



EARLY STAGE IDENTIFICATION OF RUMEN MICROORGANISMS FROM SELECTED NIGERIAN BREEDS OF CATTLE



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SUMMARY

The necessity of microbiological assessment of members of rumen community is based on the kinetics of fermentative activities.

Total heterophilic bacteria (THB) ranged between 9.1×10^7 cfu/g - 125×10^7 cfu/g for female and male Djeli (Df and Dm) respectively.

Total heterophilic fungi (THF) recorded the highest count (6.0×10^7 spores/g) for female Bunaji (Bf) and no growth was observed for female Bokolo (BkF). The pH ranged between 5.65-6.90 for male Futumi (Ff) and male Bunaji (Bm) respectively. Significant difference ($p < 0.05$) was observed for pH and total microbial counts based on sex.

Bacterial species of the genera; *Klebsiella*, *Proteus*, *Pseudomonas* and *Shigella* were isolated and identified with *Klebsiella* occurring highest (24.24%). Fungal species identified include; *Aspergillus*, *Botrytis*, *Cladosporium*, *Penicillium*, *Penicillium*, *Pullularia*, *Rhizoctonia* and *Trichophyton* with *A. glaucus* and *P. pullians* both occurring highest (17%).

Breed as a factor influenced the total counts, type and occurrence of rumen bacteria and fungi.

INTRODUCTION

Domestic Cattle: Cattle are ungulates (Grubb, 2005) with a complex stomach system (Kudo et al., 1995). They are raised for meat production except in major parts of India and Nepal (<http://www.quora.com>), milk production and as draft animals. Their by-products also serve as valuable raw materials for manufacturing industries such as; ceramics, feed mills, leather while carcasses and dung could be stabilized into organic manure and biogas production. (Rasi et al., 2007; Parawira, 2004).

Nigerian Breeds of Cattle: About twelve breeds have been reportedly found in Nigeria but extensive studies on origin, adaptation and management have been conducted on Kuri, Ndama, Muturu, Gudali and Red bororo/Fulani (DAGRIS, 2007).

Rumen: The rumen 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).

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

MATERIALS AND METHODS

Identification of breeds of cattle: Male and female sexes of Bunaji (white Fulani), Keteku (Futumi), Bokolo (Ndama) and Djeli (Sokoto Gudali) according to the description of DAGRIS, (2007) were selected.

Sampling: Content was sampled immediately after opening the rumen of each animal on weekly basis for 12 weeks at Bodija International Market Abattoir, Ibadan.

Sterilization of Materials: The methods of Jacquelyn, (1993) were adopted. Culturing Techniques: The modified methods prescribed by Cowan and Steel (1985) and Buchman and Gibbons (2000) were carefully adopted. Malt Extract (ME) agar replaced NA for total heterophilic fungi (Bornerman et al., 1992) and the plates were incubated uninvertedly (Okoro, 2004).

Isolation and identification of rumen bacteria and fungi: Colonial characteristics, cell morphology, spore formation and biochemical reactions led to the identification of resident bacteria and fungi (Cheesbrough, 2006).

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



RESULTS AND DISCUSSION

Rumen pH: The pH values ranged between 5.65 for female Keteku (Kf) and 6.90 for male Bunaji (Bm) (Table 1). Significant difference ($p < 0.05$) in pH existed 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.

Slightly acidic condition exhibited by both Bf and Kf 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).

THB: Dm and Df recorded both the highest (1.125×10^8) and the lowest (9.1×10^7) populations of THB with a significant dissimilarity ($p < 0.05$) existing between them (Table 1). Significant difference ($p < 0.05$), however, existed between male (5.8125×10^7) and female (2.4728×10^7). 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 ($10^8 - 10^{10}$ cells/ml). All the microorganisms isolated from this study differ from those identified by Czerkaski (1986) as reported by Yokoyama (1993). However, variations in microbial load have been extensively dealt with

| Sex of animal | Breed | THB | THF | pH value |
|---------------|--------|----------------------|---------------------|-----------------|
| Bm | Bunaji | 5.8125×10^7 | 1.383×10^8 | 6.80 ± 0.1 |
| Bf | Bunaji | 2.250×10^7 | 6.083×10^6 | 5.80 ± 0.1 |
| Bk | Bokolo | 4.8125×10^6 | 9.564×10^6 | 6.80 ± 0.1 |
| Ff | Futumi | 5.3024×10^6 | 3.083×10^6 | 5.60 ± 0.05 |
| Dm | Djeli | 1.125×10^8 | 1.583×10^8 | 6.80 ± 0.1 |
| Df | Djeli | 2.3024×10^6 | Nil | 6.10 ± 0.1 |
| Kf | Keteku | 1.0211×10^6 | 1.583×10^6 | 6.70 ± 0.05 |
| DF | Djeli | 9.100×10^7 | 5.383×10^8 | 6.70 ± 0.10 |

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, Bk: male Bokolo, Ff: female Futumi, Df: female Djeli, Dm: male Djeli, Bk: $\times 10^6$, Ff: $\times 10^6$, Df: $\times 10^7$, Dm: $\times 10^8$.

THF: The fungal load ranged between 1.5×10^7 - 6.0×10^8 , thus, in agreement with fungal counts obtained by Kamra, (2005), standard error based on sex revealed a significant difference ($p < 0.05$) male (2.4875×10^7) and female (2.26376×10^7), implying that cows generally, are more loaded with fungi than bulls.

Obligate anaerobic fungi found in the rumen are active in fibre degradation (Paul et al., 2003) and have additional advantage of better penetration of the lignocellulosic feeds over the cellulose-degrading bacteria due to the presence of different enzymes like proteases and esterases in combination with cellulases and hemicellulases (Fouty and Jobin, 1990).

Population variation between bacteria and fungi could be due 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 Rigby, 1987) and the parasitic effect of *Cladosporium herbarum* on other fungi (Pior, 1966).

Fungi found in rumen are involved in the hydrolysis and acidogenesis by means of extra-cellular digestion of the substrates, depending on the nature of substrate (Atlas, 1984).

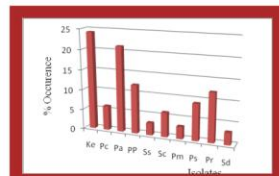


Fig. 1: % Occurrence of Bacterial Isolates in the Rumen of Selected Breeds
Kf: *Klebsiella edwardsii*, Bk: *Pseudomonas cepacia*, Bf: *Pseudomonas aeruginosa*, Ff: *Pseudomonas pseudomallei*, Dm: *Shigella sonnei*, Df: *Shigella boydii*, Sd: *Proteus morganii*, Pa: *Pseudomonas stutzeri*, Pr: *Proteus rettgeri*, Sd: *Shigella dysenteriae*.

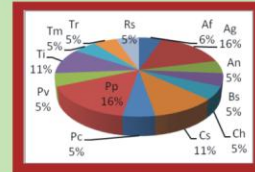


Figure 2: Percentage Occurrence of Fungal Isolates in the Rumen of Selected Breeds
Af: *Aspergillus fumigatus*, Ag: *Aspergillus glaucus*, An: *Aspergillus niger*, Bk: *Botrytis sp.*, Ch: *Cladosporium herbarum*, Cs: *Cephalosporium sp.*, Pc: *Penicillium camemberti*, Pp: *Pullularia pullulans*, Pr: *Penicillium marneffii*, Tr: *Trichophyton interdigitale*, Trs: *Trichophyton meningitropis*, Tt: *Trichophyton rufum*, Bk: *Rhizoctonia solani*.

Ruminal obligate anaerobic fungi make up only 5-10% of microbes but are absent on diets poor in fibre.

The partial digestion of microbial carcasses releases high quality protein to cattle and allows them to thrive on fibrous materials. 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 (Vinneras et al., 2008), however, *K. edwardsii* was isolated from rumen of both male and female species of all the identified breeds of cattle studied (Fig. 1). *P. aeruginosa* was found in all the breeds studied except Dm (Fig. 2), 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).

P. stutzeri, denitrifying bacterium resident in soil (Lalucet et al., 2006) which may be useful in bioremediation (Sepulveda-Torres et al., 1999).

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. It produces useful enzymes such as siderophores and pullans and has been known to be a potent biological control agent against storage disease.

CONCLUSION AND RECOMMENDATION

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, influenced the load, percentage occurrence and type of rumen residential bacteria and fungi. The use of fistulated animals should be considered for further studies at different stages without sacrificing the animals.

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