Effect of Supplementing Cowpea (Vigna unguiculata (L.) Walp) Seed hulls and Commercial concentrate on productivity of weanling Boer goats grazing during the dry season at Mantshwabisi Government ranch in Kweneng District, Botswana

John Kenneth Mthetho

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A Dissertation presented to the Department of Animal Science and Production Submitted in partial fulfilment of the academic requirement for the degree of Master of Science in Animal Science (Animal Nutrition)

By:

John Kenneth Mthetho

Main Supervisor

Dr M. Letso

Co-supervisors

Dr S.S. Ramabu

Dr K. Tshireletso

Department of Animal Science and Production

BOTSWANA COLLEGE OF AGRICULTURE/UNIVERSITY OF BOTSWANA

ABSTRACT

The study was carried out during the dry season between June and August of 2013 to evaluate chemical composition, in vitro dry matter digestibility (IVDMD), feed intake and performance by weanling Boer goats supplemented with cowpea seed hulls (CSH) and commercial concentrate/feed (CF) with natural pasture as basal diet. Weanling Boer goats (n=36) were assigned 3 treatments comprising of 4 animals each (2 bucklings and 2 females) replicated three times in a completely randomized design (CRD) matrix. The goats aged between 1 and 2 years with initial body mass ranging from 17.5 – 38 kg (mean ± SD; 26.32 ± 6.36). The animals were fed as follows: diet 1 was the non-supplemented natural browse/pasture (control), diet 2 was the natural browse/pasture supplemented with commercial concentrate and diet 3 was the natural browse/pasture supplemented with cowpea seed hulls. Each of the supplemented diets was fed at the rate of 300 g per goat per day at 08:30 a.m. before goats could be released to graze/browse on the natural pasture within the paddock.

Crude protein (CP) contents (g/100 g DM) in the Commercial concentrate was highest (P<0.05) followed by Cowpea seed hulls, browse species and grasses. The NDF, ADF and ADL were lowest (P<0.05) in the commercial concentrate followed by cowpea seed hulls, browse species and grasses, respectively.

Initial body weights of goats grazed and supplemented with commercial concentrate and goats grazed without supplementation were similar (P>0.05); the goats grazed and supplemented with cowpea seed hulls had lowest (P<0.05) weight. However, at the end of the study goats on natural pasture supplemented

with commercial concentrate gained the highest (4.74 kg; P<0.05) followed by goats grazed/browsed on natural pasture supplemented with cowpea seed hulls (0.58 kg). Goats grazed/browsed natural pasture alone lost weight (-1.12 kg; P<0.05).

The relationship between in vitro gas measured on incubation of supplemental diets, browses, grass species in buffered rumen fluid and the calculated short chain fatty acids (SCFA) showed high correlation (r = 0.999; P<0.0001) index. Due to inadequate equipment for proper methane capture, regression equation for predictive methane production from feed samples was used :(ml/200 mg DM) = (0.032xCP)-(0.057xEE)-(0.012xCF) + (0.124xNFE). Methane production (ml/200 mg DM) from various feed samples ranged between 3.2 and 6.4 from Digitaria velutina and Grewia retinervis, respectively.

The Boer-goats were also investigated for their haematological and biochemical indices to evaluate their health status before and after they were fed different diets or diet combinations. There was no variation (P>0.05) in mean values of haematological indices between the supplemented and non-supplemented goats. However, for clinical chemistry analyses, all the goats fed different diet treatments had elevated creatinine, but within the normal range attributable to recycled urea as a response to limited dietary protein intake from the basal diet. The blood metabolites did not show any differences (P>0.05) among the goats fed different diets or diet combinations.

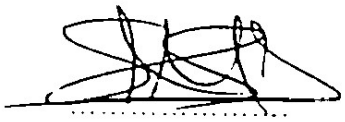
In conclusion, Cowpea seed hulls can provide adequate nutrients to sustain goat production during the extended dry season. The weight gain maintained during the

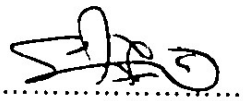
dry season easily up-surge when the conditions normalize after the first rains, hence early conception rate of goats.

Key Words: *Cowpea (Vigna unguiculata) seed hulls, Commercial concentrate/feed, Natural pasture, Goats (Capra aegagrus hircus).*

CERTIFICATION

Main supervisor's name	Date	Signature
M. LETSO	03/10/14	

Co-Supervisor's names	Date	Signatures
Dr. S S Ramba	03/10/2014	
Dr K. Tshiletso	03/10/14	

Head of Department's name	Date	Signature
S.J. Nsoso	03-10-2014	

STATEMENT OF ORIGINALITY

The work contained in this dissertation was compiled by the author at Botswana College of Agriculture/University of Botswana, during the period of May 2013 to May 2014. It is my original work and all the sources that I have used or quoted have been acknowledged by means of complete references. This dissertation has been submitted and shall not be submitted for the award of any other degree or diploma to any other university.

Author's signature ..... Date 25/09/2014

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STATEMENT OF DECLARATION

The research described in this thesis was carried out at the Department of Animal Science and Production, Botswana College of Agriculture/University of Botswana, Gaborone, Botswana, under the supervision of Drs M.Letso, S.S. Ramabu and K. Tshireletso.

I declare that this thesis is the result of my own investigation and had not been presented in any previous application for a degree. All sources of information shown in the text and listed in the references and all assistance by others have been duly acknowledged.

John K. Mthetho

BOTSWANA COLLEGE OF AGRICULTURE/UNIVERSITY OF BOTSWANA (BCA/UB)

Signed.....

Date.....25/09/2014

I, Dr M. Letso, approved the release of this thesis for examination.

Signed.....

Date..03/10/2014.....

I, Dr S.S.Ramabu, approved the release of this thesis for examination.

Signed.....

Date.....03/10/2014

I, Dr K. Tshireletso, approved the release of this thesis for examination

Signed.....

Date.....02/10/14.....

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CHAPTER 1
1.0 GENERAL INTRODUCTION

Botswana's small stock production has been described as either static or slowly growing (MoA, 2008) with a population of goats (*Capra aegagrus hircus)* estimated at 1.9 million and for sheep (*Ovis aries*) slightly over 279 237 (Statistics Botswana, 2012). Most of the small stock is kept in communal areas in small flocks of 10 – 40 animals/household (MoA, 2012); sheep and goats contribute quite significantly to poor resourced persons both in terms of nutrition and income (MoA, 2012). There are a number of factors that contribute to slow growth of the small stock ranging from feed deficits, debilitating effects of internal parasites, and death due to hypothermia, especially for kids and lambs born during winter months (MoA, 2012).

Like all other ruminants, small stock in Botswana relies on the natural feed resources, which has evidently declined. The problems surrounding the productivity of the rangeland is due to low nutritive value that is characterised by high crude fibre (CF), low crude protein (CP), low dry matter (DM) digestibility and low calcium (Ca) and phosphorus (P) contents, especially during dry seasons (Mosimanyana and Kilflewahid, 1985). The protein content of grasses in Botswana is below 6% during dry seasons (APRU, 1975), and there would be little or no browses at all. The government very often subsidizes purchases of feeds, veterinary requisites (for only a selected number of vitamin supplements, drugs and vaccines) during drought periods exclusively to cattle. This translates to annual feed deficit of livestock to be estimated at over 900, 000 tons (Mosimanyana and Kilflewahid, 1985; MoA, 2012).

The alternate livestock feed in the tropics and subtropics is the non-conventional feedstuffs, which many researchers have opined to be too costly since the feed is also consumed by human beings and serves as raw materials for agro-processing industries (Adebiyi *et al.*, 2010). In Botswana, a lot of crop residues are not sufficiently utilised as livestock feed even during drought years when the cultivated crops have not grown to the optimum size. Moreover, much of the cowpeas (*Vigna unguiculata*) that would have been cultivated, their residues (seed hulls) are either buried or burnt to avoid being spread by drafts without considering them as feed for small stock. Cowpea seed hulls could possibly maintain live-weight gain of goats since they contain 15% crude protein (Falaye *et al.*, 2012), and this value is within recommended maintenance value of between 8.9% and 16% CP (NRC, 1981; Nuru, 1985). It has been proved that animals which do not maintain weight during the dry seasons have low conception rate in the subsequent breeding season (Roche, 2006).

For the low quality crop residues to be utilized more sufficiently they need to be subjected to some physical treatments (chopping, grinding and pelleting) or chemically treated (ammoniated, use of sodium hydroxide [NaOH], sodium carbonate [$NaCO_3$] and some biological treatments (Singh and Schiere, 1995; Williams *et al.*, 1997). Forages treated in this manner are easily digested by smaller ruminants and when the level of intake is high the rate of retention can be shortened due to highly digestible components out of rumen fermentation (Van Soest and Mason, 1991).

Thus, in order to sustain the livestock industry in a bid to answer the problem of nutrient deficits, agro by—products need to be properly exploited, due to their availability and cost effectiveness (Tewe, 1997).Cowpea seed hulls are pertinent residues that can be fed goats during dry seasons and could possibly maintain their live-mass gain.

1.1 PROBLEM STATEMENT AND JUSTIFICATION

The Government of Botswana has embarked upon livestock schemes *en masse* which are geared towards poverty eradication, whereby poor resourced persons (earning an average of P135/month); disadvantaged people (woman, youth and people living with disabilities (PLWD)) are granted a minimum of fifteen small stock (sheep or goats) to rear. These animals require ample feed supply for the intended goal to be achieved, especially during the dry season. It is rational that chunks of cowpea seed hulls that are thrown away every year could be sufficiently utilized to maintain live-weight gain of small stock during dry seasons so that animals do not suffer lack of nutrition unduly. There is approximately 21,981 hectares of cultivated land on which cowpeas are annually grown (yielding 43,962 tonnes) (MoA, 2012). However, out of the cowpea harvests approximately 21, 981 tonnes of residues are obtained. These cowpea residues are either burnt or buried by farmers as they ascribe them to attraction or hibernation niches for crop pests. It is against this background that the nutritive altitude of these cowpea residues be determined.

1.1.2 LITERATURE REVIEW

The dietary choices made by domestic and wild ungulates are of great interest to both range and wildlife biologists (Hanley, 1982). Diet selection in herbivores is often a complex affair, and it can be considered a function of species-and

dividual-specific preferences (Arnold, 1981). Many workers have observed and ported that selection of diets by ungulates is generally fragmented (Iglesias and othmann, 1998; Stephens and Krebs, 1986); these observations are biased by nds, amounts, and distribution of forage on offer in a forage patch (Hanley, 982).

orage choices are often limited to local species assemblages (Iglesias and othmann, 1998; Hanley, 1982).The major influences of forage selection by erbivores are due to among others; the amount of feed on offer, vegetation ispersion patterns in time and space, season, and physiological stage of the plant pecies (Iglesias and Kothmann, 1998). Habitat complexity and heterogeneity of orage resource abundance in time and space further imply that foraging strategies nay not be constant, but also vary through time and space (Bergman *et al.*, 001).Ungulate grazers and mixed feeders face such heterogeneity, especially in Southern African landscapes, where complex grass swards and *Acacia* trees are ften associated with one another (Gordon, 1989; Van de Koppel *et al.*, 1996; Fritz nd De Garine-Wichatitsky, 1996).

Domestic goats (*Capra aegagrus hircus*) have very diverse and variable feeding nabit, and they are classified as 'classic' mixed feeders, with characteristic oraging preference of both browses and grass (Stoddart *et al.* 1975). These animals depend much more on grasses when they have reached advanced stages of maturity presumably due to lack of vigour to paddle on trees (Stoddart *et al.*, 1975). However, goats can utilize a wider variety of plant types as feed more than cattle and sheep (Peter *et al.*, 1979); they often select materials with the highest

forage on offer (Hofmann, 1989). This animal species can demonstrate and learn preferable forage items some of which have secondary metabolites (tannins) and lignin (Aldezaba and Garin, 2000).

1.1.3 CHARACTER OF FORAGE IN THE SUBTROPICS

Society of Range Management (1989), describes forage as the available browse and herbage that animals can consume or be harvested to feed livestock. Many different experts describe forage in different ways that suits the appropriation of usage: botanists and agronomists alike base their description of forage on biosynthetic point of view, whereas animal nutritionists describe forage on the basis of cells and tissue enhancement in bio-degradation to liberate nutrients for animal survival (Van Soest, 1982). Cells of young plant tissues capture, store energy, and synthesize proteins and fats in the cytoplasts and finally are utilized by animals. However, cells of older tissues have comparatively low biochemical activities as most of the photosynthates would have translocated to the seeds and roots or to other forms in the cell wall (Huston *et al.*, 1981).

Forage contain complex carbohydrates, waxes, terpenes (essential oils, saponins, etc) and phenylpropanoids (lignins, tannins, etc)(Makkar *et al.*, 1995).The structural biochemicals of forage, to large extent, determine a plant species' resilience, which is inversely proportional to nutritional value to grazing animals. Carbohydrate present in most forage is impervious to mammalian gastric and intestinal digestive enzymes and invariably accounts for less than 40% dry matter

(Huston *et al.*, 1981). Cellulose, unlike starch, it is a glucose polymer, and differs from starch in isomeric orientation or network of bonds between glucose monomers. Intestinal hydrolytic enzymes can cleave alpha linkages in starch, whereas the *beta* linkages of cellulose can only be broken down by rumen cellulolytic microbes in the presence of enough energy levels (Hungate, 1966), a condition which is often limiting in the tropics or subtropics (Huston *et al.*, 1981).It is however, important that the type of forage available should be suited to the type of animal utilizing it. Goats for example, cannot be able to digest cell walls of plants as the feed normally stays for a shorter period in their digestive systems. Therefore the type of forage relevant for their sustenance should be the one that is highly digestible although their diet selections overlap the entire array of forages. Some of the standards of goat forages are categorised as per their fibre fractions and/or chemical compositions are as shown (Table 1).

Table 1: General Quality Standards for goat diets

Quality Standard	CP (%)	ADF (%)	NDF (%)	DDM (%)	DMI (%)
Prime	>19	<31	<40	>65	>3.0
1	17-19	31-35	40-46	62-65	2.6-3
2	14-16	36-40	47-53	58-61	2.3-2.5
3	11-13	41-42	54-60	56-57	2-2.2
4	8-10	43-45	61-65	53-55	1.8-1.9
5	<8.0	>45	>65	<53	<1.8

Source: Mississippi State University (2010). CP – crude protein; ADF – acid detergent fibre; NDF – neutral detergent fibre; DDM – digestible dry matter; DMI- dry matter intake

1.1.4 COWPEA (*VIGNA UNGUICULATA* (L.) WALP)

Cowpea is a native legume to central Africa, and it is widely spread throughout the tropics between 40°N to 30°S and below 2000 m altitude (Ecopcrop, 2009). Its fresh or dried seeds, pods and leaves are used as human food. To this end,

cowpeas are rarely used as animal feeds, but their by-products such as cowpea seed wastes and cowpea seed hulls have been used to replace conventional feedstuffs in third world countries (Ikechukwu, 2000). Cowpea can do well in the savannah vegetation at day temperatures ranging from 25°C to 35°C. It tolerates a wide range of soils provided they are well drained (Madamba *et al.*, 2006). Water logging conditions may render cowpea suffer greatly although not comparable to other legumes (Ecocrop, 2009).It can withstand menace drought spells although it favours rainfalls ranging from 750 mm to 110 mm (Madamba *et al.*, 2006).

Nutritionally, cowpea can provide high biological biomass either alone as pasture or in combination with corn silage (two parts corn plus one part cowpeas) (Solainman, 2007). Its inherent nutrient quality ranges from 6 – 20% crude protein (CP) depending on whether it is fed as straw, seed-harvested vines or as seed hulls with varying degrees of crude fibre (CF) that can be as low as 24% (Solainman, 2007). Singh *et al* (2006) reported that complete replacement of groundnut cake with cowpeas increased roughage intake by lambs leading to high performance. Sheep supplemented with cowpeas fed low quality roughages resulted in higher dry matter intake and organic matter digestibility (Paduano *et al.*, 1995). On the other hand, cowpeas used as a source of urease on buffalo male calves fed urea treated straw resulted in increased body weight gain and dry matter digestibility (Sarwar *et al.*, 1995).

1.1.5 COMMERCIAL GOAT FEED

The major meat goats feed is the natural forage (Pinkerton and Pinkerton, 1989). However; in situations where the nutritional status of the natural forage is compromised in protein and energy, supplementation in adequate quantities of the

deficient nutrients is necessary. Protein feed supplements are in general meals such as soybean meal (SBM), alfalfa meal (AM), cotton seed meal (CSM), linseed meal (LSM), fish meal (FM),etc (Pinkerton and Pinkerton, 1989). Furthermore, mineral supplementation could probably be in the form of trace mineralized salts (loose or block), individual sources deficient mineral (offered separately or in combination with salt) or commercial mineral mixtures. It has long, been observed that forage invariably lacks phosphorus during dry seasons and it is also recommended that supplementation of this mineral is important (Mosimanyana and Kilflewahid, 1985), as it plays an important role in reproduction, milk production and/or enhancement of rumen microbial synthesis.

Most of the commercial feeds have protein that ranges from 17 to 85%, and are designed for achieving cost effectiveness, and more importantly to provide diverse amino acids. The commercial feeds are classified according to the growth of animal such as starter, grower and finisher with varying crude protein contents, with the highest crude protein (CP) decreasing in ascending order of the growth of an animal (Pinkerton and Pinkerton, 1989).

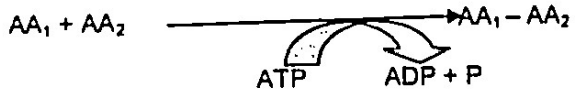
1.1.6 NUTRITIONAL REQUIREMENTS OF GOATS

Like all other herbivores, goats require energy, protein, vitamins and minerals for their bodily functions. The concept of requirements is functionally understood to be referring to "normal" metabolic activity (Huston *et al.*, 1986).The animal's requirement is thought to be met when it shows evidence of normal health and vigour, normal rate of growth, and normal reproduction (Huston *et al.*, 1988). However, "normal" cannot be misconstrued to be identical in all members of the same species at all times; the requirement of nutrients vary depending on the

physiological circumstance the animal may be found in (Huston *et al.*, 1986). Although nutrients are limiting factors in livestock production, they are utilized in hierarchical order of maintenance, reproduction, lactation and storage. In a set of animal population, reproduction and lactation may occur when the diet on offer does not provide "required" levels of these functions (NRC 1981b, 1984, 1985a). On the other hand, research has proved that a population of animals can also reproduce or lactate at nutrient levels below maintenance requirements (Huston *et al.*, 1988).

1.1.7 ENERGY REQUIREMENTS OF GOATS

Energy is required in anabolic processes, but sometimes also for the catabolic chemical bonds during animal metabolism (muscle contraction, nerve impulses and tissue synthesis). In protein synthesis, energy and amino acids are bonded together in peptide sequences. The energy that is used in this bonding process comes from a coupled reaction during which a high energy phosphate bond in adenosine triphosphate (ATP) is cleaved to yield adenosine diphosphate (ADP) and a free phosphate radical. Formation of these energy levels are captured and utilized in the formation of high energy phosphates and made available for tissue protein synthesis or any other energy requiring metabolic processes.

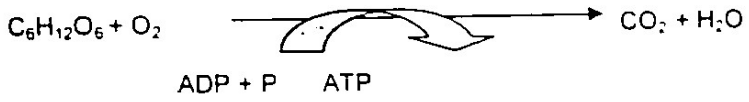


AA_1, AA_2 – amino acids are bonded in peptide sequences in the process of protein synthesis. The resultant of this process is the liberation of phosphate (P) radical that is separated from adenosine phosphate (ADP) this type of energy evolved normally occurs in respiration.

Source: Huston *et al.* 1986

Another energy requiring process is respiration, which involves capturing of energy in the formation of high energy phosphate bond. Energy captured in this

manner (respiration process) in ruminants is primarily utilized to break down volatile fatty acids (VFAs) that are liberated during fermentation and they are finally absorbed in the rumen wall. These VFAs are metabolized through a network of pathways and ultimately yield carbon dioxide (CO_2), water (H_2O) and captured energy in the form of high-energy bonds.



Source: Huston *et al.* 1986

In spite of the fact that ruminants can metabolize glucose and protein, most energy arises from either acetate, propionate or butyrate, which are the main VFAs produced during microbial fermentation. The gross energy (GE) is partitioned into digestible energy which can be depicted as Digestible energy (DE) = GE – faecal energy; metabolizable energy (ME) = DE – urinary (UE) and methane energy (ME); and finally net energy (NE) = ME – heat increment (HI). Therefore, net energy is the amount of energy available for maintenance (energy required to maintain normal health and vigour) whereas production is energy required for growth, reproduction, lactation, etc. It has been observed that metabolizability of digestible energy, ME/DE, in ruminants is constant at 82% (NRC, 1984). Moreover, the digestibility of gross energy, DE/GE, and the net availability of metabolizable energy, NE/ME, varies with the chemical composition of the diet and the metabolic function for which the net energy has been utilized (Van Soest, 1982; Fox, 1987).

1.1.8 PROTEIN REQUIREMENTS OF GOATS

The inclusion of protein in ruminant diet is to enhance the supply of nitrogen (ammonia) and amino acids for intra-ruminal microbial activity and amino acids for cellular-level tissue metabolism. During dry seasons when the supply of protein in

forage is suboptimal this can result in low rumen microbial population that may then lead to lowered fermentation rate, decreased digestibility of feed and decreased voluntary intake (Kempton and Leng, 1979). Protein requirements for goats also include the requirement of nitrogen for microbial synthesis. However, microbial protein requirement ranges from 6 – 8% crude protein in the diet whereas ruminants require protein in the ranges of 7 – 20% depending upon species, sex and physiologic state (Huston *et al.*, 1988). The sub-tropics forage has crude protein which is below 6% (APRU, 1975; MoA, 2008).

Protein is required for both as a source of nitrogen for the rumen bacteria and to supply amino acids for protein synthesis in the animal body. When the levels of protein are low in the diet, digestion of carbohydrates in the rumen will slow down and intake decreases (Houston *et al.*, 1986). The supply of protein in animal diet can affect growth rate, milk production, reproduction and disease resistance negatively since insufficient amino acids will be absorbed into the small intestines (Kempton and Leng, 1979). Protein utilization can also be affected by improper supply of other nutrients, especially vitamins or minerals in the diet of the ruminant animal (Houston *et al.*, 1988).

1.1.9 VITAMIN REQUIREMENTS OF GOATS

Vitamins are principally required by ruminants for metabolic catalytic processes. Ruminant animal has the ability to synthesize most of the vitamins through the aid of rumen microbiota with a few exceptions of vitamins such as vitamin A, D, E, and K, which have to be provided exogenously (Van Soest, 1982). However, vitamin A is the only vitamin that is likely to limit the productivity of grazing ruminants (Huston *et al.*, 1986). It has become apparent that vitamin A deficiency occurs

during an extended period of low temperature and/or drought when the green plants are no longer available in animal feeds. Moreover, the other important vitamin that often becomes deficient during the dry season is the vitamin E and its severity is often coupled with low selenium in the diet (Huston *et al*., 1988).

1.1.10 MINERAL REQUIREMENTS OF GOATS

Goats require both macro and micro minerals for their metabolic functions as cofactors for certain metabolic reactions. Macro minerals are often a problem especially for animals raised in a range setting (Huston *et al*., 1988), and the most deficient nutrients being sodium, chlorine and phosphorus. The deficiency of such minerals as sodium and phosphorus can predispose animals to secondary infection due to their indiscriminate eating of rocks, sticks, bones, etc. and reduced forage intake and productivity (Huston *et al*., 1986). The other four remaining major minerals are unlikely to be deficient under range conditions (Huston *et al*., 1988).

Micro minerals are also required but in minute amounts for the catalytic functions of the animal body cells. However, trace minerals' deficiencies are less widespread, less predictable, and more difficult to recognize and probably quantitatively less important than major elements (Fox, 1987).Toxicities from consuming trace minerals are also a serious problem even on range animals. For example, range pasture may have high selenium in the soil and this could lead to animal poisoning during grazing (Huston *et al*., 1988).

1.1.11 CHEMICAL ANALYSES OF FEEDSTUFFS

In order to ascertain the quality of feed, nutritionists invariably subject the feed to some chemical analytical procedures. Most of the information that is used in

describing the quality of livestock feeds is based on the proximate analysis, a method that was brought about by German Scientists, Henneberg and Stohmann (McDonald *et al.*, 1995). As a result of this analytical procedure having been conducted at Weende Experimental Station it has since been alternatively referred to as Weende procedure (McDonald *et al.*, 1995). Furthermore, feed nutritive value only makes sense when its digestibility is known. Scientists usually use *in vitro* methods in determining feeds digestibility as opposed to the conventional digestibility trials, which are often expensive and painstaking in conducting (Cilliers *et al.*, 1997). *In vitro* methods used are animal specific and they require some modifications whenever used from mono-gastric to ruminants or vice versa (Nheta *et al.*, 2005).The most interesting *in vitro* technique is the one that measures gas production as it enables the study of kinetics of rumen fermentations (Blummel and Becker, 1997; Getachew *et al.*, 1998; Calabro *et al.*, 2002), and it can also be used to estimate *in vivo* digestibility of feeds (Menke and Steingass, 1988; Blummel *and* Ørskov, 1993).

1.1.12 PROXIMATE ANALYSIS OR WEENDE PROCEDURE

The proximate analysis, *alias* Weende procedure, divides feed into six components: moisture, ash, crude protein, ether extract, and crude fibre and nitrogen free extract. The determination of moisture is done by drying samples to a constant weight at a temperature of 100 °C. On the other hand, ash is ascertained through burning of the sample to 550 °C until there is no more carbon. Crude protein is estimated from the calculation of nitrogen content of the feed; this is done in assumption that all nitrogen of the feed is present as protein containing 160g N/Kg (McDonald *et al.*, 1995), hence CP = total N x 6.25. Moreover, the

ether extract is obtained by subjecting the feed sample on a continuous extraction with petroleum ether for a predetermined time to establish different fractions of lipids, alcohols and pigments. With regards to crude fibre, its value is obtained by boiling feed samples in acid and alkali solutions and the organic residues is the crude fibre. Nitrogen free extract is derived by subtracting from 100 the sum of crude fibre, crude protein, ash, ether extract, and moisture.

1.1.13 DETERGENT FIBRE ANALYSIS SYSTEM (DFAS)

Despite proximate analysis procedure being widely used, it has also been criticized in some cycles as an ancient and unreliable method (Van Soest, 1982; McDonald, 1995). Van Soest (1982) developed a more unswerving method (detergent fibre analysis system) that quantifies both the cell contents and the cell wall constituents in forages. This included the quantification of neutral detergent fibre (NDF) and acid detergent fibre (ADF). The neutral detergent fibre is the residue that is obtained after the vegetal sample had been boiled in a neutral detergent solution; this consists mainly of lignin, cellulose and hemicelluloses and can be ascribed as plant cell wall. On the other hand, acid detergent fibre is the remains of plant components after it had been refluxed with sulphuric acid and cetyltrimethyl-ammonium bromide and its presence signifies the concentration of lignin, cellulose and silica. In the proximate method ash does not differentiate minerals from silica but with Van Soest (1982) method all the mineral fractions are individually separated. Some of the mineral analytical equipment used in the analysis of minerals include atomic absorption spectrophotometer and induced couple plasma apparatus.

Under the Van Soest (1982) method, NFE are referred to as "soluble carbohydrates" and these include both soluble and insoluble carbohydrates. The detergent fibre analysis system has been acclaimed to be more precise in determining the cell wall constituents (Van Soest, 1982). When cell contents are partitioned from cell wall they distinguish portions that are essentially totally digestible from those which are partially and variably digestible.

1.1.14 *IN VITRO* GAS PRODUCTION TECHNIQUE

In vitro gas production technique (IVGPT) is used to determine feed digestibility on the premise that anaerobic digestion of carbohydrates by rumen microbes lead to production of gases (CO_2, CH_4, and H_2) and volatile fatty acids (acetate, propionate, butyrate); it is from the measurement of gases produced that the rate and extent of feed degradation and energy value can be ascertained (Makkar *et al.*, 1999). In describing the operations of the *in vitro* gas production system, Menke *et al.* (1979) have indicated that a substrate is incubated in a calibrated gas-tight glass syringe fitted with a plunger wherein the gas produced can be collected and its volume read up to 96 hour period.

1.1.15 APPLICABILITY OF GAS METHOD

The *in vitro* gas production technique has been used for a variety of feed evaluation purposes (Getachew *et al.*, 2004). It has been observed that there is digestibility correlation of measured organic matter, crude protein and ash with the gas produced in feed fermentation (Getachew *et al.*, 2004). Moreover, the gas measurement can provide better estimates of the metabolizeable (ME) level of feeds, when combined with some chemical constituents, compared with calculations based on chemical

constituents only. The gas technique has been found to be the only method that is repeatable in predicting the energy value of feeds as compared to other techniques of similar assessment (Getachew *et al.*, 2002).

In determining the nutritive value of feeds, it has become apparent that the rate at which feed chemical constituents are digested in the rumen is just as important as the extent of digestion. The kinetics of fermentation influence voluntary intake by ruminants. The fermentative process that occurs in the gas method reflects the microbial growth and accessibility of the feed to microbial enzymes (Getachew *et al.*, 2002).

The gas method can also be used in measuring how microbial activity lowers feed digestibility. A number of vegetal feeds, such as forage legumes and cottonseed, contain phenolics, alkaloids and saponins that have negative biological effects on microbial activity in the rumen. Tannins are such natural occurring anti-nutritional factors that may form complexes with feed and microbial proteins leading to depressed feed digestibility. The effects of tannins on the nutritive value of feeds can be studied using polyethylene glycol (PEG), which binds with tannins thereby inhibiting their biological effects. Therefore, the percentage increase in gas production when PEG is present indicates the rate at which tannins depress rumen fermentation of feeds (Makkar *et al.*, 1999).

The gas method can also be used to study feed additives and rumen fermentation modifiers such as *monensin* sodium (Getachew *et al.*, 2000). For example, some rumen modifiers are used to reduce methanogenic bacteria so as to reduce methane emissions. By studying the impact of rumen modifiers on microbial

fermentation effects is important to milk production and can be quantified (Getachew *et al.*, 2000). It is common that the nutrient composition of feeds is determined by chemical analyses. However, this procedure does not provide sufficient information to determine the true feed nutritive value. The efficiency at which an animal utilizes feed nutrients has an impact on its productive performance (Getachew *et al.*, 2004). The gas method can sufficiently quantify nutrient utilization and its accuracy in describing digestibility in animals. *In vitro* gas production can also be used to predict animal performance at lower costs (Getachew *et al.* 2004).

1.1.16 DRY MATTER INTAKE AND GROWTH PERFORMANCE

Forage intake is a function of digestibility and it indicates how effective the reticulo-ruminal rates of fermentation and passage are. Furthermore; digestion can be described as the balance between what the animal would have consumed and the amount of waste generated. The variability among animals fed the same diet is less in digestibility than in intakes. However, digestibility can be predicted with great precision than intake (Solaiman, 2007). Some workers (Minson, 1990; Moore, 1994; Coleman *et al.*, 1999) have described intake as the most important parameter for estimating forage quality and animal performance. On the other hand, feed conversion efficiency is important in determining the economics of feed utilization by animal. Feed conversion ratio entails the amount of feed ingested per unit of weight gained and therefore lower feed conversion ratio (FCR) is the most ideal (Coleman *et al.*, 1999). Getahun (2001) observed that there was significantly different ($P<0.05$) results in feed intakes of stall-fed Somali goats (572.1g) than Mid-Rift Valley goats (523.4g). The feed intakes in g/kg $W^{0.75}$ per day were 68 and

64.7 for Somali and Mid-Rift Valley goats, respectively. It became apparent that the feed conversion ratio was better in Somali (19.5) than in the other Mid-Rift Valley (33.8).

1.1.17 RUMINAL FERMENTATION AND DEGRADATION PATTERNS

The fermentation process of dietary carbohydrates and protein in the rumen enhances the production of adenosine triphosphate (ATP), which is the major source of energy for microbial growth (Ruiz *et al.*, 2004). From the rumen fermentation reactions volatile fatty acids and microbial cells evolve. The former are a primary source for metabolizable energy and the latter sources of metabolizable amino acids for maintenance and milk synthesis. However, the efficiency with which the dietary nutrients are converted to energy and protein for tissues and milk synthesis vary is quite minimal. Emphasis of the efficiency seems to occur in the rumen due to the efficacy of the microbial community as a result of the nutrient variability and/or conditions in the rumen. It is important that the basic aspects of rumen fermentation be understood as they are the basis of identifying strategies for improving efficiency of converting feed energy and protein into end products (Houston *et al.*, 1988)

1.1.18 CARBOHYDRATE USE AND ADENOSINE TRIPHOSHPATE GENERATION

There is virtually small amount (10 – 12%) of aerobic ATP that is yielded from the rumen as a result of anaerobic fermentation and its inherent losses (Hungate, 1966). Microbial synthesis and their maintenance, VFA and other products that emanate from fermentation play an important role in supplying nutrients to the animal but also place a limit on the productive efficiency (Russell *et al.*, 1986).

Ruminant nutritionists are therefore faced with challenges of maximizing nutrients in the rumen by manipulating rumen function to increase nutrient capture and promote rumen by-pass to optimize nutrient supply. Carbohydrates are major sources of energy supply in the rumen and much is known about the pathways at which the carbohydrates are metabolized, but estimation of ATP liberated remains arbitrary (Hungate, 1966). It is due to the diversity of the microbial populations, competitive metabolic pathways and their ability to adapt to the environment that makes quantitative estimation difficult. Diet is one of the variables that seem to affect microbial fermentation. The effects of changing dietary forage to concentrate ratio on digestion kinetics is widely reported (Bach *et al.*, 2005). Changes in VFA profiles and shifts in rumen pH that occur in ruminants and subsequent impact on animal function are cases in point. Microbial growth structure normally follows nature of the substrate, pH and rate of passage from the rumen. The microbiota may choose less efficient metabolic pathways as a way of reducing high maintenance costs associated with energy extraction, thereby increasing their growth rate (Bach *et al.*, 2005).

1.1.19 MICROBIAL PROTEIN, METHANE PRODUCTION AND FERMENTATION

Although it is debatable as to what could be the true cause of inherent fermentative energy losses; heats of fermentation and methane production have registered the most focus (Bach *et al.*, 2005). Heat of fermentation is a lost energy estimate due to inefficiencies in metabolic activities (Bach *et al.*, 2005). On the other hand, methane comprises 20 – 30% of total gases produced in the rumen and it can represent a significant feed energy loss (Johnson and Johnson, 1995). Both of these losses are as a result of either feed intake or feed quality. Microbial

growth is not directly proportionally to ATP liberated from energy substrates in the rumen, but depends on the growth rate and maintenance requirements of the microbes (Stouthamer and Bettenhaussen, 1973; Isaacson *et al.*, 1975). Therefore, microbial efficiencies are increased due to rapid growth conditions (increased dilution rate) and reduction in maintenance energy (Isaacson *et al.*, 1975).

1.1.20 HAEMATOLOGY AND SERUM BIOCHEMISTRY

Clinical haematology is a scientific procedure used in medicine to evaluate health status of animals (Sowande *et al.*, 2008). Animal nutritionists undertake haematological and serum biochemistry studies to investigate feed production and utilization to ascertain the cyclical pattern of weight gain and loss between seasons (Sowande *et al.*, 2008). Indeed, haematological parameters together with plasma metabolites can provide information that could show effects of ingested diets (Jenni-Elermann, 1998; Spinu *et al.*, 1999), such as tannin rich diets. Further more, effects of dietary treatments on performance and physiological functions of the animal can be monitored through blood examination (Church *et al.*, 1984). Some blood metabolites such as minerals (sodium, potassium and chlorine) especially those involved in acid-base balance may be lowered or increased in the blood stream depending on the diet offered animals. Excess of potassium in the feed, for example, can interfere with the absorption of magnesium (McDowell, 2002). Therefore, clinical haematology and blood chemistry are usually influenced by diseases, nutritional stress, body condition, sex, age, diet, circadian rhythms, captivity, etc.(Woerpel *et al.*, 1984; Palomeque *et al.*,1991; Spinu *et al.*, 1999; Quintavalla *et al.*, 2001).

1.2 OBJECTIVE OF THE STUDY

The main objective of the study is to assess the potential of Cowpea (*Vigna unguiculata*) seed hulls as a supplemental feed for maintaining live-weight gain in goats during the dry season.

1.2.1 SPECIFIC OBJECTIVES:

(i) To determine chemical composition and *in vitro* dry matter digestibility (IVDMD) in six selected natural forages (grass and browses), commercial feed (CF) and cowpea seed hulls (CSH) which were fed Boer-goats in the study.

(ii) To establish feed intake and performance of goats supplemented with cowpea seed hulls and commercial concentrate during the dry season.

(iii) To determine haematological and biochemical parameters of grazing/browsing goats supplemented with either commercial feed or cowpea seed hulls.

1.3 HYPOTHESES

(i) Different feed treatments have similar *in vitro* organic matter digestibility (IVOMD), short chain fatty acids (SCFA) and metabolizable energy (ME).

(ii) Probability level ($P<0.05$) weight gain by goats does not occur only when natural pasture is supplemented with either commercial concentrate or cowpea seed hull.

(iii) Supplementing natural pasture with either commercial feed or cowpea seed hulls has no significant ($P>0.05$) effect on the haematological and mineral profile of the goats.

CHAPTER 2
2.0 MATERIALS AND METHODS

2.1 EXPERIMENTAL AREA

The study was conducted at Mantshwabisi Government ranch, located about 95 km from Gaborone (Capital City) in the north-eastern Kweneng District, Botswana. The local climate of the area is classified as hot and semi-arid with mean daily temperature of 30°C; the area has mean rainfall of 153.4 mm per annum, which is received in summer months (December– April)(Meteorology, 2013). The geographic position of the ranch is between the coordinates: S 24^0 10' 47.4" and E 25^0 16' 36.1" at an altitude of 1174 m above sea level. The ranch is estimated at 430 hectares and it has been subdivided into four paddocks with mean area of 86 hectares each. The vegetation of the ranch can be described as woody or savannah grassland with plenty of *Acacia*, *Terminalia*, *Grewia* species and a variety of grass species such as *Digitaria*, *Stipagrostis*, etc.

2.2 RANGE ASSESSMENT

The double sampling method was used in the estimation of forage production in a 97.2 hectare paddock (Bonham, 1989). The paddock was divided into seven transects (500 m each) on which a 0.25 m^2 quadrant was thrown in every 10 metres (making a total of 50 transects) of walking distance in an attempt to estimate cover which was partially or fully covered by either the vegetation as a whole or a single species. In these small estimated plots the vegetation was independently clipped and weighed. Herbage weight drawn from the quadrats was multiplied by allowable factor (0.5) to obtain kg/ha of biomass as suggested by Hussain and Durrani (2009). From the small samples, the relation between

estimated and actual dried weight was calculated and used to adjust the estimate of the large sample (Platis and Papanastasis, 2003).

This type of evaluation/assessment was done based on plant-animal accessibility height and leaf growth available to the intended animals, within 2.0 m from the ground. Measurements of browses were done on representative plants of each woody species in the paddock, and heights were measured. Those within a lower height of 2.0 metres were considered to be highly available to the goats whereas those more than 2.0 m were considered less available or whenever available to the animals (when leaves have fallen to the ground) would be of lower nutritive value.

A total of 60 samples were randomly taken for measuring forage production. In an effort to determine the number of quadrats required in estimating standing crop forage production within 10 and 20 g per quadrat of the population mean at 90 percent probability level, the quadrat weight (total of grazeable dry matter) was taken as a sample unit. The sample mean and standard deviation for different quadrat weights were calculated from the data.

2.3 FEED SAMPLES AND SAMPLES ORIGINS

Dried samples of cowpea seed hulls, six different natural forages, and commercial feed were collected from Kgatleng ploughing fields, Mantshwabisi ranch and local commercial supplier, respectively. These samples were milled through 1 mm screen after having been oven-dried at 65°C for 24h.

2.4 CHEMICAL ANALYSES

The dry matter (DM) was determined by drying the samples at 65°C for 24h and ash obtained by igniting the samples in a muffle furnace at 550°C for 6hours. Nitrogen content was measured by the Kjeldahl method (AOAC, 1990) and crude protein calculated as N x 6.25. Acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL) and ether extract (EE) content of the forage/feeds were determined using the method by Van Soest *et al.* (1991).

2.5 *IN VITRO* GAS PRODUCTION STUDY

Rumen fluid was obtained from three female Tswana-goats. The method used for the rumen liquor collection was as described by Fievez *et al.* (2005), whereupon suction tube was used to draw the rumen fluid from goats which had previously been fed with 60% hay-Lucerne and 40% concentrate at 3% body weight. The rumen fluid was collected into thermo flasks that had been pre-warmed to a temperature of 39°C before morning feeding. The incubation procedure was as reported by Menke and Steingass (1988), using 120 ml calibrated transparent glass tubes fitted with needles at the bottoms. Sample weighing 0.2 g (n=3) were put on the tubes and 30 ml of inoculums of strained rumen liquor and buffer (g/l) of 9.8 $NaHCO_3$ + 2.22 Na_2HPO_4 + 0.57 KCl + 0.47 NaCl + 2.16 $MgSO_3$ + $7H_2$) + 16 $CaCl_2.2H_2O$) was used. Incubation was carried out under the temperature of 39°C, and the gas volumes were measured at 2, 4, 8, 20, 24 and 48 hours. Gas production was not recorded after 48h because of the concentrate characteristics of the experimental diets (Wallace *et al.*, 2001). Due to failure to secure appropriate equipment used to quantify methane from trapped gas, a regression equation of methane production (ml/200 mg) = (0.032xCP)-(0.057xEE)-(0.012xCF)

+ (0.124xNFE) by Lee *et al* (2003) was used to estimate methane produced by each feed sample. Such parameters as metabolizable energy (ME), organic matter digestibility (OMD) and short chain fatty acids (SCFA) were estimated at 48h post gas collection as according to Menke and Steingass (1988). The average of the volume of gas produced from the blanks was deducted from the volume of gas produced per sample.

After 48h of digestion, the samples were transferred into test tubes and centrifuged for 1h in order to obtain the residues which were filtered using Whitman No. 4 filter paper by gravity and the residues placed in an oven for drying at 65°C for 24 h. The residues obtained were weighed and digestibility calculated using the equation shown below:

$$\text{IVDMD}(\%) = \frac{(\text{Initial DM Input} - \text{DM residue} - \text{Blank})*100}{\text{Initial DM Input}}$$

Metabolisable Energy (ME) was calculated as ME = 2.20 + 0.136 GV + 0.0057 CP + 0.00029 EE (Menke and Steingass, 1988). Organic matter digestibility (OMD %) was determined as OMD = 14.88 + 0.889 GV + 0.45 CP + 0.651 XA (Menke and Steingass, 1988). Short chain Fatty Acids (SCFA) were assessed through the calculation of 0.0239 GV – 0.0601 (Getachew *et al.*, 1999). Where GV, CP, CF and XA are total gas volume, crude protein, crude fibre and ash, respectively.

2.6 EXPERIMENTAL ANIMALS, FEEDING AND MANAGEMENT

A total of 36 Boer-goats aged 15±2.3 months old with body weights (BW) 26.32 ± 6.36 kg and of different mixed sex were used in the study. The Boer-goats are also found in other drier areas of Botswana in fairly large numbers and are known to occupy all the agro-pastoral ecotones. These goats are known to be adapted to

arid and semi-arid areas and could survive drought by utilizing little browses (MoA, 2012).

Natural pasture was mainly used as basal diet for all the goats. However, experimental goats were supplemented with either commercial feed or cowpea seed hulls at the rate 300 g per goat before they were released to graze/browse. The control group grazed/browsed the natural pasture without supplementation. Furthermore, all the goats were given dicalcium phosphate x salt licks and fresh clean water on free choice basis. The experimental goats were also de-wormed before the start of the experiment with Nalsacur anthelmintic (after 14d of having been immunized with enterotoxaemia vaccine) at 2.5 ml per 10 kg BW. The animals were housed in sufficiently ventilated pens with concrete floors at night after grazing during the day. For the three different treatments, different ear tag colour codes were used for ease of identification of the animals. The colour code for the control was red, commercial supplement, was green and for cowpea seed hulls supplement, was yellow. The experiments were carried out by following Research Council guidelines approved by Ethical Committee on Animal Experimentation of the Botswana College of Agriculture/University of Botswana, which is in compliance with the world animal welfare statute.

2.7 EXPERIMENTAL STUDY DESIGN

Thirty-six Boer-goats were partitioned into live-weight groups of four animals (two males and two females), with three replicates for each treatment, and within a live-weight group, animals were assigned dietary treatments in a completely randomized design (CRD). The first group was grazed/browsed with no supplemental diet (control); the second group of goats was offered 1.2 kg of

commercial feed at 08h30 and then released to graze/browse in the natural pasture; the third group of goats was also offered 1.2 kg of cowpea seed hulls at 08h30 and also released to graze/browse in the natural pasture. The study comprised of a 14-day preliminary period of feed adjustment which was started on 15 June to 29 June; the actual study trials started on 30 June and finished on 26 August, 2013.

Both at the beginning and end of the feeding trial (60 d), two sets of blood samples were collected from each animal by jugular vein puncture using a 10 ml 20 gauge syringe and 10 gauge needles. One set of the blood samples (5ml) was collected into plastic tubes containing the anti-coagulant ethylene diamine-tetra-acetic acid (EDTA) for the determination of haematological parameters. The other set of blood samples (10 ml) was collected into anti-coagulant free tubes, and the blood coagulated at room temperature thereafter centrifuged for 5 minutes at 3000 rpm. The supernatant sera were stored in a freezer for biochemical analysis.

2.8 BLOOD ANALYSIS

The blood was analysed using the QBC® Vet Test analyser (IDEXX Laboratories inc. Westbrook, Maine, USA) for blood parameters including haematocrit, differential cell count and haemoglobin concentration. Briefly, fresh blood was filled into venous tubes and the samples were centrifuged for about 10 minutes before reading with the QBC® VetAutoread haematology™ analyser. In addition, the analyser reported the following parameters; mean corpuscular volume [MCV], mean corpuscular haemoglobin [MCH] and mean corpuscular haemoglobin concentration [MCHC] all calculated from values of red blood cells [RBC] count, and haemoglobin [Hb] concentration. Serum total protein, serum urea, creatinine,

albumen, serum calcium and phosphorus were determined by the use of Pentra C200, Horiba Medical equipment, clinical chemistry analyzer (Co. Ltd., France).

2.9 DATA COLLECTION

2.9.1 SUPPLEMENTAL FEED RESIDUES

Daily supplemental diets offered and refusals were recorded for each set of animals. All the animals were weighed at the start of the experiment thereafter weighed every 2-week after overnight fasting using suspended weighing scale. Furthermore, the forage value of the range was ascertained through the range assessment that was carried out in the paddock on which the goats grazed/browsed. The weight gain by goats was inferred from both the range condition and/or combination of the supplemental feeds offered.

2.9.2 COLLECTION AND PRESERVATION OF RUMEN LIQUOR

About two hundred millimetres of rumen liquor was collected from each of the nine goats randomly selected after having been fed each of the three diet treatments. The rumen liquor was collected within two hours of animals having been fed supplemental diets/or grazed/browsed natural pasture. Samples of rumen fluid were drawn using a suction tube inserted through the oesophagus to the rumen (Babayemi and Bamikole, 2006). The fluid samples were then strained through four layers of cheesecloth, and then preserved as described by Han *et al.* (1989). The collected rumen fluid was placed in a 250 ml Erlenmeyer flask and acidified with 25% metaphosphoric acid (1 part acid and 5 part rumen liquor). Then the flask was put in a freezer at -20°C until analyses were done.

2.9.3 ENUMERATION OF PROTOZOA IN RUMEN LIQUOR

About 1 ml of rumen liquor was mixed with 9 ml of Lugol's Iodine solution before a small sample from the mixture was put on a dry slide. After covering the slide counting of the protozoa was done under low power of microscope in a *zig-zag* manner.

2.9.4 ENUMERATION OF TOTAL RUMINAL BACTERIA

A quantity of 5 ml supernatant from centrifuged rumen liquor was placed in a test tube and also 5 ml of 10% formalin was added to kill the bacteria. A mixture of rumen fluid and formalin (2 ml mixture) was added to 8 ml of distilled water to give 1×10^{-1} dilution and thereafter serial dilutions were made up to 1×10^{-4} on a clean glass slide. A small amount from this dilution was put on a slide and then a loopful of saturated nigrosine was added. The observation of bacteria was carried out under the oil immersion lens.

2.9.5 DETERMINATION OF pH AND AMMONIA-NITROGEN CONCENTRATIONS

The pH values were determined immediately after collection by the use of Corning electronic pH electrode. The ammonia-nitrogen concentration was also determined by direct distillation and titration using the UDK Kjedahl Automatic Distillation Unit.

2.9.6 DETERMINATION OF TOTAL VOLATILE FATTY ACIDS IN RUMEN LIQUOR

A small proportion (1 ml) of rumen liquor was placed into the cup of distillation whereupon 0.5 ml of 5% oxalic acid together with 0.5 ml of 10% potassium oxalate solutions was added. The mixture was distillated for three minutes. The distillate was collected and a few drops of phenolphthalein indicator were added. The distillate was further titrated with 0.01 N sodium hydroxide.

2.10 STATISTICAL ANALYSIS

The dependent variables analysed were *in vitro* organic matter digestibility (IVOMD), weight, haematological profile (RBC, WBC, PCV, Hb, MCV, MCH, and MCHC), blood chemistry (total protein, albumin, creatinine, urea, calcium and phosphorus). Data were analyzed using the MIXED procedures of the Statistical Analysis Systems Institute (2008).

Performance characteristics, haematological parameters and blood chemistry were analysed with initial body weight (BW) used as covariate in analyzing performance variables whereas pre-experimental haematological and clinical chemistry of the sera values were also used as covariates in analyzing post-experimental haematological and biochemical values. Means were compared by Dunnette t-test in the general linear model (GLM) procedure. Differences among means with $P<0.05$ were accepted as representing statistically significant differences. Repeated measures were taken on individual goats from the start of the trial and at the end of the study. The random variable fitted was the effect of experimental animals.

The effects of sexes and their interactions were fitted for independent variables. Interactions among the main effects were retained in the final models whether found significant ($P<0.05$) or not in preliminary analyses. Measurements of dates were used as a within subjects factor for repeated measures taken on individual animals. The fortnightly weight readings of the animals were fitted in the models; mean measurement dates indicated different intervals between measurement dates. In analyzing these repeated measures data, the repeated statement was

used to model the covariate structure within subjects. For the *in vitro* gas production experiment, data obtained were subjected to general linear model (GLM) procedure. Where significant differences occurred, the means were separated using least significant difference (LSD) of the SAS.

3.1 BIOMASS PRODUCTION AND CARRYING CAPACITY DETERMINATION

Table 2 describes the six forage species that are available among other forages in the area of the study. The total dry matter (DM) of the selected forage species was 1,646.6 kg per hectare. This showed grazeable/browseable dry matter of 823.3 kg per hectare at 50 percent utilization level (0.5 allowable factors). The mean live-body weight of goats was 25 kg. In view of these animals' live-body weights, DM requirement of livestock unit (LSU) was calculated at 6 kg per hectare. Thus, the annual DM requirement of LSU came to be 2,190 kg.

Table 2: Grazeable/browseable Dry Matter Production (kg ha^{-1}) by different forage species at Mantshwabisi Government Ranch, Kweneng District of Botswana.

FORAGE NAME	DRY MATTER PRODUCTION (kg/ha)	FORAGE VALUE
Grewia retinervis	41.88±0.07	Good
Ochna pulchra	17.92±0.27	Good
Terminalia sericea	34.58±2.01	Good
Digitaria eriantha	561.77±2.09	Intermediate
Digitaria velutina	393.47±3.00	Intermediate
Stipagrostis.uniplumis	596.98±3.30	Fair
Total	1646.6±0.90	

*Forage value based on goat preference according to Holechek *et al.* (2004).

Based on this information, the carrying capacity of the paddock on which goats were grazed/browsed was calculated as 1.3 hectares per livestock unit (1.3 ha/LSU). On live-body weight basis 6 goats are equivalent to one livestock unit (based on mature cow of 450 kg mass). So the paddock is capable of being utilized by 438 goats in its entire capacity of 97.2 hectares. The browses have

been classed as having good fodder value whilst the grasses as either having intermediate or fair forage values.

3.2 COMPOSITION OF THE DIETS

Nutrient composition of both supplemental diets and the six selected goat forages are presented in Table 3. Among the diets fed goats, commercial feed contained (g/100g DM) maximum ($P<0.05$) crude protein followed by *Grewia retinervis*, *Ochna pulchra*, cowpea seed hulls and *Terminalia sericea* had similar ($P>0.05$) crude protein and the grasses (*Digitaria* eriantha, *Digitaria velutina* and *Stipagrostis uniplumis*) had the lowest ($P<0.05$) protein mean values. Lignin (ADL) was similar ($P>0.05$) in grass species followed by browses and lastly, cowpea seed hulls. Lignin was lowest ($P<0.05$) in commercial feed. The dry matter (DM) of the grass specie were highest ($P<0.05$) followed by browses, cowpea seed hulls and commercial concentrate being the lowest. The neutral detergent fibre (NDF) of the grasses were highest ($P<0.05$) compared to browses, cowpea seed hulls and commercial feed. In quantifying acid detergent fibre (ADF) among the feeds, commercial concentrate proved to have the lowest ($P<0.05$), followed by cowpea seed hulls, browses and the grasses being the highest. Ether extract was highest ($P<0.05$) for supplemental commercial concentrate followed by cowpea seed hulls, browses and the grasses had the lowest mean values. Ash content of commercial concentrate was highest ($P<0.05$) followed by *Digitaria velutina*, Cowpea seed hulls, *Digitaria eriantha*, *Grewia retinervis*, *Stipagrostis uniplumis*, *Ochna pulchra* and *Terminalia serecia* having the lowest.

Table 3: Chemical composition (g/100 grams dry matter) of cowpea seed hulls, commercial feed/concentrate and natural forages fed goats at Mantshwabisi Government ranch.

FORAGE/ FEED	DM	CP	EE	ASH	NDF	ADF	ADL	NFE
Cowpea seed hull	93.70^{d}	6.50^{g}	3.59^{b}	8.89^{c}	47.31^{bc}	37.23^{f}	35.03^{d}	34.53^{f}
Commercial feed	92.20^{e}	12.52^{a}	5.11^{a}	22.4^{a}	45.04^{c}	10.40^{h}	7.96^{f}	31.13^{g}
Grewia retinervis	93.75^{cd}	9.13^{b}	0.59^{d}	5.50^{ef}	22.53^{d}	32.33^{g}	28.60^{e}	52.07^{a}
Digitaria eriantha	95.39^{a}	3.64^{e}	0.11^{g}	6.33^{d}	48.55^{ab}	46.00d	39.80^{c}	39.33^{c}
Digitaria velutina	95.35^{a}	2.41^{f}	0.60^{d}	15.0^{b}	51.29^{a}	46.89^{c}	43.00^{b}	29.60^{h}
Ochna pulchra	94.71^{b}	7.40^{c}	0.50^{e}	4.57^{de}	47.7^{bc}	48.00^{b}	38.77^{c}	36.90^{e}
Terminalia sericea	94.32^{bc}	6.55^{d}	0.81^{c}	4.40^{e}	48.54^{ab}	39.17^{e}	27.40^{e}	43.50^{b}
Stipagrostis uniplumis	94.91^{ab}	2.84^{f}	0.29^{f}	4.93^{de}	49.25^{ab}	49.47^{a}	47.87^{a}	37.90^{d}
Mean	94.29	6.37	1.45	9.00	45.03	38.68	33.55	38.20
SEM	0.22	0.67	0.3 6	1.27	1.84	2.52	2.43	1.41

DM – dry matter; CP- crude protein; EE – ether extract; NDF – neutral detergent fibre; ADF – acid detergent fibre; ADL – acid detergent lignin; SEM – standard error of mean; IVDMD – *in vitro* dry matter digestibility; NFE- nitrogen free extract (=100-mo+ash+CP+CF+EE); mo- MO – moisture; ^{abcdefh} values in the same column with different superscript differ (P<0.05). *Protein analysis is on dry matter basis.

3.3 PERFORMANCE CHARACTERISTICS OF BOER-GOATS

Mean performance of Boer-goats on natural pasture (browse/graze), supplemented with either commercial concentrate or cowpea seed hulls are presented in Table 4. Initial body weights of goats on natural pasture were different (P<0.05); however, goats on natural pasture supplemented with commercial concentrate gained the highest (4.74 kg [17.6%]; P<0.05) followed by goats grazed/browsed on natural pasture and supplemented with cowpea seed hulls (0.58 kg [2.4%]). Goats grazed/browsed on natural pasture alone lost weight (-1.12 kg [4.1%]; P<0.05). There was however, no significant difference (P>0.05) between Boer goats grazed/browsed natural pasture and supplemented with cowpea seed hulls and those grazed/browsed natural pasture alone.

Table 4: Performance characteristics of Boer-goats grazed/browsed natural pasture and grazed/browsed natural pasture supplemented with either commercial concentrate or cowpea seed hulls at Mantshwabisi Government ranch.

VARIABLES	NP X CF	NP X CSH	NP
NO. OF GOATS	12	12	12
Initial body weight (kg)	27.0[a]	24.5[ab]	27.5[a]
Final body weight (kg)	31.74[a]	25.08[b]	26.38[b]
Total gain (kg)	4.74[a]	0.58[b]	-1.12[b]
Average daily gain (g/day)	79.0[a]	9.7[b]	-18.7[b]

NP x CF – natural pasture supplemented with commercial feed; NP x CSH – Natural pasture supplemented with Cowpea seed hulls; NP – natural pasture alone; [ab]Means within the same row with different letters differ (P<0.05).

The results showed that the gain or loss of body weight was gradual over the 42 day feeding period (Figure 1); although the goats lost some amount of weight none died.

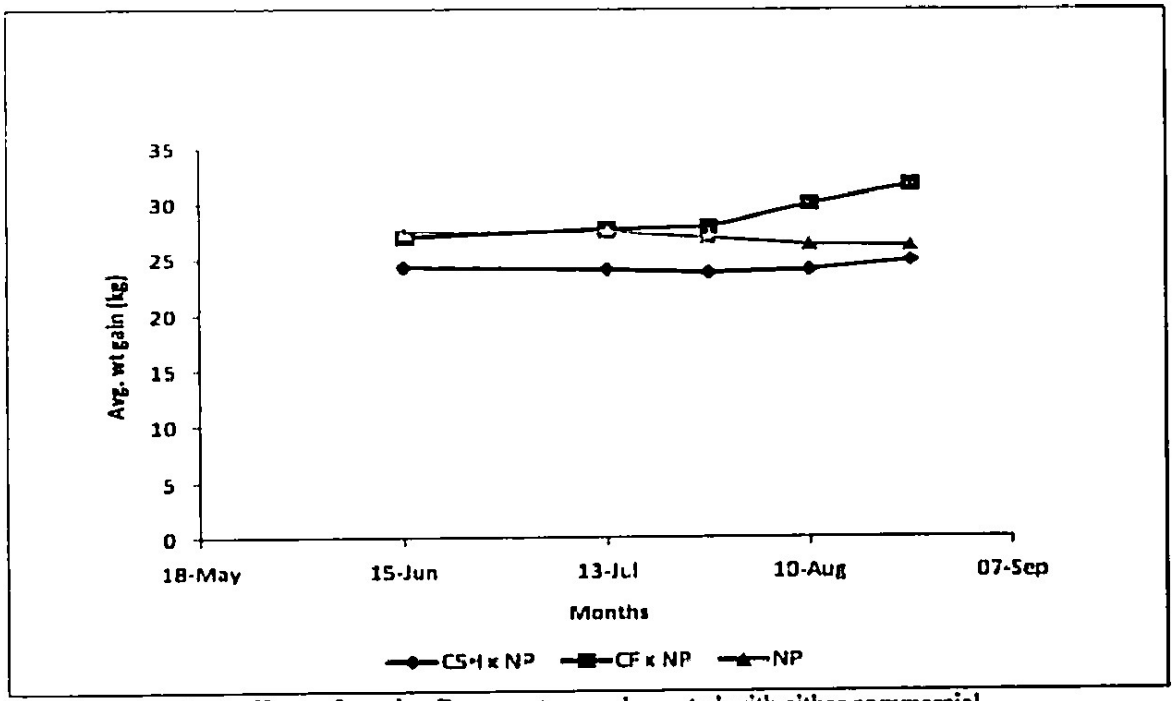


Figure 1:Total effects of grazing Boer-goats supplemented with either commercial concentrateor cowpea seed hulls on weight gain at Mantshwabisi Government Ranch, Botswana.
Key: CSH x NP – natural pasture supplemented with cowpea seed hulls; CF x NP – natural pasturesupplemented with commercial feed; NP – natural pasture unsupplemented

3.4 COMPARISON OF INTACT MALE AND FEMALE GOATS

Table 5 describes the performance results of intact male (Fig.2) and female Boer-goats (Fig.3) grazed/browsed natural pasture alone and Boer-goats grazed/browsed natural pasture supplemented with either commercial concentrate or cowpea seed hulls. The initial mean body weights of intact males fed control diets and natural pasture supplemented with commercial concentrate were higher ($P>0.05$) as compared to female counterparts fed similar diets. Furthermore, the initial mean body weights of intact male goats grazed/browsed natural pasture and supplemented with cowpea seed hulls were lower ($P<0.05$) than those for their female counterparts fed similar diets. By the end of the study, intact males grazed/browsed and supplemented with commercial concentrate had gained mean BW of 4.1 kg while females gained mean BW of 5.4 kg. Also, the mean BW gains by intact male goats fed basal diet and basal diet supplemented with cowpea seed hulls were not significantly different ($P>0.05$) from their female counterparts, respectively.

Table 5: Comparison of performance of intact male and female goats fed natural pasture and natural pasture supplemented with either cowpea seed hulls or commercial concentrate at Mantshwabisi Government ranch.

PARAMETER	INTACT MALE GOATS ± SD			FEMALE GOATS ± SD		
	NP	NPxCF	NPxCSH	NP	NPxCF	NPxCSH
Initial BW(kg)	28.8±5.9^{a}	29.2±5.1^{a}	23.0±2.5^{c}	26.2±6.3^{b}	24.8±7.5^{c}	25.7±7.2^{b}
Final BW(kg)	26.9±4.6^{c}	33.3±8.4^{a}	23.6±1.8^{c}	25.8±6.8^{c}	30.2±7.2^{b}	26.3±7.0^{c}
Daily Gain(g/d)	-31.7±1.3^{c}	68.3±3.3^{b}	10.0±0.7^{c}	-6.7±0.5^{c}	90.0±0.3^{a}	10.0±0.2^{c}

^{abc} Means within the same row with different letters differ ($P<0.05$). NPxCF – natural pasture supplemented with commercial feed; NPxCSH – Natural pasture supplemented with cowpea seed hulls; NP – natural pasture alone; ± SD - standard deviation.

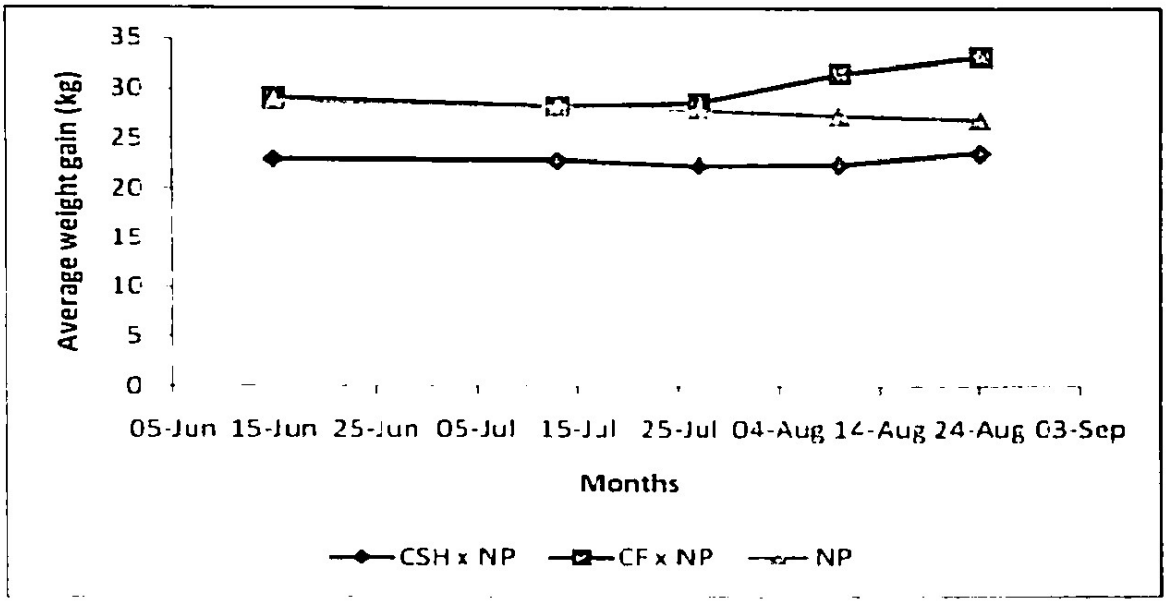


Figure 2. Effect of feeding intact male Boer-goats with natural pasture and natural pasture supplemented with either commercial concentrate or cowpea seed hulls on weight gain at Mantshwabisi Government Ranch, Botswana.

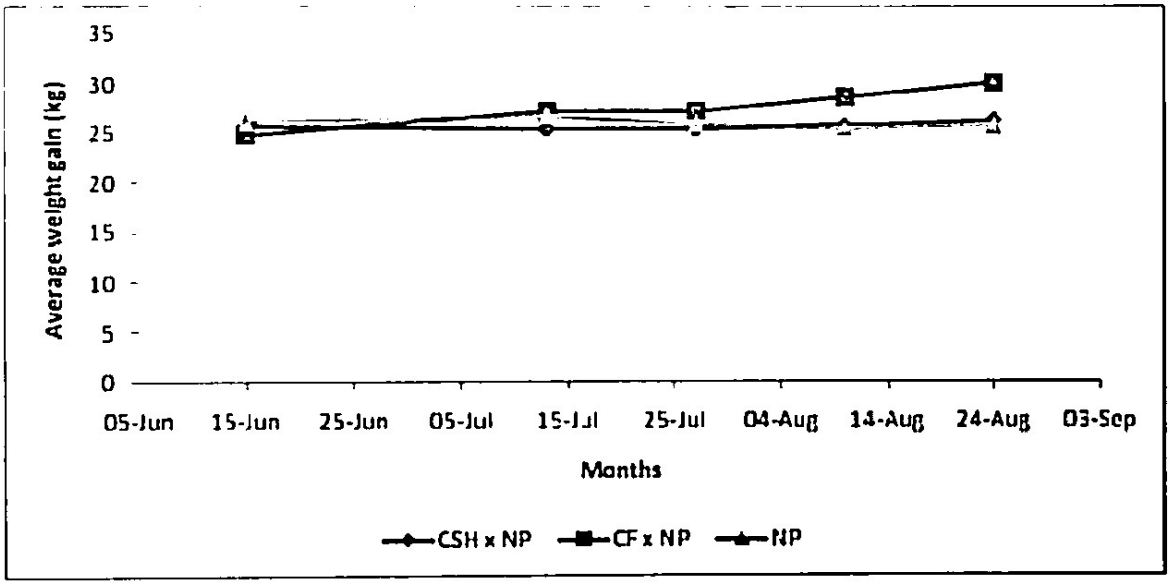


Figure 3. Effect of feeding female Boer-goats with natural pasture and natural pasture supplemented with either commercial feed or cowpea seed hulls on weight gain at Mantshwabisi Government Ranch, Botswana.

3.5 *IN VITRO* GAS PRODUCTION CHARACTERISTICS

Table 6 and Fig. 4 show the *in vitro* gas production characteristics by commercial feed, cowpea seed hulls, natural browses and grasses. There were varied rates of increase in the gas production in the first 6h. Among the goats' diets at 48h, *Stipagrostis uniplumis* had the lowest ($P<0.05$) gas production (ml/200 mg)

followed by *Digitaria velutina*, *Digitaria eriantha*, *Ochna pulchra*, *Grewia retinervis*, *Cowpea seed hulls*, *Terminalia sericea* and Commercial feed being the highest. Furthermore, the lowest and highest values for metabolizable energy (MJ/kg DM), Organic matter digestibility (%), methane (ml/200 mg), short chain fatty acids (µmmol) and *in vitro* dry matter digestibility (IVDMD) (%) ranged from 2.8 in *Stipagrostis unimplumis* to 5.2 in Commercial feed; 23.3 in *Stipagrostis uniplumis* to 54.6 in commercial concentrate; 3.2 in *Digitaria velutina* to 6.4 in *Grewia retinervis*; 0.37 in *Stipagrostis uniplumis* to 0.78 in commercial feed; 44.7 in *Stipagrostis uniplumis to* 79.6 in Commercial feed, respectively.

Table 6: Net Gas Volume, Methane, Metabolizable Energy, Organic Matter Digestibility, Short Chain Fatty Acid and *In vitro* dry matter digestibility of goats feeds at Mantshwabisi Government ranch.

FEED/FORAGE	NGV	CH_4	ME	OMD	SCFA	IVDMD
Cowpea seed hull	11.48^{c}	3.80^{f}	3.80	33.82^{ab}	0.54	62.10^{c}
Commercial feed	21.81^{a}	3.68^{g}	5.24	54.56^{a}	0.78	79.85^{a}
Grewia retinervis	10.81^{d}	6.37^{a}	3.73	32.19^{ab}	0.52	60.70^{c}
Digitaria eriantha	5.14^{f}	4.53^{c}	3.11	26.38^{b}	0.42	50.63^{e}
Digitaria velutina	5.20^{g}	3.21^{h}	2.92	30.32^{b}	0.39	47.53^{f}
Ochna pulchra	10.5^{e}	4.31^{d}	3.67	30.54^{b}	0.51	57.90^{d}
Terminalia sericea	17.15^{b}	5.11^{b}	4.57	35.98^{ab}	0.67	66.10^{b}
Stipagrost.uniplumis	4.46^{h}	4.20^{e}	2.83	23.33^{b}	0.37	$44,70^{h}$
Mean	10.98	4.40	3.73^{NS}	33.39	0.53^{NS}	58.69
SEM	1.17	0.19	0.38	2.93	0.07	2.22

NGV – net gas volume (ml/200 mg DM); CH_4 – methane (ml/200 mg DM); ME – metabolizable energy (ME = $MJ.Kg^{-1}$ DM); OMD – organic matter digestibility (OMD = %); SCFA – short chain fatty acid (µmmol) of goats feeds/forages; NS – not significant; IVDMD – *in vitro* dry matter digestibility; ^{abcdefgh} values in the same column with different superscript are different ($P<0.05$)

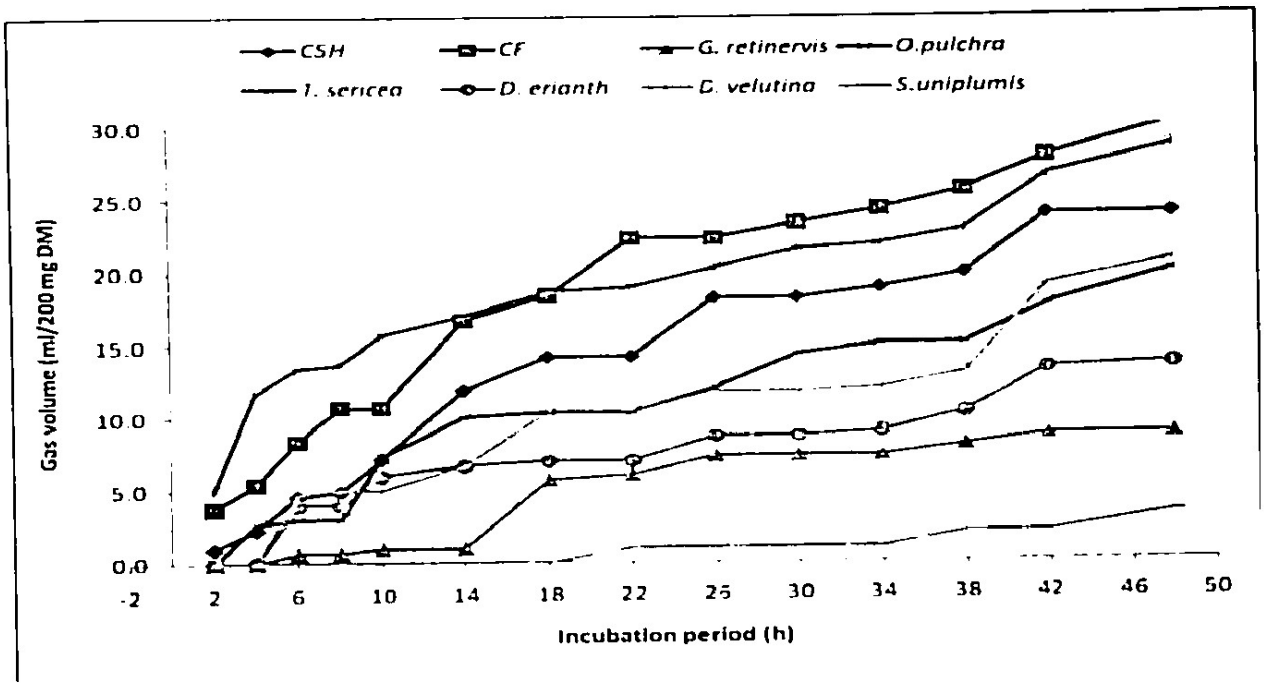


Figure 4: *In vitro* gas production by commercial feed, cowpea seed hulls, natural browses and grasses at Mantshwabisi Government ranch, Botswana

3.6 RUMEN LIQUOR PH AND AMMONIA-NITROGEN CONCENTRATION

3.6.1 RUMEN LIQUOR PH

Table 7 shows comparison of rumen pH and ammonia-nitrogen production after goats were fed different diets. The mean pH of rumen liquor for goats fed natural pasture (control) was the highest (pH 6.94 ± 0.17; $P<0.05$) followed by the cowpea seed hulls supplemented goats (pH 6.70 ± 0.33) and lowest (pH 6.34 ± 0.06; $P<0.05$) being for commercial concentrate supplemented goats.

Table 7: Rumen fluid measurements from natural pasture-fed, natural pasture-fed plus commercial feed and natural pasture-fed plus cowpea seed hulls supplemented weanling Boer-goats at Mantshwabisi Government ranch.

ATTRIBUTE	NP	NPxCF	NPxCSH
Animals used	3	3	3
Rumen fluid pH	6.94 0.17[a]	6.34 ±0.06[c]	6.70±0.33[b]
NH_3-N produced within 2h (NH_3/100 ml of rumen fluid)	97[b]	120[a]	104[b]
		TVA(mEq/l)	
	50	49.2	46.3[NS]

NP – natural pasture (grass and browses) alone; NP x CF – natural pasture supplemented with commercial feed; NP x CSH – natural pasture supplemented with cowpea seed hulls; TVFA – total volatile fatty acids; mEq/l – mill equivalent per litre; NS - not significant; [abc] Means within the same row with different letters differ (P<0.05).

3.6.2 RUMEN LIQUOR AMMONIA-NITROGEN

The ammonia-nitrogen concentration in rumen liquor of the goats supplemented with commercial concentrate was highest (120 mg/l; P<0.05) followed by goats fed natural pasture supplemented with cowpea seed hulls (104 mg/l; P<0.05) and goats grazed/browsed without supplementation (97 mg/l; P<0.05). However, there was no significant difference (P>0.05) between the ammonia-nitrogen concentration obtained from goats fed natural pasture alone and the goats fed natural pasture and supplemented with cowpea seed hulls. The mean concentration of ammonia-nitrogen increased correspondingly with increasing level of crude protein content of the supplemental diets.

3.7 TOTAL VOLATILE FATTY ACIDS CONCENTRATION (TVFA)

The rumen liquor for goats grazed/browsed on natural forage showed highest (50 mg/l; P<0.05) total volatile fatty acid concentrations followed by commercial feed x natural forage (49.2 mg/l; P<0.05) and cowpea seed hulls x natural forage (46.3 mg/l; P<0.05). However, there were no significant differences (P>0.05) in the

production of volatile fatty acids among the goats fed different diets or diet combinations.

3.8 PROTOZOA COUNTS

The mean protozoal counts were highest ($48x10^5$/ml; $P<0.05$) in rumen liquor of goats fed natural pasture x commercial concentrate followed by natural pasture x cowpea seed hulls ($44x10^5$/ml; $P<0.05$) and natural pasture alone ($19x10^5$/ml; $P<0.05$) (Table 8). There was however, no significant difference ($P>0.05$) between the protozoal counts from goats fed either of the supplemental diets. But the microorganisms count were significantly lower ($P<0.05$) in goats fed natural pasture alone. The morphology of the dominant protozoal species showed *Entodiomorphs* and a lesser number being of *Holotrichs*.

Table 8: Protozoa and bacteria concentration in goats' rumen supplemented with cowpea seed hulls,
Commercial concentrate and non- supplemented goats (natural pasture alone).

ATTRIBUTE	NP x CF	NP x CSH	NP
Protozoa x 10^5/ml	48^a	44^a	19^b
Bacteria x 10^9/ml	38.3	37	19.3

^{a,b,c} means within the same row with different superscripts differ ($p<0.05$); SEM- standard error of mean; NPxCF-natural pasture supplemented with commercial feed; NPxCSH-natural pasture supplemented with cowpea seed hulls; NP-natural pasture.

3.9 BACTERIAL ACTIVITY

Goats grazed/browsed and supplemented with commercial concentrate proved to have had higher population of total viable bacteria (38.3×10^8/ml; $P<0.05$) compared to those supplemented with cowpea seed hulls (37×10^8/ml; $P<0.05$) and the control group (19.3×10^8/ml; $P<0.05$) (Table 8). However, there were no

significant differences (P>0.05) of bacterial counts among the goats fed either natural pasture with supplements or natural pasture alone.

3.10 HAEMATOLOGIC PROFILE OF THE GOATS

Table 9 shows the mean values of white blood cells (WBC), haemoglobin (Hb), red blood cells (RBC), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) in Boer-goats grazed/browsed natural pasture and Boer-goats grazed/browsed natural pasture supplemented with either cowpea seed hulls or commercial concentrate. There was no significant variation (P>0.05) in mean values of haematological parameters of goats grazed/browsed and supplemented and those that were grazed/browsed without supplementation, except for the values of MCV and MCH, which were above the normal range. Both values increased significantly (P<0.05).

3.11 CLINICAL CHEMISTRY

The results for biochemical indices of Boer-goats grazed natural pasture alone and Boer-goats grazed natural pasture supplemented with either cowpea seed hulls or commercial concentrate are presented in Table 10. There were no statistically significant variations (*P*>0.05) in the mean biochemical parameters in Boer-goats grazed natural pasture alone and those grazed natural pasture and supplemented with either cowpea seed hulls or commercial concentrate, except in the mean values of calcium (P<0.05). There was a slight increase in the calcium level of goats that were not supplemented; this was evident at the beginning of the trial and at the end of the study. Moreover, phosphorus levels also slightly increased in all the goats offered supplements together with those offered basal diet alone

whereas the albumin showed a slight decline after supplementation with cowpea seed hulls. Although creatinine level was within normal ranges in all the goats fed different diets or diet combinations, it was observed to be on the high side.

Table 9: Effect of natural pasture and natural pasture supplemented with cowpea seed hulls and commercial feed on clinical haematology of Boer-goats

Parameter	Before treatment with CSH	After treatment with CSH	Before treatment with CF	After treatment with CF	Initial treatment with NP	End of treatment with NP	Reference range
WBC x106/µl	10.26 ± 1.64	10.64 ± 1.76	12.81 ± 2.45	8.34 ± 0.80	9.26 ± 0.60	8.46 ± 0.52	(4 - 13)
Hb (g/l)	8.29 ± 1.02^{a}	8.25 ± 0.90	7.34 ± 0.53	7.36 ± 0.44	7.90 ± 0.15	8.09 ± 0.53	(8 - 12)
RBCx1012/l	9.87 ± 1.23	9.12 ± 0.01	9.18 ± 2.23	8.27 ± 0.25	9.46 ± 0.24	7.98 ± 0.08	(8 - 18)
PCV (%)	28.11 ± 3.51	26.10 ± 2.83	26.6 ± 1.14	23.22 ± 0.74	26.73 ± 0.71	22.27 ± 0.24	(22 - 38)
MCV (f/l)	28.4 ± 3.36^{a}	28.70 ± 3.04^{a}	29.0 ± 0.60^{ab}	28.11 ± 0.11^{a}	28.27 ± 0.20^{a}	28.00 ± 0.00^{a}	(16 - 25)
MCH (pg)	8.41 ± 1.00^{a}	9.08 ± 1.00^{b}	8.06 ± 0.59^{a}	8.96 ± 0.57^{a}	8.37 ± 0.13^{a}	10.13 ± 0.68^{c}	(5 - 8)

NP – natural pasture (grass and browses); CF – commercial feed; WBC – white blood cells; Hb – haemoglobin; RBC – red blood cells; PCV – packed cell volume; MCV – mean corpuscular volume; MCH – mean corpuscular haemoglobin; MCHC – mean corpuscular haemoglobin concentration; ^{a,b,c} Values within the same row with different letters differ ($P<0.05$)

Table 10: Some biochemical profiles (Mean ± SEM) of Boer goats grazed natural pasture and grazed natural pasture supplemented with either cowpea seed hulls or commercial concentrate

Parameter	Before treatment with CSH	After treatment with CSH	Before treatment with CF	After treatment with CF	Initial treatment with NP	End of treatment with NP	Reference range
Protein (g/l)	61.65 ± 1.72	59.72 ± 1.27	61.15 ± 0.74	62.03 ± 0.72	61.40 ± 1.27	61.02 ± 0.61	(59 - 74)
Calcium (mg)	2.53 ± 0.03^{a}	2.43 ± 0.01^{a}	2.53 ± 0.04^{a}	2.44 ± 0.03^{a}	2.66 ± 0.49^{b}	2.58 ± 0.05^{b}	(2.13- 2.55)
Urea (mg/l)	5.61 ± 0.58	4.55 ± 0.25	6.51 ± 0.39	4.57 ± 0.41	5.86 ± 0.49	2.58 ± 0.05	(5.4 - 11.8)
Creatinine(g/l)	73.91 ± 2.75	97.87 ± 2.43	77.56 ± 3.03	90.96 ± 2.59	78.07 ± 2.48	102.06 ± 3.26	59.7 - 134.8
Phosphorus(mg)	1.69 ± 0.12	2.11 ± 0.12	1.85 ± 0.17	2.98 ± 0.10	1.40 ± 0.14	2.12 ± 0.13	(2.42- 3.97)

CSH – cowpea seed hulls; CF – commercial concentrate; NP – natural pasture; ^{ab} values within the same row with different superscript are different

CHAPTER 4
4.0 DISCUSSIONS

4.1 THE CARRYING CAPACITY OF THE PADDOCK

The total grazeable/browseable dry matter (DM) for the paddock was determined as 1,646.6 kg per hectare. Holechek *et al.* (2004) reported daily dry matter intake of moose, bighorn sheep, mule deer, white tailed deer, elk and pronghorn antelopes as two percent of their body weight. Since the DM intake for the goats at Mantshwabisi ranch was not determined, DM intake was taken as two percent of their live-body weight as suggested by Holechek *et al.* (2004). However, the total number of goats grazed/browsed in this study stood at thirty-six. This shows that the paddock had ample pasture to support extra 402 goats.

4.2 FORAGE SAMPLES COMPOSITION

The crude protein (CP) findings in different diets had somewhat similar range as those reported from West Africa (Rittner and Reed, 1992). Most of the browse forages/feeds had crude protein above 6% except for the grasses which had the lowest mean crude protein content of about 3%. The results of this study indicate that most of the browse species have considerable crude protein contents that can stimulate effective microbial synthesis in the rumen. Non-woody parts of these dicots (shrub browses) have higher quantities of cell solubles than the monocots (grasses) and lower levels of structural carbohydrate and lignin. However, this apparent advantage in the browses is often overcome by some biologically significant proportions such as tannins, volatile oils and alkaloids which have deleterious effects on the microbial fermentation (Hegarty, 1982).

Furthermore, the grasses have lower crude protein content which often results in loss of weight by goats during the dry seasons. Forage species that have crude protein lower than 6% do not support rumen microbial synthesis (Ørskov and Ryle, 1990). These grasses take a longer period to be degraded in the rumen and with that reducing intake by animals, hence their loss of weight. The nutritive status of these grasses exemplifies the characteristic nature of the C_4 grasses as they contain less *mesophyll* and greater proportions of *sclerenchyma*, epidermis and vascular tissue. The vascular bundles found in monocot grasses are often densely packed and the *parenchyma* bundle sheaths are thick-walled denoting high neutral detergent fibre and as such inhibiting microbial digestion in the rumen.

4.3 BODY WEIGHT CHANGES OF THE GOATS

The mean daily live body weight gain and mean final body weight obtained in the present study were higher for the goats that grazed/browsed natural pasture and supplemented with commercial concentrate compared to the goats supplemented with either cowpea seed hulls and/or the control treatment (Table 4 and Fig. 1). The final weight gain of the goats grazed and supplemented with cowpea seed hulls was not statistically different from goats fed basal diet (control). The low weight gain for the goats supplemented with cowpea seed hulls may be partly attributed to no feed intake by the animals when the supplemental feed was first introduced. When the goats started feeding on the cowpea seed hulls they had already lost some weight.

The weight loss from the goats that grazed basal diet (control) alone was due to less nutrient density extracted from the basal diet that did not meet the daily

requirements of the animals. The mean crude protein (5.3%) of the browsed forage was lower than the appreciable extractive crude protein (6-8%) by the rumen microbes (Huston *et al.*, 1986). This agrees with the findings by Ørskov and Ryle (1990) who explain that feeds which have lower than 6% crude protein do not support microbial activity in the rumen as a result limiting the rate of digestion in the rumen. Devendra and McLeroy (1982) also reported that it was important for animals fed fibrous diets to be supplemented with feeds rich in crude protein.

4.4 INTACT MALES AND FEMALE GOATS PERFORMANCE

The findings of the mean weight gain of female goats were significantly higher than that of the intact males fed corresponding diets or diet combinations. This suggested that the female weanlings were at their fastest growing period than their counterpart intact males (Gubartella *et al.*, 2002). However, the values for body gain in the present study were in consonant with that reported by Gubartella *et al.* (2002) for Nubian male and female weanling goats (ranged between 56 and 80 g/day). The study showed that although the weanling male goats were not castrated the effect of testosterone was low in influencing their weight gain. Testosterone is the male hormone that is known to increase efficiency of dietary nitrogen utilization (Garray, 2005). It is this male hormone that increases muscle protein accretion in intact male weanling goats (Morgan *et al.*, 1993).

4.5 METABOLIZABLE ENERGY, METHANE PRODUCTION, ORGANIC MATTER, SHORT CHAIN FATTY ACIDS AND *IN VITRO* DRY MATTER DGESTIBILITY

The values of metabolizable energy (ME), organic matter digestibility (OMD) and short chain fatty acids (SCFA) obtained in the current study were higher than those reported on tropical forages (Njidda and Ikhimioya 2010). There are many variables that can influence metabolizable energy, organic matter digestibility and short chain fatty acid production in the feed ingredients such as anti-nutritional factors, the presence or absence of prebiotics (fermentable dietary carbohydrates) and the probiotics (rumen bacteria) (Lee *et al.* 2003).

Among the feed treatments, commercial concentrate, cowpea seed hulls and browses had high considerable organic matter digestibility effects (MSU, 2010). This agrees with Gazaneo *et al.* (2003), and could be as a result of the microbes having degraded the easily fermentable carbohydrates of the feeds. Finally, it became apparent from the results that gas production increased with organic matter digestibility.

There were no significant differences in SCFA among the feedstuffs. The gas production in feeds is closely related to the production of short chain fatty acids which are also based on carbohydrate fermentation (Blummel and Ørskov, 1994). It is therefore indicative that gas volumes produced in this study were produced quantitatively and qualitatively as a result of SCFA production (the amount of fermentative CO_2 and CH_4 could be accurately calculated from the amount and proportion of acetate, proportionate and butyrate present in the incubation medium).

Thus, the more feedstuff increased in SCFA the more it resulted in gas production which ultimately heightened digestibility and energetic value (Maheri-Sis *et al.*, 2011).

Although predicated metabolizable energy profiles among feedstuffs showed variations in quantity, they were not statistically different. There was a positive correlation between ME calculated from *in vitro* gas production together with crude protein and crude fat with metabolizable energy obtained from conventional feeds (Fievez *et al.*, 2005).

As shown in the results (Table 6) *in vitro* dry matter digestibility of feedstuffs was highest in the commercial concentrate. Among the feeds, commercial concentrate did not have leaves and possibly that is the reason why its digestibility was highest. Leaves tend to have tannins in both the NDF and ADF fractions tightly bound to the cell wall and cell protein and they decrease digestibility (Reed *et al.*, 1990). Furthermore, cowpea seed hulls have shown quite convincing dry matter digestibility compared to either grasses or browse forages. This suggests that the cowpea seed hulls have low tannins and as a result could be valuable protein supplement in ruminant diets (Aganga and Mosase, 2001). The acceptable character of this supplement was further showed by its low methane production. Methane is known to be responsible for energy losses in ruminants especially in tropical forages orchestrated by methanogenic bacteria (Babyemi and Bamikole, 2006)

4.6 RUMEN PH AFTER FEEDING GOATS

The mean pH values for the rumen liquor of goats fed natural pasture and natural pasture with supplemental combinations were slightly lower than the pH values

described in a similar study elsewhere (Islam, 1999). The ARC (1984) described the most nutritionally desirable diet to be having a pH of 7.32, which was never the case with the current findings. The low pH value for the rumen liquor obtained from the goats fed natural pasture supplemented with commercial concentrate was due to lower physically effective neutral detergent fibre (*pe*NDF) of the supplemental diet. Ruminant feeds with lower physically effective NDF have the problem of stimulating low production of saliva which could otherwise increase the buffering process, hence elevating the rumen fluid pH (Mirzaei-Aghsaghali and Maheri-Sis, 2011).

Physically effective neutral detergent fibre (*pe*NDF) of a feed is related to the physical properties of its fibre content (primarily particle size) that stimulates chewing activity and establishes biphasic stratification of ruminal contents (Mirzaei-Aghsaghali and Maheri-Sis, 2011). The suggestion of smaller particle size of feed leading to lowered ruminal pH was also observed by Ørskov and Ryle (1990), who reported that pelleted roughage (higher *pe*-NDF) could show a lowered pH level than coarse roughage. The duo further explained that ground or pelleted roughage could show depression in pH than with long roughage, suggestive of high salivary secretion with the latter. Slightly higher pH in both the natural pasture and natural pasture x cowpea seed hulls fed goats was due to effective neutral detergent fibre (*e*-NDF) (effective chewing and ruminal activity) contents of the forage consumed. The cowpea seed hulls and the natural pasture forage parts were much elongated although less coarse.

The overall observation of the rumen liquor mean pH values from the goats were maintained at a higher level than the critical level of the rumen pH (6.0) for fibre digestion (Ørskov and Ryle, 1990). The cellulolytic microbes need a rumen pH between 6.2 and 7.0 (Ørskov and Ryle, 1990), and in the current study the pH values for rumen liquor of goats fed either sole natural pasture or natural pasture with supplemental diets were in the range of 6.34 to 6.94.

4.7 AMMONIA-NITROGEN CONCENTRATION

The ammonia-nitrogen (NH_3-N) concentrations of rumen liquor from goats fed basal diet (control) alone and goats that grazed natural pasture and supplemented with cowpea seed hulls were not significantly different. However, ammonia-nitrogen concentration obtained from goats fed natural pasture supplemented with commercial concentrate was significantly higher than those for either goats fed basal diet or basal diet supplemented with cowpea seed hulls. Although the goats fed either natural pasture alone or natural pasture supplemented with cowpea seed hulls had relatively low ammonia-nitrogen concentrations, these values were above the critical level of 50 mg/litre level suggested by Preston (1986) and Leng (1990). The critical level of ammonia-nitrogen concentration is that level beyond which fibre digestion of a feed is adversely affected (Ørskov and Ryle, 1990).

There is acclaimed hypothesis that for most fibrous diets, the major limiting factor to the growth of microbes is due to low ammonia-nitrogen concentrations (Islam *et al.*, 1999). For ammonia-nitrogen concentration to support microbial synthesis it has to be above the critical level for a considerable period of the day (Satter and Slyter, 1974). The deficiency of ammonia-nitrogen can result in the reduction of

microbial populations (Satter and Slyter, 1974). Ørskov and Ryle (1990) reported that the decrease in microbial synthesis may as well affect feed intake of ruminant animals. The ammonia-nitrogen concentration observed from all the dietary treatments were within 2h of sampling. Ammonia-nitrogen concentration levels for better digestion have been speculated by different workers: some estimated to be within 50-70 mg/litre (Satter and Slyter, 1974) while others said it should be in the range of 150 to 200 mg/litre (Krebs and Leng, 1984; Preston 1986). However, Boniface *et al.* (1986) argued that for better optimal rumen performance, the ammonia-nitrogen concentration should be within a range of 45 to 120 mg/litre. The ammonia-nitrogen values in the present study were above those reported by Satter and Slyter (1974), lower than those reported by Krebs and Leng (1984) and Preston (1986), but within values reported by Boniface *et al.* (1986).

4.8 MICROBIAL COUNTS AND TOTAL VOLATILE FATTY ACIDS

The predominant protozoal species found in the rumen fluid of goats fed natural pasture supplemented with commercial concentrate were suggestive (morphology) of *Holotrichs* (Table 8). However, rumen liquor from goats fed natural pasture supplemented with cowpea seed hulls and from those fed natural pasture alone showed evidence of a combination of *Entodiniomorphs* and traces of *Holotrichs.* The present study of protozoal counts corroborates findings by Fenn and Leng (1990). The duo reported protozoal enumeration of 3.7 – 5.6 x 10^5/ml in sheep which were fed either basal diet supplemented with *bentonite* or basal diet without *bentonite.* However, the protozoal counts in the present study were higher but showing similar trend observed by other workers elsewhere (Fenn and Leng,

1990). Mughetti *et al.* (2007) have also recorded highest occurrence of *Entodiniomorphs* species in rumen liquor of sheep fed rumen degradable diet which is in agreement with the current findings. It is widely acclaimed that the *Entodiniomorphs* are largely active protozoal species in the degradation of tree leaves supplemented diets whereas *Holotrichs* play major role in degradation of supplemental concentrates (Odenyo *et al.* 1997). However, ruminal protozoa are not as important as the bacteria, since ruminants can live well without them and fermentation could proceed normally as long as bacteria are present (Ørskov, 1992).

Although there was no significant difference in bacterial counts on goats fed different diets or diet combinations, the rumen liquor from goats supplemented with a commercial concentrate had the highest bacteria count (Table 8). The mean population of the bacteria observed in this study are in consistent with the report by Prasad and Pradhan (1990), recorded in sheep on different straw-concentrate diets. The high bacterial counts from commercial concentrate may be attributed to its high crude protein and better degradability (Singh, 2004), resulting in optimum metabolites concentration for bacterial proliferation.

Total volatile fatty acid (TVFA) concentration in ruminal liquor of the experimental goats in this study were higher than the TVFA concentrations reported by Yanez-Ruiz *et al.* (2004), but within ranges reported by Antoniou and Hadjipanayiotou (1985) and Fondevilla *et al.*(1994) in goats and sheep, respectively. High total volatile fatty acid concentrations indicate protein degradability. Forages/feeds in

this study may have provided considerable activity to the fibrolytic microorganisms (Hume, 1970).

4.9 HAEMATOLOGICAL INDICES

The packed cell volume (PCV) and mean corpuscular haemoglobin (MCHC) mean values observed in this study are in agreement with what other workers have established (Mason *et al.*, 1989; Mbassa and Poulsen, 1993). The mean values of the blood components were observed to have had no significant differences to those of goats that were solely fed on natural pasture and those grazed natural pasture and supplemented with either cowpea seed hulls or a commercial concentrate.

The goats fed different treatments showed significantly higher levels of both mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) than the normal reference range. Mean corpuscular haemoglobin evaluates the concentration of haemoglobin present in the blood whereas the mean corpuscular volume determines the cell size. Haemoglobin plays an important role in supplying oxygen in the animal body. The over production of MCH is called macrocytosis, which is often caused by either megablastic anaemia due to dietary deficiencies in foliate vitamin or heat stress (Borges *et al.*, 2013). Although investigation on vitamins deficiency was never carried out in this study, it is hypothesized that the over production of mean corpuscular haemoglobin and mean corpuscular volume were reflecting deficiency of foliate or heat stress (Borges *et al.*, 2013). The mean daily temperature of the area is higher than 30°C (Meteorology, 2013), which ensures high ambient temperature.

The observations of leucocyte counts in this study were slightly lower than those reported by Waziri *et al.* (2010), except for goats supplemented on cowpea seed hulls. This showed that most of the goats were on healthy condition and the cowpea seed hulls had some deleterious effects or increased animals bone marrow activities (Waziri *et al.*, 2010). An increase of leucocytes can be an indication of either stressful condition such as disease development or some increment of bone marrow activities (Waziri *et al.*, 2010). Dellmann and Brown (1987) reported that stress stimulates the release of leucocytosis inducing factor (LIF) and colony stimulating factors (CFS) which are known to increase haemopoeitic activities and blood cells mobilization into the circulatory system, hence elevation of the leucocytes. This was due to lack of feed intake by the goats at the beginning of the study.

The mean values of plasma proteins were also observed to be on high side than those reported in Sahel goats (Kamalu *et al.*, 1988), but within normal ranges. There was no significant variation in the calcium levels between the goats grazed/browsed basal diet at the beginning of the trial and at the end of the study. Calcium regulates phosphorylation of endogenous proteins in the nervous system through the activation of calmodulin-dependent protein kinases and it also controls excitability of nerves and muscles. Overproduction of calcium has the effect of reduced excitability of pre-and post-ganglionic nerve fibres (Garel, 1987). Moreover, a high level of calcium in the blood tends to interfere with phosphorus absorption, a condition that may lead to deficiency of phosphorus in animals.

Although phosphorus levels appeared low in all goats fed the three treatments before supplementation, but at the end of the study it was slightly increased to the high side. Thus, there was subclinical hypophosphotaemia in the goats before treatments. Hypophosphotaemia is an electrolyte disturbance condition in which an animal abnormally has low level of phosphorus in the blood. This often occurs in malnourished animals which may have consumed large quantities of carbohydrates thereby removing phosphates from the blood creating high phosphorus demands by cells (Barcia *et al.* (1997). Therefore, the elevation of phosphorus level in goats at the end of the study was due to replenishment of phosphorus through controlled release of parathyroid hormone from parathyroid gland and calcitonin from the thyroid gland (homeostatic process).

The creatinine, albumin and urea levels in goats differed from the report by Kamalu *et al.* (1988). The steadily increase of creatinine levels was attributed to the recycled urea as a response to limited dietary protein intake from the natural pasture (Gwaze *et al.*, 2010). Incremental creatinine in the blood stream is as a result of reduced filtration effects in the kidneys and increased production due to muscle catabolism (Wisloff *et al.*, 2003). The creatinine levels seemed high in all the goats given three different feed treatments or diet combinations. However, the levels were within the normal limits. The difference in creatinine levels was also influenced by crude protein content of the diets or diet combinations fed on by the goats (Gwaze *et al.*, 2010).

CHAPTER 5
5.0 CONCLUSION

Cowpea seed hulls provided adequate protein and energy levels to sustain goat production during the extended dry season. Supplementing goats with cowpea seed hulls improved their performance (weight gain) compared to those which were grazed only. This improvement in weight gain was due to relative high dry matter digestibility (>60%) of cowpea seed hulls and its low methane production compared to grasses and browses. In addition, supplementing with cowpea seed hulls resulted in higher ammonia-nitrogen in rumen liquor compared to the goats that grazed without being supplemented. Male and female goats supplemented with cowpea seed hulls were found to have similar daily weight gains. Some blood metabolites (protein, calcium and urea) slightly decreased when goats were supplemented with cowpea seed hulls while creatinine and phosphorus increased. Despite this, the blood metabolites were within the normal range.

Due to their effect on body weight gain in grazing goats, cowpea seed hulls are valuable feed resource for farmers who have no access to commercial feed.

CHAPTER 6
6.0 FUTURE INVESTIGATION

The findings about cowpea seed hulls in this study have provided a stepping stone to the future of agro-industrial by-products or agricultural wastes as ruminant feeds. The following aspects regarding the cowpea (*Vigna anguiculata*) seed hulls require further investigation:

- The best form at which the cowpea seed hulls could be processed and stored without their nutritional status being spoilt or compromised.
- Best ways in which the cowpea seed hulls can be incorporated into total mixed ration (TMR).

CHAPTER 7
7.0 LITERATURE CITED

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APPENDIX I

DETERMINATION OF PROTOZOAL COUNTS IN RUMEN FLUID

Different types of protozoa in the rumen liquor can be determined by the following method:

APPARATUS/REQUIREMENTS:

Glass slide, cover slip, microscope, rumen liquor, test tube, test tube rack, pipette, muslin cloth, and funnel. Lugol's iodine solution was prepared by dissolving 5 g of iodine and 10 g of potassium iodide into 60 ml of distilled water, 10 ml of formalin was added together with 30 ml of glycerol to make the volume of 100 ml.

PROCEDURE

Rumen liquor was collected and filtered through 2 layers of muslin cloth. After having shaken the strained rumen liquor, 1 ml of sample was placed through a wide bore pipette into a test tube. Then 9 ml of Lugol's iodine solution was added into the solution. The mixture was gently swirled and 0.1 ml of sample was transferred swiftly to a dry clean slide and spread under a glass cover of known area (22 mm x 32 mm).

Counting of protozoa was done under low power of microscope in a zig-zag manner. Thirty fields were counted per slide for ease of accuracy and average counts per field were calculated.

Total protozoa count per ml can be calculated by following formula:

Total protozoa/ml = Average x microscope x Distillation factor
Protozoa (1000)(100) Counted/field

*Normal population of rumen protozoa varies from 10^5 to 10^6 per ml of rumen liquor.

APPENDIX II

DETERMINATION OF TOTAL RUMEN MICROBIOTA

The enumeration of rumen bacteria can be determined by a simple method as shown below:

APPARATUS/REQUIREMENT:

Rumen liquor, test tube, pipettes, centrifuge,funnel, muslin cloth, glass slide, microscope, wire loop. 10% formaldehyde solution was prepared by mixing 10 ml of formaldehyde with 90 ml of water. Saturated solution of Nigrosine was also prepared i.e. 5 g of water soluble Nigrosine was dissolved in 20 ml of distilled water and 80 ml of methyl alcohol added. It was thoroughly mixed before use.

PROCEDURE:

Rumen liquor was collected from Boer-goats through a mouth probe tube and put into a thermo flask whereupon it was then filtered through double layered muslin. The rumen liquor filtrate was centrifuged at 3000 rpm for 5 minutes. 5 ml of supernatant from centrifuged rumen liquor was poured into a test tube and 5 ml of 10% formalin was added to kill the bacteria. 2 ml of formalin mixed with rumen liquor was transferred into a test tube and 8 ml of distilled water added to give 1 x 10^{-1} dilution which made serial dilutions of up to 1 x 10^{-4} on a clean glass slide. A 0.01 ml of sample was taken from 1 x 10^{-4} dilution and put on clean glass slide on a marked area of 2 x 2 cm. A loopful of saturated solution of nigrosine was placed on a glass slide. It was then mixed thoroughly and stained with the use of loop wire, spread on a slide as thin as possible. The slide was also passed on a hot

plate for 2 seconds to dry the smear. The slide was then observed under the oil immersion of the microscope where the counting was also done.

The bacteria appeared colourless against the black background.

The bacteria were counted in 10 different fields in a zig-zag manner and the mean number of bacteria per field taken. Total counting of bacteria per ml of rumen liquor was calculated by using the following formula:

*Ruminal bacteria/ml of rumen liquor = Average number of bacteria per field x microscopic factor (1000) x dilution factor (10^6).

DETERMINATION OF TOTAL VOLATILE FATTY ACIDS IN RUMEN LIQUOR

Volatile fatty acids are the end product of carbohydrate fermentation and amino acid catabolism in the rumen. They are the main source of energy in the ruminants. Principal volatile fatty acids present in the rumen liquor are acetic acid, propionic acid and butyric acid occurring in molar proportion of 68-70%, 16-18% and 12-14%, respectively. Total concentration of volatile fatty acids varies from 80 t0 120 mEq/l.

Acetic acid mainly provides energy and milk fat in lactating animals, whereas propionic acid maintains blood glucose and butyric acid forms ketone bodies as is metabolized in rumen epithelium.

APPARATUS/REQUIREMENTS

UDK Kjedahl Automatic distillation unit, hot plate, burette, pipette. Rumen liquor, funnel, test tube, muslin cloth, phenolphalein, 5% oxalic acid solution, 10% potassium oxalate solution and 0.01 N sodium hydroxide solution.

PROCEDURE:

The rumen liquor was collected from Boer-goats and filtered through 2 layers of muslin cloth. 1 ml of strained rumen liquor (SRL) was transferred into the cup of distillation apparatus. 0.5 ml of 5% oxalic acid and 0.5 ml of 10% potassium oxalate solutions were added together into the sample (rumen liquor). UDK Kjedahl Automatic distillation unit was adjusted to distillate the samples for 3

minutes. Collection of the distillate was done through the distillation cup. The distillate was then removed and a few drops of phenolphthalein indicator were added. The distillate was titrated with 0.01 N sodium hydroxide until the pink colour developed.

The volume of 0.01N sodium hydroxide was recorded and it was used to calculate the total concentration (total volatile fatty acids) by multiplying by 10.

TVFA in mEq/l of rumen liquor = volume of 0.01N sodium hydroxide used x 10.

APPENDIX IV

DETERMINATION OF AMMOINIA-NITROGEN IN RUMEN LIQUOR

APPARATUS

Tecator, Kjeltec System 1002 Distilling Unit

REAGENTS

Magnesium oxide powder (H_2SO_4 free) MgO

Sulphuric acid H_2SO_4

Boric acid H_2BO_4

Octylalcohol(antifoamingagent)

$CH_3CH(OH).(CH_2)_5C$

H_3

Indicator: 5 parts methyl red (0.1% in alcohol) - 2 parts methylene blue (0.1% in alcohol).

Borax $Na_2B_4O_7.10H_2O$

Ethyl alcohol

Ether

Glass beads

SOLUTIONS

0.01 Normal H_2SO_4 was standardized with borax. This was done by pouring 0.28 ml of sulphuric acid into a 1 litre of distilled water. The solution was allowed to stand for 48 hours before it could be standardized in triplicate using borax. Standardization of borax was done by placing 0.5 g of crystallized borax into 250

ml Phillips beaker. The crystals were dissolved in 50 ml of distilled water. Two drops of methyl red were then dropped before it was titrated with sulphuric acid. The burette used was rinsed first using newly prepared sulphuric acid before the commencement of the titration. The changeover point was carefully observed as it was supposed to be from yellow to pink before the titration could be done in triplicate.

HOW THE NORMALITY WAS CALCULATED (N) OF H_2SO_4:

$$N\ H_2SO_4 = \frac{\textit{Mass of borax} \times 1000}{\textit{Titration value} \times 190.72}$$

The calculated N of the three replicates differed less than 0.0003, and as such there was no need to repeat the procedure.

Both methyl red and methylene blue were weighed to the mass of 1 g each and put into large containers. Each of the indicators was dissolved into a 1 litre of 60% ethyl alcohol. They mixed into ratios of 2 parts of methylene blue to five parts of methyl red. A volume of 120 ml of the indicator solution was added to a 10 litre of boric acid solution.

In preparing the boric acid, a saturated solution was made by heating the boric acid and it was allowed to crystallize.

PROCEDURE

1.0 Preparation for rumen samples

1.1 Rumen fluid was collected with an oesophageal tube, filtered through a double layer of cheesecloth and acidified with 0.5 ml concentrated sulphuric acid aliquots of 60 to 70 ml were stored in plastic bottles at -15°C.

1.2 The frozen rumen was taken out of the freezer and was allowed to thaw over night at room temperature.

1.3 The thawed bottles were vigorously shaken and were let to stand for a few minutes as the solid fractions settled at the bottom.

1.4 A 50 ml of distilled water together with five glass beads and 0.4 ml of octyl alcohol were added to each Kjeltec tubes.

1.5 A 20 ml of rumen fluid (in triplicate) was paced in tubes and analyzed. The rumen fluid was siphoned.

2.0 Distilling process

2.1 A 250 ml Erlenmeyer flask was prepared by adding 50ml boric acid/indicator solutions.

2.2 The samples were prepared for distillation as the technical procedure dictates.

2.3 Five heaped spatulas of Magnesium oxide (MgO) were added to a Kjeltec tube (see 1.4 above) and fitted to the distilling unit. A 250 ml Erlenmeyer flask was placed under the distillate tube (submerged in the boric acid) and distilled for 10 minutes.

2.4 The distillation was completed after 10 minutes. The blue colour of the solution in the Erlenmeyer flask turned green as expected. The flask was removed and while still under the tube it was rinsed clean with distilled water.

2.5 A blank sample solution was introduced in the analysis once a day.

2.6 The distillate in the Erlenmeyer flask was then titrated with the standard H_2SO_4 solution (0.01 N) until purple colour developed. The volume of the titration was recorded and the answered was computed. The blank sample solution was treated in the same way.

CALCULATIONS/COMPUTATIONS:

14g nitrogen (N)/litre = 1 Normal H_2SO_4

0.00014 g N/ml = 0.01 Normal H_2SO_4

Or

0.14 mg N/ml rumen fluid = 0.01 Normal H_2SO_4Thus, 0.14 x titration value = mg NH_3-N in rumen fluid.

The molecular mass of NH_3 = 17

And the atomic mass of N = 14.

For converting the calculated amount of NH_3-N in the rumen fluid to NH_3, the molecular mass is divided by atomic mass: 17/14 = 1.2143

A 100 ml rumen fluid thus contains:

(0.14 x N of H_2SO_4) x (titration value x 1.2143)*100 = $\%NH_3$

In the formula above, N of H_2SO_4 is the Normality of the H_2SO_4 as determined in the standardization with borax.

APPENDIX V

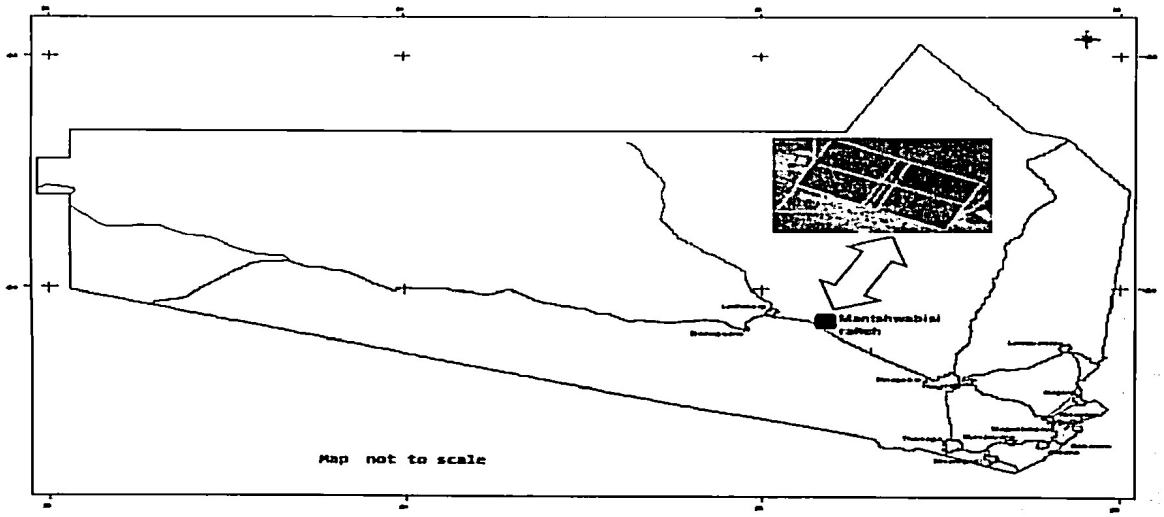

Figure 5: Study area map: Mantshwabisi ranch, Kweneng District, Botswana

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Atriplex Nummularia (Old Man Saltbush) : A Potential Forage Crop for Arid Regions of Botswana

A. A. Aganga, J. K. Mthetho and S. Tshwenyane
Animal Science and Production, Botswana College of Agriculture, Gaborone

Abstract: *Atriplex nummularia* (Old man saltbush) is widely planted on salt affected land to provide a vegetative cover, which can be used as fodder reserve. Such plantations are also perceived as having the capacity to use saline groundwater and hence affect the extent of shallow water tables. *Atriplex spp.* contains high concentrations of nitrogen (N) in winter as compared to summer when it has high concentrations of sodium. The sum of soluble protein-N, amino acid-N, nucleic acid-N and nitrate-N is about half of the total nitrogen. The remainder includes non-soluble protein-N and other N associated with cell membranes and walls. Phosphorus is known to uniformly distribute among pools of inorganic-P, phytate-P, nucleic acid-P and other (residual) fractions. This paper reviews the potentials of *A. nummularia* as a forage for arid areas of Botswana with saline ground water.

Key words: *Atriplex nummularia*, salt affected land, halophytes

Introduction

Animal production practice in Botswana is mainly based on grazing natural vegetation. The native rangelands of the country is greatly affected by annual rainfall precipitation which is irregular and poorly distributed. The rangelands are open shrubs vegetation with a lot of Acacia species. They vary in their green biomass production, distribution and nutritive value from year to year due to mainly, environmental changes. The rangelands are characterized by a short wet/rainy season usually not more than three to four months per year. The palatable and good quality forage always deteriorate or disappear as a result of overgrazing. Therefore, forage scarcity is prevalent and there is an urgent need for increase in feed resources in the arid zones of the country.

Extensive areas of subtropical Botswana are degraded through activities associated with agriculture and grazing, and, to lesser extent mining. Rehabilitation of the degraded land is generally limited by low rainfall, unfavourable soil physical conditions and often by salinity. In these desert terrains or degraded areas there is inadequate pasture or areas where there is some sizeable amounts there is a problem of phosphorus deficiency. It is suggested that *Atriplex spp.* may be more suitable for revegetating very saline soils and also be a good source of productive feed (Hopkins and Nicholson, 1999; Osman and Ghassaeli, 1997).

Atriplex nummularia (old man saltbush) is a halophyte shrub that grows to an average height of 2.0 m. Sheep fed on *Atriplex* species alone will at best maintain live weight, despite the high nitrogen levels found in the leaves (Atiq-Ur-Rehman *et al.*, 1994). When supplemented with hay however, sheep fed *Atriplex* exhibit increase in live weight. The complementary interactions of saltbush and hay have been extensively studied and results have shown that the *Atriplex* can be effectively used not just to regenerate saline soils, but also as a source of productive feed (Hopkins and Nicholson, 1999).

Old man saltbush was tried in Bokspits in the Kgalagadi desert in Botswana to stabilize sand dunes and as fodder crop with successful results. *Atriplex nummularia* is known not to affect organoleptic characteristics of meat when fed either alone or supplemented with other sources of nutrition (Hopkins and Nicholson, 1999). *Atriplex spp.* is known to have high levels of nitrogen and phosphorus, characteristic nutrient elements involved in protein synthesis. This paper is to review the importance of *Atriplex nummularia* as forage crop for possible cultivation in saline areas of Botswana.

Plant Description: *Atriplex spp.* originated from Australia and had spread to arid and semi arid parts of the world (Osman and Ghassaeli, 1997). *Atriplex spp.* is an erect shrub belonging to the family *chenopodiaceae*, grows up to 2m high and spreads to 2.4m wide, has white branches, oval to almost round grey leaves up to 2 cm long, small green terminal flowers, and triangular, laterally compressed fruits 1-2 cm. *Atriplex spp.* is often grown as fodder plant in drier areas because of its great resistance to drought and salt tolerance (Abou El Nasr *et al.*, 1996). It grows well in deep soils with only 150-200 mm of rainfall annually, but can survive for a year with 50mm of rainfall. Resists temperatures as low as −10 °C. *Atriplex spp* are not affected by heavy textured and high salinity soils and water. Their frost resistance is high (El Aich, 1987).

Propagation: *Atriplex nummularia* can efficiently be propagated from stem cuttings especially during spring as opposed to summer as it would be affected by

Table 1: Mean levels of ash, crude protein(CP), acid detergent fibre(ADF), Neutral detergent fibre(NDF), lignin, and crude fat (CF) in Atriplex species.

Species	Ash (g/kg^{-1})	CP (g/kg^{-1})	ADF (g/kg^{-1})	NDF (g/kg^{-1})	lignin (g/kg^{-1})	CF (g/kg^{-1})
A nummalana						
Cut 1	181	92	337	497	104	22.1
Cut 2	247	131	243	405	92	22.2
Cut3	220	91	317	489	93	19.8
Cut4	223	85	306	472	84	22.6
Ave regrowth	230	103	289	455	90	21.5

Source Watson and O'Leary, 1993

Table 2 Mean levels of sodium(Na), calcium(Ca), potassium (K), magnesium (Mg) and Phosphorus(P) and ratios of Na to K(ionic equivalents) in *Atriplex* species

Species	Na g/kg^{-1}	Ca g/kg^{-1}	K g/kg^{-1}	Mg g/kg^{-1}	P g/kg^{-1}	Na/K Ratio
A nummalana						
Cut1	64 2	4.9	19.8	3.6	2.2	5.5
Cut2	75 3	6.8	23.2	4.3	2.6	5.5
Cut3	71 1	4.9	20.4	4.6	2.0	5.9
Cut4	68 8	4.8	17.4	4.9	1.5	6.7
Ave regrowth	71 7	5 5	20.3	4.6	2.0	6.0

Source Watson and O' leary, 1993

Table 3 Mean *in vitro* apparent digestibility (± SEM) of *Atriplex nummalaria, Atriplex canascens* and *Cassia sturtii*

			In vitro apparent digestibility	
Species	Crude protein	Ash content	DM(%)	OM(%)
Atriplex nummalana	18 7±0.5	28.3±1.4	73.5±1.2	58.7±1.1
Atriplex canescens	17.3±0.4	18.4±3.3	62.0±1.3	46.7±1.2
Cassia sturtii	13.0±0.3	5.2±0.5	50.9±0.5	47.9±0.4

Source: Benjamin *et al.*, 1995

Table 4: Least square means (S.E.D) for muscle pH, meat color measurements (l* lightness, a* redness, b* yellowness) and b* values(b_2*) for subcutaneous fat from lambs fed saltbush/ grain(SG; n=14), saltbush/hay (SH; n=14) and Luceme (L; n=14)

	SG	SH	L	SED
PH colour measures	5 53*	5 62*	5 62*	0 06
l*	36.2*	35 7*	36 7*	0 72
a*	17.9*	17 2*	17 8*	0 68
b*	6 6*	6 3*	6 8*	0 42
Fat colour (b_2*)	7.0*	6 8*	7.6*	0.72

Values followed by the same letters are not significantly different at ($p< 0.05$). Source: Hopkins and Nicholson, 1999.

effective disease pathogens (Malan and Rethman, 1997). Rooting of the new growth plant has also been reported to be more viable than older ones. Arya *et al.*, 1993, have shown that *Atriplex spp.* terminal cuttings propagated and treated with IBA have quick rooting and can be transplanted into pots in nurseries. *A. nummualria* may be spaced at 1 m * 5 m and can be grazed by animals when it's 1.5 m high i.e. second or third year of growth (Malan and Rethman, 1997). This kind of propagation has to be done in nurseries and seedlings transplanted when they have grown to the size of a pencil (10-15 cm).

Nutrient composition: Mean levels of ash, crude protein (CP), acid detergent fibre (ADF), Neutral detergent fibre(NDF), lignin and crude fat (CF) in *Atriplex* species are shown in Table 1. *Atriplex nummalaria* is relatively high in protein and ash. The crude protein and ash contents of *A. nummalaria* average 18.2 and 22.7 percent respectively. (EL Aich, 1987).

Mean levels of sodium (Na), calcium (Ca), potassium (K), magnesium (Mg) and Phosphorus (P) and ratios of Na to K (ionic equivalents) in *Atriplex* species are shown in Table 2.

The digestibility of *Atriplex spp* averages 59% in spring and 46% in Summer. The intake of *atriplex spp* varies in the Interval 50-55g Dm/Kg $LW^{0.75}$. Increased consumption of *Atriplex spp* is accompanied by higher water intakes because of the increased water required for urinary excretion of sodium (El Aich, 1987). Sheep grazing *Atriplex vesicaria* consume 7-7.5 Kg/day of water in comparison to 3.2 kg/day on grasslands. *A. nummalria* has high digestibility, the high digestibility is

due to the salt concentration in their leaves (Benjamin *et al.*, 1995).

Mean *in vitro* apparent digestibility (± SEM) of *Atriplex nummalaria*, *Atriplex canascens* and *Cassia sturtii* is shown in Table 3.

Palatability and preference: Palatability is defined as the ratio between the amounts of feed ingested by herbivores and the amount on offer, for a given period of time. Preference is the order in which forage species are selected by herbivores within a given plant community or population, or at a given site, at a given time (Le Houerou, 1991). These concepts, however, are liable to wide variability in time and space, depending, on many variables and parameters that may change with season, site, animals and other local conditions. Some of these variables are linked to the plant, others to the animal, environmental factors. For a given species palatability for a given type of animal varies with phenological stage, the organ concerned and the season (Squires and Ayoub, 1992). As a rule of thumb, the content of crude fibre in forage plays an important role in its selection by livestock. Forages with high fibre content are usually better accepted by cattle, than sheep and goats; also this depends on the high levels of protein in the overall diet (Meyer and Karazov, 1991). The stage of growth and maturity considerably affect the nutritive value, palatability and utilization of *atriplex spp.* Such plants are nutritious in wet season while they are poor during dry season (El Shaer *et al.*, 2000). As a supplementary fodder, *atriplex spp* should not take more than 25-30% of the sheep's diet. Casson *et al* (1996) suggested that the high salt content of saltland forage plants is likely to be the major determinant of palatability and that dilution of salt content through the availability of other feed resource would be necessary to improve intake and performance. Hopkins and Nicholson (1999) reported that there was no effect of feeding *Atriplex* to lambs on tenderness or juiceness and overall panelists ranked the meat samples similarly for acceptability. Finishing lambs on saltbush and either supplemented with hay or grain as used in their study did not present any apparent meat quality problems compared to Lucerne fed lambs (Hopkins and Nicholson, 1999). Table 4 shows least square means for muscle pH, meat colour measurements for lambs from Hopkins and Nicholson (1999).

Atriplex contains considerable amounts of protein (15.5 and/or 21.3%) and crude fibre (20.5%) with digestibility of 52.0 and 39.4%, respectively. Hopkins and Nicholson (1999) reported that sheep fed on saltbush had significant weight gain in western Australia, where the soil is very saline. It has also been observed however, that although animals may maintain live weight while grazing *Atriplex spp.*, they invariably loose condition (Casson *et al.*, 1996). This is attributed to a large increase in water intake (Atiq-Ur-Rehman *et al.*, 1994), to counter the high amount of sodium and potassium salts found in *Atriplex species* (Wilson, 1996).

Conclusion: *Atriplex nummularia* can be an effective fodder component in mixed diets for livestock. The principal advantages would be that adverse effects due to the high mineral content of the halophyte tissues could be minimized, that animal performance and economic returns might be higher than direct grazing of the shrub species, or in-adequate feed of the dry season in the arid areas . However, the main disadvantage of using *A. nummularia* as one of the feed ingredients would be reduced feed conversion efficiency, due to the dilution effect of minerals on energy density.

Recommendations: Vast areas of salt-affected land will remain unproductive unless efforts are made to rehabilitate them with highly salt-tolerant plants (halophytes). It goes without saying that salty areas such as Kgalagadi and Gantsi areas of Botswana can benefit from being re-vegetated by planting saltbush and farmers can also obtain fodder for their livestock from this halophytic shrub.

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