both male and female species of all the identified breeds of cattle studied, thus occurring highest (24.2%) (Fig.1). *Pseudomonas aeruginosa* was found in all the breeds studied except Dm, the presence of *P. aeruginosa* is as a result of its ability to form acid from simple sugars (Ugoji and Bolarinwa, 2010) and its protease enzyme helps in decomposing protein in the rumen (Meirelles *et al.*, 1996). *P. morganella* has been reported to have a commensally relationship within the intestinal tracts of mammals and reptiles as normal flora (eMedicine, 2014). *Pseudomonas stutzeri*, denitrifying bacterium resident in soil (Lalucat *et al.*, 2006) which may be useful in bioremediation (Sepulveda-Torres *et al.*, 1999).

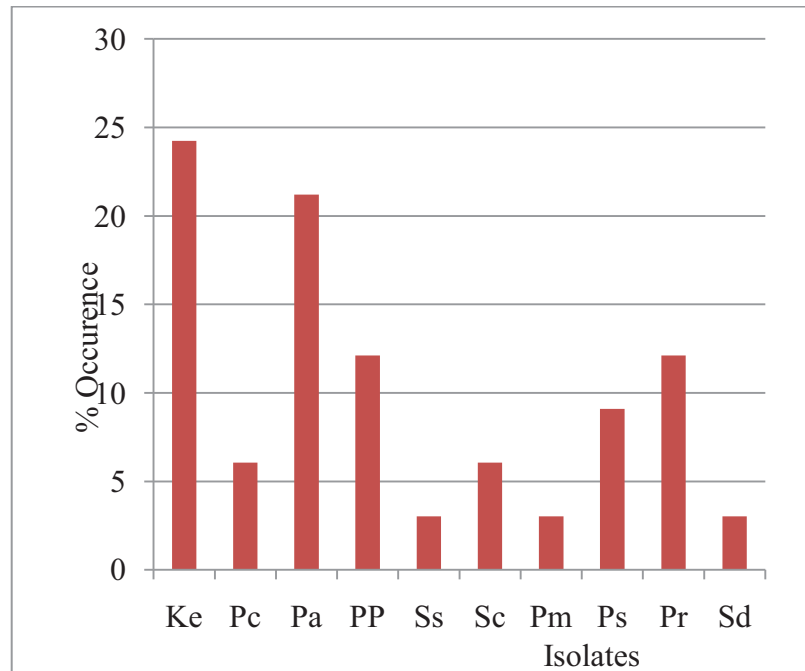


Fig. 1: % Occurrence of Bacterial Isolates in the Rumens of selected Breeds

Ke: *Klebsiella edwardsii*, Pc: *Pseudomonas cepacia*, Pa: *Pseudomonas aeruginosa*, Pp: *Pseudomonas pseudomallei*, Ss: *Shigella sonnei*, Sc: *Shigella ceylonensis*, Pm: *Proteus morganella*, Ps: *Pseudomonas stutzeri*, Pr: *Proteus rettgeri*, Sd: *Shigella dysenteriae*.

Both *Pullularia pullulans* and *Aspergillus glaucus* occurred highest (16%) (Fig.2). *A. glaucus* is primarily found in Arctic marine environment (Hubka *et al.*, 2013; Cai *et al.*, 2012). *P. pullulans* is a ubiquitous black yeast-like fungus and well known as naturally occurring epiphyte or endophyte of a wide range of plant species such as apple, grape, cucumber, green beans and cabbage without any disease symptoms. It produces useful enzymes such as siderophores and pullans and has been known to be a potent biological control agent against storage diseases.

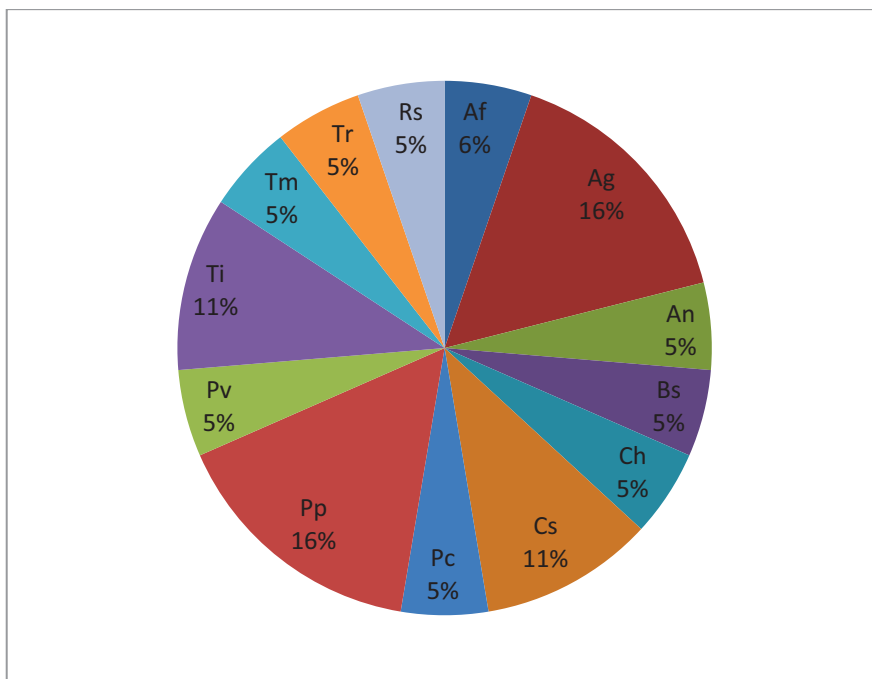


Figure 2: Percentage Occurrence of Fungal Isolates in the Rumens of selected Breeds

Af: *Aspergillus fumigatus*, Ag: *Aspergillus glaucus*, An: *Aspergillus niger*, Bs: *Botrytis sp.* Ch: *Cladosporium herbarium*, Cs: *Cephalosporium sp.*, Pc: *Penicillium camemberti*, Pp: *Pullularia pullulans*, Pv: *Paecilomyces variotii*, Ti: *Trichophyton interdigitale*, Tm: *Trichophyton mentagrophytes*, Tr: *Trychophyton rubrum*, Rs: *Rhizoctonia solani*.

CONCLUSIONS AND RECOMMENDATIONS

It can be concluded from this study that microorganisms as normal micro-flora of rumen, play a vital role in sustaining and maintaining a balance in the rumen ecosystem. Symbiotic relationship that exists between rumen microbes and cattle enables the animals to enjoy adequate supply of nutrients released by the microbial activities, which eventually results in bio-degradation of fibrous feedstuff abundantly available in Nigeria.

Breed as a factor, was found to have significant effects on the load, percentage occurrence and type of rumen bacteria and fungi at the early stage of fermentation.

The use of fistulated animals is recommended for microbial screening at different stages of fermentation without the need to sacrifice the animals. Further investigations on rumen microbes from Nigerian breed of cattle should be directed towards molecular assessment of the prospective isolates at different stages of fermentation.

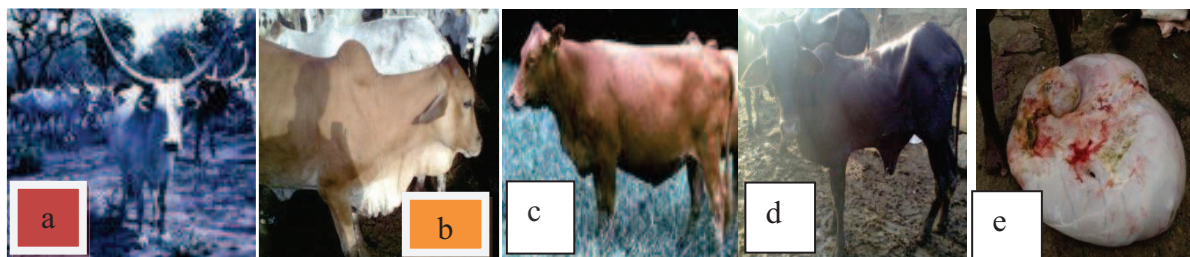


Plate 1 Different cattle breeds used in this study as well as photo of a rumen: a: Bunaji, b: Bokolo, c: Djeli, d: Keteku, e: rumen

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