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Comparison of plant cell wall degrading community in the rumen of N'Dama and N'Dama x Jersey crossbred cattle in relation to *in vivo* and *in vitro* cell wall degradation

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1. Introduction

Livestock in sub-Saharan Africa acts as the keystone in millions of rural livelihoods; it also contributes significantly to regional and national economies (Sumberg, 2002). In addition, human needs for livestock products are predicted to increase significantly in all developing countries by 2020 (Delgado et al., 1999). This continued rapid increase in demand, dubbed the “livestock revolution” is attributed to rapid growth in human population, rising incomes and rapid urbanisation, with accompanying changes in lifestyle and preferences for foods of animal origin.

However, the contribution of livestock to the rural economy in Africa has not been commensurate with the number of animals or the extent of land resources available. Amongst the constraints faced by livestock productivity in Africa, inadequate feeding and low dairy productivity of local breeds more adapted to the prevailing conditions are listed. Options to improve livestock productivity are better feeding and management of African livestock or the exploitation of crossbred animals. Breeds of European origin with higher milk production have been imported to upgrade the local breeds or to be used as pure breeds (Cunningham and Syrstad, 1987; Akinbamijo et al., 2003) and this has resulted in higher incomes for smallholder dairy producers. In order to make more efficient use of the introduced breeds and available feed resources, there is a need to evaluate the performance of the native local breeds and their crosses with European breeds under the local production environment and several scenarios of feed supply.

In the Greater Banjul area (The Gambia) where crossbreeding is being promoted, crop and horticultural residues and agro-industrial by-products are the main if not the only feed resource available. With few exceptions (leguminous residues), crop and horticultural residues are often limited by the low nitrogen content, high fibre and low non-structural carbohydrate content; Supplementation with conventional concentrates (oilseed cake, cereal bran) and/or with unconventional feed such as tree legumes or other non-leguminous browses have been proposed to improve the nutritive value (digestibility) of crop residues and thereby, animal productivity. In the Greater Banjul area

conventional concentrates are often non available and not affordable, therefore *Moringa oleifera* Lam. (moringa) a promising non-leguminous multipurpose tree with high crude protein and negligible tannins content (Makkar and Becker, 1996) offers a good alternative source of protein to ruminant.

The breed difference in forage utilization is still inconsistent. Studies comparing fibre digestibility between breeds have either attributed the differences observed to eating behaviour (Cruywagen et al., 2001), reduced rate of feed passage through the digestive tract or increased nitrogen recycling to the rumen (McAllister, 2000). With the current stage of molecular techniques (ribosomal RNA) and the availability of probes to track the most important cell wall degrading microbes (Stahl et al., 1988) it is possible to assess the differences in microbial community structure of native and crossbreeds cattle.

On a separate development, microbial digestion within the rumen has always caused difficulties with prediction of nutrient supply to ruminant animals. *In vivo* studies are rather very expensive, laborious and time consuming. Therefore, other less animal-based techniques have been developed, both *in situ* and *in vitro* to predict the rumen degradation of feedstuffs. The *in vitro* gas production technique has been developed as a predictive tool by which the kinetics of fermentation can be assessed. Blümmel and Ørskov (1993), Blümmel and Becker (1997) and Nherera et al*.* (1999) reported the accuracy with which this method predicts feed intake, digestibility, microbial nitrogen supply and animal performance. In addition, the technique has been used to assess the effect and optimum level of supplementation (Rymer and Givens, 2002; Muetzel et al*.,* 2003). The former authors observed positive correlations between *in vitro* and *in vivo* parameters in terms of energy production (total short chain fatty acids–SCFA) and energy partitioning (molar proportion of SCFA). The latter authors used the *in vitro* 16S rRNAs concentration to predict the optimum level of supplementation. However, no *in vivo* trial was conducted to confirm their observations. The question here is if the *in vitro* techniques can be used to predict optimum level of supplementation *in vivo* to what extent the breed and the diet of the fistulated donor animal affect the *in vitro* gas production parameters and, thereby, the prediction of *in vivo* results. Therefore the objectives of the present study were:

1. To compare the animal responses of pure N'Dama and N'Dama x Jersey crosses to two basal diets (baby corn stover and groundnut hay) with graded levels of supplementation.

2. To evaluate the potential of moringa leaves as an alternative to conventional concentrate for cattle production in The Gambia.

3. To assess the effect of breed and donor diets on the *in vitro* gas production parameters.

4. To test the accuracy of the *in vitro* gas production techniques in predicting the optimum level of supplementation for cattle production.

5. To compare the cell wall digesting community of N'Dama and F1 crosses using phylogenetically based hybridization probes.

2. Literature review

2.1 Crop residue as animal feed

The main constraint to livestock production in sub-Saharan African is the limited availability of suitable feeds. Especially in the dry season (November to June), the digestibility, concentration of crude protein and edibility of rangeland forages are very low. The concentration of crude protein may fall to well below 6% (<1% N), furthermore, the quantity of forage available decreases by 25±50% as compared to the rainy season (Wolf et al., 1991). Crop residues are an important alternative to overcome shortages in that period.

The main crop residues are stover of cereals, such as sorghum, maize and millet, haulms of leguminous crops such as cowpea, groundnut and Bambara groundnut and other straws, such as rice and cotton. The amount produced in Sub-Saharan West Africa ranges from 2 to 3 tons per ha (Sansoucy, 1992; Smartt, 1994; Williams et al., 1997). In most parts of Africa, crop residues provide 45 to 80% of the total annual feed intake for cattle (McDowell and Hildebrand, 1980; Sandford, 1989).

In The Gambia, groundnut hay is the only crop residue stored as animal feed (Njie and Reed, 1995) and its sale is a major source of income for many farmers. During the last years efforts have been made to use horticultural residues as ruminant feed especially in the peri-urban areas where there is growing interest in the horticultural sector. Among theses residues is Baby corn stover; Baby corn is the variety *Pacific 421* of maize, grown mainly for export in large-scale horticultural farms. It is harvested at 60 days, 10 days after flowering. After removal of the cobs, the residual stover is ploughed back in the soil as manure. Baby corn is harvested from early December to late June. Within this period 50 tons DM of stover (30% DM at harvest) is available every week (Akinbamijo et al., 2003).

The utilization of straws depends on their nutritive value, feeding strategies and the eating behaviour of animals. Under conditions of *in situ* grazing, about 60% of the straws may be wasted due to trampling, termites and nutrient depletion by weathering (Sansoucy, 1992). Hand feeding is now becoming a widespread

practice in many areas. Much work has been done to improve the nutritive value of crop residues, including studies on physical, chemical or biological treatment of these materials. The major treatments are chopping and treatment with urea or ammonia. An alternative is to supplement low-quality feeds with concentrates.

2.2 Supplementation with conventional concentrates

Conventional concentrates used in sub-Saharan Africa are mainly agro-industrials by-products. They include cereal bran and/or oilseed cake or meal. The former are used mainly as a source of energy and the latter as source of protein. They are either fed alone or in mixture. However oilseed cake when not produced locally is unavailable and expensive to small-scale farmers. In such situations cereal bran is used alone. Supplementations of low-quality forages with high nitrogen (N) supplements such as oilseed meals have often resulted in large increases in intake and animal productivity (Abidin and Kempton, 1981; Kellaway and Leibholz, 1983; Coombe, 1985; Coombe et al., 1987). Supplements may decrease, have no effect, or increase both the voluntary intake of forages and the digestibility of the fibrous components of the roughage, thus affecting ME intake (Hunter, 1988; Leng, 1990; Dixon and Stockdale, 1999).

In the rumen the negative effect of supplementation appears to be: a decrease in fibrolytic activity of the solid-associated micro organisms (Nozière et al*.,* 1996), without modification in their concentration (Martin and Michalet-Doreau, 1995; Michalet-Doreau et al., 2002), not the ability of fibrolytic bacteria to adhere to fibrous particles (Leedle et al., 1986; Roger et al*.,* 1990; Martin et al*.,* 2001).

The extent of the effect of a concentrate on digestion of fibre depends on the nature and the proportion of the concentrate as well as the quality of the roughage (Archimede et al*.*, 1995; Matejousky and Sanson, 1995) and on the management strategy for feeding concentrate (Ørskov, 1999). For instance most of the farmers in sub-Saharan Africa have been sensitised to feed the concentrate in two discrete feedings, one in the morning and one in the

evening. In severe instances low pH that occurs 2 to 3 hours after feeding causes acidosis resulting in the animals going off-feed. He suggested that concentrate be fed in four to six portions per day rather than two will reduce fluctuations in rumen pH.

Despite the impressive response on station, the rate of adoption by farmers remains low. Some of the reasons of this low adoption are the availability and the high costs of concentrates in the present production systems (Schiere and Nell, 1993; Williams et al., 1997; Sumberg, 2002).

2.3 Supplementation with tree fodder

The use of forage legumes and other non-leguminous browse as supplement has been suggested as an alternative to the use of concentrate because of their beneficial effect on increasing energy intake, nitrogen intake and feed efficiency and improving animal performance (Jones, 1979; Osuji et al., 1995; Ndemanisho, 1996; Roothaert and Paterson, 1997; Aregheore, 2002). Fodder trees are playing an increasingly important role in agricultural production system in sub-Saharan Africa. Apart from their beneficial effect on soil fertility, they provide firewood, shade which help to reduce heat stress of cattle and are an important alternative as forage source, due to their high production of edible, high acceptable biomass and drought resistance (Navas-Camacho et al., 1993).

Tree leaves have high protein content and low fibre content (Devendra, 1994; Topps, 1992; Nsahlai et al., 1998), and high digestibility (Norton, 1994; Teferedegne, 2000). Calculating protein-energy interrelationship of browse species, Nsahlai et al. (1998) concluded that crude protein content was twice of the optimum ratio required for efficient rumen fermentation. Supplementation with browses invariably alleviates nitrogen deficiency (Bonsi et al., 1994) and also sulphur and other mineral deficiencies, thus increasing the intensity of rumen microbial activity (Said and Tolera, 1993; Bonsi et al., 1994; Merkel et al., 1999; Hove et al., 2001). As it is the case with conventional concentrates, the amount of foliages needed to provide effective supplementation varied with the quality of basal diets, the rate of fermentation of the foliage (Osuji et al., 1995; Kiatho, 1997; Nsahlai et al., 1998) and the level of animal production

expected (Teferedegne, 2000). Abule et al. (1995) and Bonsi et al. (1994) observed an almost twofold increase in the rates of degradation and passage when low nitrogen feeds were supplemented with foliages. However with good quality roughages, Nsahlai et al. (1995) and Umunna et al. (1995) did not observe a positive response suggesting that responses to supplementation with foliage from forage trees increase with decreasing of the quality of the basal feed. Muetzel et al. (2003) reported that at higher levels of supplementation with the *Sesbania pachycarpa* leaf, the growth of the anaerobic rumen fungi (a major group of cell wall degrading organisms) was negatively influenced. This was possibly due to saponins.

The limited potential of tropical fodder legumes as supplement is often attributed to secondary compounds generally termed anti-nutritional factors that interfere with their utilization by livestock (Reed et al., 1990; Reed, 1995; Bonsi et al., 1994; Teferedegne, 2000). The common anti-nutritional factors that have been implicated in limiting the utilization of tropical fodder legumes include: non-protein amino acids (mimosine and indospicine), glycosides (cyanogens and saponins) and polyphenolic compounds (tannins, Kumar, 1992; Makkar, 1993; Leng, 1997; Teferedegne, 2000).

The most widely occurring anti-nutrient in plants is a group of polyphenolic compounds commonly known as tannins. Tannins are divided into hydrolysable tannins and condensed tannins. Hydrolysable tannins are more susceptible to enzymatic hydrolysis than condensed tannins. Hagerman and Robbins (1993) found that hydrolysed tannins did not affect nutrient digestibility in ruminants.

One of the most well known and most highly recommended tree forage is *Leucaena leucocephala* (Shelton and Brewbaker, 1994). Unfortunately, this tree is prone to devastating attacks by a psyllid (*Heteropsylla cubana*). *Moringa oleifera,* a pantropical multipurpose tree, offers therefore an alternative. It is characterized by high biomass yield and can tolerate unfavourable environmental conditions (Foidl et al., 2001). It has high crude protein and negligible tannin content (Makkar and Becker, 1996). However, data on its use to assess the performance of cattle *in vivo* are still scarce. To develop and disseminate feeding package for cattle production it becomes an imperative to

determine the level of supplementation that will optimise the utilization of the basal diet and economically viable.

2.4 Feed intake and digestibility in relation to animal genotypes

Many attempts to introduce crossbreed animals have simply failed or not lived up to the expectations (FAO, 2001), because crossbreds are only adopted when they perform better than the local parent breeds under conditions the latter are predominantly kept. Ample evidence exists in the literature that indicates that indigenous breeds compared more than favourably with exotic breeds and crossbred types in very arid, arid to semi arid, and tsetse infested humid lowlands (Udo, 2002). It is therefore of paramount importance that the performance of crossbreeds compared to the local breeds, is evaluated under the local production environment. Such studies are essential to guide livestock development programmes.

The consumption of feed is the first step in the process, which converts feed into products such as milk and meat for human consumption. Voluntary intake of ruminants is not strictly governed by a single factor; rather it is influenced by interplay of external and internal factors (Ketelaars and Tolkamp, 1992; Schlecht et al., 1999; Forbes, 2003).

Ketelaars and Tolkamp (1992) stated that feed conversion in herbivores is dependent of the size because it is directly proportional to maintenance requirements. As with increasing size, maintenance requirements per unit body weight decreases, feed intake relative to bodyweight will decrease to the same extent.

In general, breeds that have developed in a particular environment are well adapted for survival and production. For instance zebu (*Bos indicus*) and zebu-influenced cattle have been recognized for their ability to be productive in poor nutrition environments.

Kariuki et al. (1999) comparing Sahiwal and Friesian under semi arid tropical conditions attributed the difference in feed intake to the difference in time spent eating. This was in agreement with Schlecht et al. (1999); they reported the

adaptation of local breed to maintain high levels of intake under high ambient temperatures.

McDowell et al. *(*1996) in a review assessing the economic viability of crosses of *Bos taurus* and *Bos indicus*, reported higher intake in zebu cattle compared to its crosses (Jersey and Holstein). His observations were made on a pasture-based system and he attributed the difference in favour of the improved breeds to the morphological difference (zebus are smaller in muzzle circumference) and the difference in feeding behaviour (zebus are more selective). The composition of the weight gain also appears to be related to species differences in feed intake: highest intakes are recorded in breeds with highest fat/protein ratio in their weight gain (Ketelaars and Tolkamp, 1992).

Unlike feed intake, little attention has been given to the difference in digestibility between breeds or genotypes in the literature. Givens and Moss (1994) with dried grass found a significant (although small) difference in digestibility between two breeds of sheep; Ranilla et al. (1998) found no significant differences in dry matter and fibre digestibility of good quality forage between two breeds of sheep. Akinbamijo et al. (2003), comparing three breeds of cattle, found that there was a significant difference in the digestibility with the local breed eating more and digesting better than the crosses. Norton et al. (1979) explain such difference by the better sparing of nitrogen in breeds adapted to their environment than in exogenous breeds. But in the cited study, all the three breeds were well adapted to their environment. Doreau and Diawara (2003) found no difference in the digestibility of good and medium quality hay between Holstein and Charolais confirming the previous findings of Ahn et al. (1986) comparing the two breeds. It can also be assumed that zebu cattle with the higher rate of passage compared to its crosses with Jersey or Holstein (McDowell et al., 1996) would digest less than the latter. The breed difference in forage utilization is still inconsistent. Ketelaars and Tolkamp (1992) indicated that quantities and arrays of absorbed digestion end-products might influence the ratio of heat production to metabolized energy to impact feed intake. It is therefore conceivable that the anticipated breed differences in forage utilization be partially attributed to rumen microbial community structure and /or activity.

The present study is an attempt to explain the breed difference in fibre digestibility in terms of microbial community composition.

2.5 The Rumen ecosystem

Digestion in ruminants is achieved by one of the most fascinating but also extremely complex microbial ecosystems: the rumen. The rumen is a large pregastric fermentation chamber present in the digestive tracts of all ruminants. It has a volume of up to 250 L in an adult cow and contains a microbial community consisting of about 10^{11} microbes per ml of rumen fluid.

The microbiota comprises mostly:

Anaerobic bacteria and archaea (about 10^{8} – 10^{11} ml^{-1}) belonging to more than 200 species, but only about 20 of these species occur in numbers greater than $10^{6.}$ It is the bacterial team that is responsible for the majority of feed digestion in the rumen. The fibrolytic bacteria *Fibrobacter succinogenes*, *Ruminococcus flavefaciens* and *Ruminococcus albus*, are generally considered as the primary organisms responsible for degradation of plant cell walls in the rumen (Cheng et al., 1991; Forsberg and Cheng, 1992). Cellulolytic *ruminococci* (*R. albus* and *R. flavefaciens*) are gram-positive (Hespell et al., 1997). It is difficult to divide these two species on the basis of morphology or phenotype but the ability of *R. flavefaciens* to produce succinate as a major end-product seems to be consistent with its phylogenetic position (Krause et al., 1999). Total cellulolytic *Ruminococci* have been estimated to be between 4% and 11% of the total ruminal population (Krause et al., 1999). *Fibrobacter succinogenes* is gram-negative and is probably the best examined rumen organism; its population size varies widely (0.1 to 3% of the total RNA, Muetzel et al., 2003).

Anaerobic ciliated protozoa (10^{4} to 10^{6} ml^{-1}). They are the second micro organism's community in the rumen and over one hundred different species are identified. Protozoa are thought to be responsible for one quarter to one third of the fibre digestion in the rumen. However, the number of protozoa in the rumen fluctuates inversely with the number of bacteria and ruminants can survive without any protozoa in the rumen. In the last two decades attempts have been made to ascertain the exact role of protozoa in the rumen microbial ecosystem

and generate information on the role of rumen protozoa in the metabolism and the performance of animals (Jouany et al., 1988; Coleman, 1988; Demeyer, 1992; Santra and Jakhmola, 1998). The available reports on the role of protozoa and their contribution in animal production are contradictory (Ramprasad and Raghavan, 1981; Bird and Leng, 1985; Demeyer, 1992).

On the one hand it is clearly shown that defaunation (absence of protozoa in the rumen) decreases rumen methane production (Kreuzer et al., 1986; Santra et al., 1994), and increases the intestinal protein supply to the animals (Ushida et al., 1984; Rowe et al., 1985; Kayouli et al., 1983) resulting in better growth performance and feed conversion efficiency (Bird et al., 1994; Chaudhany et al., 1998; Santra and Karim 2000). On the other hand, Wang and McAllister (2002) reported that all the major fibrolytic enzyme activities could be detected in the rumen protozoa population. Santra and Karim (2002) reported a decrease in digestibility of fibre in defaunated lambs. They related this to the elimination of large Entodiniormorphid ciliates, which have high cellulolytic activity (Ushida and Jouany, 1990). Moreover, better digestibility of cell wall constituents was also attributed to increase retention time of feed particles in the rumen of faunated lambs (Kayouli et al., 1983; Ushida and Jouany, 1990), stabilization of rumen environment (ingestion of starch grain thus reducing acidosis) favouring development of cellulolytic bacteria (Jouany and Martin, 1997; Hegarty et al., 1991) and the stimulatory effects of rumen ciliate protozoa on rumen bacteria (Onodera and Koga, 1987). Bonhomme (1990) and Williams and Coleman (1988) concluded that the activities of ruminal protozoa contribute significantly to the degradation of plant cell polymers and their absence from the rumen may have a negative effect on the extent of fibre digestion.

Anaerobic fungi (10^2 to 10^4 zoospores ml^{-1}). They are the most recently recognized group of ruminal micro organisms. It has been estimated that fungi may contribute up to 8% of the total microbial biomass. Although anaerobic fungi appear to colonize plant materials rapidly (Preston and Leng, 1987), their significance in degradation depends strongly on the composition of the diet (Orpin and Joblin, 1988). With a generation time of 24h-30h fungi are rapidly depleted if they are unable to attach to feed particles to delay their passage through the rumen (Wang and McAllister, 2002). Ruminal fungi produce a broad

array of enzymes and generally degrade a wider range of substrates than do the ruminal bacteria (Wubah et al., 1993; Trinci et al., 1994; Wang and McAllister, 2002). Ruminal fungi are able to degrade the most resistant plant cell wall polymers (Forsberg and Cheng, 1992; Wubah et al., 1993; Wang and McAllister, 2002) and the cellulases and xylanases they produce are among the most active fibrolytic enzymes described to date (Trinci et al., 1994). Growth of fungi is apparently restricted to the recalcitrant sclerenchymal fraction of the plant cell walls (Wang and McAllister, 2002) and they appear to be superior over rumen bacteria in their ability to break down and degrade the structural barriers in plant materials (Akin, 1989). When fungi were removed from the rumen, both feed intake and fibre digestibility were decreased, however total viable bacteria, cellulolytic bacteria or ciliate protozoa concentrations were not affected (Dehority and Tirabasso, 2000).

A complex network of interactions exists among the rumen microbial community and these interactions are essential for sustaining the community. Examples of these interactions include:

Synergy: the synergistic act of fungi in the digestion of forage by physically disrupting the lignified stem of straw tissue and allowing entrance of the rumen microbes into plant stems thereby accessing the digestible portions of plants (Wang and McAllister, 2002).

Predation: the predation of bacteria by protozoa and protozoa themselves, whereby bacteria represent the most important source of nitrogenous compounds for protozoa growth (Williams and Coleman, 1988).

Competition: the inhibitory effects of *Ruminococcus albus* and *Ruminococcus flavefaciens* on the cellulolytic activity of ruminal fungi (Fonty and Joblin, 1991; Stewart et al., 1992).

Factors such as composition of feed, the degree of physical processing and the presence of feed additives all affect the numbers, proportions and digestive activity of rumen micro organisms. Because of this confounding effect of diet on the composition of rumen microbes, the differences in microbial community structure between animal breeds have been difficult to assess. Because of this difficulty, the higher ability of certain breeds of ruminants to digest poor-quality

feeds is mainly attributed to reduced rate of feed passage within the digestive tract and increase recycling of nitrogen to the rumen rather than superior rumen bacteria (McAllister 2000).

The identification of rumen bacteria has been carried out using several techniques such as gram positive reaction and cell morphology, motility, growth media, fermentation products, substrates fermented (Table 1). In the eighties, a new system for the classification of micro organisms based on sequence comparisons of the ribosomal RNA gene was developed (Woese, 1987; Woese et al., 1990). The ribosomal RNA gene fulfils several requirements as an evolutionary marker gene. First it is ubiquitous, and apparently no horizontal gene transfer occurred (Pace et al., 1986; Olsen et al., 1986). Recent studies of 16S rRNA and sequencing indicate that the diversity of ruminal bacteria has been greatly underestimated because many ruminal species have strains with little DNA or RNA similarity (Krause and Russell, 1996).

Table 1: Typical rumen bacteria identified by gram positive reaction and morphology and source of energy *in vitro*

Species	Description	Typical energy source
Fibrobacter succinogenes	Gram negative, rods	Cellulose
Ruminococcus flavefaciens	Catalase negative, Streptococci with yellow colonies	Cellulose
Ruminococcus albus	Single or paired cocci	Cellobiose
Streptococcus bovis	Gram positive, short chain of cocci capsulated	Starch
Prevotella ruminicola	Gram negative oval or rods	Glucose
Megasphaera elsdeni	Large cocci paired or in chain	Lactate

Source: McDonald et al., 1995

Comparative sequencing of the rRNAs, principally the 16S-like rRNAs, has yielded the most complete understanding of microbial phylogeny (Stahl et al., 1988). The analysis of the rRNA gene not only revolutionised bacterial taxonomy, but also allows the tracking of organisms with rRNA-targeted probes in a complex microbial ecosystem (Gray and Herwig, 1996; Muetzel et al.,

2003). Despite the availability of probes for the evaluation of cell wall degrading, methanogenic and protein degrading communities (Table 2) the effects of animal's genetics on rumen micro organisms have not been extensively explored. Our challenge in the present study is the first attempt to compare the cell wall degrading community of two breeds of cattle fed the same diets (three different compositions) in consecutive experiments.

Table 2: Probes available for studies of cell wall degradation, protein degradation and methanogenesis in the rumen

Group		Reference
All organisms	Universal	Zheng et al., 1996
Bacteria	Domain	Amann et al., 1990
Eukarya	Domain	Hicks et al., 1992
Archaea	Domain	Amann et al., 1990
Cell wall degrading organisms		
Fibrobacter	Genera	Stahl et al., 1988
Ruminococcus albus	Species	Odenyo et al., 1994
R. flavefaciens	Species	Odenyo et al., 1994
Lachnospira multiplarus	Species	Stahl et al., 1988
Chytridiomycetes	Family	Dore et al., 1993
Methanogens		
Methanobacteriaceae	Family	Raskin et al., 1994
Methanosarcchina	Family	Raskin et al., 1994
Methanomicrobiaceae	Family	Raskin et al., 1994
Protein degrading organisms		
Peptostreptococcus anaerobius	Species	Krause and Russell, 1996
Clostridium sticklandii	Species	Krause and Russell, 1996
C. aminophilum	Species	Krause and Russell, 1996
Butyrivibrio	Genus	Forster et al., 1997
Prevotella	Family	Avgustin et al., 1994

2.6 *In vitro* gas production technique

There is a long history of characterising ruminant feeds by incubating them *in vitro* with buffered rumen fluid to simulate rumen fermentation (Tilley and Terry, 1963; Czerkawski and Breckenridge, 1977). The gas production technique has been developed using this methodology (Menke et al., 1979; Menke and Steingass, 1988; Pell and Schofield, 1993; Theodourou et al., 1994; Cone et al., 1996; Davies et al., 2000). The gas production technique also generates kinetic information, but rather than measuring the disappearance of dietary components, it measures the appearance of fermentation gases notably carbon dioxide and methane. Compared to the *in situ* degradability technique, the gas production method is less animal dependent.

The technique allows to generate also information on the volatile fermentation products (Rymer and Givens 2002; Muetzel et al., 2003) and the microbial biomass production (Blümmel and Becker, 1997; Blümmel et al., 1997; Muetzel et al., 2003). The gas production has also been used to estimate the metabolisable and net energy content of feeds (Menke and Steingass, 1988; Cottyn et al., 1990), to predict feed intake, digestibility, microbial nitrogen supply and animal performance (Blümmel and Ørskov, 1993; Blümmel and Becker, 1997; Nherera et al., 1999).

The primary short chain fatty acids (SCFA) in descending order of abundance are acetic, propionic, butyric, isobutyric, valeric, isovaleric and traces of various others acids (McDonald et al., 1995; Beever and Mould, 2000).

The energy supplied from the production of SCFA has been estimated to make up as high as 70-80% of the total energy required by ruminants (Houtert, 1993). Pyruvate is the key intermediate molecule (McDonald et al., 1995; Van Houtert, 1993). It is from pyruvate that the different volatile fatty acids are derived. Pyruvate is converted to acetate through an enzymatic pathway that results in the cleavage of pyruvate to form acetate and formate (Leng, 1990).

Acetic acid can constitute 50-70% of the total SCFA. It is predominant in high-forage diet. Acetate is a precursor for fat synthesis and production of adequate levels of acetate in the rumen is essential to maintain adequate quantities of milk fat.

Propionate can make up to 18-20% of the total SCFA. It reaches its highest concentration in a high-grain diet. Propionate is a potent stimulant for insulin release in ruminants (Harmon, 1992; Sano et al., 1993) and it decreases feed intake (Leuvenink et al., 1997).

The overall stoichiometry of carbohydrates utilization by fermentation in the rumen has been well established (Baldwin et al., 1977; Murphy et al., 1982; Beever, 1993) and it shows that the proportion of SCFA is greatly influenced by diet and is largely pH dependent.

There is some evidence that changes in the molar proportions of SCFA in the rumen can alter energy partitioning, thereby affecting milk production and composition (Rymer and Givens, 2002). Increasing the molar proportion of propionate for example tends to increase the efficiency of energy utilization, and it tends to partition energy toward body tissue synthesis and away from milk production (Journet et al., 1976). The fat concentration in milk increased when acetic acid was infused into the rumen of dairy cows (Ørskov and MacLeod, 1982), while infusing propionic acid into the rumen resulted in a decrease of milk fat concentration (Hurtaud et al., 1992). A relative large proportion of propionate in the rumen is associated with a depression in milk fat content and a small increase in milk protein (Dijkstra, 1994; Van Vuuren et al., 1995). However, the physiological role and significance of propionate in ruminants has not been fully clarified (Lee and Hossner, 2002). Rymer and Givens (2002) reported a good relationship (R^2=0.81) between the *in vitro* estimates and the mean concentration of total SCFA *in vivo*, using hay based diet supplemented with various levels of micronised maize.

More important, the gas production technique can be used to test different supplementation strategies by incubating not only the pure components, but also the entire diets. By so doing it can allow an initial screening of combinations and reduce the number of combinations to be tested *in vivo*. Muetzel et al. (2003) studied the effect of *Sesbania pachycarpa* leaf supplementation on barley straw and reported differences ranging from 6.2 to 14.7% in the microbial biomass production estimated by the total rRNA concentration between the interpolated values (calculated by the multiplication of the value from the pure substrates with their inclusion levels) and the

observed values. Although their study demonstrated that the *in vitro* system has potential to predict an optimal supplementation strategy it was not followed by an *in vivo* study.

Nevertheless, several factors affect the accuracy on the *in vitro* gas technique. Mainly the activity of micro organisms in the rumen fluid collected that can vary based on the time of collection after feeding, diet and species of the donor animal and animal-to-animal variation within species (Kitessa et al., 1999). Some investigators (Horton et al., 1980; Ayres, 1991; Holden, 1999) have found that the fermentative activity of rumen inocula is affected by diet of the donor, although no effect of donor diet has been reported in other studies (Nik-Kahn and Tribe, 1977; Jung and Varel, 1988). In the present study the effect of diet and breed of donor animal on the fermentation parameters *in vitro* will be examined and the accuracy of the technique to predict optimum level of supplementation will also be tested.

3. Materials and Methods

3.1 *In vivo* study

3.1.1 Animals and housing

The study was carried out at the International Trypanotolerance Centre (ITC) station in Kerr Serigne (The Gambia) from January 2002 to June 2003; twelve bulls (six N'Dama 152 ±12 kg and six Jersey x N'Dama 295±10 kg) aged between 5 and 6 years were used for this study. They were individually housed in pens with concrete floors on an open-air platform. They had free access to saltlick and to water four times a day. Prior to the experiment, animals were dewormed with albendazole (Albenol®- 100, oral Inter chemie, Holland) at a dose of 12 ml/kg BW and were sprayed against ticks with Decatix (Cooper® Zimbabwe Pvt. LTD). They were also tested for trypanosomosis infection using the dark ground buffy coat technique and all infected animals were treated with diminazene aceturate (Berenil® Hœchst A.G., Frankfurt Am Main, Germany) at a dose of 3.5 mg /kg BW. During the experimental period the animals were monitored for tick infection on a weekly basis, and for trypanosomosis infection fortnightly.

3.1.2 Experimental feeds

Chemical compositions of the different feed components used in the study are shown in Table 3.

Table 3: Chemical composition of the different feedstuffs (as % DM, OM: Organic matter, CP: Crude protein, NDF: neutral detergent fibre, ADF: acid detergent fibre)

Feed	OM	CP	NDF	ADF
Baby corn stover	92.4	6.0	65.7	34.9
Groundnut hay 1st batch	90.7	12.8	51.2	43.1
Groundnut hay 2nd batch	85.0	15.2	37.6	29.4
Concentrate mixture*	84.0	29.8	41.1	34.4
Moringa oleifera meal	89.3	23.3	18.7	16.1

*Concentrate Mixture = 50% groundnut cake + 50% rice bran

3.1.2.1 Roughage

Baby corn stover (*Zea mays L*.)

The stover used during the experiment was from early maturing maize the variety *Pacific 421*. The stover were cut after harvesting and allowed to wilt in the field for 2-3 days. Thereafter, the maize stover was bailed, carted to the station and sun-dried naturally in open air. To facilitate intake, the stover was then mechanically chopped to a length of between 5 and 7 cm before being offered to animals.

Groundnut hay (Arachis hypogaea L.)

The hay used in the present study was collected and carted at the station by dealers in two batches, the first one in January 2002 and the second in January 2003. Representative samples of each batch were taken for analysis. On average, (Table 3) the groundnut hay of the second batch was of better quality compared to that of the first batch, it had 2.4% more crude protein (CP) and 13.7% less neutral detergent fibre (NDF) content.

3.1.2.2 Supplements

Concentrate mixture

This was made from locally available agricultural by-products, i.e. rice bran and groundnut cake (1:1, w:w). The high fibre content of our concentrate mixture

was mainly attributed to the high fibre content of the rice bran used for the mixture (65% NDF, 51% ADF), due to the high proportion of husks.

Moringa leaf meal

The moringa fodder was obtained from the ITC plots (between latitudes 13^{o} N and 14^{o} N). Moringa in this plot is planted at 20 cm interval in and between the rows (around 36 plants/m^{2}). The field was irrigated during dry season and 50 to 60 tons per ha of organic manure was applied at the beginning. On a weekly basis, 50 kg/ha of inorganic fertilizer NPK (15: 15: 15) was applied throughout the year. The plants were cut at about 30-40 cm above the ground 60 days after plantation or re-growth. The sun dried leaves and less lignified parts of the branches were then ground and mixed in an industrial mixer to obtain a homogeneous meal. The chemical composition of the moringa meal (Table 3) showed that it had almost two times less NDF and around 6% less CP than the concentrate mixture.

3.1.3 Treatment

Animals from each breed were assigned to 15 different diets (Table 4) in three different periods in a simple cross over design. During the first period, baby corn stover were used as basal, and concentrate mixture as supplement at 0%, 10%, 20%, 30% and 40%. For each level of supplementation, the supplement replaced equal proportion of the basal diet in the estimated intake.

After this period the animals were allowed two months resting period and were taken back on the platform for the second period with groundnut hay as basal and concentrate mixture as supplement. The hay used during this phase was from the 2002 batch. After another resting period they were taken back for the third period with groundnut hay and moringa meal as supplement were tested. The hay in this phase was from the 2003 batch. Each diet (roughage and one level of supplementation) was tested in 24 days with 14 days adaptation period and 10 days data collection period. The combination roughage: supplement was considered as a diet.

Table 4: Combinations of feeds tested *in vivo* (a diet consists of roughage and one level of supplementation)

Roughage	Supplement	Level
Baby corn stover	Concentrate mixture	0%, 10%, 20%, 30%, 40%.
Groundnut hay	Concentrate mixture	0%, 10%, 20%, 30%, 40%.
Groundnut hay	Moringa leaf meal	0%, 10%, 20%, 30%, 40%.

The following abbreviations were used: baby corn stover: concentrate mixture = BCS:Co; groundnut hay: concentrate = GNH:Co and groundnut hay : moringa meal =GNH:Mo

Table 5: Chemical composition of test diets (as % DM, OM: Organic matter, CP: Crude protein, NDF: neutral detergent fibre, ADF: acid detergent fibre).

BCS:Co	0%	10%	20%	30%	40%
OM	92.4	91.6	90.7	89.9	89.0
CP	6.0	8.4	10.8	13.1	15.5
NDF	65.7	63.2	60.8	58.3	55.9
ADF	34.9	34.9	34.8	34.8	34.7

GNH:Co	0%	10%	20%	30%	40%
OM	90.7	90.0	89.4	88.7	88.0
CP	12.8	14.5	16.2	17.9	19.6
NDF	51.2	50.2	49.2	48.2	47.2
ADF	43.1	42.2	41.4	40.5	39.6

GNH:Mo	0%	10%	20%	30%	40%
OM	85.0	85.4	85.9	86.3	86.7
CP	15.2	16.0	16.8	17.6	18.4
NDF	37.6	35.7	33.8	31.9	30.0
ADF	29.4	28.1	26.7	25.4	24.1

Table 6: Summary of the *in vivo* experimental procedures

Day	Activity
1	Weigh- in animals and start feeding experimental diet
2 to 14	Adaptation period
15	Collection of representative feed samples
16	Collection of representative feed samples and uneaten feed
17 to 23	Faecal collection period
24	Weigh- out animals

3.1.4 Feed intake measurement

The feed was offered at 2.5% body weight in two equal daily portions in the morning and in the afternoon to ensure constant availability. During the adaptation period the average feed intake was measured individually and the bulls were offered this quantity plus 10-20% during the data collection period. Cleaning of the pens, removal and weighing of leftovers from the previous day were done daily before feeding. The roughage (basal diet) was only offered after the animal had completely eaten the supplement. Representative samples of feed offered and of feed refused were taken daily for dry matter determination and bulked per breed for crude nutrients analysis.

The dry matter intake (DMI) on a daily basis was calculated as the difference between the quantity of feed dry matter offered and refused.

3.1.5 *In vivo* digestibility

Faeces were manually collected immediately after voided and sub samples weighed at 8.00 h and 20.00 h. The dry matter was determined on a daily basis, and 5% of the representative faeces voided were sampled from each animal kept in a freeze and bulked on a weekly basis until required for further analysis. The percentage dry matter digestibility (DMD) was determined as:

DMD= ((DMI- faeces)/DMI)*100.

Where DMI is the total dry matter intake.

Organic matter, NDF and ADF in the faeces were then determined for nutrients digestibility.

For organic matter determination faecal samples were incinerated in a muffle furnace at 550 °C for 3 hours.

Neutral detergent fibre (NDF) and acid detergent fibre (ADF) determination:

0.5 g of sample were weighed directly into filter bags (ANKOM F57) and placed in the ANKOM fibre analyser (ANKOM FIBER ANLYZER ^{II} ANKOM technology, Fairport, New York, USA); for NDF determination, 24 filter bags were boiled in 2 L of neutral detergent solution for 75 min then rinsed three times with hot water and oven dried. ADF was determined by boiling 24 bags in 2 L of acid detergent solution for 60 min.

3.1.6 Weight gain

Body weight was determined at the beginning and the end of each period using an electronic scale (JR200 Trust-Test limited New Zealand). Weighing was done after 12 hours of complete starvation. The difference of the two weights was divided by the number of days of the period (24) and the results taken as the average daily weight gain.

3.2 Microbial community analysis

3.2.1 Sample collection

Samples for microbial community analysis were collected from *in vitro* donor animals at the day of incubation Samples were collected before morning feeding from each of the fistulated animals. 300 µl of the rumen fluid was then taken into a 2 ml screw cap cup containing 600 µl of pH 5 phenol, stored at –25 °C and sent every two months to the University of Hohenheim where they were stored at –80 °C.

3.2.2 RNA extraction

Total RNA was extracted by a modification (Muetzel and Becker, 2002) of the low pH hot phenol extraction procedure described by Stahl et al. (1988). To the sample containing phenol, 270 µl of buffer pH 5.1 (50 mM sodium acetate, 10 mM EDTA, pH 5.1), 30 µl SDS (20% w/v) and 1 g zircona silica beads (0.1 mm), were added. Cells were then lysed by beating the samples in a beat mill (Bartlesville, OK, USA) and shaken at for 2 min. Samples were placed into a water-bath at 60 °C for 10 min and the beating was repeated. 300 µl of chloroform were then added and samples were shaken vigorously and incubated at room temperature for 5 min. The incubation was repeated after another vigorous shaking. Aqueous and organic phases were separated by centrifugation (10000 g, 5 min, 4 °C), and the aqueous phase was transferred into a new vial containing 300 µl of 7.5 M-ammonium acetate and 900 µl of isopropanol. Nucleic acids were precipitated at –20 °C overnight and recovered by centrifugation (16000 g, 10 min, 4 °C). Supernatant was discarded and the samples were washed once in 80% ethanol. Nucleic acids were dissolved in 100 µl double distilled H_2O and samples were stored at –80 °C.

3.2.3 Agarose gel electrophoresis

The quantification and the integrity of the RNA extracts were evaluated by agarose gel electrophoresis. 10 µl of nucleic acids were mixed with 10 µl of sample loading buffer (40% saccharose and 0.05% bromophenol blue) and 4 µl of the mixture were loaded onto a 1.4% agarose gel. 16s ribosomal RNAs were separated in a horizontal unit at 200 V for 2 hours. The electrophoresis buffer TBE was composed of 0.1 M tris base, 0.083M boric acid and 1mM EDTA pH 8. After separation, the gels were stained for 30 min in the electrophoresis buffer containing 1 µg/ml ethidium bromide, and destained for 30 min in the electrophoresis buffer. Pictures of the gels were thereafter taken with a Ray test product Diana (Figure 1). For the evaluation of gels the program AIDA (Raytest Isotopenmeßgeräte GmbH, Straubenhardt, Germany) was used. Each gel contained 6 lines of known RNA as standard that served as calibration for the estimation of RNA concentrations.

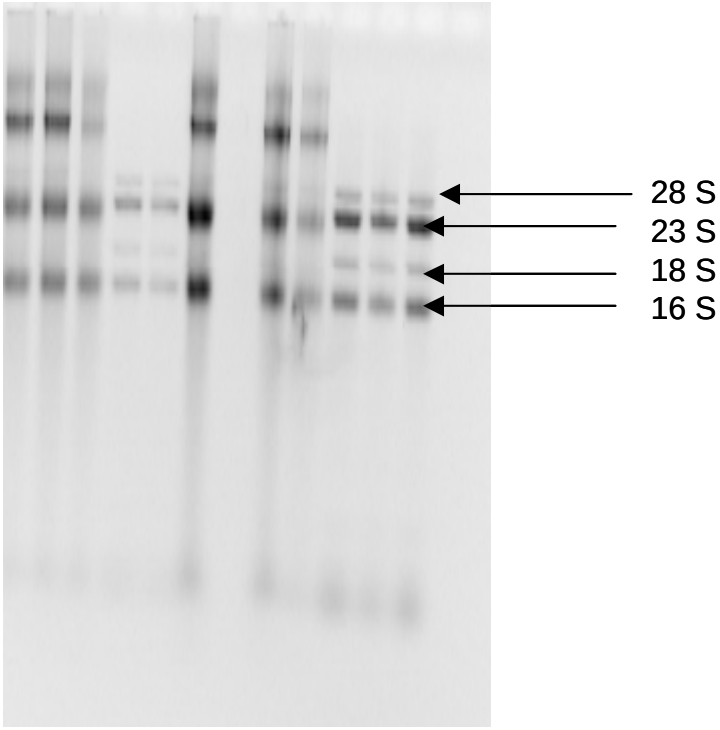

Figure 1: Gel for quantification and quality check of RNAs after electrophoresis. Each gel contained 6 profiles of known RNAs as standard

3.2.4 Membrane hybridisation

The membrane hybridizations were carried out as described in Stahl et al. (1988). RNA samples were denatured with 3 volumes glutaraldehyde (2% v/v) and diluted to approximately 2 ng/µl with dilution water (0.2 ng/ml BPB) 10 µg poly A. 50 µl of these dilutions were applied to a positively charged nylon membrane (Magna Charge, Micron Separation Inc., Westboro, Ma) under slight vacuum using a Minifold II™ slot blotter (Schleicher and Schüll, Horb, Germany) in triplicate. For quantification purpose each membrane contained standards (reference series) with known amounts of target RNA (Figure 2). Membranes were air-dried and RNA was fixed by baking at 80 °C for 1 hour. Membranes were transferred to hybridization bottles and the hybridization buffer was added (3 ml per membrane). Membranes then were prehybridised at 40 °C for 1 hour.

The oligonucleotide probes used were custom synthesized form Amersham PharmaciaBiotech, Freiburg, Germany. Sequences and wash temperature are given in Table 7. Probes were labeled with ^{32}P-ATP (MP Biomedicals Inc.,

Eschwege, Germany) using T4 NucleaseTransferase (Amersham Pharmacia Biotech, Freiburg, Germany). The labeled probes were purified with spin columns (QIA Quick spin, Queen GmbH, Holden, Germany). The purified labeled probes were then added to the appropriate amounts of hybridization buffer (3 ml per membrane). Pre-hybridization buffer was discarded and the hot buffer was added to the membranes. Hybridization was carried out over night at 40 °C. Membranes then were removed from the bottles and placed into wash solution (SSC and 1% SDS), which was preheated to the probe specific wash temperature. After 15 minutes membranes were transferred for another 15 min in fresh wash solution. Afterwards membranes were air dried covered with saran film and exposed to imaging plates (Type BAS-III, Fuji Photo Co., LTD., Japan).

Table 7: Oligonucleotide probe sequences with empirically determined thermal denaturation temperatures (td) used in the experiments

Target	Sequence (‘5-‘3)	td (°C)
All organisms	GAC GGG CGG TGT GTA CAA	44
Bacteria	GCT GCC TCC CGT AGG AGT	54
Eukarya	TAC AAA GGG CAG GGA C	42
Archaea	GTG CTC CCC CGC CAA TTC CT	56
Chytridiomycetes	GTA CAC ACA ATG AAG TGC ATA AAG G	43
Fibrobacter	AAT CGG ACG CAA GCT CAT CCC	56
R. flavefaciens	AAC GGC AGT CCC TTT AG	46
R. albus	GTC AAC GGC AGT CCT GCT A	46

Source: Muetzel et al. (2003)

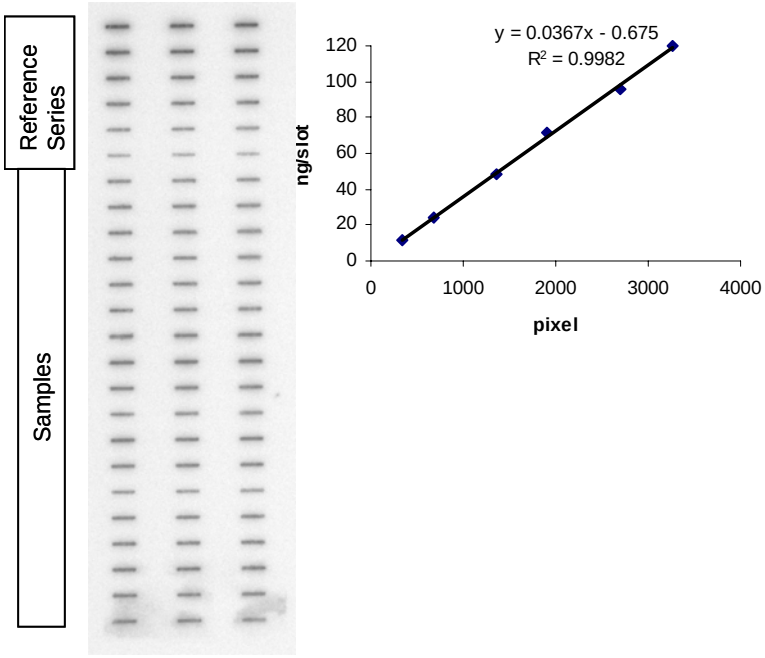

Figure 2: Membrane layout used for hybridisations. Each membrane contained 18 slots where RNA from a member of the target group was blotted. The membrane shown was hybridised with the Universal probe using *E. coli* as reference series

The imaging plates were scanned in a phosphor imager (BAS 1000, Raytest Isotopenmeßgeräte GmbH, Straubenhardt, Germany). The digital images were analyzed using the program AIDA

3.3 *In vitro* study

3.3.1 Donor animals and housing

Three N'Dama x Jersey crossbred bulls aged 6 years and three pure N'Dama bulls aged between 4 and 6 years all fitted with permanent rumen canulae were used. They were housed in a fly proof house with concrete floor. They had free access to saltlick and to water four times a day.

3.3.2 Donor diets

The donor animals were fed three different diets at three different periods equally distributed between February 2002 and June 2003 (18 months). The diets consisted of:

Baby corn stover (BCS) and 20% of the concentrate mixture (Co),

Groundnut hay (GNH) and 20% concentrate mixture

Groundnut hay and 20% moringa leaf meal (Mo)

The roughage and the supplement were offered in two equal meals in the morning and in the afternoon.

3.3.3 Treatments

For each of the three diets, 6 incubations were performed using individual animals as donors (Table 8). This incubation strategy was designed to assess firstly the effect of donor breed and donor diet on fermentation parameters, secondly the effect of increasing level of supplementation in the substrate on fermentation parameters and lastly the effect of donor diet on the correlation of *in vivo* and *in vitro* parameters. The substrates incubated consisted of all diets tested *in vivo* and the supplements alone and individual animals were used as true replicate.

For example: incubation 1. Three donor animals (crossbred) were fed BCS:Co 20% and the substrates were BCS:Co at 0%, 10%, 20%, 30%, 40% and 100%. Rumen fluid from each animal was used individually making a total of 20 syringes per animal (including 2 blanks)

Table 8: Incubation structure (BCS: Baby corn stover and concentrate mixture, GNH: Groundnut hay and concentrate mixture, GNH:Mo: Groundnut and moringa meal powder, Crossbred= N'Dama x Jersey, N'Dama= Pure breed. For each incubation, rumen fluid from 3 different animals were used separately)

Incubation	Donor Breed	Donor diet	Substrate[1]
1	Crossbred	BCS:Co	BCS:Co
2	Crossbred	BCS:Co	GNH:Co
3	Crossbred	BCS:Co	GNH:Mo
4	N'Dama	BCS:Co	BCS:Co
5	N'Dama	BCS:Co	GNH:Co
6	N'Dama	BCS:Co	GNH:Mo
7	Crossbred	GNH:CO	BCS:Co
8	Crossbred	GNH:Co	GNH:Co
9	Crossbred	GNH:Co	GNH:Mo
10	N'Dama	GNH:Co	BCS:Co
11	N'Dama	GNH:Co	GNH:Co
12	N'Dama	GNH:Co	GNH:Mo
13	Crossbred	GNH:Mo	BCS:Co
14	Crossbred	GNH:Mo	GNH:Co
15	Crossbred	GNH:Mo	GNH:Mo
16	N'Dama	GNH:Mo	BCS:Co
17	N'Dama	GNH:Mo	GNH:Co
18	N'Dama	GNH:Mo	GNH:Mo

[1]: Level of supplementation in the substrate: 0%, 10%, 10%, 20%, 30%, 40%, and 100%

3.3.4 *In vitro* incubation

Rumen liquor from each individual animal was used separately for the incubations in a water bath at 39 °C. About 400 mg of each test diet (substrate) was weighed into three 100 ml calibrated glass syringes; each syringe was fitted with a plunger as described by Menke and Steingass (1979). The syringes

and the double strength buffer (Blümmel et al., 1995) solution were prewarmed (39 °C) overnight prior to incubation.

Rumen liquor and digesta were collected from each animal in a prewarmed thermos flask in the morning before feeding. Before adding to the reduced buffer solution, the rumen liquor and digesta were strained and squeezed though a 100 µm pore size nylon bag. All laboratory handlings of rumen liquor were done under continuous flushing with CO_2 so as to maintain anaerobic conditions. The buffered rumen fluid (inoculum) was gassed for 10 minutes with CO_2 to equilibrate the solution and 30 ml was injected in the syringes using dispenser. Two syringes without substrate were incubated at the same time as blank. The syringes were shaken by hand twice in the first hour of incubation and regularly during the incubation period to prevent the plunger from picking up substrate as it rose. After 24 hours, the gas volume was recorded. The syringes were then transferred into a cold-water bath to stop the fermentation.

3.3.5 Sample collection from *in vitro* incubation

The residues from the fermentation were filtered in 50 µm pore size polyester (nitrogen free) bags (pore size 40 µm, ANKOM F57) into 100-ml beakers. 20 ml of the filtrate was taken into a 30ml tube for rumen ammonia and 1.5 ml into a 2 ml Eppendorf cup for SCFA determination. The syringes were then rinsed with 30 ml tap water into the bags until the entire residues were collected (three rinses); the bags were then air-dried and heat-sealed.

In vitro true digestibility determination

The dried sealed bags were transferred in the Ankom fibre analyser for neutral detergent fibre (NDF) determination. The difference of the sample DM and the incubation residue after neutral detergent treatments reflects the *in vitro* true digestibility assuming that no neutral detergent soluble plant material is present after 24 hours of incubation (Blümmel and Becker, 1997).

Rumen ammonia analysis

For the determination of ammonia, 15 ml of centrifuged filtrate were treated with two drops of concentrated sulphuric acid and frozen at –25 °C to wait for

analysis. For the analysis, 10 ml of the filtrate were mixed with 10 ml of 5% sodium tetra borate solution and the mixture was distilled using the semi automat Kjeldahl. The distillate was collected in a flask containing 25 ml of 4% boric acid with indicator solution and titrated with 0,1N H_2SO_4.

Short Chain Fatty Acids

720 µl of rumen fluid supernatant were collected into a vial containing 80 µl of internal standard (1 ml 100%methylvaleric acid (1/1 v/v methyl in ferric acid) made up with formic acid to 100 ml). Proteins were precipitated over night and recovered by centrifugation (30000 g, 10 min 4 °C). The supernatant was transferred into a GC vial and closed tightly. The SCFA were then determined using a gas chromatograph GC14A (Shimazu Corp, Kyoto, Japan), with a stainless steel column packed with GP 10% SP 1000 1% H_3PO_4, Chromosob W Aw (Suppelco Inc, Bellafonte, PA).

3.4 Statistical analysis

Data regarding intake, nutrients digestion and growth were analysed using the mixed model procedure of SAS, which estimates the variance components using the residual maximum likelihood method. The model used evaluated the effects due to breed, diet, level and their interactions. For *in vitro* parameters, the GLM procedure was used to evaluate the effects of donor breed, donor diet, level of supplementation and their interactions. In both studies, orthogonal contrasts were used to determine whether the increased level of supplementation had a linear or quadratic effect on the parameters. The optimum level of supplementation for organic matter digestibility (OMD) and total organic matter intake was estimated using the single-slope broken–line model (Robbins, 1986) using the NLIN procedure of SAS.

Pearson's correlation coefficients were used to determine the relationship between *in vitro* digestibility and *in vivo* organic matter digestibility.

Data on microbial community were subjected to an analysis of variance, with breed and diet as the factors in the model.

4. Results

4.1 *In vivo* study

The objectives of this study were: a) to compare the responses (feed intake, digestibility and daily weight gain) of local N'Dama breed and N'Dama x Jersey crosses (crossbred) to two basal diets (baby corn stover and groundnut hay) supplemented with graded levels of concentrate mixture and moringa meal; b) To evaluate the potential of moringa leaf as an alternative to conventional concentrate for cattle production in The Gambia. Twelve animals (6 for each breed) were used; the crossbred were significantly heavier than the N'Dama at the beginning of the study (292 ±10 kg and 152 ±12 kg respectively), all the diets were fed in a sequence manner (BCS:CO, GNH:CO and GNH:Mo) to the same animals, which implied that the weights increased linearly.

The general effects of breed, diet and their interaction on total organic matter intake (TOMI g/kg$^{.75}$ d^{-1}), organic matter digestibility (OMD) and digestible neutral detergent fibre (DNDF) are shown in Table 9.

Table 9: Least square means (Lsmeans) estimates and p values of the main effects on total organic matter intake (TOMI), organic matter digestibility (OMD), digestibility of neutral detergent fibre (DNDF) and daily weight gain (ADG) observed in two cattle breeds (N'Dama and Crossbred) fed three different diets

Parameters	Breeds		Diets			Probability (p)		
	N'Dama	Cross bred	BCS:Co	GNH:Co	GNH:Mo	Breed	Diet	BxD
TOMI (g/kg$^{.75}$d^{-1})	87.6	94.0	95.8^{a}	88.5^{b}	88.1^{b}	<0.001	<0.001	<0.001
OMD (%DM)	64.6	60.7	60.0^{a}	64.0^{b}	63.9^{b}	<0.001	<0.001	<0.001
DNDF (%DM)	45.2	38.2	50.5^{a}	46.2^{b}	28.4^{c}	<0.001	<0.001	<0.001
DOMI (g/kg$^{.75}$d^{-1})	56.4	56.8	57.0^{a}	56.6^{a}	56.4^{a}	NS	NS	<0.001
ADG (g/kg$^{.75}$ d^{-1})	9.1	9.4	11.1^{a}	7.8^{b}	8.9^{b}	NS	NS	NS

BCS:Co= Baby corn stover and concentrate mixture; GNH:Co =Groundnut hay and concentrate mixture; GNH:Mo=Groundnut hay and moringa meal. BxD= Breed x Diet; NS= Non significant (p>0.05) %DM =percent dry matter; ^{a} figure with the same letter in the same row are non significant

4.1.1 Organic matter and digestible organic intake

Total organic matter intake (TOMI) was on average higher in crossbred. They ingested 6.4 g/kg$^{.75}$ d^{-1} more than the local N'Dama (Table 9). TOMI was also higher with baby corn stover diet compared to groundnut hay diets; there was no difference between moringa meal and concentrate mixture supplementation (Table 9) in both breeds.

The diet x breed interaction was significant ($p<0.001$). On BCS:Co diet there was no significant difference in TOMI between N'Dama and crossbred although it tended to be higher with the former (97 and 95 g/kg$^{.75}$ d^{-1} respectively); on groundnut hay based diets (GNH:Co and GNH:Mo), crossbred had higher TOMI (93 against 83 g/kg$^{.75}$ d^{-1} for N'Dama (Figure 3). It should be noted that there was no refusal of supplement during the entire study; therefore the different effects observed in TOMI were the same with roughage organic matter intake.

The interaction breed x diet x level of supplementation (Figure 3) was significant. When the diet consisted of baby corn as roughage, at low level of supplementation (0 and 10%) the N'Dama ingested about 8 g/kg$^{.75}$ d^{-1} more than the crossbred ($p>0.05$) while on groundnut hay based diet, the crossbred ingested significantly more than N'Dama only at higher level of concentrate mixture supplementation (≥20%). With moringa meal supplementation, crossbred ingested more than N'Dama at all levels of supplementation (Figure 3).

Increasing level of supplementation increased TOMI (quadratic $p< 0.05$) with both breeds and all the diets, although there was a sudden increase with groundnut hay at 40% level of concentrate supplementation (Figure 3). The optimum level estimated with the single slope broken line model, depended on the diet and on the breed. For instance the optimum level was estimated for the two breeds at 10% and 20% with BCS:Co and GNH:Co respectively while with GNH:Mo it was estimated at 30% and 10% for N'Dama and crossbred respectively.

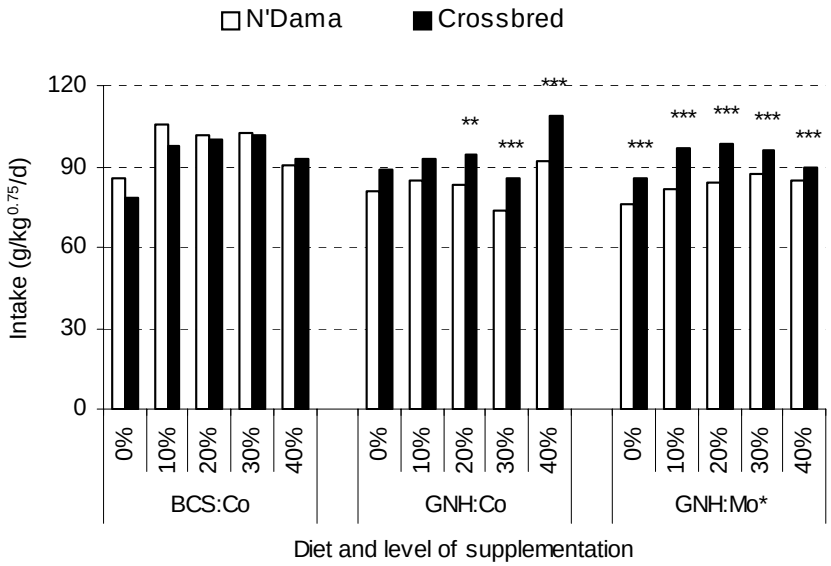

Figure 3: Effect of increasing level of supplementation on total organic matter intake (TOMI) of three different diets fed to two cattle breeds. (BCS:Co= baby corn stover and concentrate supplementation; GNH:Mo* groundnut hay(2nd batch) and moringa meal. *** Breed difference $p<0.001$, **$p<0.01$ if not mentioned $p>0.05$)

The digestible organic matter intake (DOMI $g/kg^{.75}d^{-1}$) was neither affected by the breed nor by the diet fed to animal (Table 9). However, there was a significant breed x diet interaction. On BCS:Co, N'Dama consumed 8 $g/kg^{.75}d^{-1}$ digestible organic matter more than crossbred (61 and 53 $g/kg^{.75}d^{-1}$ respectively). On groundnut hay based diet crossbred had the advantage, the DOMI was with GNH:Co 55 and 59 $g/kg^{.75}d^{-1}$ and with GNH:Mo 54 and 59 $g/kg^{.75}d^{-1}$ for N'Dama and crossbred respectively.

Increasing level of supplementation quadratically increased DOMI of BCS:Co in both breeds (Table 10) and the optimum level of supplementation for DMOI was estimated at 9% for the two breeds. With GNH:Co, increasing level of supplementation linearly increased DMOI in crossbred whereas it quadratically increased in N'Dama with an optimum estimated at 6% (Table 10). On GNH:Mo diet, increasing level of supplementation quadratically increased DOMI in the two breeds with the optimum level estimated at 20 and 16% for N'Dama and crossbred respectively.

Table 10: Effect of increasing level of supplementation on DOMI ($g/kg^{.75}d^{-1}$) in two cattle breeds

Diet	Breed	Level						Contrast	
		0%	10%	20%	30%	40%	SEM	L	Q
BCS:Co	N'Dama	52.3	67.7	63.5	61.8	59.8	0.6	NS	<0.05
	Crossbred	44.5	57.5	54.5	55.7	52.0	0.8	<0.05	<0.001
GNH:Co	N'Dama	53.3	57.6	54.3	46.7	60.6	0.6	NS	<0.01
	Crossbred	50.5	57.7	62.4	56.8	66.1	0.8	<0.001	NS
GNH:Mo*	N'Dama	47.9	54.3	55.9	58.8	54.4	0.5	<0.001	<0.001
	Crossbred	49.7	60.3	61.7	63.9	59.2	0.8	<0.001	<0.001

BCS:Co= baby corn stover and concentrate GNH:Co= groundnut hay and concentrate; GNH:Mo*= groundnut hay (second batch) and moringa meal; SEM= standard error of the mean; L=Linear; Q=Quadratic; NS= Non significant

4.1.2 Organic matter digestibility

As shown in Table 9 organic matter digestibility (OMD) was highly affected by the breed of animal and the diet fed. OMD was about 4% higher with N'Dama compared to crossbred and 4% lower with BCS:Co compared to other diets. Like with TOMI there was no difference in OMD between concentrate mixture and moringa meal supplementation on groundnut hay. The interaction breed x diet was significant; on average the difference between the two breed in advantage of N'Dama was higher with BCS:Co (7%) compared to groundnut hay diets (2.8 and 2.1% with concentrate and moringa supplementation respectively). The diet x level interaction on OMD was only significant at $p<0.01$ (Figure 4); on baby corn based diet, N'Dama digested more than their crossbred counterparts irrespective of the level of supplementation, while when groundnut hay was fed with either concentrate or moringa meal as supplement, the difference in favour of N'Dama was only significant at low levels of supplementation (less than 20 and 30% with concentrate and moringa supplementation respectively). After these levels the difference between the breeds was not significant although the crossbred tended to have an advantage (Figure 4). Increasing level of concentrate supplementation did not affect the OMD in both breeds fed baby corn based diets. However when animals were fed groundnut hay, on the one hand increasing level of concentrate

supplementation did not affect OMD in N'Dama whereas it significantly increased (quadratic: p< 0.001) in crossbred with a peak at 20% (Figure 4). On the other hand, with moringa supplementation there was a quadratic increase in OMD with a peak at 20% in N'Dama and at 30% in crossbred.

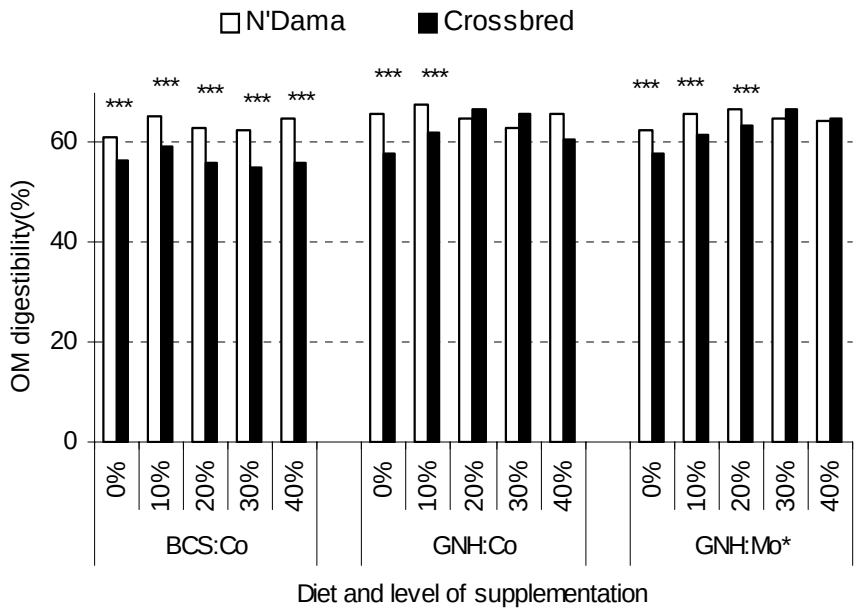

Figure 4: Effect of increasing level of supplementation on organic matter digestibility (OMD) of three different diets fed to two cattle breeds. (BCS:Co= baby corn stover and concentrate supplementation; GNH:Mo* groundnut hay (second batch) and moringa meal. *** breed difference p<0.001, **p<0.01 if not mentioned p>0.05)

The digestibility of neutral detergent fibre (DNDF) showed the same trend with regard to breed difference but this difference was more pronounced and ranged from 10% with BCS:Co to 5% with groundnut hay diets in advantage of N'Dama; DNDF was higher with BCS:Co compared to groundnut hay diet and was very low with GNH:Mo.

The diet x level interaction was also highly significant (Figure 5). When animals were fed baby corn stover as basal diet, NDF digestibility was significantly higher in N'Dama compared to crossbred at all level of supplementation. On groundnut hay based diets the difference (advantage of N'Dama) in NDF digestibility was only significant at level of supplementation less or equal to 10%

with concentrate supplementation and to 20% with moringa supplementation (Figure 5).

Increasing level of concentrate supplementation did not significantly affect NDF digestibility of BCS:Co and GNH:Co in the two breeds (Figure 5). Nevertheless with GNH:Co it tended to reach a peak at a level of 30% in crossbred and 10% in N'Dama. On the other hand graded levels of moringa supplementation linearly increased NDF digestibility in both breeds.

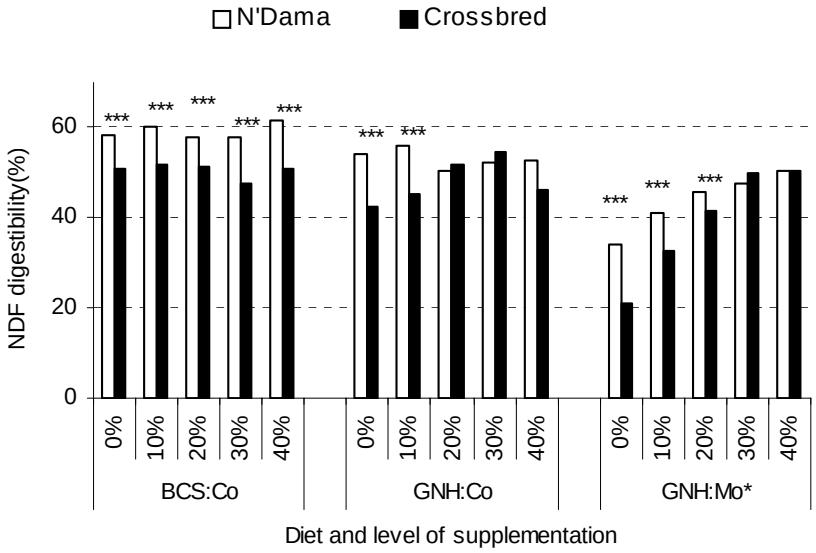

Figure 5: Effect of increasing level of supplementation on digestibility of Neutral detergent fibre (DNDF) of three different diets fed to two cattle breeds. (BCS:Co= baby corn stover and concentrate; GNH:Co= groundnut hay and concentrate GNH:Mo*= groundnut hay (second batch) and moringa meal. *** breed difference $p<0.001$, **$p<0.01$ if not mentioned $p>0.05$)

4.1.3 Daily weight gain

There was no effect of breed on average daily weight gain expressed in $g/kg^{0.75}$. The overall effect of diet was not significant (Table 9); however, the between diets comparison showed that animals gained significantly ($p<0.05$) more weight when they were fed BCS:Co compared to GNH:Co and GNH:Mo and the difference between the latter diets was not significant. There was a significant diet x level interaction. With groundnut hay diets there was no effect of level of

supplementation on ADG (Figure 6), while with BCS:Co there was a quadratic ($p<0.01$) effect of level of supplementation on ADG with the two breeds.

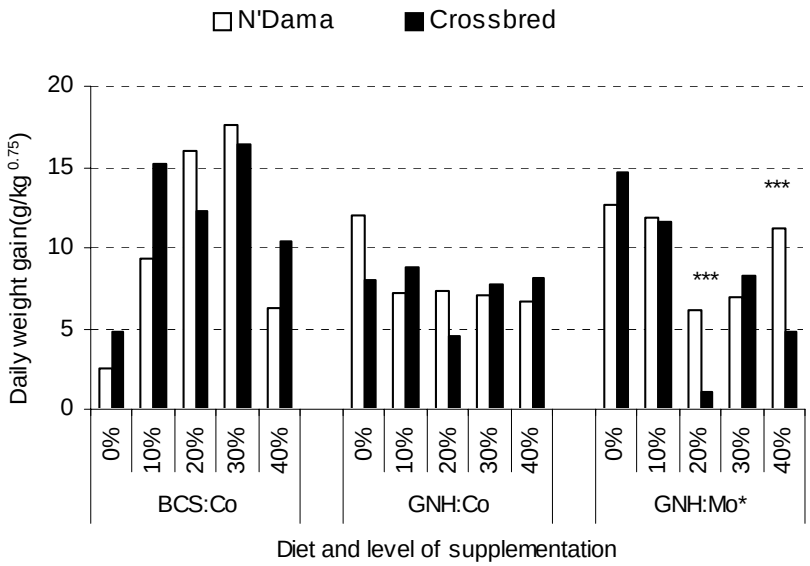

Figure 6: Effect of increasing level of supplementation on average daily weight gain observed in two cattle breeds fed three different diets. (BCS:Co= baby corn stover and concentrate; GNH:Co= groundnut hay and concentrate; GNH:Mo*= groundnut hay (2nd batch) and moringa meal; ***: Breed difference $p<0.001$; where not mentioned $p>0.05$)

4.2 Microbial community analysis

The results presented here compared the microbial community structure of two breeds of cattle fed three different diets. For each diet rumen fluid was collected during the *in vitro* study and the results presented here were from three different collection periods.

The probes used in this study included a probe targeting all rumen bacteria (Table 2). There was a significant effect ($p<0.05$) of animal diet on the bacterial RNA concentration (µg/ml of rumen fluid), the bacteria RNA concentration was higher in groundnut based diets as compared to baby corn stover based diet (Figure 7). There was no significant difference between the two breeds.

The probe used to target all rumen eukaryotes (Table 2) does not differentiate between protozoa and anaerobic rumen fungi. For the *Chytridiomycetes* a separate probe was used. On average the eukaryotic RNA concentrations (µg/ml of rumen fluid) was significantly ($p<0.05$) higher in crossbred compared to N'Dama (7 and 4 µg/ml respectively); however within the diets, the difference between the breeds was insignificant (Figure 7). There was no effect of diets though it tended to be higher for GNH:Mo and no significant breed x diet interaction was observed.

Contrary to the eukaryotes concentration, the *Archaea* (methanogens) RNA concentration was affected by the diet of the animals ($p<0.01$), it was lower in baby corn stover diet vs. groundnut hay diet (1 and 2 µg/ml respectively) and was different between GNH:Co and GNH:Mo diets; however the difference between the two breeds was not significant and no breed x diet interaction was observed

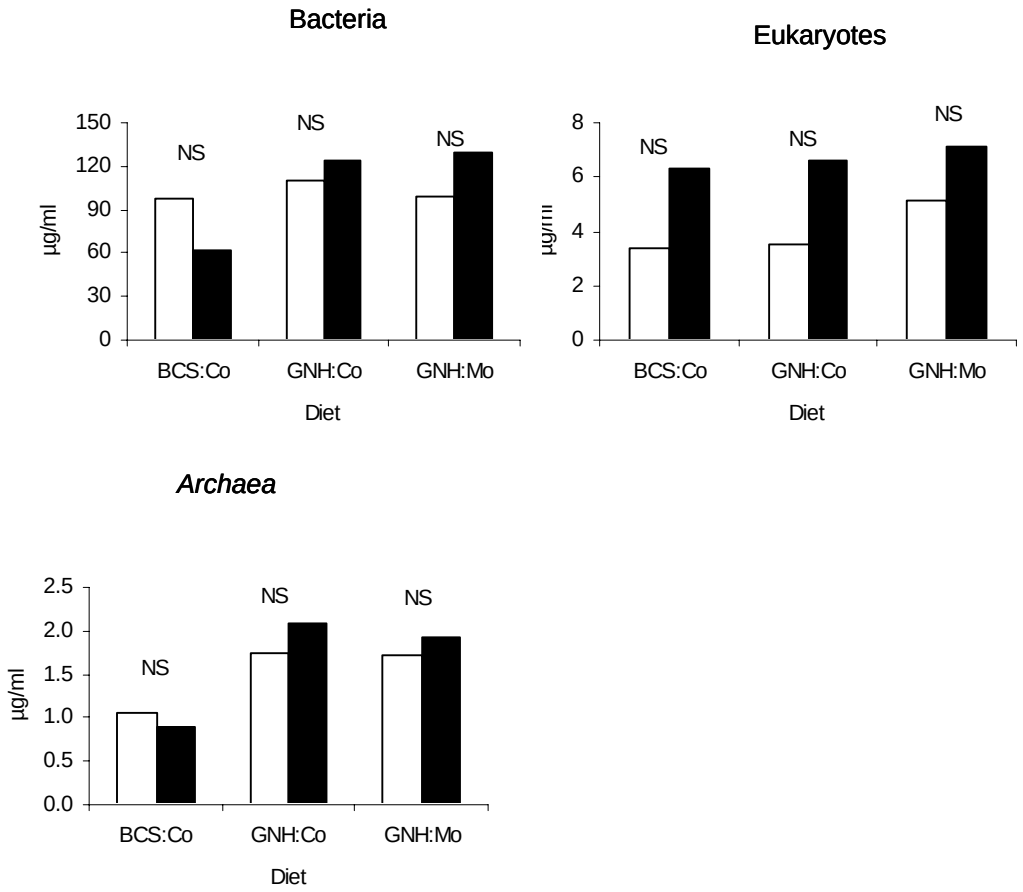

Figure 7: Bacteria, Eukaryotes and *Archaea* RNA concentrations (µg/ml) in rumen fluid of two cattle breeds fed different diets (Black columns=Crossbred, White columns = N'Dama; BCS:Co= Baby corn stover plus 20% concentrate mixture; GNH:Co= Groundnut hay plus 20% concentrate mixture; GNH:Mo=Groundnut hay plus 20% moringa meal NS= non significant).

Four cell wall degrading organisms were also targeted *(Chytridiomycetes, Fibrobacter* sp., *R. albus* and *R. flavefaciens*). The *Fibrobacter and R. flavefaciens* RNA concentrations (µg/ml) were higher in rumen fluid of N'Dama compared to crossbred (Figure 8). These concentrations were also significantly affected by diet where they were higher on Baby corn stover diet compared to groundnut hay based diet. There was a significant breed x diet interaction with the *Fibrobacter* and *R. flavefaciens* concentrations; when the animals were fed BCS:Co N'Dama had higher *Fibrobacter* ($p<0.05$) and *R. flavefaciens* ($p<0.05$) RNA concentrations compared to crossbred; on groundnut based diets no significant difference in *Fibrobacter* and *R. flavefaciens* RNA concentrations (µg/ml) was observed between the two breeds. No difference in *R. albus* RNA

concentration was observed between the different diets. The difference in *R. albus* RNA concentration was also in favour of N'Dama although not significant (p=0.06). On groundnut hay based diets the differences were not significant although the crossbred tended to have higher *R. albus* concentration.

The RNA concentration of *Chytridiomycetes* (Figure 8) was not significantly affected by animal breed; when GNH:Mo was fed, *Chytridiomycetes* concentration in the rumen fluid from crossbred was extremely high although it was not statistically different with concentrations from other diets, *Chytridiomycetes* constituted 3% to 5% of the total eukaryotic population.

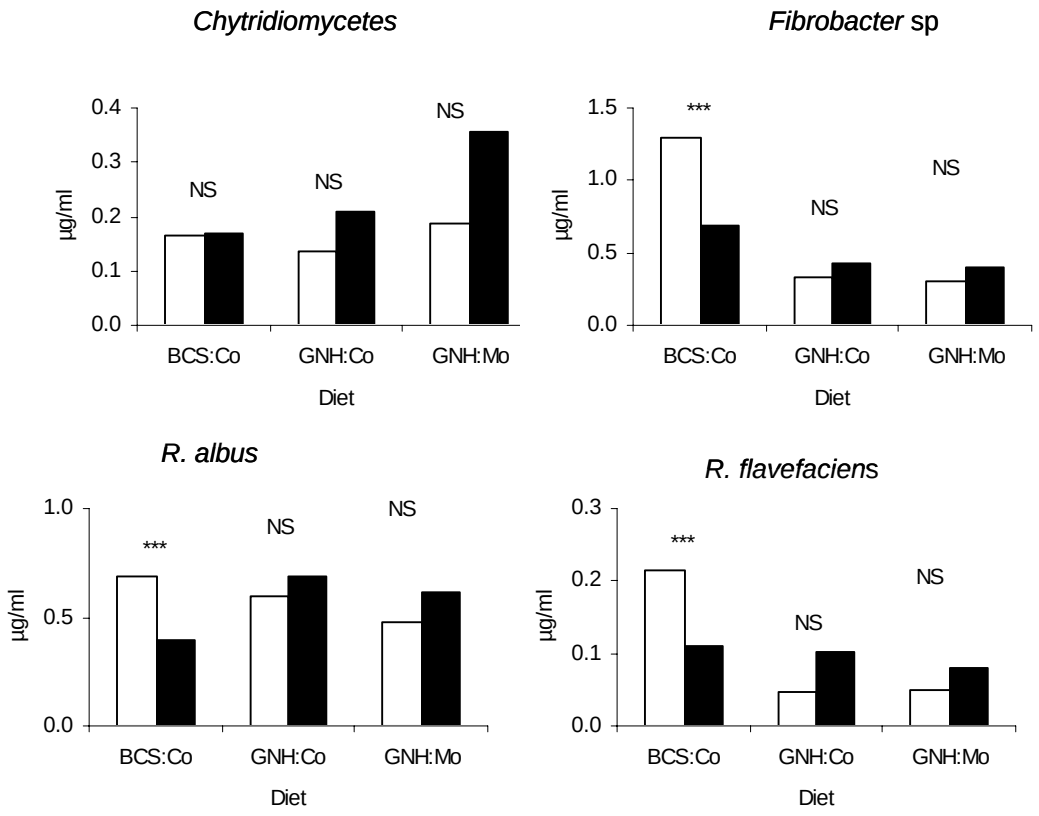

Figure 8: *Chytridiomycetes, Fibrobacter* sp., *R. albus* and *R. flavefaciens* RNA concentrations (µg/ml) in rumen fluid of two cattle breeds fed different diets (Black columns= Crossbred, White columns = N'Dama; BCS:Co= Baby corn stover plus 20% concentrate mixture; GNH:Co= Groundnut hay plus 20% concentrate mixture; GNH:Mo=Groundnut hay plus 20% moringa meal; breed difference: NS= non significant ***p<0.001)

4.3 *In vitro* experiments

The *in vitro* study was conducted to assess: a) the effects of donor animal breed, donor diet and their interaction on substrate fermentation parameters, b) the effect of increasing level of supplementation in the substrate on fibre digestibility.

Rumen fluids from six donor animals (3 N'Dama and 3 crossbred) fed 3 different diets were used individually (18 incubations). The diet comprised roughage and supplement at a ratio of 80:20.

The first hypothesis tested was that gas production would be higher if the substrate incubated is the same as donor diet. This hypothesis was not true as shown in Table 11. Gas production was not significantly different when animals were fed either BCS:Co or GNH:Co but decreased but not significantly ($p>0.05$) by about 6 ml/g with BCS:Co substrate and significantly ($p<0.001$) increased by 41 ml /g with groundnut hay based substrate when animals were fed Moringa meal as supplement

Table 11: Average 24 hours gas production (ml/mg) when substrate incubated and donor diets are the same (roughage: supplement 80:20)

Diet	Substrate		
	BCS:Co	GNH:Co	GNH:Mo
BCS:Co	$175^{a} \pm 11$	$132^{a} \pm 4$	$147^{a} \pm 3$
GNH:Co	$176^{a} \pm 10$	140 ± 5^{a}	$150^{a} \pm 6$
GNH:Mo	$169^{a} \pm 6$	$181^{b} \pm 4$	$191^{b} \pm 4$

BCS:Co= Baby corn stover and concentrate mixture, GNH:Co= Groundnut hay and concentrate mixture, GNH:Mo= Groundnut hay and moringa meal. [a] values in the same column with different superscripts are significantly different ($p<0.05$)

4.3.1 Gas production and short chain fatty acids (SCFA)

Gas production

There was no difference in gas production between the breeds whatever the diet or the substrate incubated with an average of about 160ml/g of substrate

fermented. There was however, a significant ($p<0.001$) effect of donor diet on gas production after 24 hours with all the substrate incubated. When donor animals were fed GNH:Mo, Gas production (GP) was higher on groundnut hay based substrate and lower with BCS:Co substrate; whereas when they were fed BCS:Co or GNH:Co, GP was higher on BCS:Co substrate (Table 12). The breed x substrate interaction was significant ($p<0.05$): on BCS:Co substrate gas production was on average higher with rumen fluid from crossbred compared to N'Dama (169 and 163 ml/g respectively) whereas with other substrate there was no difference. There was no significant ($p>0.05$) breed x diet interaction (Table 12).

Table 12: Average gas production (ml/g) from the fermentation of three substrates incubated with rumen fluid from two cattle breeds (N'Dama and crossbred) fed three different diets.

Diet	Breed		Substrate			Probability (p)		
	N'Dama	Crossbred	BCS:Co	GNH:Co	GNH:Mo	Breed	Substrate	BxS
BCS:Co	149[a]	151[a]	169[a]	132[a]	150[a]	NS	<0.001	<0.05
GNH:Co	153[b]	154[a]	168[a]	138[a]	154[a]	NS	<0.001	
GNH:Mo	174[c]	174[b]	161[b]	173[b]	189[b]	NS	<0.001	
Average	159	160	166	148	164	NS	<0.001	

BCS:Co= Baby corn stover and concentrate mixture, GNH:Co= Groundnut hay and concentrate mixture, GNH:Mo= Groundnut hay and moringa meal BxS= breed x substrate interaction. [a] values in the same column with different superscripts are significantly different ($p<0.05$)

Short chain fatty acid

Rumen fluid from crossbred significantly produced more SCFA than that of N'Dama (Table 13) with one exception when animals were fed GNH:Co. Rumen fluid from animals fed GNH:Mo yielded more SCFA (64 mM against 52 and 54 mM BCS:Co and GNH:Co respectively).

Table 13: Short chain fatty acids (mM) produced *in vitro* from the fermentation of three substrates incubated with rumen fluid from two cattle breeds (N'Dama and crossbred) fed three different diets.

Diets	Breeds		Substrate			Probability (p)		
	N'Dama	Crossbred	BCS:Co	GNH:Co	GNH:Mo	Breed	Substrate	BxS
BCS:Co	50.9[a]	53.8[a]	55.4[a]	47.8[a]	53.8[a]	<0.05	<0.001	<0.05
GNH:Co	55.1[b]	53.0[a]	62.7[b]	49.4[b]	50.1[b]	NS	<0.001	
GNH:Mo	60[c]	69[b]	58[c]	63[c]	73[c]	<0.001	<0.001	
Average	55	59	59	54	59	<0.001	<0.001	

BCS:Co= Baby corn stover and concentrate mixture, GNH:Co= Groundnut hay and concentrate mixture, GNH:Mo= Groundnut hay and moringa meal BxS = breed x substrate interaction. [a] values in the same column with different superscripts are significantly different ($p<0.05$)

There was a significant difference between the substrate where SCFA was lower with GNH:Co (Table 13). When moringa was fed SCFA increased by 14 mM with groundnut hay as a substrate; this is not surprising when compared to the trend observed in GP since these two parameters are stoichiometrically linked. There was a significant breed x diet interaction on short chain fatty acid production. When animals were fed GNH:Mo SCFA was higher with rumen fluid from crossbred (60 mM against 69 mM) and on GNH:Co it was the opposite although the difference was only of 2 mM; with BCS:Co diet there was no difference between the breeds. There was also a significant breed x substrate interaction whereby SCFA was higher with rumen fluid from crossbred compared to N'Dama when BCS:Co was incubated (63 and 55 mM/ml respectively) while the other substrates yielded the same amount of SCFA whatever the donor breed.

The lipogenic: glycogenic (acetate + butyrate: propionate) ratio was on average higher with rumen fluid from N'Dama compared to that from crossbred (Table 14) reflecting the lower propionate produced from N'Dama rumen fluid. However, when GNH:Mo was included as donor diet there was no difference between donor breeds. This ratio was lower with BCS:Co as substrate (Table 14), while no difference was observed between groundnut hay substrates.

Table 14: Acetate + butyrate: propionate ratio *in vitro* from the fermentation of three substrates incubated with rumen fluid from two cattle breeds (N'Dama and crossbred) fed three different diets.

Diets	Breeds		Substrate			Probability (p)		
	N'Dama	Crossbred	BCS:Co	GNH:Co	GNH:Mo	Breed	Substrate	BxS
BCS:Co	3.7[a]	3.4[a]	3.1[a]	3.9[a]	3.7[a]	<0.001	<0.001	NS
GNH:Co	4.0[b]	3.6[b]	3.3[b]	4.1[b]	4.0[b]	<0.001	<0.001	
GNH:Mo	3.9[c]	3.9[c]	3.5[b]	4.0[b]	4.1[b]	NS	<0.001	
Average	3.9	3.6	3.3	4.0	3.9	<0.001	<0.001	

BCS:Co= Baby corn stover and concentrate mixture, GNH:Co= Groundnut hay and concentrate mixture, GNH:Mo= Groundnut hay and moringa meal BxS = breed x substrate interaction. [a] values in the same column with different superscripts are significantly different ($p<0.05$)

4.3.2 *In vitro* true fibre digestibility (IVTD) and partitioning factor (PF)

The effect of donor breed on *in vitro* fibre digestibility (IVTD) was highly significant ($p<0.001$, Table 15). *In vitro* digestibility was on average 4% higher when rumen fluid from N'Dama vs. crossbred was used as inoculum independent of the donor diet (breed x diet interaction insignificant $p>0.05$), and remained constant for all substrates (breed x substrate insignificant). However, when donor animals were fed GNH:Mo, IVTD increased by 5 to 8%. The IVTD was lower with fibre rich substrate (BCS:Co) by about 12% compared to GNH:Mo substrate (Table 15).

Table 15: *In vitro* fibre digestibility (IVTD %DM) of three substrates incubated with rumen fluid from two cattle breeds (N'Dama and crossbred) fed three different diets.

Diets	Breeds		Substrate			Probability (p)		
	N'Dama	Crossbred	BCS:Co	GNH:Co	GNH:Mo	Breed	Substrate	BxS
BCS:Co	73.9[a]	68.8[a]	66.9[a]	70.4[a]	76.7[a]	<0.001	<0.001	NS
GNH:Co	74.8[a]	70.2[b]	66.9[a]	71.1[a]	79.6[a]	<0.001	<0.001	
GNH:Mo	80.8[b]	77.3[c]	71.9[b]	79.6[b]	85.6[b]	<0.001	<0.001	
Average	76.5	72.1	68.6	73.7	80.6	<0.001	<0.001	

BCS:Co= Baby corn stover and concentrate mixture, GNH:Co= Groundnut hay and concentrate mixture, GNH:Mo= Groundnut hay and moringa meal BxS = breed x substrate interaction. [a] values in the same column with different superscripts are significantly different ($p<0.05$)

IVTD of supplements (concentrate or moringa meal) alone were not affected by the diet of donor animals; however IVTD of roughage alone (baby corn stover or groundnut hay) was higher when donor animals were fed GNH:Mo. The effect of graded level of *in vitro* supplementation in the substrate was highly depended on the source of inoculum. When BCS:Co was incubated as substrate increasing level of concentrate supplementation linearly increased IVTD when donor animals were fed GNH:Mo. However, with other diets this linear increase was only observed when rumen fluid was taken from crossbred (Figure 9). Graded level of supplementation did not affect IVTD of GNH:Co substrates independent of donor breed when animals were fed GNH:Co (Figure 10), with GNH:Mo diet it tended to decrease although insignificant. IVTD of GNH:Mo increased linearly with increasing level of supplementation with rumen fluids from the two breeds when animals were either fed BCS:Co or GNH:Co, with GNH:Mo diet, the change in IVTD was not perceptible although statistically it linearly increased (Figure 11).

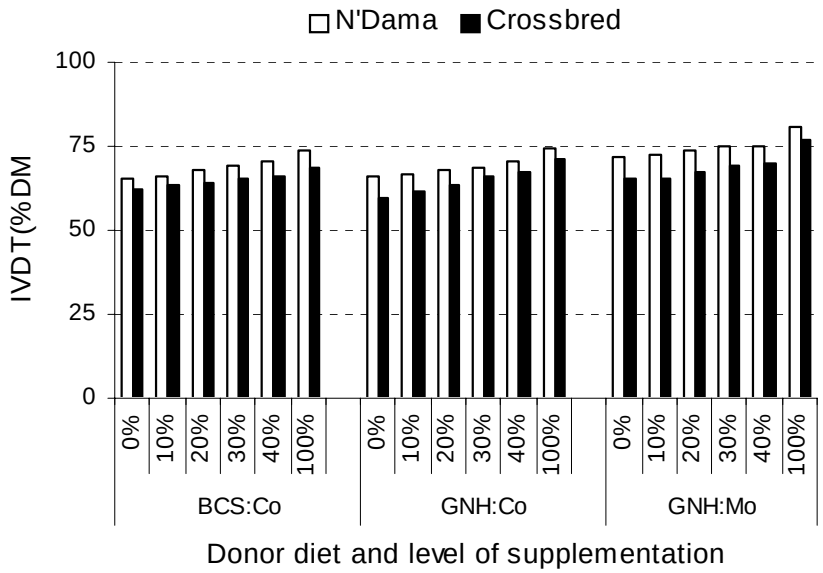

Figure 9: Effect of increasing level of supplementation in the BCS:Co based substrate on IVTD using rumen fluid from two donor breeds fed three diets. BCS:Co= Baby corn stover and concentrate mixture, GNH:Co= Groundnut hay and concentrate mixture, GNH:Mo= Groundnut hay and moringa meal

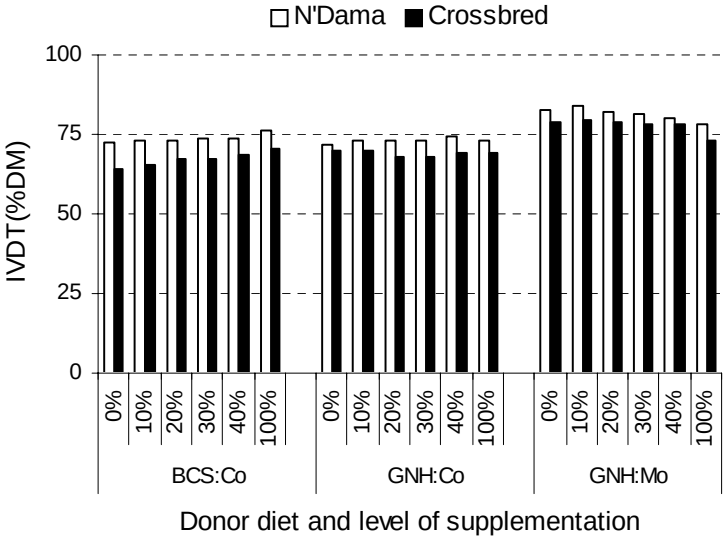

Figure 10: Effect of increasing level of supplementation in GNH:Co based substrate on IVTD using rumen fluid from two donor breeds fed three diets. BCS:Co= Baby corn stover and concentrate mixture, GNH:Co= Groundnut hay and concentrate mixture, GNH:Mo= Groundnut hay and moringa meal

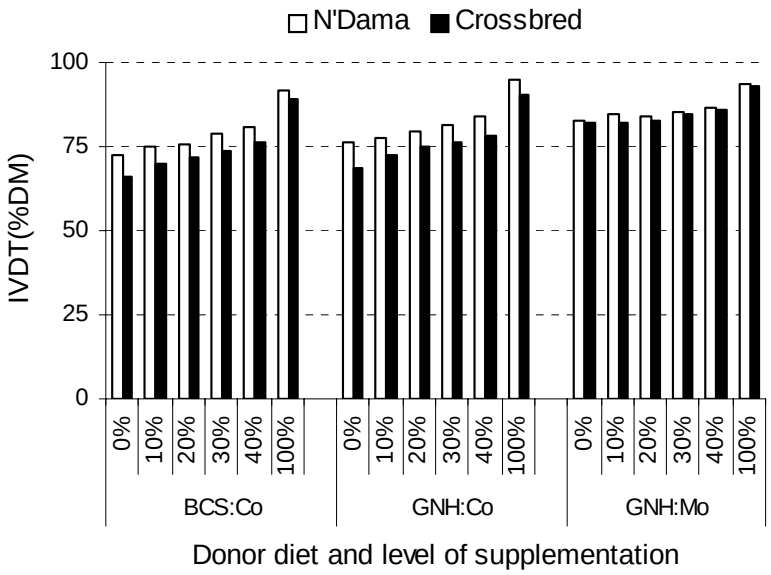

Figure 11: Effect of increasing level of supplementation in GNH:Mo based substrate on IVTD using rumen fluid from two donor breeds fed three diets. BCS:Co= Baby corn stover and concentrate mixture, GNH:Co= Groundnut hay and concentrate mixture, GNH:Mo= Groundnut hay and moringa meal

The ratio substrate truly degraded (mg): gas thereby produced (ml), termed the partitioning factor (PF) (Blümmel et al., 1997) was calculated as an indication for efficiency of microbial production. In general *in vitro* PF was higher in N'Dama when animals were fed concentrate as supplement (Table 16); however for GNH:Mo diet the difference between the breeds was not significant. BCS:Co substrate resulted in lower PF than groundnut hay based substrate and the difference between groundnut based substrates was insignificant. Again when donor animals were fed GNH:Mo there was no significant difference between the substrate. No interactions between breed x substrate and breed x diet were observed.

Table 16: Partitioning factor (PF) observed with the fermentation of three substrates incubated with rumen fluid from two cattle breeds (N'Dama and crossbred) fed three different diets.

Diets	Breeds		Substrate			Probability (p)		
	N'Dama	Crossbred	BCS:Co	GNH:Co	GNH:Mo	Breed	Substrate	BxS
BCS:Co	5.0[a]	4.6[a]	4.0[a]	5.3[a]	5.1[a]	<0.001	<0.001	NS
GNH:Co	5.0[a]	4.6[a]	4.1[a]	5.2[a]	5.2[a]	<0.001	<0.001	
GNH:Mo	4.7[b]	4.5[a]	4.5[b]	4.7[b]	4.5[b]	NS	NS	
Average	4.9	4.6	4.2	5.1	4.9	<0.001	<0.001	

BCS:Co= Baby corn stover and concentrate mixture, GNH:Co= Groundnut hay and concentrate mixture, GNH:Mo= Groundnut hay and moringa meal, BxS= breed x substrate interaction. [a] values in the same column with different superscripts are significantly different ($p<0.05$)

4.4 Correlation between *in vitro* parameters and animal responses

Another objective of the present work was to find out if optimum level of supplementation *in vivo* could be predicted from *in vitro* data. Two parameters one *in vivo* (organic matter digestibility) and one *in vitro* (IVTD) were used for this purpose. Increasing level of supplementation *in vivo* and in the substrate *in vitro* affected OMD (Figure 4) and IVTD (Figure 9, Figure 10 and Figure 11) in the same manner, with exception of GNH:Co.

The overall correlation matrix showed that *in vitro* digestibility was positively correlated to *in vivo* dry matter digestibility (DMD, $r^2 = 0.58$ $p<0.001$). However, when diets were taken individually, DMD and IVTD were strongly correlated only when BCS:Co was the tested diet, and were weakly correlated when groundnut hay based diets were tested (Table 17). Moreover, when donor animals were fed BCS:Co, IVTD and DMD with groundnut hay diets were higher correlated than when the donor animals were fed these diets.

Table 17: Correlation coefficients (r^2) between *in vitro* fibre digestibility (IVTD) following increasing level of supplementation in the substrate and *in vivo* dry matter digestibility (DMD) of the tested substrates

Donor diet	Tested diets		
	BCS:Co	GNH:Co	GNH:Mo
BCS:Co	0.88**	0.70*	0.50
GNH:Co	0.80**	0.37	0.45
GNH:Mo	0.83**	0.68*	0.28

BCS:Co = Baby corn stover and concentrate; GNH:Co = Groundnut hay and concentrate ; GNH:Mo= Groundnut hay and moringa meal; **p<0.01; *p<0.05; non significant if not mentioned.

5. Discussion

The first study (*in vivo*) was conducted to compare feed utilization (intake, digestibility and growth) in two cattle breeds (a local breed: N'Dama and its crossbred: N'Dama x Jersey) fed two different roughages (baby corn stover and groundnut hay) supplemented with various levels of concentrate or moringa meal. The results clearly showed that feed utilization and the subsequent animal performance depended on the breed of animal, the type of diet (roughage: supplement) and the level of supplementation.

5.1 Feed intake as affected by the cattle breed

Biological types of ruminants with high milk production or growth potential generally have greater maintenance energy requirements (ME required for energy stasis; fasting heat production plus heat increment) than those with lower potential (Ferrell and Jenkins, 1987; Frisch and Vercoe, 1991; Goetsch, 1998; Goetsch and Johnson, 1999). Also it has been generalised that high production potential of some biological types is expressed only with no stressful nutritional environments and high quality diets. High quality diets elicit high peripheral tissue energy availability relative to absorbed energy, which with high capacity for peripheral tissue energy accretion or secretion in milk apparently allows a level of intake more than compensatory for high maintenance energy demand. High splanchnic bed energy use relative to digestible energy (DE) intake with low quality diets corresponds to a low quantity of energy used by extra-splanchnic tissues and with high production potential a high proportion of this energy is devoted for maintenance. Thus digestible OM intake per kg $BW^{0.75}$ with very low quality forage based diets would be greater for biological types with low vs. high production potential and as forage quality increases feed intake and energy accretion or secretion in milk should change more for biological types with high potential.

In accordance with the aforementioned rationale, in the present study there was a significant breed x forage interaction. Crossbred had higher OM and DOM intake per kg $BW^{0.75}$ compared to N'Dama when animals were fed groundnut

hay based diets while baby corn stover based diets N'Dama had the highest. These observations are in accordance with the results of Goetsch and Johnson (1999), they reported a forage x breed interaction when comparing four breed groups of sheep. During our study animals consumed the entire supplement offered and the difference observed in total organic matter intake was driven by the difference in roughage (basal) organic matter intake.

5.2 Effect of cattle breed on *in vivo* digestibility

In the literature, few digestion experiments have been devoted to between-breed comparisons in cattle. There was a significant difference in OM and NDF digestibility between the two breeds and this difference was highly dependent on the diet (roughage: supplement) and the level of supplementation. When the diets consisted of a cereal stover as basal, dry matter OM and fibre digestibility were higher in the local breed whatever the level of supplementation was, while on groundnut hay based diets the local breed was superior only at low levels of supplementation. Such between-breed differences are in contrast with the observations of Doreau and Diawara (2002), Ahn et al., (1986), Xue and Han (1997), Kennedy (1982 and 1995), Grimaud et al. (1998) who compared either two breeds of *Bos taurus* (Charolais vs. Holstein) or bovine genotypes more different (*Bos taurus* vs. yaks (*Bos grunniens*), *Bos taurus* vs. swamp buffaloes (*Bubalus bubalis), Bos taurus* vs. *Bos indicus*).

However in cattle, between-breed differences have been mentioned in the literature (Norton et al., 1979) but it corresponded to a better sparing of N in breeds adapted to their environment than in exotic breeds or to the difference in the anatomy where *Bos indicus* have been reported to have smaller digestive tracts (Schneider and Flatt, 1975) and faster passage rates of digesta (Preston and Leng, 1987) than *Bos taurus* breeds. Hunter and Siebert (1985b) reported that zebu (*Bos indicus*) is better able to utilize lower quality feed than the temperate breeds; this superiority was attributed to their superior ability to recycle endogenous nitrogen in the rumen. Zebus are also reported to have higher true digestibility, more extensive ruminal digestion, more efficient protein

synthesis and lower metabolic faecal nitrogen excretion than most temperate breeds (Hunter and Siebert, 1985a, and 1985b).

The crossbred animals used in this study were between 5 and 6 years old and could be assumed to be as well adapted to the environment as the local breed. Therefore for the between-breed difference observed in our study, our hypothesis was that there was a difference in the microbial community composition/structure.

5.3 Comparison of cell wall degrading microbial community composition in two cattle breeds

Rumen microbes play the key role for the digestibility of a given feed and thus also for feed intake and finally animal performance. Obviously, the community composition and activity is highly dependent on the diet. With the present set-up, however, with identical external conditions and three different, well defined diets fed to both, N'Dama and crossbred cattle, a comparison of microbial community structure between breeds could be attempted. For the evaluation of the microbial community, rumen samples were taken from animals fed at 20% supplementation level. The bacteria and *archaea* RNA concentration were highly dependent on animal diet. The high concentration of *archaea* in groundnut hay diets could not be explained by the short chain fatty acids composition. The rumen fluids from animals fed BCS:Co contained 30 mM and 5 mM of total SCFA and propionate respectively against 30 and 4 mM with rumen fluid from animals fed either GNH:Co or GNH:Mo. There was no significant difference in RNA concentration of bacteria and *archaea* between the two breeds. However there was a trend of higher RNA concentration of eukaryotes with rumen fluid from crossbred. Protozoa have been reported to reduce intestinal protein supply. It can therefore be speculated that the low concentration of protozoa observed in rumen fluid of native N'Dama will increase protein utilization efficiency in the native cattle and this could support the better sparing of nitrogen reported by Norton et al. (1979).

The absolute amount of cell wall degrading community (*Fibrobacter* sp., *R. albus* and *R. flavefaciens*) was significantly higher in N'Dama compared to crossbreed when animals were fed baby corn stover. This difference

corresponded to the difference (in advantage of N'Dama) in *in vivo* organic matter and NDF digestibility; on groundnut hay based diet there was no significant difference in all the organisms targeted between the two breeds; this is also in accordance with the absence of significant differences in organic and NDF digestibility *in vivo* of GNH:Co at the tested supplementation level (20%). However with moringa as supplement N'Dama had in contrast the highest digestibility at 20% level of supplementation despite the absence of significant differences in RNA concentrations (µg/ml) of the targeted cell wall degrading microbial community between the two breeds. Stahl et al. (1988), Weimer et al. (1999) and Michalet-Doreau et al. (2002) did not find any relationship between digestion results and microbial ecosystem composition determined using oligonucleotide probes to rRNA. This absence of relationship may be due to the low identified bacterial proportion with the rRNA probes used (Michalet-Doreau et al., 2002). Therefore the highest ability of N'Dama to digest GNH:Mo could be attributed to their superior ability to recycle endogenous nitrogen in the rumen (discussed earlier with respect to the *archaea* concentration) as reported by Hunter and Siebert (1985a, and 1985b) comparing digestibility in zebus and temperate breeds of cattle.

Within the cell wall degrading community RNA concentration of *Chytridiomycetes* was not affected by the diet nor by the breed of the animals; however, *Chytridiomycetes* was very high when animals were fed moringa as supplement represented 19 and 25% of the of the cellulolytic rRNA in N'Dama and crossbreed respectively. The *Chytridiomycetes* are known for their high activity in cell wall breakdown and were expected to be higher on groundnut hay diets with low NDF digestibility (Figure 5).

The RNA concentration of *Fibrobacter* showed elevated amounts on stover based diet almost four times more than in the groundnut hay based diet; this is not surprising because these organisms are recognized as the most active cell wall degrading community species (Malburg and Forsberg, 1993; Li and Heath, 1993). *Fibrobacter* is probably the best examined rumen organism and the basic work with 16 rRNA targeted probes was done by Stahl et al. (1988) using a probe specific for this cell wall degrading organism. The proportion of *Fibrobacter* in the rumen fluid from our study showed a large variability ranging

from 0.3 to 1.3% of the total bacterial RNA, the lowest proportion being observed in groundnut hay based diet. These proportions are within the range of Muetzel et al. (2003) and much lower than those of Stahl et al. (1988). The difference between the data could be explained by the difference in the diet and more important the difference in the collection time. Our samples were collected before morning feeding (about 12 hours after the evening feeding), while those of Stahl et al. (1988) were collected 6 hours after feeding. The *Fibrobacter* appeared to be the dominant cellulolytic species targeted on stover based diet, they presented 50 to 55% of the cellulolytic rRNA) while the *Ruminococci* (*R. albus* and *R. flavefaciens*) were dominant (48 to 58%) on groundnut hay based diet. Weimer et al. (1999) and Martin et al. (2001) reported a higher proportion of the 2 *Ruminococci* compared to *Fibrobacter* in the ruminal microbial ecosystem of dairy cows, with *R. albus* as the dominant species. In our study *R. albus* appeared also to be the dominant *Ruminococci.*

5.4 Feed intake and digestibility as affected by the level of supplementation

A supplement should primarily provide critical nutrients lacking in the basal diet and create an environment conducive to optimising the release and utilization of other nutrients in the basal diet (Ngwa et al., 2002). Numerous reviews have discussed the responses of growing cattle fed low quality roughage and it appears from these reviews that the responses to supplementation depend on the type and level of the supplement and the quality of the basal; the results of the study are in agreement with the previous observations.

Increasing levels of concentrate supplementation decreased intake of groundnut hay as basal while the total intake did not change substantially, although the greatest total intake was observed at 10% and the lowest at 30% compared to the non-supplemented groups. Allden (1981) and Sanson (1993) reported that increases in intake due to protein based concentrate are not consistent when forages contain more than 7% CP. The groundnut hay used in the present study had 12% CP (first batch, Table 3). When the basal diet consisted of baby corn (6% CP), intake of the roughage increased at the first level of supplementation (10%) then decreased with subsequent levels. The

total organic matter intake followed the same trend and the optimum intake estimated at 10% level of supplementation in both breeds. Decline in roughage and total intake with high level of supplementation (40%) agrees with other studies (Archimede et al., 1995; Dixon and Stockdale, 1999). Concentrate at low level acts as a true supplement and improve forage utilization.

Organic matter and NDF digestibility were not affected by concentrate supplementation of either baby corn stover or groundnut hay. These feeds contained 6 and 12% CP (Table 3) necessary for optimal rumen fermentation.

In general crossbred gained more weight but the feed conversion efficiency averaged for all diets was better with N'Dama compared to crossbred (9.6 and 10 respectively). On BCS:Co N'Dama were less efficient (14 against 9) while on groundnut hay based diets N'Dama were more efficient (11 vs. 13 and 9 vs. 27 GNH:Co and GNH:Mo respectively). These results suggested that on low quality roughage native breeds would use proportionally more of their energy intake for maintenance (McDonald et al., 1995) and exotic breeds will consume more feed relative to their maintenance energy requirements, thereby gaining faster and more efficiently (Krehbiel et al., 2000). With feed of better quality however, native breeds might economically be suitable for fattening purposes.

5.5 *Moringa oleifera* as an alternative supplement for cattle production

Moringa meal was included in the study to be assessed as an alternative supplement to cattle production. Little information on the use of moringa leaf as sole supplement to ruminant production exists in the available literature. Aregheore (2002) using moringa as a supplement to batiki grass (CP=8.3%) in growing sheep observed highest intake and digestibility at levels of supplementation between 20 and 30%; in his study moringa was fed up to 80% of the daily ration. Groundnut hay as a basal diet could not yield any animal response in terms of weight gain, however the linear increase observed with NDF digestibility suggested high microbial activity in the rumen. Moreover no negative effect was observed on animals.

Groundnut hay is widely used in other parts of West Africa as a supplement to low quality cereal straw. But in the Gambian context or in southern Senegal

where groundnut is the main cash crop, groundnut hay constitutes the main basal diet for ruminants and non-ruminant herbivore livestock. Supplementation of this roughage with concentrate mixture is common practice in ruminant production in the region. Although in our study supplementation did not affect daily weight gain, in other production levels (e.g. dairy) where protein and energy requirements are higher, groundnut might need to be supplemented. In this line previous study with milking crossbred and local cows (Nouala et al., 2003) revealed that supplementation of groundnut hay with concentrate mixture substantially increased milk off-take and reduced weight loss during lactation in the two genotypes; therefore the most promising combinations obtained in our study should be tested on dairy cows.

5.6 Effect of source of inoculum (donor breed and donor diet) on *in vitro* fermentation parameters

The *in vitro* study was conducted to assess the effect of the diet and the breed of donor animals on *in vitro* parameters as a cheap method for quality prediction.

The activity of micro organisms in the rumen fluid used for *in vitro* incubation can alter the *in vitro* fermentation pattern as reported by Huntington et al. (1998) and Tejido et al. (2002), where the donor diet influenced the gas production, the *in vitro* digestibility, and the short chain fatty acid composition. The first authors noted that rumen fluid from animals fed roughage resulted in a lower rate of *in vitro* fermentation compared to rumen fluid from animals fed a roughage: concentrate mixture. In their study, the second authors reported lower *in vitro* digestibility with rumen fluid from animals fed 80:20 (forage: concentrate ratio) vs. rumen fluid from animal fed 20:80(forage: concentrate ratio). In our study three diets (BCS:Co, GNH:Co and GNH:Mo) were used as diets of donor animals of two breeds (N'Dama and crossbred). The results showed a clear effect of donor diet and donor breed on 24 hour gas production and *in vitro* digestibility, where on the one hand *in vitro* digestibility was higher with rumen fluid from N'Dama regardless the diet of donor animals and the substrate incubated and a trend of lower gas production with rumen fluid from N'Dama

only when the donor diet consisted of the tested substrate. On the other hand, *in vitro* digestibility (with all the substrate) and gas production (with exception of baby corn based substrate) tended to be lower with rumen fluid from animal fed BCS:Co and higher when moringa meal was fed as supplement to donor animals.

On groundnut hay based substrate, gas production was lower with rumen fluid from N'Dama fed BCS:Co. With the high density of cell wall degrading community in this inoculum, it was expected to yield a high rate of fermentation. The lower gas production observed from the fermentation of groundnut hay based substrate could be explained by an adaptation to the substrate of the microbial community as observed and reported by Bonsi et al. (1995) and Muetzel et al. (2003).

Moreover, the higher trend of *in vitro* digestibility observed with rumen fluid from N'Dama vs. crossbred fed GNH:Co and GNH:Mo, despite the absence of difference in the density of cell wall degrading community, might be hard to explain and we can speculate that digestibility is not only affected by the density of microbial community but also by their activity and interactions between them (synergy) as suggested by Dehority (1998). Contrasting to *in vitro* digestibility, SCFA tended to be higher in crossbred vs. N'Dama with one exception (when GNH:Co was incubated with rumen fluid from animals fed GNH:Mo). The relative higher protozoa community density observed in crossbred could be an explanation for this higher SCFA production. In the rumen environment, protozoa engulf and digest bacteria and are the major factor of rumen nitrogen turnover (Frikins et al., 1992; Itabashi et al., 1984). Fermentation of microbial biomass by protozoa would lead to end products (SCFA) without an increase in substrate disappearance. Regarding the effect of donor diet on SCFA, rumen fluid from animals fed moringa as supplement resulted in higher SCFA production which reflected the higher *in vitro* digestibility observed with diet and most probably the higher microbial growth (measured indirectly by the partitioning factor) due to the supply of animo acids by moringa meal as reported with other tree leaves (Nsahlai et al., 1998).

5.7 Prediction of level of supplementation from *in vitro* fibre digestibility

In a previous study Muetzel et al. (2003) demonstrated that it was possible to predict the optimum level of supplementation from *in vitro* parameters; however no *in vivo* data could support their conclusions. In the present study, the effect of increasing levels of supplementation on certain *in vitro* and *in vivo* parameters was tested using the same tested materials with rumen fluid from different sources. Our results confirmed their observations. *In vivo* dry matter digestibility and *in vitro* digestibility were affected in the same manner and were positively correlated; nevertheless, the correlation average for the two breeds (r^2 =0.90) was very strong only when a fibre containing diet was tested and the weakness of the correlation (r^2 =0.20) with high quality roughage. If a change in feed digestibility occurs as a shift in the ruminal fermentation pattern, the 24-hour period used in our study may have allowed time for the *in vitro* culture to establish an adequate fibre fermentation with increasing level of supplementation and this might explain the linear increase in IVTD rather than a quadratic response that one would have expected. Sanson et al. (1990) observed a decrease in ruminal pH and an increase in ruminal ammonia for only the first 6 to 8 hours after supplementation with cereal grain; therefore decreasing the fermentation time to 12 hours might mimic closely the fermentation pattern in the rumen.

6. Conclusion

Rapid urbanisation in sub-Saharan African countries has become a major motivating force in the transfer of animal science technology, which has expanded into dairy production leading to the need for cattle with higher potential for milk yield than the indigenous breeds. However, adaptation to a specific environment is essential for ruminants to thrive.

The present study was carried out first to compare feed utilization in two cattle breeds (a local breed and its crosses with Jersey) under different feeding regimes. It appeared from our results that in terms of organic matter intake high quality forage (groundnut hay) yielded greater differences among the breeds than forage of lower quality (baby corn stover). Crossbred cattle ingested more of high quality forage than the local N'Dama, whereas with lower quality forage there tended to be an advantage for the local breed. Data on weight gain showed that N'Dama might be as equally efficient as their crossbred counterparts in converting feed to weight.

The genetic make up of cattle appeared to have some effect on the rumen microbial community especially in a nutritionally restrictive environment. When animals were fed low quality forage, the total RNA concentration of the cell wall degrading community was higher in the local breed and thus in agreement with the higher fibre and organic matter digestibility observed with this breed.

Moringa was included in our study to be tested as an alternative supplement to a concentrate mixture. When moringa leaf was used to supplement GNH, results on daily weight gain were not conclusive. Results on digestibility and intake, however, were promising. Moreover, no negative effects were observed on animals fed moringa meal. A study using poor quality roughage is needed to fully assess the potential of Moringa as supplement. Finally, an economics study will be important to evaluate the economic feasibility of using moringa residues (after processing for human consumption) for animal nutrition.

Although diets and breeds of donor animals affected *in vitro* parameters, the fermentation patterns were more influenced by the donor diets. It could be concluded that for routine tests emphasis should be put on donor diets when comparing results across studies. The correlation observed between *in vitro*

fibre digestibility and *in vivo* organic matter digestibility suggested that *in vitro* digestibility could be used as a screening tool for optimum level of supplementation, thereby reducing the number of combinations to be tested *in vivo*. However, to substantiate these predictions and to make them more reliable, one of the combinations should be fed to donor animals.

7. Summary

This thesis presents a unique combination of an *in vivo* feeding trial, the analysis of the microbial community structure in the rumen, and *in vitro* fermentation studies, in order to assess the impact of breeds and diets on animal performance in a West African production setting. Pure N'Dama and N'Dama x Jersey crossbred cattle were fed two basal diets, baby corn and groundnut hay, supplemented with graded levels of either conventional concentrate or moringa leaf meal, to compare animal responses in productivity. In this context, *Moringa oleifera* leaf meal constitutes a locally available, potential alternative to commercial concentrate for cattle production. The cell wall digesting community of N'Dama and its crosses was analysed using phylogenetically based hybridisation probes to account for the contribution of rumen microbes to differences in fermentation patterns and animal response. *In vitro* fermentation studies were carried out using the same diets and supplementation levels as fed *in vivo*, to test the accuracy of the *in vitro* gas production technique in predicting the optimum level of supplementation.

The *in vivo* feeding experiment focussed on the comparison of breed performance with diets relevant for local production conditions. Six N'Dama and six N'Dama x Jersey (crossbred) animals were used in a cross over design. They were fed consecutively three combinations of roughage and supplement, baby corn stover and concentrate (BCS:Co), groundnut hay and concentrate (GNH:Co) and groundnut hay and moringa meal (GNH:Mo), each at 5 levels of supplementation (0, 10, 20, 30 and 40%). Results from this study showed that there was a clear difference in animal response to different feeding regimes between the two breeds. When averaged over all diets organic matter intake (OMI) was higher in crossbred compared to N'Dama (94 and 87.6 g/kg $^{0.75}$ d^{-1}, respectively). When analyzed for the diets and averaged over the breeds OMI was higher when animals were fed the baby corn based diet compared to groundnut based diets (95 against 88 g/kg $^{0.75}$ d^{-1}). Only when the diet consisted of BCS:Co, and at low levels of supplementation, N'Dama ingested more than crossbred, but the difference was not significant. With GNH:Co crossbred ingested significantly more at levels of supplementation less or equal to 20%. With GNH:Mo crossbred ingested more, whatever the level of supplementation.

The optimum level of supplementation *in vivo*, estimated with the single slope broken line model, was 10% and 20% for both breeds when they were fed BCS:Co and GNH:Co respectively, but 30% for N'Dama and 10% for the crossbreds when animals were fed GNH:Mo.

Organic matter digestibility (OMD) was higher in N'Dama (64.6% against 60.7% in crossbreds) when animals were fed BCS:Co and supplementation had no effect on OMD of BCS:Co whatever the breed. When animals were fed groundnut hay as basal diet, OMD was also significantly higher in N'Dama at low levels of supplementation, but the differences became insignificant beyond 10% and 20% of concentrate or moringa, respectively. With GNH:Co OMD showed a quadratic response ($p<0.001$) with increasing level of supplementation when it was fed to crossbreds and was not affected when it was fed to N'Dama. Increasing levels of moringa meal supplementation increased OMD in both breeds up to a peak at 20 and 30% for N'Dama and crossbred, respectively.

Average daily weight gain (ADG) was not affected by the breed, however it was higher on BCS:Co compared to other diets. On BCS:Co ADG increased with the level of supplementation, reaching a peak at 30%, whereas supplementation had no effect on ADG when animals were fed groundnut hay based diets.

As N'Dama could take in and digest more of the low quality BCS:Co diet, they were less efficient in feed conversion under this feeding regime (FCE: 14 vs. 9 for the crossbreds). On GNH based diets, however, N'Dama surpassed the crossbreds in feed conversion efficiency with ratios of 11 vs. 13 for GNH:Co and 9 vs. 27 on GNH:Mo.

Rumen microbes play the key role for the digestibility of a given feed and thus also for feed intake and finally animal performance. Obviously, the community composition and activity is highly dependent on the diet. With the present set-up, however, with identical external conditions and three different, well defined diets fed to both, N'Dama and crossbred cattle, a comparison of the microbial community structure between breeds could be attempted. The *in vivo* and *in vitro* data taken in the other parts of the study allow a sensible interpretation of potential changes in microbial composition. Rumen fluid was collected from three fistulated N'Dama and three crossbred animals adapted to the

experimental diets at medium supplementation level. The cell wall degrading community was analyzed using the phylogenetically based 16S rRNA hybridisation probes. The results showed that on BCS:Co diet the *Fibrobacter* and *R. flavefaciens* RNA concentrations were higher in rumen fluid of N'Dama compared to crossbred. These concentrations were also significantly affected by the diet, such that they were higher on baby corn stover compared to groundnut hay based diets. The results of the microbial community analysis suggested that the differences between breeds observed in digestibility could be partially explained by the composition of the cell wall degrading community.

Parallel to the *in vivo* experiment, *in vitro* fermentation studies were undertaken to evaluate the predictability of the *in vivo* response to supplementation by the *in vitro* data. Rumen fluid from 3 N'Dama and 3 crossbred donor animals was used for 24 hour *in vitro* fermentations. The donor animals were fed consecutively the same three diets used *in vivo* (BCS:Co, GNH:Co and GNH:Mo) at 20% level of supplementation. Each of these inocula was incubated with *in vitro* substrates consisting of all the combinations tested *in vivo* (i.e. 3 diets, 5 levels of supplementation) plus supplement alone. This design should allow to analyze for both, the impact of donor breed as well as that of the donor diet and to conclude which factors may be varied while maintaining predictability. The breed of the donor animals did not significantly affect 24 hour gas production, but short chain fatty acid concentration was higher with rumen fluid from crossbreds when donors were fed BCS:Co and GNH:Mo. Moringa meal as supplement to donor animals changed the fermentation pattern of all the substrates, such that gas production and SCFA increased substantially in groundnut hay based substrates, whereas gas production of BCS:Co substrates decreased and SCFA did not substantially change. *In vitro* digestibility was higher with rumen fluid from N'Dama whatever the diet of donor animals and the substrate incubated. GNH:Mo as donor diet also increased IVTD of all the substrates. Even though there was no clear response *in vivo*, this indicates a general stimulation of microbial activity in the rumen and renders moringa leaf meal a promising supplement.

Averaged over all data there was a positive correlation (r^2=0.53 $p<0.001$) between IVTD and *in vivo* OMD. This correlation was much stronger when

calculated for a specific diet (e.g. r^2=0.90 $p<0.001$ for BCS:Co, averaged over the breeds). Analyzing the data for the individual breeds affected correlations only to a minor degree. Thus, when testing a supplementation strategy *in vitro*, it should be important that donor animals are fed the same components (roughage and supplement) that will be combined at different levels *in vitro*, whilst the breed of donor animals may be of second importance.

This work provides conclusive evidence that *in vitro* incubations may be used to design supplementation strategies, thus reducing the need for *in vivo* experiments. Moringa leaf meal is a promising local resource to substitute for conventional concentrate. Differences in productivity between breeds could be correlated to (and may be partially manifested through) a divergent community structure of rumen microbes. That, in turn, indicates that animals of different breeds might have a ‘genetic background’ that favours the establishment of a certain community, even if the animals are kept under identical conditions. This relationship should be investigated by more advanced molecular techniques.

8. Zusammenfassung

Die vorliegende Arbeit kombiniert mit einem *in vivo*-Fütterungsexperiment, einer molekularbiologische Analyse der mikrobiellen Populationsstruktur im Pansen, und einer Serie von *in vitro*-Fermentationsstudien auf einzigartige Weise drei methodische Ansätze, um den Einfluß von Tierrasse und Diät (auf die Rindermast) unter Produktionsbedingungen im westlichen Afrika zu erfassen.

Um die Produktivität der Tiere zu vergleichen, wurden reine N'Dama-Rinder und N'Dama X Jersey Kreuzungstiere mit Ernterückständen von babymais (BCS) oder Erdnüssen (GNH) als Grunddiät gefüttert, supplementiert mit steigenden Anteilen von konventionellem Konzentrat oder moringa Blattmehl. Letzteres stellt in diesem Kontext eine lokal verfügbare Ressource dar, die kommerzielles Konzentratfutter ersetzen könnte. Die zellulolytische Population im Pansen der N'Dama und der Kreuzungstiere wurde mittels phylogenetischer 16S rRNA-Sonden analysiert, um den Beitrag der Pansenmikroben zu den Unterschieden im Fermentationsmuster und somit letztlich der Produktivität der Tiere einzuschätzen. Die *in vitro*-Inkubationen wurden mit denselben Substraten und Supplementen durchgeführt wie der *in vivo*-Versuch, um zu testen, inwieweit sich die optimale Supplementierungsstrategie aus *in vitro*-Daten vorhersagen lässt.

Im Zentrum des Fütterungsexperimentes stand der Vergleich der Produktivität der beiden Rinderrassen mit verschiedenen, unter den lokalen Bedingungen relevanten Diäten. Sechs N'Dama und sechs Kreuzungstiere wurden in einer Überkreuz-Anordnung eingesetzt. Nacheinander wurden drei Kombinationen von Rauhfutter und Supplement verfüttert, und zwar Babymais und Konzentrat (BCS:Co), Erdnussheu und Konzentrat (GNH:Co), und Erdnussheu mit moringa Blattmehl (GNH:Mo), jeweils in 5 Supplementationsstufen (0, 10, 20, 30 und 40%). Die Ergebnisse dieser Studie zeigen unter allen Fütterungsstrategien einen klaren Unterschied zwischen den Rassen im Masterfolg. Über alle Diäten gemittelt, war die Aufnahme der organischen Substanz (OMI) bei Kreuzungstieren höher als bei den N'Dama (94 versus 87.6 g/kg 0.75 d-1). Im Vergleich der Diäten und über die Rassen gemittelt, war die OMI für die auf Babymais basierenden Diäten höher als für die auf Erdnussheu basierenden

(95 versus 88 g/kg 0.75 d-1). Nur von der Kombination Babymais-Konzentrat und auf niedrigen Supplementierungsstufen nahmen die N'Dama mehr auf als die Kreuzungstiere, aber dieser Unterschied war nicht signifikant. Wurden Erdnussheu-Konzentrat Mischungen mit 20% Supplement oder weniger angeboten, nahmen die Kreuzungstiere signifikant mehr Futter auf. Erdnussheu-Moringa Mischungen wurden unabhängig von der Supplementierung von den Kreuzungstieren besser aufgenommen. Die optimale Supplementierungsstufe *in vivo*, geschätzt nach dem „single slope broken line"-Modell, lag für beide Rassen bei 10% bzw. 20% für BCS:Co und GNH:Co. Mit GNH:Mo wurden Rasse-bedingte Unterschiede sichtbar indem das Optimum für N'Dama bei 30% lag, aber bei 10% für die Kreuzungstiere.

Die Verdaulichkeit der organischen Masse (OMD) war in N'Dama höher, wenn BCS:Co verfüttert wurde. Bei dieser Diät hatte die Supplementierung, unabhängig von der Rasse, keinen Einfluß auf die OMD. Mit Erdnussheu als Grundfutter und auf niedrigen Supplementierungsstufen war die OMD ebenfalls bei den N'Dama höher, diese Unterschiede waren jedoch mit mehr als 10% Konzentrat oder 20% moringa in der Diät nicht mehr signifikant.

In den GNH:Co-Kombinationen zeigte die OMD bei den Kreuzungstieren mit steigendem Supplementanteil einen quadratischen Verlauf ($p<0.001$), während sie bei den N'Dama nicht beeinflusst war. Eine steigende Supplementierung mit moringa Blattmehl auf GNH erhöhte die OMD in beiden Rassen bis zu einem Optimum bei 20% für N'Dama und 30% für die Kreuzungstiere.

Der durchschnittliche tägliche Zuwachs (ADG) unterschied sich nicht zwischen den Rassen, war aber mit BCS:Co höher als mit den anderen Kombinationen. Auf BCS:Co stieg der ADG mit der Supplementierunsstufe bis zu einem Maximum bei 30%, während die Supplementierung bei den anderen Diäten keinen Einfluß auf den ADG hatte.

Da die N'Dama von der Diät niederer Qualität (BCS:Co) aufnehmen und verdauen konnten, waren sie unter diesem Fütterungsregime weniger effizient in der Futterverwertung (FCE: 14 versus 9 bei den Kreuzungstieren). Mit Erdnussheu als Grundfutter übertrafen hingegen die N'Dama die Kreuzungstiere hinsichtlich der Verwertungseffizienz mit Koeffizienten von 11 versus 13 für GNH:Co und 9 versus 27 für GNH:Mo.

Die Pansenflora spielt eine Schlüsselrolle für die Verdaulichkeit eines Substrates und somit letztlich auch für die Futteraufnahme und die Produktivität der Wiederkäuer. Die Zusammensetzung der mikrobiellen Population ist in erster Linie abhängig von der Diät. Der hier gewählte Ansatz jedoch, in dem unter identischen Haltungsbedingungen drei verschiedene, gut definierte Diäten an zwei verschiedene Rinderrassen verfüttert wurden, erlaubt einen Vergleich der Populationsstruktur zwischen den Rassen. Die *in vivo* und *in vitro* Daten, die in den anderen Teilen der Studie erhoben wurden, ermöglichen eine sinnvolle Interpretation der molekularbiologischen Befunde zu den Populationsgrößen. Pansensaftproben wurden von jeweils drei fistulierten Tieren beider Rassen gesammelt, die an die experimentellen Diäten auf mittlerem Supplementierungsniveau angepasst waren. Membranhybridisierungen mit 16S rRNA-Sonden für unterschiedliche phylogenetische Gruppen wurden benutzt, um die zellulolytische Population zu analysieren. Es konnte gezeigt werden, dass auf der BCS:Co-Diät die Konzentration von *Fibrobacter*- und *R.flavefaciens*-RNA im Pansensaft der N'Dama höher war als bei den Kreuzungstieren. Auch in Abhängigkeit von der Diät war die Konzentration dieser Gruppen auf BCS Grundlage stets höher als auf den GNH basierten Diäten. Diese Befunde korrelieren gut mit der Beobachtung, dass die N'Dama die BCS-Diäten besser verdauen konnten als die Kreuzungstiere. Es liegt also nahe, dass die Pansenflora einen signifikanten Beitrag zu den *in vivo* beobachteten Unterschieden leistet.

Parallel zum Fütterungsexperiment wurden *in vitro* Fermentationsstudien ausgeführt, um zu testen, inwieweit sich der Supplementierungseffekt *in vivo* aus *in vitro*-Daten vorhersagen lässt. Hierzu wurde Pansensaft von 3 N'Dama und 3 Kreuzungstieren für 24 Stunden inkubiert. Die Spendertiere erhielten nacheinander die auch *in vivo* verfütterten Diäten (BCS:Co, GNH:Co und GNH:Mo) mit jeweils 20% Supplement. Jedes dieser Inocula wurde mit allen *in vivo* getesteten Kombinationen (d.h. 3 Diäten mit je 5 Supplementierungsstufen) inkubiert und mit dem Supplement allein. Dieser Ansatz erlaubt eine Analyse der Daten sowohl hinsichtlich des Einflusses der Rasse als auch der Diät der Spendertiere, und somit Rückschlüsse darauf, welche Faktoren (in welchem Ausmaß) variabel sein dürfen, ohne die Vorhersagbarkeit zu beeinträchtigen. Die Rasse des Spendertieres hatte keinen signifikanten Einfluß auf die 24h

Gasproduktion; die Fettsäuren-Konzentration war jedoch mit dem Pansensaft der Kreuzungstiere erhöht, wenn die Spender BCS:Co oder GNH:Mo gefressen hatten. Moringa-Blattmehl als Supplement in der Donor-Diät veränderte das Fermentationsmuster aller Substrate, so dass Gas und Fettsäureproduktion aus Substraten mit GNH Grundlage deutlich erhöht waren, während aus BCS:Co weniger Gas freigesetzt wurde und die Fettsäuren unverändert blieben. Die *in vitro* Verdaulichkeit (IVTD) war mit Pansensaft von N'Dama stets höher, egal welche Donor-Diäten gefüttert und welche Substrate inkubiert wurden. Auch mit GNH:Mo als Donor-Diät war die IVTD aller Substrate erhöht. Obwohl die Supplementierung mit moringa *in vivo* keine eindeutigen Effekte erzielt hatte, deutet letzteres auf eine allgemeine Stimulation der mikrobiellen Aktivität im Pansen hin, und somit auf ein hohes Potential von moringa-Blattmehl als Alternative zu herkömmlichem Konzentratfutter.

Über alle Daten gemittelt, gab es eine positive Korrelation zwischen IVTD und *in vivo* OMD (r^2=0.53 p<0.001). Diese war noch deutlicher, wenn wenn sie für eine spezifische Diät-Substrat Kombination berechnet wurde (z.B. r^2=0.90 p<0.001 für BCS:Co, gemittelt über die Rassen). Eine Analyse hinsichtlich der Tierrasse beeinflußte die Korrelationskoeffizienten nur unwesentlich. Um eine Supplementationsstrategie *in vitro* zu testen, sollte also das Spendertier möglichst mit denselben Komponenten gefüttert werden, die *in vitro* in unterschiedlichen Mischungsverhältnissen inkubiert werden, während die Rasse des Spendertieres weniger kritisch ist.

Diese Arbeit belegt anhand schlüssiger Daten, dass *in vitro*-Inkubationen herangezogen werden können, um Supplementierungsstrategien zu entwerfen, und so den Umfang von *in vivo* Experimenten deutlich reduzieren können. (insbesondere wenn fistulierte Spendertiere derselben Rasse zur Verfügung stehen und Komponenten der Spenderdiät den zu testenden Substraten entsprechen?). Moringa Blattmehl ist eine vielversprechende, lokal verfügbare Alternative zu konventionellem Konzentratfutter. Unterschiede in der Produktivität zwischen zwei Rassen wurden mit einer divergenten Populationsstruktur der Pansenflora korreliert, die somit wohl zur Manifestation der Unterschiede beiträgt. Dies wiederum könnte man als Hinweis darauf interpretieren, dass es abhängig von der Rasse eine genetische Disposition für

die Ausbildung einer typischen Pansenpopulation gibt, selbst wenn Tiere unter identischen Bedingungen gehalten werden. Diese Beziehung sollte mit molekularen Methoden, die ein höheres Auflösungsvermögen haben, weiter untersucht werden.

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10. Appendix

Abbreviations

BCS:Co: Baby corn stover and concentrate

GNH:Co: groundnut hay and concentrate

GNH:Mo: groundnut hay and moringa meal

SEM Standard error of the mean

L: linear

Q: quadratic

NS non significant p>0.05

***p<0.001

**p<0.01

*p<0.05

Appendix 1: Effect of increasing level of supplementation on average roughage organic matter intake in two cattle breeds

Diet	Breed	Level					SEM	Contrast	
		0%	10%	20%	30%	40%		L	Q
BCS:Co	N'Dama	85.8	96.4	82.0	77.8	58.6	0.85	***	**
	Crossbred	78.7	87.5	81.3	71.1	53.8	1.15	***	***
GNH:Co	N'Dama	80.8	76.0	69.6	53.8	67.2	0.76	***	**
	Crossbred	88.7	83.6	76.0	63.1	75.9	1.18	***	***
GNH:Mo	N'Dama	76.3	73.8	68.7	62.1	54.5	0.76	***	*
	Crossbred	85.5	87.9	81.0	68.7	58.0	1.18	***	***

Appendix 2: Effect of increasing level of supplementation on total organic matter intake (TOMI) in two cattle breeds

Diet	Breed	Level					SEM	Contrast	
		0%	10%	20%	30%	40%		L	Q
BCS:Co	N'Dama	85.8	105.8	101.3	102.2	90.4	1.73	NS	**
	Crossbred	78.7	97.5	99.8	101.7	92.7	1.49	**	***
GNH:Co	N'Dama	80.8	84.5	83.3	73.3	92.2	1.60	*	**
	Crossbred	88.7	93.0	94.4	85.4	108.6	1.60	***	***
GNH:Mo	N'Dama	76.3	81.6	83.6	87.4	84.5	1.59	***	*
	Crossbred	85.5	96.7	98.1	96.4	89.8	1.59	**	***

Appendix 3: Effect of increasing level of supplementation on organic matter digestibility in two cattle breeds

Diet	Breed	Level					SEM	Contrast	
		0%	10%	20%	30%	40%		L	Q
BCS:Co	N'Dama	61.4	65.4	63.2	62.6	64.7	0.48	NS	NS
	Crossbred	56.4	59.4	55.9	55.2	55.8	0.42	NS	NS
GNH:Co	N'Dama	65.7	67.5	64.9	63.1	65.7	0. 43	*	NS
	Crossbred	57.9	61.9	66.6	65.8	60.6	0.44	*	***
GNH:Mo	N'Dama	62.6	65.9	66.8	65.1	64.2	0. 44	NS	***
	Crossbred	57.7	61.7	63.5	66.7	64.8	0.44	***	***

Appendix 4: Effect of increasing level of supplementation on digestible neutral detergent fibre in two cattle breeds

Diet	Breed	Level					SEM	Contrast	
		0%	10%	20%	30%	40%		L	Q
BCS:Co	N'Dama	58.3	60.4	58.0	57.6	61.8	0.70	**	NS
	Crossbred	51.1	51.9	51.5	47.8	50.7	0.61	***	NS
GNH:Co	N'Dama	54.0	56.0	50.2	52.5	52.9	0. 64	***	NS
	Crossbred	42.4	45.5	52.0	54.6	46.3	0. 64	NS	NS
GNH:Mo	N'Dama	33.9	40.9	45.9	47.7	50.6	0. 65	***	NS
	Crossbred	21.1	32.8	41.7	49.8	50.6	0. 65	***	***

Appendix 5: Effect of increasing level of supplementation on digestible acid detergent fibre in two cattle breeds

Diet	Breed	Level					SEM	Contrast	
		0%	10%	20%	30%	40%		L	Q
BCS:Co	N'Dama	44.5	48.1	40.8	40.8	47.4	0.86	NS	*
	Crossbred	39.3	38.3	35.9	20.9	28.2	0.76	***	NS
GNH:Co	N'Dama	50.0	55.4	43.6	45.1	41.2	0.79	***	NS
	Crossbred	42.6	37.7	43.1	45.3	28.2	0.79	*	*
GNH:Mo	N'Dama	29.0	30.4	29.8	25.2	23.7	0.79	**	NS
	Crossbred	15.9	17.3	25.4	26.3	21.0	0.79	***	***

Appendix 6: Effect of increasing level of supplementation daily weight gain in two cattle breeds

Diet	Breed	Level					SEM	Contrast	
		0%	10%	20%	30%	40%		L	Q
BCS:Co	N'Dama	2.6	9.4	16.0	17.6	6.2	2.2	**	***
	Crossbred	4.8	15.2	12.3	16.4	10.4	2.2	NS	**
GNH:Co	N'Dama	12.0	7.2	7.4	7.1	6.7	2.2	NS	NS
	Crossbred	8.1	8.8	4.6	7.7	8.1	2.2	NS	NS
GNH:Mo	N'Dama	12.6	11.9	6.1	7.0	11.2	2.2	NS	NS
	Crossbred	14.7	11.5	1.1	8.3	4.9	2.2	NS	NS

Appendix 7: RNA concentration (µg/ml) of rumen micro organisms in the rumen fluid of two cattle breeds

Organism	Breed	Diet		
		BCS:Co	GNH:Co	GNH:Mo
Total organisms	Crossbred	53.36	97.90	105.70
	N'Dama	76.49	89.69	79.37
Bacteria	Crossbred	62.52	124.36	128.85
	N'Dama	97.69	110.69	98.89
Archaea	Crossbred	0.89	2.08	1.93
	N'Dama	1.06	1.75	1.71
Eukaryotes	Crossbred	6.30	6.62	7.14
	N'Dama	3.36	3.56	5.13
Chytridiomycetes	Crossbred	0.17	0.21	0.36
	N'Dama	0.16	0.14	0.19
Fibrobacter sp	Crossbred	0.68	0.42	0.39
	N'Dama	1.29	0.33	0.31
R. albus	Crossbred	0.40	0.69	0.61
	N'Dama	0.68	0.59	0.47
R. flavefaciens	Crossbred	0.11	0.10	0.08
	N'Dama	0.22	0.05	0.05

Appendix 8: Effect of increasing level of supplementation in the substrate on *in vitro* gas production using rumen fluid from two breeds of cattle fed three different diets

Donor Diet	Substrate	Donor Breed	Level						SEM	Contrast	
			0%	10%	20%	30%	40%	100%		L	Q
BCS:Co	BCS:Co	N'Dama	176	172	167	164	163	133	2.1	***	NS
		Crossbred	182	184	184	179	176	142	2.4	*	NS
	GNH:Co	N'Dama	133	135	134	133	134	137	0.7	NS	NS
		Crossbred	127	130	130	131	131	131	2.6	NS	NS
	GNH:Mo	N'Dama	138	144	148	150	155	170	0.7	***	NS
		Crossbred	138	142	145	150	151	167	1.6	***	NS
GNH:Co	BCS:Co	N'Dama	182	178	171	168	165	134	2.1	**	NS
		Crossbred	188	185	181	173	163	132	2.4	***	*
	GNH:Co	N'Dama	139	140	138	135	134	130	0.7	***	NS
		Crossbred	140	143	143	142	139	132	2.6	NS	*
	GNH:Mo	N'Dama	146	150	153	156	158	175	0.7	***	NS
		Crossbred	139	142	147	151	155	173	1.6	***	NS
GNH:Mo	BCS:Co	N'Dama	177	172	170	163	160	128	2.2	***	NS
		Crossbred	175	171	168	163	157	128	2.4	***	NS
	GNH:Co	N'Dama	194	189	181	175	168	131	0.7	***	NS
		Crossbred	196	188	181	175	169	128	2.6	***	NS
	GNH:Mo	N'Dama	194	193	190	189	187	175	0.7	***	NS
		Crossbred	195	194	193	190	188	177	1.6	***	NS

Appendix 9: Effect of increasing level of supplementation in the substrate on *in vitro* acetate (mM/ml) using rumen fluid from two breeds of cattle fed three different diets

Donor Diet	Substrate	Donor Breed	Level						SEM	Contrast	
			0%	10%	20%	30%	40%	100%		L	Q
BCS:Co	BCS:Co	N'Dama	33.4	32.0	32.4	29.0	29.8	28.7	1.4	NS	NS
		Crossbred	35.2	36.6	37.0	36.9	36.6	31.9	1.6	NS	NS
	GNH:Co	N'Dama	35.2	29.1	28.8	29.2	33.7	31.3	0.8	NS	*
		Crossbred	31.6	33.0	33.2	33.6	32.8	29.4	0.9	NS	NS
	GNH:Mo	N'Dama	32.6	38.3	34.2	36.9	41.2	44.4	1.3	*	NS
		Crossbred	38.0	39.4	39.5	32.8	36.0	32.3	1.5	NS	NS
GNH:Co	BCS:Co	N'Dama	42.9	43.1	40.4	40.5	38.5	29.9	1.4	*	NS
		Crossbred	44.3	43.7	44.3	38.8	37.1	35.9	1.6	**	NS
	GNH:Co	N'Dama	36.5	33.8	37.9	30.3	31.6	32.8	1.0	**	NS
		Crossbred	34.2	33.9	34.1	31.6	33.4	30.3	1.0	NS	NS
	GNH:Mo	N'Dama	35.9	34.4	35.2	37.8	38.0	42.9	1.4	NS	NS
		Crossbred	27.7	28.9	33.7	30.6	32.4	38.0	1.5	NS	NS
GNH:Mo	BCS:Co	N'Dama	35.8	32.9	35.0	30.5	32.6	28.0	1.5	NS	NS
		Crossbred	36.9	50.0	45.1	38.1	39.4	36.7	1.6	NS	NS
	GNH:Co	N'Dama	43.5	43.4	45.1	43.1	38.8	35.7	1.0	NS	Ns
		Crossbred	46.3	47.0	43.7	45.6	42.2	30.6	0.9	NS	NS
	GNH:Mo	N'Dama	44.6	44.0	49.8	44.6	44.9	43.8	1.4	NS	NS
		Crossbred	50.1	54.8	53.7	56.0	51.7	58.0	1.5	NS	NS

Appendix 10: Effect of increasing level of supplementation in the substrate on *in vitro* propionate production (mM/ml) using rumen fluid from two breeds of cattle fed three different diets

Donor Diet	Substrate	Donor Breed	Level						SEM	Contrast	
			0%	10%	20%	30%	40%	100%		L	Q
BCS:Co	BCS:Co	N'Dama	13.7	13.2	13.3	11.9	12.0	9.8	0.5	NS	NS
		Crossbred	14.6	15.2	16.1	15.1	14.6	11.6	0.6	NS	**
	GNH:Co	N'Dama	8.8	7.4	7.2	7.6	9.0	9.6	0.8	NS	*
		Crossbred	9.8	10.3	10.5	10.7	10.8	11.6	0.9	NS	NS
	GNH:Mo	N'Dama	9.2	10.9	10.1	11.3	13.0	16.7	0.4	**	NS
		Crossbred	10.0	10.5	10.8	9.5	10.7	12.1	0.4	NS	NS
GNH:Co	BCS:Co	N'Dama	14.6	14.8	13.8	13.6	12.9	9.9	0.5	**	NS
		Crossbred	16.5	16.1	16.1	14.3	13.9	11.9	0.6	***	NS
	GNH:Co	N'Dama	8.5	8.1	9.5	7.9	8.4	10.5	0.8	NS	Ns
		Crossbred	9.2	9.4	9.6	9.1	9.9	10.8	0.9	NS	NS
	GNH:Mo	N'Dama	9.0	8.8	9.2	10.2	10.5	13.8	0.4	*	NS
		Crossbred	7.2	7.7	9.1	8.6	9.2	12.3	0.4	**	NS
GNH:Mo	BCS:Co	N'Dama	11.9	11.2	11.5	10.2	10.7	9.0	0.6	NS	NS
		Crossbred	12.7	16.6	15.1	12.5	13.0	11.9	0.6	NS	*
	GNH:Co	N'Dama	11.6	11.6	12.1	11.9	10.9	11.3	0.8	NS	NS
		Crossbred	12.5	12.7	12.1	12.4	11.8	10.0	0.9	NS	NS
	GNH:Mo	N'Dama	11.8	11.8	13.4	12.2	12.4	13.2	0.4	NS	NS
		Crossbred	13.5	14.7	14.6	15.1	14.1	17.2	0.4	NS	NS

Appendix 11: Effect of increasing level of supplementation in the substrate on *in vitro* butyrate production (mM/ml) using rumen fluid from two breeds of cattle fed three different diets

Donor Diet	Substrate	Donor Breed	Level						SEM	Contrast	
			0%	10%	20%	30%	40%	100%		L	Q
BCS:Co	BCS:Co	N'Dama	7.1	7.1	7.2	6.6	7.0	6.8	0.2	NS	NS
		Crossbred	6.1	6.3	6.5	6.4	6.6	6.2	0.3	NS	NS
	GNH:Co	N'Dama	3.1	2.9	3.1	3.4	4.3	6.2	0.6	*	NS
		Crossbred	3.2	3.9	4.4	4.7	5.3	7.5	0.7	***	NS
	GNH:Mo	N'Dama	2.2	2.9	2.7	3.0	3.4	5.6	0.3	***	NS
		Crossbred	3.1	3.3	3.6	3.2	3.7	5.0	0.3	NS	NS
GNH:Co	BCS:Co	N'Dama	6.3	6.6	6.2	6.3	6.3	5.8	0.2	NS	NS
		Crossbred	6.4	6.4	6.5	5.9	6.0	6.0	0.3	NS	NS
	GNH:Co	N'Dama	4.0	4.3	4.6	3.7	4.5	6.6	0.6	NS	NS
		Crossbred	2.8	3.4	3.5	3.5	3.8	4.9	0.7	***	NS
	GNH:Mo	N'Dama	3.2	3.3	3.6	4.0	4.4	6.1	0.3	*	NS
		Crossbred	2.3	2.9	3.5	3.5	3.8	5.7	0.3	***	NS
GNH:Mo	BCS:Co	N'Dama	5.3	5.1	5.5	5.0	5.4	5.5	0.3	NS	NS
		Crossbred	5.9	8.7	7.6	6.3	7.0	6.2	0.3	NS	NS
	GNH:Co	N'Dama	4.3	4.9	5.2	5.0	4.9	6.5	0.6	NS	NS
		Crossbred	5.2	6.0	5.3	6.4	6.2	5.6	0.7	*	NS
	GNH:Mo	N'Dama	4.5	4.7	5.6	5.3	5.4	6.8	0.3	**	NS
		Crossbred	4.5	5.4	5.7	6.2	5.7	8.7	0.3	*	NS

Appendix 12: Effect of increasing level of supplementation in the substrate on *in vitro* valerate production (mM/ml) using rumen fluid from two breeds of cattle fed three different diets

Donor Diet	Substrate	Donor Breed	Level						SEM	Contrast	
			0%	10%	20%	30%	40%	100%		L	Q
BCS:Co	BCS:Co	N'Dama	0.5	0.6	0.7	0.7	0.8	1.4	0.05	*	NS
		Crossbred	0.5	0.6	0.7	0.8	0.9	1.5	0.06	***	NS
	GNH:Co	N'Dama	0.7	0.7	0.7	0.8	0.9	1.4	0.04	*	NS
		Crossbred	0.7	0.9	1.0	1.1	1.1	1.6	0.05	***	NS
	GNH:Mo	N'Dama	0.5	0.6	0.6	0.7	0.7	0.9	0.04	**	NS
		Crossbred	0.7	0.8	0.9	0.8	0.8	0.9	0.05	NS	NS
GNH:Co	BCS:Co	N'Dama	0.6	0.7	0.7	0.8	0.9	1.4	0.05	*	NS
		Crossbred	0.6	0.7	0.8	0.8	0.9	1.7	0.06	***	NS
	GNH:Co	N'Dama	0.7	0.7	0.9	0.7	0.9	1.5	0.04	NS	NS
		Crossbred	0.5	0.6	0.7	0.7	0.8	1.5	0.05	***	NS
	GNH:Mo	N'Dama	0.7	0.6	0.7	0.8	0.8	1.0	0.04	NS	NS
		Crossbred	0.5	0.5	0.6	0.6	0.7	0.9	0.05	***	NS
GNH:Mo	BCS:Co	N'Dama	0.5	0.5	0.6	0.6	0.8	1.3	0.05	***	NS
		Crossbred	0.5	0.8	0.8	0.8	1.0	1.7	0.06	**	NS
	GNH:Co	N'Dama	1.8	1.7	1.8	1.7	1.5	1.5	0.04	*	NS
		Crossbred	1.9	1.9	1.8	1.8	1.7	1.4	0.05	**	NS
	GNH:Mo	N'Dama	2.1	1.8	1.9	1.6	1.5	0.9	0.04	**	NS
		Crossbred	2.1	2.2	2.0	2.0	1.8	1.2	0.05	***	NS

Appendix 13: Effect of increasing level of supplementation in the substrate on *in vitro* isoacids production (mM/ml) using rumen fluid from two breeds of cattle fed three different diets

Donor Diet	Substrate	Donor Breed	Level						SEM	Contrast	
			0%	10%	20%	30%	40%	100%		L	Q
BCS:Co	BCS:Co	N'Dama	0.7	0.8	1.0	1.0	1.3	2.7	0.06	***	NS
		Crossbred	0.5	0.6	0.9	0.9	1.2	2.7	0.06	***	NS
	GNH:Co	N'Dama	1.1	1.1	1.1	1.4	1.7	2.8	0.07	NS	NS
		Crossbred	0.7	0.9	1.0	1.1	1.4	2.5	0.06	***	NS
	GNH:Mo	N'Dama	0.9	1.1	1.0	1.1	1.3	1.6	0.06	**	NS
		Crossbred	1.3	1.4	1.5	1.3	1.3	1.5	0.07	***	NS
GNH:Co	BCS:Co	N'Dama	0.8	1.1	1.3	1.5	1.6	3.0	0.06	***	*
		Crossbred	0.8	1.1	1.3	1.4	1.6	3.5	0.06	***	NS
	GNH:Co	N'Dama	1.5	1.5	1.9	1.5	1.9	3.3	0.06	***	*
		Crossbred	0.9	1.1	1.3	1.4	1.7	2.9	0.06	***	NS
	GNH:Mo	N'Dama	1.3	1.3	1.3	1.6	1.6	2.1	0.06	NS	NS
		Crossbred	0.9	0.9	1.2	1.1	1.3	1.7	0.06	***	NS
GNH:Mo	BCS:Co	N'Dama	0.8	0.8	1.2	1.3	1.4	2.6	0.06	NS	NS
		Crossbred	0.7	1.3	1.4	1.4	1.8	3.5	0.06	**	NS
	GNH:Co	N'Dama	1.4	1.6	2.0	2.0	2.1	3.6	0.06	NS	NS
		Crossbred	1.7	2.0	2.0	2.4	2.5	3.2	0.06	***	NS
	GNH:Mo	N'Dama	1.3	1.7	2.0	1.6	1.6	2.3	0.06	NS	*
		Crossbred	1.5	1.6	1.9	2.0	1.8	2.7	0.06	NS	NS

Appendix 14: Effect of increasing level of supplementation in the substrate on *in vitro* total short chain fatty acid production (mM/ml) using rumen fluid from two breeds of cattle fed three different diets

Donor Diet	Substrate	Donor Breed	Level						SEM	Contrast	
			0%	10%	20%	30%	40%	100%		L	Q
BCS:Co	BCS:Co	N'Dama	55.5	53.8	54.6	49.3	50.9	49.5	1.74	NS	NS
		Crossbred	57.0	59.3	61.1	60.2	59.9	53.9	2.02	NS	NS
	GNH:Co	N'Dama	48.9	41.2	41.0	42.3	49.6	51.4	1.74	NS	NS
		Crossbred	46.0	48.9	50.2	51.2	51.4	52.5	2.02	**	NS
	GNH:Mo	N'Dama	45.4	53.8	48.7	52.9	59.6	69.1	1.90	NS	NS
		Crossbred	53.1	55.4	56.2	47.5	52.6	51.8	2.03	NS	NS
GNH:Co	BCS:Co	N'Dama	65.3	66.2	62.5	62.7	60.1	50.0	1.61	NS	NS
		Crossbred	68.6	67.9	68.9	61.2	59.5	59.1	1.41	NS	NS
	GNH:Co	N'Dama	51.2	48.4	54.8	44.1	47.4	54.8	1.55	NS	NS
		Crossbred	47.7	48.4	49.3	46.3	49.6	50.5	1.61	NS	NS
	GNH:Mo	N'Dama	50.1	48.6	50.0	54.3	55.3	65.8	1.54	NS	NS
		Crossbred	38.7	41.0	48.1	44.3	47.4	58.5	1.62	NS	NS
GNH:Mo	BCS:Co	N'Dama	54.2	50.5	53.8	47.6	50.8	46.4	1.87	**	NS
		Crossbred	56.7	77.5	70.1	59.1	62.2	60.0	1.62	NS	NS
	GNH:Co	N'Dama	62.6	63.2	66.2	63.7	58.2	58.5	1.87	NS	NS
		Crossbred	67.7	69.6	65.0	68.7	64.4	50.9	1.62	*	NS
	GNH:Mo	N'Dama	64.3	63.9	72.7	65.4	66.0	67.1	1.87	NS	NS
		Crossbred	71.7	78.9	77.9	81.3	75.1	87.8	1.62	NS	NS

Appendix 15: Effect of increasing level of supplementation in the substrate on *in vitro* digestibility using rumen fluid from two breeds of cattle fed three different diets

Donor Diet	Substrate	Donor Breed	Level						SEM	Contrast	
			0%	10%	20%	30%	40%	100%		L	Q
BCS:Co	BCS:Co	N'Dama	65.4	66	68	69.4	70.5	73.6	3.72	NS	NS
		Crossbred	62.1	63.2	63.9	65.6	66.1	68.8	3.05	***	NS
	GNH:Co	N'Dama	72.4	73.1	73.2	73.6	73.5	76.3	3.76	*	NS
		Crossbred	64.1	65.4	67	67.6	68.3	70.7	3.07	***	NS
	GNH:Mo	N'Dama	72.6	75.1	75.7	78.7	80.6	91.5	4.04	***	NS
		Crossbred	65.8	69.9	71.5	73.9	76.2	88.8	3.27	***	NS
GNH:Co	BCS:Co	N'Dama	65.8	66.9	67.9	68.9	70.4	74.3	1.68	NS	NS
		Crossbred	59.9	61.5	63.3	65.8	67.1	71.5	1.94	**	NS
	GNH:Co	N'Dama	71.8	73	73.1	73.4	74.4	73.4	1.69	NS	NS
		Crossbred	69.7	70.2	67.8	67.9	69.1	69.1	1.94	NS	NS
	GNH:Mo	N'Dama	76.5	77.6	79.5	81.2	83.9	94.6	1.69	**	NS
		Crossbred	68.6	72.3	75.2	76.5	78.5	90.4	1.94	NS	NS
GNH:Mo	BCS:Co	N'Dama	71.7	72.4	73.5	75.3	75.2	80.9	1.38	**	NS
		Crossbred	65.3	65.1	67.5	69.2	69.9	76.9	1.6	***	NS
	GNH:Co	N'Dama	82.4	83.7	82.2	81.2	80.4	78.1	1.4	***	NS
		Crossbred	79	79.2	78.8	78.2	78.1	73.3	1.6	***	NS
	GNH:Mo	N'Dama	82.8	84.6	84.1	85.4	86.5	93.4	1.4	***	NS
		Crossbred	81.9	82.2	82.8	84.5	85.7	93.2	1.6	***	NS

Appendix 16: Effect of increasing level of supplementation in the substrate on *in vitro* ammonia production (mM/ml) using rumen fluid from two breeds of cattle fed three different diets

			Level						SEM	Contrast	
Donor Diet	Substrate	Donor Breed	0%	10%	20%	30%	40%	100%		L	Q
BCS:Co	BCS:Co	N'Dama	0.2	0.2	0.2	0.3	0.3	0.6	0.05	***	NS
		Crossbred	0.3	0.4	0.5	0.5	0.5	1.2	0.04	NS	NS
	GNH:Co	N'Dama	0.2	0.3	0.3	0.3	0.3	0.6	0.05	**	NS
		Crossbred	0.2	0.2	0.3	0.3	0.3	0.5	0.04	***	NS
	GNH:Mo	N'Dama	0.3	0.3	0.3	0.2	0.3	0.3	0.04	***	NS
		Crossbred	0.2	0.2	0.2	0.2	0.3	0.3	0.05	***	NS
GNH:Co	BCS:Co	N'Dama	0.2	0.3	0.3	0.3	0.4	0.6	0.03	***	NS
		Crossbred	0.1	0.2	0.2	0.3	0.3	0.5	0.03	***	NS
	GNH:Co	N'Dama	0.3	0.3	0.4	0.4	0.4	0.6	0.03	*	NS
		Crossbred	0.2	0.2	0.3	0.3	0.3	0.6	0.03	***	NS
	GNH:Mo	N'Dama	0.3	0.3	0.3	0.3	0.4	0.4	0.03	***	NS
		Crossbred	0.3	0.3	0.3	0.3	0.3	0.3	0.03	***	NS
GNH:Mo	BCS:Co	N'Dama	0.2	0.3	0.3	0.3	0.4	0.6	0.04	NS	NS
		Crossbred	0.2	0.2	0.2	0.3	0.3	0.6	0.04	***	NS
	GNH:Co	N'Dama	0.4	0.4	0.4	0.4	0.5	0.6	0.04	NS	NS
		Crossbred	0.3	0.3	0.4	0.4	0.4	0.6	0.04	***	NS
	GNH:Mo	N'Dama	0.4	0.4	0.4	0.4	0.4	0.4	0.04	NS	NS
		Crossbred	0.3	0.3	0.3	0.3	0.3	0.3	0.04	NS	NS

Appendix 17: Effect of increasing level of supplementation in the substrate on *in vitro* fibre bound nitrogen(NDF-N) using rumen fluid from two breeds of cattle fed three different diets NDF-N

Donor Diet	Substrate	Donor Breed	Level						SEM	Contrast	
			0%	10%	20%	30%	40%	100%		L	Q
BCS:Co	BCS:Co	N'Dama	12.7	9.3	5.8	7.2	7.2	9.3	0.7	**	*
		Crossbred	10.3	8.3	9.6	10.1	9.6	10.9	1.0	NS	NS
	GNH:Co	N'Dama	19.6	19.0	18.4	18.0	16.1	13.1	0.7	*	NS
		Crossbred	21.0	14.1	15.5	14.2	18.4	14.2	0.8	NS	NS
	GNH:Mo	N'Dama	18.7	20.1	18.4	16.7	21.5	33.6	1.07	NS	NS
		Crossbred	14.2	15.3	14.8	16.8	17.3	23.2	1.2	NS	NS
GNH:Co	BCS:Co	N'Dama	12.4	12.2	12.7	9.6	13.3	11.5	0.7	NS	NS
		Crossbred	13.9	13.6	14.9	16.2	19.4	15.9	1.0	*	NS
	GNH:Co	N'Dama	16.7	15.4	15.0	15.0	12.4	9.1	0.9	**	NS
		Crossbred	17.5	16.9	14.7	13.6	15.2	10.3	0.8	*	NS
	GNH:Mo	N'Dama	19.2	16.1	19.1	18.0	19.1	34.2	0.7	NS	NS
		Crossbred	19.0	19.9	11.8	16.3	19.3	38.2	1.0	NS	NS
GNH:Mo	BCS:Co	N'Dama	8.5	9.2	9.6	10.2	10.8	13.0	1.4	*	NS
		Crossbred	10.5	12.4	11.9	12.2	11.9	12.5	1.2	NS	NS
	GNH:Co	N'Dama	16.6	16.3	15.4	13.5	13.0	10.2	0.8	**	NS
		Crossbred	14.2	16.1	13.9	13.0	12.4	9.6	0.8	NS	NS
	GNH:Mo	N'Dama	14.0	16.5	15.4	15.6	18.6	28.2	1.2	NS	NS
		Crossbred	14.1	13.6	13.4	14.8	15.3	23.9	1.2	NS	NS