CHEMICAL COMPOSITION, RUMEN DEGRADABILITY AND POST RUMINAL DIGESTIBILITY OF SELECTED SOYA BEAN *(GLYCINE MAX)* CULTIVARS HARVESTED AT DIFFERENT GROWTH STAGES

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DECLARATION

I, Rendani Mukosi, hereby declare that this dissertation is submitted in fulfillment of the requirement of the degree of Master of Science in Agriculture (Animal Science) at the University of Venda. This dissertation is my work and has not been submitted previously for any degree at this or any other university. It is an original in design and execution, and all reference material contained in this dissertation has been acknowledged.

Rendani Mukosi: Mukosi Date : 11th August 2020

DEDICATION

I dedicate this to my mother Mrs Lufuno Hellen Mukosi, my husband Abednico Nyoni, my sister, Shandukani Sharon, my brothers, Vhonani, Dakalo Kenneth, and Marubini Justice Mukosi.

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ABSTRACT

Soya bean (*Glycine max*) is a legume that is mostly cultivated for food grain which can be used as high-protein forage for grazing, haying or ensiling. The use of forage soya bean by small holder farmers is currently very limited. The objective of the current study was to evaluate the nutritive value of three trifoliate forage Soya bean *cultivars (Locally denoted as 4-LF, PAN, and TGX*). The study was carried out at the University of Venda where the soya beans were planted in 63 25L pots (21 pots for each cultivar) which were randomly placed on the floor of an open, wire-net protected house. Forage harvested at three growth stages (pre-anthesis, anthesis and postanthesis). Samples of the forage were analyzed for dry matter (DM), ash, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) micro and macro minerals. Ruminal DM and CP degradability were evaluated *in situ* by incubation of samples within nylon bags (external dimension: 6 × 12 cm, pore size of 46 μm) in the rumen of three Bonsmara steers for 0, 6, 12, 16, 24, 48, 72, and 96 hours. Estimates of rapidly degradable fraction "*a"*, slowly degradable fraction "*b",* constant outflow rate 'c' and the DM or CP degradability (p) at time (t) were estimated by fitting the degradability data into the exponential equation $P = a + b\,(1 - e^{-ct})$ using the NEWAY computer programme. Parameters were subjected to ANOVA for a 3 X 3 factorial treatment arrangement using the General Linear Model procedures of MINITAB software (version 17 of 2014). Effective degradability ED) was estimated as $\mathrm{ED} = \mathrm{a} + \frac{\mathrm{bc}}{(\mathrm{k}+\mathrm{c})}$ at fractional outflow rates of k= 2%, 5% and 8%. *In vitro enzymatic* DM and CP digestibility of rumen undegradable residues collected after 24 and 48-hour incubation was determined by simulating sequential gastro-small intestinal digestion. Cultivar PAN harvested post anthesis had significantly higher ($p < 0.05$) CP than other cultivars. The CP content increased with growth stage. Cultivar 4LF harvested preanthesis had significantly highest ($p < 0.05$) NDF. The cultivar had no significant effect ($p > 0.05$) on DM, ash, CP, NDF, ADF and minerals. Cultivar PAN harvested pre-anthesis had significantly highest ($p < 0.05$) Mg. The harvest stage significantly affected ($p < 0.05$) mineral content other than ($p > 0.05$) Zn and Cu. Cultivar TGX harvested pre-anthesis had significantly highest ($p < 0.05$) effective degradability of dry matter at k=0.08. Fraction 'c' and ED at k= 0.08 were lower ($p > 0.05$) in cultivar * growth stage interaction in dry matter degradability. Fraction 'a' for CP was highest ($p < 0.05$) for cultivar TGX harvested post-anthesis. Fraction 'c' was lower ($p > 0.05$) for cultivar 4LF harvested at anthesis stage. There was a significant effect ($p < 0.05$) on crude protein soluble fraction 'c' and effective degradability k=0.08 in cultivar and growth stage interaction. There was no significant interaction (p> 0.05) of the cultivar X growth stage on crude protein degradability at 48 hours, IVCPD at 24 and 48 hours with significant effect on crude protein degradation at 24 hours caused by cultivar TGX at pre-anthesis growth stage. In conclusion, growth stage increases the chemical composition of soya bean but does not affect digestibility. **Keywords: *In situ, In vitro, pre anthesis, anthesis, post anthesis, fertilizer.***

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LIST OF ABBREVIATION

A	Soluble fraction
ADF	Acid detergent fiber
AOAC	Association of official analytical chemists
B	Insoluble but potentially degradable fraction
C	Outflow rate of degradation per hour
Ca	Calcium
°C	Degree Celsius
CP	Crude protein
Cu	Copper
DM	Dry matter
ED	Effective degradability
FAO	Food and agricultural organization
Fe	Iron
G	Grams
g/kg	Grams per kilogram
IVDMD	*In vitro* dry matter digestibility
IVCPD	*In vitro* crude protein digestibility
K	Potassium
L	Litre
Mg	Magnesium
Mg/kg	Milligram per kilogram
ME	Metabolized energy
mm	Millimeter
N	Nitrogen
NDF	Neutral detergent fiber
RUP	Rumen undergraded protein
RUR	Rumen undegraded residues
SEM	Standard error of means
TDN	Total digestible nutrients
Zn	Zinc

CHAPTER ONE: INTRODUCTION

1.1 Background

Annual legumes can contribute to the feed flow when perennial crops undergo winter dormancy (Carrauthers *et al*., 2000; Sheaffer *et al*., 2001). However, their use as forages is currently very limited and, in most cases, restricted to situations where climatic conditions limit both growth and quality (Sheaffer *et al*., 2001). Soya bean (*Glycine max*) is a multi-purpose, largely grain legume species native to East Asia (Pier *et al.*, 2018). It is an annual legume which can be grown as either a cold or a warm-season crop (Martin *et al*., 1990; Mislevy *et al*., 2005).

Small holder farmers in Vhembe district are significant contributors to the country's agricultural value chain (Wolfgang *et al.*, 2018). However, they experience feed supply and quality constraints especially during winter seasons (Rambau, 2017). This is because, in the tropics, natural pastures do not provide adequate nutrients for livestock during winter seasons due to frost (Suttie, 2000) and dormancy. As a result, animals go through cycles of weight gain during wet summer months, and weight loss in the dry winter seasons (Clatworthy, 1998).

Soya bean is important in animal nutrition because of the protein (45%) in the grain (Popovi, 2016). Soya bean may also be grown for foraging, haying or ensiling, either alone or in grass mixtures (Spanghero *et al*., 2015). High forage yielding soya bean varieties were developed (Rao *et al*, 2005).

Grain varieties have lower forage yields compared to forage varieties when harvested at a similar stage of maturity (Sheaffer *et al*., 2001). The minimal reduction in forage quality coupled to increased forage dry matter yield associated with late-maturity makes forage soybean a better choice for forage production (Hintz *et al*., 1992). Late maturing forage cultivars harvested at anthesisto post-anthesis stages are recommended (Jacobs *et al.,* 2009). The forage generally has a superior combination of low fiber, high protein content, and digestible energy (Darmosarkoro *et al.,* 2001). Limited research has been conducted on forage soya bean cultivars despite the potential as a forage crop (Abdurrahim *et al.*, 2019).

The usefulness of a particular feed ingredient for animal feeding is not only determined by the amount of nutrients contained in it, but also by the proportion of utilizable nutrients (Yulong *et al*., 2011). The important nutrients in this study are dry matter and protein as they play a major role in determining the forage value (Cecilia *et al.,* 2007). The protein content is considered the most important factor in evaluating the nutritional quality. However not all protein contained in the feed is available to the animal for metabolic functions (Yulong *et al*., 2011).

1.2 Problem statement

Most farmers in Limpopo province rely on native pasture as the main feed for their livestock throughout the year (Mapiye *et al*., 2010). This is because they cannot afford commercial feeds (Mapiye *et al*., 2010). The problem with pasture being the only feed for livestock is that it may not have enough nutrients to meet the maintenance requirements of grazing ruminants (Baloyi *et al*, 2008). During winter seasons, there is insufficient pasture for the livestock and the little that is available has low protein and high lignin content (Baloyi *et al.,* 2008). Poor nutrition limits productivity. There is, therefore need to balance the abundance of pasture in summer and shortage of pasture in winter by preserving it during the time of abundance for use during the time of shortage (Undersander *et al.,* 2002). It is important to find alternative supplementary protein sources to ensure adequate feed flow for the livestock throughout the year. Currently, there is not much information on the forage value of tri-foliate soya bean varieties in terms of the chemical composition and degradability of DM and protein, with a limitation to their efficient supplementary feeding to ruminants. Forage soya bean has a delayed maturity which is favorable to fodder quality (Snavely, 2012). Little is known about variation of morphological traits, DM yields and plant components of different soya bean genotypes (Acikgoz *et al.,* 2007).

1.3 Justification of the study

There is a need to evaluate the forage quality of potential forage soya bean cultivars to help increase forage Soya bean production (Altinok *et al*., 2004).Due to inadequatepublic and private research, farmers are forced to use a century old cultivars such as Williams 82' and Clark 63 (Abdurrahim *et al.*, 2019). The location and maturity stage at harvest are known to differentially affect both forage yields and quality among grain-type cultivars (Altinok *et al*., 2004). There is limited published information regarding the ruminal degradability and *in vitro* digestibility of soya bean leaves. Mustafa and Seguin, (2005) stated that chemical composition and ruminal nutrient degradabilities are influenced by the cultivar. There is no published information on the nutritive value of forage from soya bean cultivars 4LF, TGX and PAN. Improving the main available feed resource by identifying alternative feeds is necessary to alleviate the prevailing nutritional problems of livestock. Improvement in the availability and nutritional quality of feed resources can be achieved through the identification of high quality forage of high yield that are adapted to local conditions, such as soya beans and study them. The results obtained from the study will provide information that will help local small-scale farmers in improving the production value of animals which are becoming less productive due to insufficient nutritional feeds and lack of knowledge. Using soya bean as an intercrop with staple cereals will also help maintain soil fertility, thereby reducing the need for expensive N fertilizers.

1.4 Objectives

1.4.1. Broad objective

The main objective was to evaluate the forage nutritive value of three tri-foliate varieties of Soya bean.

1.4.2. Specific objectives

The specific objectives were to evaluate the comparative nutritive value and determine ideal harvest stages of novel, tri-foliate forage cultivars *(4-LF, PAN, and TGX)* of Soya beans in terms of the following nutritional parameters;

i. The chemical composition dry matter (DM), Ash, crude protein (CP), Neutral detergent fiber (NDF) Acid detergent fiber (ADF), macro and micro minerals.
ii. *In sacco* rumen degradability of dry matter and protein
iii. *In vitro* post ruminal digestibility of dry matter and protein

1.5 Null Hypothesis

The Soya bean cultivar *(4-LF, PAN, and TGX)*and stage of harvesting do not affect their forage:

i) Chemical composition ii) In *sacco* rumen degradability of dry matter and protein iii) In vitro post ruminal digestion of dry matter and protein

CHAPTER TWO: LITERATURE REVIEW

2.1 General introduction

Soya bean (*Glycine max*) is a member of the *Fabaceae* family. It is an annual, leguminous, warm temperature, short-day plant, normally bushy and of upright growth habit. Plant height varies from 40 to 100 cm, more-branched with well-developed roots. The plant produces several small pods containing one to four seeds, usually yellow to black. The optimum temperature is 25 ºC for all growth stages, with rainfall requirement of 500 to 900 mm (Blignaut and Taute, 2010). Deep, welldrained, fertile soil with high, water-holding capacity is ideal for high yields (Blignaut and Taute, 2010).

Soya bean is a protein and oil-seed crop mostly cultivated for grain (Spanghero *et al*., 2015). It may also be used as forage for grazing, haying or ensiling, either alone or in mixtures with grass because of its high protein content (Spanghero *et al*., 2015). Substantial nitrogen fixation is obtained when soya bean is planted in rotation with grasses or cereal crops (Sinclair and Vadez, 2012).Soya bean grain and the hay are high in protein, which makes it a protein ideal supplement to low-quality forages, particularly those deficient (Albro *et al*., 1993).

Before the 1935, soya beans used to be grown mostly for fodder, but to this date its value as an oilseed and protein crop started to outweigh its value as forage. However, there is a resurgence of interest in soya bean forage especially when the economic return of soya bean grain decreases due to drought or frost (Wright, 2013). Some high yielding soya bean varieties have also been developed specifically for forage production (Rao *et al*., 2005). Devine and Hatley 1998 and Devine *et al*., 1998 developed several improved cultivars (Derry, Donegal and Tyrone) of forage soya bean. One of the top performing forage soya beans was introduced in the USA as PI40658. Derry, Donegal, and Tyrone were among the top performing forage soya beans.

Forage soya bean yield and nutritive value varies depending on genotype, location and stage of maturity at harvest (Bilgili *et al.,* 2005). One of the top performing forage soya bean seed was introduced in USA in 1914 as PI40658. Early breeds of forage soya bean were Derry, Donegal and Tyrone. They produced DM yields varying from 5216 to 13900kg per hectare depending on location and year (Nayigihugu *et al.,* 2000). In the study done at UK, the weather conditions allowed cultivar Derry and Donegal to reach 7.95 ton per hectare (Koivisto *et al.,* 2003).

Munoz *et al.,* (1981) showed that when soya beans pods were filled and leaves began to turn yellow, the percentage of leaves, stem and pods were 28%, 36% and 36% respectively with a total DM yield of 12.4 ton per hectare. Soya beans harvested for grain type and forage type's dry matter was not different which averaged 8.8 ton per hectare according to a study conducted by Sheaffer *et al,* (2001).

Forage yields of forage soya beans double cropped following barley, winter wheat and winter rye ranged from 60 to 105% of the main cropping soya bean (LeMahieu and Brinkman, 1990). Forage soya bean is not ideal for grazing as animals cause damage due to trampling to the growing plants (Morse *et al.,* 1952) but small animals such as sheep and goats can graze without damaging the plant (Luginbuhl, 2006). Soya bean breed specifically for forage is tolerant to drought and thrives when other forage legumes like alfalfa are not available (Ecoport, 2010).

2.2 Soya bean production in South Africa

Local soya beans production increased by 20% annually from 2010 indicating a growing local capacity to process Soya bean (Dlamini*et al.*, 2014). Therefore, there is substantial potential to expand the soya bean value chain by promoting the production of multipurpose varieties, to meet the demand. Soya beans are produced nearly in all the provinces in South Africa, albeit with varying magnitudes. In 1976, 22 000 hectares were planted with soya beans and gave an output of 17 900 tons, in 1993, soya beans output stood at 63 100 tons (Dlamini *et al.,* 2014). Dlamini *et al.,* (2014) reported that the area planted with soya beans has also increased quite considerably ever since then. Between1997/8 and 2012, land set aside for soya beans increased from 93 790 ha to 472 000 ha (Dlamini *et al.,* 2014).

2.3 Productivity

Due to several agronomical features, Soya bean has great potential as a forage crop (Einar, 2007). This is because it has more branches and nodes with leaves about 6-15 cm. The recommended planting space for forage soya bean is 35 cm as opposed to 70 cm when planting for seeds (Rena, 2009). Forage Soya beans are unique in growing to remarkable heights than grain soya beans and produce more than triple the amount of biomass (Sheaffer *et al.,* 2001). Zhai *et al*., (2008) reported dry matter yields of forage soya bean of 2.3 to 6.5 tonnes per hectare depending on the maturity at harvest. EunJa Lee *et al.,* (2014) reported average fresh forage yields of 4.4 ton per hectare (pre-anthesis stage), 12.7 ton per hectare (anthesis stage), and 16.3 ton per hectare (post-anthesis stage), with average dry matter of 0.9 t ha^{-1} (pre-anthesis stage), 3.4 t ha^{-1} (anthesis stage), and 4.9 t ha^{-1}(post-anthesis stage). Unlike grain soya bean that can easily be stunted and even killed off by hungry animals (Jungman, 2018), forage soya beans withstand heavy browsing pressure.

The best forage soya beans cultivars were Derry, Donegal and Tyrone which was developed in the USA (Shah *et al.,* 2017). But because of the need for the cultivars to do well in different environmental conditions, many cultivars were developed and registered making it hard to get the original or best forage producing soya beans (Snavely, 2012). The most popular cultivar is Williams 82 as it produces more biomass, protein and oil content compared to other cultivars (Shah *et al.,* 2017). It is also one of the two (the other cultivar being Forrest) soya beans providing the majority of genomic tools for forage soya beans (Lightfoot, 2008).

2.5. Nutritive value of soya beans

Soya beans provide a high protein and high energy forage crop for livestock (Perez, 2009). CP concentrations of soya bean hay generally range from 120 to 140 g/kg for stems, 190 to 200 g/kg for leaves, and 120 to 270 g/kg for pods, depending on the plant's stage of development (Miller *et al*., 1973). According to Lee *et al*., (1993), the CP contained in the leaves and the seeds of soya beans were 280 g/kg and 460 g/kg, respectively and around 170 g/kg in the whole plant. The NDF concentrations were 500 g/kg, 480 g/kg, and 620 g/kg in the leaves, seeds and whole plants, respectively. This variation between the stem, leaves, and pods is because when the plant matures, the stem becomes fibrous containing less nutrients and more fiber, the leaves will lose more of their nutrients to the pods for the development and growth of the seed and thus the seeds will have more nutrients than the leaves. Comparing the results of Miller *et al.,* (1973) and EunJaLee*et al.,* (2014), the variation can be due to environmental conditions and management practice used as these affect the growth of the crop.

From the results obtained in the study of EunJa Lee *et al.,* (2014), it showed that the protein content is high in the pre-anthesis stage and then starts to decrease when it continues to grow but then it starts to increase again when it begins to mature. The opposite is in NDF, as the plant matures, the NDF increases. It is therefore important to find the optimum growth stage where the protein would be high and the NDF low.

Table 2.1 below shows the chemical composition of different soya bean cultivars. These cultivars have CP ranging from 153 g/kg to 205 g/kg this shows that CP content will differ according to cultivar. The wild soya bean in the study conducted by EunJa Lee *et al.*, (2014) had higher levels of protein, with an average protein content of 465.4 g/kg and the growth stages had an average protein content of 350.6 g/kg. Higher than average protein content may be due to the application of fertilizers used for wild soya bean. Wild soya bean is reported to have higher (44.9%) protein content than normal soya beans.

Table 2.1. Chemical composition of soya bean forage harvested at different growth stages

Stage	CP	NDF	ADF	ADL	EE
Blooming	201	386	282	5.9	-
Podding	181	431	319	6.6	-
Seeding	182	457	337	7.1	0.9
Mature	192	407	297	6.2	105

CP; Crude protein, neutral detergent fiber (NDF) acid detergent fiber (ADF) acid detergent lignin (ADL) ether extract (EE) (Source: Hintz, *et al.*, 1992)

EunJa Lee *et al., (*2014) reported an increase of CP from 179 g/kg in pre-anthesis stage to 213 g/kg in post-anthesis stage for cultivar G. max, however for cultivar G. *soja*the protein content increased from pre-anthesis to anthesis then decrease at post-anthesis stage. This shows that the growth stage had effect on the CP content. As the plant matures the CP content increases for cultivar G. *max*but decreases for cultivar G. *soja*. This trend may indicate that cultivar G. *soja* reach maturity faster than cultivar G. *max.*

Devine and Hatley (1998) conducted similar experiment to that of the current study. The soya bean cultivars that was used in their study were; Derry, Donegal, Tyrone, Corsoy and Pella. Devine and Hatley (1998) estimated the average concentration of crude protein (CP) in five different Soya bean cultivars to be 153 g/kg for cultivar, Derry, 162 g/kg for cultivar Donegal, 165 g/kg for cultivar Tyrone, 205 g/kg for cultivar Corsoy, 190 g/kg for cultivar Pella and 187 g/kg for cultivar Williams.

Peiretti, *et al.*, (2017) did a study on chemical composition of soya bean at different growth stages. For pre-anthesis growth stage, 197.7 g/kg was observed for DM, 96.5 g/kg for Ash, 228.5 g/kg for CP, 454.2 g/kg for NDF and 371.8 g/kg for ADF. For anthesis growth stage, 181.8 g/kg was for DM, 92.5 g/kg for Ash, 205.7 g/kg for CP, and 592.2 g/kg for ADF. Post-anthesis growth stage, 199.8 g/kg was observed for DM, 95.8 g/kg for Ash, 153.9 g/kg for CP, 662.7 g/kg for NDF and 425.4 g/kg for ADF. When comparing the different growth stages for DM, post-anthesis growth stage had higher level and anthesis growth stage had lower levels. For CP, anthesis growth stage had higher level compared to the other two growth stages. Higher NDF and ADF levels were on post-anthesis growth stage. DM, NDF and ADF increases with growth stages while CP and Ash decreases.

There is not enough information regarding the cultivars in the current study. However for cultivar PAN, the developer company of the seeds reported top performance rankings in all production regions over the past five years (Pannar, 2016; Jarvie, 2020). The developer reported that there are three different PAN cultivars (PAN1532R, PAN1521R and PAN 1623R) that are developed for early, medium and late growing season respectively (Pannar, 2016). Cultivar PAN has dominated the industry in South Africa and their seeds are good because they mix old and new cultivar when developing a new breed (Jarvie, 2020).

A study was conducted by Ikeogu and Nwofia (2013) in 2009 and 2010 for different soya beans cultivars of TGX (1440, 1448, 1485, 1835 and 1910). The cultivars were classified according to maturity (early, medium and late maturing) with 1440, 1448 and 1910 being medium; 1485 and 1835 being early maturing. There was a significant difference in yield and number of seeds in a plant within the genotype. Yields ranged from 1128.35 kg/ha to 1880.97 in 2009 with medium maturing cultivar TGX 1910 with higher (1880.97 kg/ha) biomass yield. When the same cultivars were planted again the following year, different results were obtained. And it was observed that the yields were lower than that of the previous year, the yields of 2010 ranged from 461.25 kg/ha to 992.59 kg/ha. It was then concluded that the difference were due to change in soil and weather conditions. The chemical composition of these cultivars were not determined in their study.

Minerals are chemical compounds that occur naturally in pure form. They are essential in plants, animals and people. These minerals are required in different quantities in all living organisms depending on their age. Soya bean is rich in minerals such as Iron, Zinc and copper (Ogbemudia, 2018). Most of these minerals are well known and are essential in the formation of red blood cells, bones and teeth. In SA, there has been report on mineral deficiency during wet season (McDowell, 2012). This is because during wet season, livestock gain weight rapidly since energy and protein supplies are adequate and thus mineral requirements are high (McDowell *et al.*, 2012). Concentration of minerals in the plant depends on; soil, plant species and stage of maturity. All the other minerals (calcium, phosphorus, sulfur, magnesium) get them from the forages (McDowell *et al.*, 2012). Minerals for livestock are not supplemented except for salt; they get all the other minerals from forages (McDowell *et al.*, 2012). For grazing livestock, the prevalent mineral deficiency throughout the world is the lack of phosphorus (P) with reports of deficiencies in at least 38 tropical developing countries (McDowell *et al.,* 2012). Minerals are often classed as macro minerals Calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K) and sodium (Na). Others that are required in much smaller amounts are known as micro minerals or trace elements zinc (Zn), copper (Cu), manganese (Mn) andiron (Fe). Soya beans contain 277 g/kg Ca, 1.66 mg/kg Cu, 15.7 mg/kg Fe, 280 g/kg Mg, 704 g/kg P and 4.89 mg/kg Zn. The mineral content observed by Gibson and Mullen (2001) were 3.67g/kg Ca, 51.8 g/kg Mn, 35.7 g/kg Na, 8.85 g/kg P, 25.16 g/kg K, 3.67 g/kg Ca and 3.77 g/kg Mg.

2.6. *In sacco* rumen degradation

The ruminants require energy for maintenance and production (Chumpawadee *et al.*, 2005). The rate and extent of degradation of different feed sources is largely different (Chanjula *et al.*, 2003). This is because the rate and extend of ruminal fermentation depends on the substrates, which depend on type feed and degree of processing (Arieli *et al.,* 1995). Protein degradability is an important factor when assessing the value of feedstuffs (Visagie, 2010). Rumen degradation of protein is regarded a major descriptor of forage quality (Øskov and Mc'Donald, 1979). Important characteristics of forage digestion in the rumen are the effective degradability, which is a function of the rate of nutrient digestion, including the digestion of DM, organic matter, protein, fibre and minerals (Larbi *et al.,* 1997).

2.6.1. The nylon bag technique

The *in sacco* nylon bag technique is widely used to estimate the rate and extend of degradation and digestion of feed in the rumen (Øskov and Mc'Donald 1979). The size of bags micro pores is important to prevent feed from falling out and allow bacteria to enter the bags. Due to the small feed sample, the test feed will not affect the rumen fermentation, while the conditions within the bag assumed to be like the rumen content (Mahwasane, 2018).

2.6.2. Soybean forage degradation kinetics

Legumes can produce their own nitrogen if they are inoculated with the proper *Rhizobium* bacterium at planting (Bruce, 2008). Inoculated forage legume plants can meet their own nitrogen fertilization needs as well as those associated with pasture grasses. The rhizobium bacterium is found in the soil but many soils do not normally have enough of this rhizobium to form nodules naturally (Grossman *et al.,* 2011). Many top quality legumes are pre-inoculated with a seed coating that contain rhizobium bacteria to ensure good nodule formation (Grossman *et al.,* 2011). When the seed is inoculated, soil type will not have effect on the forage quality and yield; therefore the seed will do well in almost all soil types (Mothapo *et al.,* 2011). Inoculation is recommended when the field has no past history of growth of legumes or when there is a high value crop to ensure successful growth (Grossman *et al.,* 2011). Fields history that includes legumes can increase the soil rhizobium population and ultimately improve nodulation (Mothapo *et al.,* 2011). Proper plant growth ensures high nutritive compositions and inoculated seeds produce higher biomass and plant tissue N (Mothapo *et al.,* 2011)

In few studies available of forage soya bean, less attention has been paid to assessment of ruminal degradability. The knowledge of degradation in the rumen is very important because the rate of degradation level can give an idea of how the quality of individual feed is reflected (Rambau, 2016). However, the digestibility of forage soya beans differs due to cultivars and environmental condition, including temperature, light intensity, total rainfall, and stage of maturity (Kasuya *et al*., 2008)

Peiretti *et al.,* (2018) reported dry matter soluble fraction of soya bean silage of 338 g/kg which was lower than that reported by Mustafa and Seguin (2003) (450 g/kg). Peiretti *et al.,* (2018) reported higher soluble fraction 'b' (395 g/kg) than that reported by Mustafa and Seguin (2003) (373 g/kg). The degradability dry matter of Mustafa and Seguin was relatively higher than that of Peiretti *et al.,* (2018). For degradability crude protein , Peiretti *et al.,* (2018) had higher level of soluble fraction 'a' (601 g/kg) compared to that of Mustafa and Segun (2003) (594 g/kg). Mustafa and Seguin (2003) reported higher soluble fraction 'b' (320 g/kg) when compared to the results reported by Peiretti *et al.,* (2018) of (304 g/kg). The main cause in variation of the two studies may be attributed by the type of soil. The study carried out by Peiretti *et al.,* (2018) was done in sandy soil while that done by Mustafa and Seguin (2003) was in loamy soil. Another factor that may have contributed to variation in the results was different drying methods used. Peiretti *et al.,* (2018) dried the sample in the forced draft oven. Mustafa and Seguin (2003) placed the sample in the sun to wilt to get 30% DM.

2.7. *In vitro* post ruminal digestion

The digestibility of a feed determines the amount that is absorbed by an animal and therefore the availability of nutrients for growth and reproduction (FAO, 2013). Digestibility data can offer an insight into the proper feeding and management of animals (Awoke, 2018).The mobile bag technique is usually used to measure crude protein digestibility. Because protein may be degraded in the rumen, less digestible dietary protein reaches the abomasum and subsequently the small intestine.

The use of estimates of intestinal digestion in combination with estimates of protein degradation in the rumen may provide estimated values of intestinally absorbable dietary protein derived from individual ingredients (Calsamiglia and Stern, 1995). Feed intake is relatively more important than digestibility in determining overall nutritive value because highly digestible feeds are of little value (Awoke, 2018). Forage with Low NDF content is highly digestible and forage with high lignin contents are digested slowly (Goodchild, 1994). However, digestibility usually provides a reliable index of nutritive value because more digestible feeds are normally consumed to a greater extent than less digestible feeds. Unlike the fibrous fractions that are fermented more slowly and retained in the rumen longer, forages with high NDF digestibility are degraded more rapidly in the rumen which will allow for faster passage and disappearance from the rumen (Oba and Allen, 2000).

Forage soya bean cultivars selected for late maturity have 590 to 640 g/kg *in vitro* OM digestibility and 150 to 180 g/kg CP (Sollenberger *et al.*, 2003). Similar results are reported for forage soya bean were *in vitro* OM digestibility ranged from 470 to 600 g/kg and CP concentration from 114 to 189 g/kg (Mislevy *et al.*, 2005).Heitholt *et al.*, (2004) reported that soya beans harvested at full seed has optimal quality with an average *in vitro* DM digestibility between 71.9 and 75.7%. Acikgoz *et al*., (1998) reported that as the crop matures, the *in vitro* DM digestibility decreases from 750 g/kg vegetative stage 500 to 583 g/kg full seed stage. Seiter *et al*., (2004) reported a mean content of 133 g/kg CP, 820 g/kg degradable protein and 606 g/kg in digestibility of soya bean.

Approximately 31% of untreated soya bean meal protein disappeared almost immediately from the rumen (Mir *et al.,* 1984). No data was available for forage soya bean. The evaluation is important as the protein provided for the animal may not be available for the animal to absorb and utilize in the small intestine. Most of the soya bean meal used in other study where only available in the abomasum and small intestine only if it was treated with heat or other protein inhibitors. Because naturally soya bean have tannins, the current study assumed that there will be amino acids in the abomasum and small intestine due to the tannins present in the soya beans.

Amino acids absorbed from the small intestine of ruminants animals are supplied by microbial protein synthesized in the rumen, undegraded or protected food proteins and amino acids which bypass the rumen (Walli *et al.,* 1995). Probably little can be done to influence directly the amino acids, but amino acids supplied by microbial protein and materials which bypass the rumen can be controlled (Walli *et al.,* 1995). Bacterial proteases are constitutive enzymes which do not appear to be subject to metabolic control. Therefore the enzymatic machinery necessary for ruminal degradation can be anticipated to be present under most conditions (Sampath *et al.,* 1993). Proteolytic enzymes enable rumen protozoa to digest bacterial protein which is the major source of amino acids for growth of these microbes the best methods to protect protein is chemical reagents or heat treatment to reduce the degradability of highly degradable proteins in the rumen (Sampath *et al.,* 1993). Some methods used to treat soya bean meal are extrusion, roasting, expeller, lignosulfonate and formaldehyde has been successfully used to protect it from ruminal degradation (Tice *et al.,* 1993). With regard to heat treatment, the temperature and period of treatment is critical. Heat treatment of soya bean meal at 150 ^{0}c for two hours seems to give sufficient protection (Walli *et al.,* 1995). The heat treatment may be costly because of equipments and electricity.

Tannins are also available in soya beans, and their presence depends on the sensitivity of the bond to the PH (Jones and Hayward, 1975). On the rumen, the pH is normal and therefore the bond does not break (Jones and Hayward, 1975). But in the abomasums the pH is low which break the bond and the protein is released (Jones and Hayward, 1975). Thus the protein is effectively protected from degradation in the rumen but becomes available in the abomasums and small intestine (Jones and Hayward, 1975).

The in vitro methods of feeds have numerous advantages over in vivo methods. They are less expensive, less time consuming, and allow incubation to be maintained more precisely than the in vivo. In vivo techniques utilize small amount of test feed making them applicable to screening feeds that are not available in sufficient quantity for I vivo experiments. The in vitro gas production system help to better quantify nutrient utilization, and its accuracy in describing digestibility in animals has been validated in numerous experiments.

2.8 Summary

Soya bean is not widely used as forage crop. Because it is popularly used as an oil seed, there is limited information regarding forage soya beans. Hence the proposed chemical composition, degradability and digestibility of forage soya beans for different cultivars at different growth stages can provide information on different forage soya beans. For regular supply of feed rich in protein throughout the year as the forage soya beans can be planted in summer and winter. The evidence of other studies showed that harvesting after the forage soya beans have bloomed may increase yield and nutritive value. The effectiveness of growth stage and cultivar to alter the chemical composition, ruminal degradability and digestibility is limited and inconsistent in the literature. Therefore further investigation is necessary to determine the effects of growth stage and growth stage of different forage soya beans and chemical composition, ruminal degradability and in vitro digestibility.

CHAPTER 3 CHEMICAL COMPOSITION OF SELECTED SOYA BEAN CULTIVARS HARVESTED AT DIFFERENT GROWTH STAGES

Abstract

The objectives of the study were to determine the chemical composition of three tri-folate soya bean cultivars harvested at different growth stages. Soya beans were planted in a shaded house in 63* 25L pots which were randomly arranged in a 3 (cultivars; locally denoted 4-LF, PAN and TGX)) by 3 (stage of harvest; pre-anthesis, anthesis, post-anthesis) factorial design. Standard procedures were used for triplicate forage sample analyses for dry matter (DM), crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF), calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K), iron (Fe) sodium (Na), zinc (Zn), copper (Cu), and manganese (Mn). Cultivar PAN harvested at post anthesis had significantly higher ($p< 0.05$) CP than others. The CP content increased ($p <0.0.5$) with growth stage. Cultivar 4-LF harvested postanthesis had significantly highest ($p< 0.05$) ADF. ADF content is increasing ($p< 0.05$) with growth stages. There was significant interaction ($p< 0.05$) of cultivar and growth stage on DM, ash, CP, NDF, and ADF. Cultivar PAN harvested pre-anthesis had significantly highest ($p< 0.05$) Mg. Cultivar PAN harvested pre-anthesis had significantly highest ($p< 0.05$) K. There was significant interaction ($p < 0.05$) of cultivar ^{x} growth stage on Ca, Mg, K, P, Mn and Fe. Cultivar had significant effect ($p< 0.05$) on Ca, K, P, Mn and Fe. Stage in the mineral composition significantly affected ($p< 0.05$) minerals except for Zn and Cu. In conclusion, chemical composition of all soya bean cultivars increased with growth stages.

Keywords Soya bean, Forage, nutrients, cultivar, chemical composition

3.1. Introduction

Dry matter is what remains when water (moisture) is removed from the feed (Heguy, 2015). Cattle and sheep generally consume between 2-3% of their live weight in DM daily (Singhal *et al.*, 2005). Factors that affect DM intake included the following: amount of water, digestibility, neutral detergent fibre (NDF), Nitrogen (Rumen Degradable Protein), sodium, and palatability (Barber *et al.*, 2010). Changes in the weight of a feed due to changes in moisture alter the nutrient concentrations supplied to the animal.

Animals either have macro-minerals present at large in their body or require them in large amount in the diets than the micro-minerals which are often referred to as trace minerals because they are present at low levels in the body or require a small amount (Costa *et al.*, 2015). Legumes tend to have more calcium and phosphorus than grass (Costa *et al.*, 2015). Young dark green forage tends to have more minerals than old, dry and yellow forages (Liz *et al.,* 2014).

Forages provide a good alternative to grain and seed crops because of greater water use efficiency and less susceptibility to potentially devastating yield reductions due to severe water stress during critical growth stages (Nielsen, 2011). Forage cultivar of soya beans uses less space compared to grain type of soya bean. Few studies have reported quality of forage soya bean (EunJa Lee *et al.,* 2014), hence this study aim at finding the quality of forage soya beans.

Each cultivar of soya beans has indeterminate growth habit (Raquel *et al.,* 2016). Either has good composition or high yield potential and optimal plant structure (Raquel *et al.,* 2016). Growth stage has effect on the chemical composition (Raquel *et al.,* 2016). Cultivar has no effect on chemical composition. (Rezende et al., 2011; Spanghero *et al.,* 2015 and Raquel *et al.,* 2016) suggested that soya bean should be harvested at full pod as it shows high chemical composition.

Soya bean has high levels of protein during each growth stage than grasses which quickly loses its nutrients content when matured (Aganga and Tswenyane, 2003). The protein in soya bean leaves suggests that they have the potential to be used as a protein supplement for ruminants.

The objective of the study was to evaluate chemical composition of 4LF, TGX, and PAN soya bean cultivars (locally denoted) harvested at pre-anthesis, anthesis and post-anthesis growth stages.

3.2. Materials and methods

3.2.2. Description of the Study Area

The study was conducted at the University of Venda (22.9761º S, 30.4465º E) in Thohoyandou. The area is characterized by deep well-drained red clay soils (Soil Classification Working Group, 1991).The average maximum and minimum temperatures are 31ºC and 18ºC, respectively (Tadross *et al.*, 2005). Rainfall is highly seasonal with 95% occurring between October and March (M'marete, 2003), often with a mid-season dry spell during critical periods of growth (FAO, 2009). The plants were planted in the School of agriculture's shaded house and chemical analysis was done in the Animal Science laboratory.

3.2.1. Experimental design and soya bean forage production

The experimental was conducted in completely randomized, 3 (cultivar) by 3 (stage of harvest) design whereby three Soya bean cultivars (locally denoted as 4LF, TGX and PAN) were planted at 21 25l pots per cultivar and harvested at three (pre-anthesis, pre-anthesis, post-anthesis) growth stages. The planting date was [29 January 2019]. Soil was composited from different sites fertilized with 5g of NPK fertilizer per pot before planting. Eight seeds were planted in each pot and the pots were randomly placed in open, wire-net protected house for the entire experiment. Planting was done by poking 8 holes in the pot using the index finger for the seeds to be in same height, which is not too deep or too shallow. After planting, the plants were irrigated with 3.5 L of water per pot per day. There was no thinning done for the whole experiment. Harvesting was done on 11th March (pre-anthesis), 25th March (anthesis) and 8th April (post-anthesis). For chemical analyses one random plant from each pot of each cultivar was uprooted and the roots chopped at 25 mm for each growth state. Harvested plants were placed in brown bags and weighed fresh to determine moisture, and then oven dried before determination of the chemical components.

Figure 3.1 shows the randomized layout of the pots in the shaded house. Figure 3.2-4 shows the trifoliate leaf morphology of soya bean cultivars TGX, PAN and 4LF, respectively.

Figure 3.1: Arrangements of pots

Figure 3.2: Cultivar TGX

Figure 3.3: Cultivar PAN

Figure 3.4: Cultivar 4LF

3.2.2. Chemical analysis

The harvested samples were oven-dried at 60^{o}C for 48 hours and milled through a 1mm sieve. To determine dry matter (DM), the method of (AOAC, 2000: method number 976.05) was used. Ash was determined by igniting samples overnight at 550°C (AOAC, 2000: method 923.03). Nitrogen was analyzed using the Kjeldahl method (AOAC, 2000: method 984.13), and was converted to crude protein as N x 6.25. The neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents were determined using the method of Goering and Van Soest (1970). To determine minerals, soya bean plants were ashed in a muffle furnace at a temperature of 550°C overnight. The ashed materials were then digested in 1M nitric acid. Digested material was then made up to a volume of 100ml volumetric flask with distilled water and introduced to the auto sampler for analyses of calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K), iron (Fe), sodium (Na), zinc (Zn), copper (Cu) and manganese (Mn). The inductively coupled plasma optical emission spectrometry (ICP-OES) followed by Standard Operating Procedure (SOP) (2005).

3.2.5. Statistical analysis

Chemical composition data was analyzed using Two Way analysis of variance (ANOVA) for a 3x3 factorial experiment using the General Linear Model (GLM) procedures of MINITAB software version 19 (2019). The statistical analysis model that was used is as follows:

$$Y_{ijk} = \mu + S_i + G_j + (SG)_{ij} + \varepsilon_{ijk}$$

Where;

Y_{ijk}= the kth observation on the ith cultivar and jthstage of growth at harvesting µ
= the overall mean
G_i=effect of the jth cultivar
S_j= effect of the ith growth stage at harvesting
SG_{ij}= the interaction between the ithcultivar and jth stage of growth at harvesting
ε_{ijk}= random error

Where the main effects were found to be significant, treatment means were compared using the Tukey's test at P<0.05

3.3. Results

3.3.1 Chemical composition of soya bean at different growth stages

The organic nutrient composition of the 3 cultivars of soya bean at different growth stages are presented in Table 3.1. There was significant interaction ($p < 0.05$) of the cultivar ^{X} growth stage on DM, ash, CP, NDF, and ADF. Cultivar had no significant effect ($p > 0.05$) on ash and CP. Growth stage significantly affected ($p < 0.05$) DM, ash, CP, NDF and ADF. All three cultivars harvested post-anthesis had significantly higher CP. Cultivar PAN harvested post-anthesis had significantly highest ($p < 0.05$) CP. The CP and ADF content increased ($P<0.05$) with growth stages. Cultivar 4LF harvest pre-anthesis had significantly highest ($p < 0.05$) NDF.

The mineral composition of the 3 cultivars of soya bean at different growth stages are presented in Table 3.2. There was significant interaction ($p < 0.05$) of the cultivar ^{X} growth stage on Ca, Mg, K, Na, P, Zn, Mn, Cu and Fe. Cultivar had significant effect ($p < 0.05$) on Ca, Mg, Na, P, Zn, Mn and Cu. Growth stage had significant effect ($p < 0.05$) on Ca, Mg, K, P, and Fe. The Ca, Mg, K, P, Mn, and Fe content decreased ($p > 0.05$) with growth stages. Cultivar PAN harvested pre-anthesis had significantly highest ($p < 0.05$) Mg.

Table 3.1:The mean chemical composition (g/kg DM)of Soya bean cultivar 4LF, TGX and PAN harvested at pre-anthesis, anthesis and post-anthesisgrowth stages.

	(g/kg)	(g/kg DM)

Pre-anthesis	4LF	3	909.8^{c}	99.1^{a}	189.1^{c}	574 .3^{a}	229.5^{c}
	TGX	3	911.5^{c}	99.1^{a}	180.2^{c}	443.5^{c}	199.1^{c}
	PAN	3	909.9^{c}	99.3^{a}	181.1^{c}	437.5^{c}	208.5^{c}
Anthesis	4LF	3	919.2^{a}	96.9^{b}	191.8^{b}	523.3^{b}	308.2^{b}
	TGX	3	921.3^{a}	97.7^{b}	189.1^{b}	475.1^{b}	238.8^{b}
	PAN	3	920.1^{a}	95.1^{b}	187.7^{b}	449.2^{b}	220.1^{b}
Post-anthesis	4LF	3	913.2^{b}	81.2^{c}	198.8^{a}	467.7^{c}	381.1^{a}
	TGX	3	918.3^{b}	85.9^{c}	195.2^{a}	563.7^{a}	299.5^{a}
	PAN	3	915.9^{b}	84.6^{c}	214.5^{a}	563.4^{a}	339.2^{a}
SEM			14.04	1.32	5.5	24.84	15.37
Cultivar mean							
	4LF	9	914.1^{c}	92.6	193.2	521.8^{a}	306.3^{a}
	TGX	9	915.3^{b}	93.0	194.2	483.3^{b}	255.9^{b}
	PAN	9	917.0^{a}	94.2	188.2	495.2^{b}	245.7^{b}
SEM			7.02	0.66	2.75	12.42	7.69
Stage means							
Pre-anthesis		9	910.4^{c}	99.4^{a}	183.3^{b}	485.1^{c}	212.4^{c}
Anthesis		9	920.2^{a}	96.6^{b}	189.5^{b}	482.5^{b}	255.7^{b}
Post-anthesis		9	915.8^{b}	83.9^{c}	202.8^{a}	531.6^{a}	339.9^{a}
SEM			9.02	0.81	3.33	14.25	9.64
Significance							
Cultivar			**	NS	NS	*	*
Stage			**	**	*	**	**
cultivar×stage			**	**	**	**	**

**: *P* <0.01; *: *P* <0.05; (ns) not significant: *P* >0.05. ^{abc}For different effects, means with different superscripts differ significantly at *P* <0.05. DM: Dry Matter, CP: Crude Protein, NDF: Neutral Detergent Fibre, ADF: Acid Detergent Fibre, N: Number of Replications, g/kg: Grams per Kilogram, g/kg DM: Grams per Kilogram Dry Matter and SEM: Standard Error Mean.

Table 3.2 Effect of cultivars at different growth stages macro-(g kg$_{-1}$) and micro-(mg kg$_{-1}$) minerals of fresh soya bean.

Pre-anthesis	4LF	14.8^{a}	6.1_{b}	28.0^{a}	0.3_{a}	3.8_{a}	65.5^{a}	98.0^{a}	6.5_{b}	260.0^{b}
	TGX	15.0^{a}	6.0_{b}	28.5^{a}	0.4_{a}	4.1_{a}	62.5^{b}	72.5^{c}	6.5_{b}	321.5^{b}
	PAN	12.7^{b}	8.0_{a}	30.5^{a}	0.3_{a}	4.1_{a}	64.0^{a}	72.0^{c}	9.0_{a}	418.0^{b}
Anthesis	4LF	13.6^{b}	5.5_{b}	26.6^{b}	0.2_{a}	3.6_{b}	65.0^{a}	91.0^{a}	6.5_{b}	486.5^{a}
	TGX	13.1^{b}	5.3_{b}	25.1^{b}	0.3_{a}	4.0_{a}	60.5^{c}	72.0^{c}	7.0_{a}	364.5^{b}
	PAN	12.4^{b}	6.1_{b}	26.1^{b}	0.2_{a}	4.1_{a}	63.0^{b}	72.0^{c}	8.5_{a}	446.5^{b}
Post-anthesis	4LF	10.6^{b}	4.2_{c}	19.1^{c}	0.0_{b}	3.2_{c}	64.0^{a}	78.5^{c}	6.5_{b}	606.5^{a}
	TGX	11.4^{c}	4.5_{c}	21.9^{c}	0.3_{a}	3.6_{b}	60.5^{c}	81.5^{b}	6.5_{b}	586.5^{a}
	PAN	10.9^{c}	4.5_{c}	21.7^{c}	0.1_{b}	4.2_{a}	64.5^{a}	81.5^{b}	7.5_{a}	512.5^{a}
SEM		10.6	0.42	1.02	0.09	0.09	1.88	0.68	3.3	26.81
				g/kg			Mg/kg			

Cultivar mean										
	4LF	12.9^{a}	5.2^{b}	24.5	0.02^{b}	0.36^{b}	64.83^{a}	89.17^{a}	6.50^{b}	451.00^{a}
	TGX	13.2^{a}	5.2^{b}	25.1	0.03^{a}	0.39^{a}	61.17^{b}	75.33^{b}	6.67^{b}	424.17^{a}
	PAN	11.9^{b}	6.6^{a}	26.1	0.02^{b}	0.41^{a}	63.83^{a}	71.83^{b}	8.33^{a}	459.00^{a}
SEM		0.53	0.21	0.51	0.05	0.05	0.94	0.34	1.65	13.41
Stage means										
Pre-anthesis		14.1^{a}	6.6_{a}	29.0^{a}	0.03	0.40^{a}	64.00	80.83	7.33	333.17^{b}
Anthesis		13.0^{b}	5.6_{b}	25.9^{b}	0.02	0.39^{a}	62.83	78.33	7.33	506.5^{a}
Post-anthesis		10.9^{c}	4.4_{c}	20.9^{c}	0.02	0.36^{b}	63.00	77.33	6.83	494.50^{a}
SEM		1.01	0.35	1.00	0.07	0.07	1.14	0.48	2.07	16.32
Significance										
Cultivar		*	*	NS	*	*	*	**	*	NS
Stage		**	**	**	NS	*	NS	NS	NS	**
cultivar×stage		**	**	**	*	**	**	**	*	**

**: P <0.01; *: P <0.05; (ns) not significant: P >0.05. ^{abc}For different effects column means, different superscripts differ significantly at P <0.05. Ca: Calcium, Mg: Magnesium, K: Potassium, Na: Sodium, P: Phosphorus, Zn: Zinc, Cu: Copper, Mn: Manganese, Fe: Iron, N: Number of Replications, g/kg DM: Grams per Kilogram Dry Matter, mg/kg DM: Milligrams per Kilogram Dry Matter and SEM: Standard Error Mean.

3.4. Discussion

Cultivar and growth stage had effect on the chemical composition of soya beans on DM, ash, CP, NDF, and ADF. DM is an important nutrient that plays a vital role when determining the quality of forage. Similar cultivar ^{X} growth stage interaction on DM, CP, NDF and ADF was observed by Hintz *et al.,* (1992). All three cultivars harvested post-anthesis had significantly higher CP as the CP was increasing with growth stages. This shows that soya bean increases its protein content as it grows. This may be attributed to the Rhizobial bacterial which will not form until when the roots have fully developed. In the study of EunJa Lee *et al.,* (2014), one cultivar was increasing with growth stage (179g/kg-213g/kg) and the other cultivar increased from pre-anthesis to anthesis and then decreased at post-anthesis. The increase and decrease may be attributed by environmental condition. Hintz *et al.,* (1992) reported a decrease (201g/kg-192) in CP with growth stages. The decrease may be attributed by the early maturing soya bean cultivars as the highest (201g/kg) CP content was recorded at pre-anthesis. Early maturing cultivar progresses through different stages at a faster rate, especially when planted late.

The ADF content in this study increased with growth stages. Similar results of increase (297g/kg315g/kg) in ADF content with growth stages was reported by Hintz *et al.,* (1992). EunJa Lee *et al.,* (2014), reported a decrease in ADF content with growth stage of soya bean cultivar G.soja. The ADF values found in this study were considerably lower than that 400–420 g/kg value reported by Sheaffer *et al*. (2001). The values of ADF reported by Seiter *et al*., (2004) consistently increase from about 300g/kg to about 370g/kg as growth stage increased from pre-anthesis to post-anthesis stage which was comparable to the findings of the current study. Seiter *et al.,* (2004) reported NDF increase (400g/kg-490g/kg) with growth stage increased. [Hintz *et al.,* (1992); EunJa Lee *et al.,* (2014)], reported an increase in NDF content with growth stage. NDF in the current study decreased from pre-anthesis to anthesis and then increased at post-anthesis. The fluctuation in the NDF content may be attributed by spacing, competition for establishment and water holding capacity of the pots.

The cultivars are very different as they are bred for certain environmental conditions and because soya beans are bred as either early, medium or late maturing which affects the nutrient content and forage quality. Because there is no information/study done with the same cultivar as those of the current study, there was no data to compare it with. However, based on the study of other soya bean cultivars, it can be concluded that the cultivars used in the current study are a good source of protein and quality forage. And the overall results of this study followed similar trends/ patterns with the study done with different cultivars

There was significant interaction ($p < 0.05$) of the cultivar ^{x} growth stage on Ca, Mg, K, Na, P, Zn, Mn, Cu and Fe. Cultivar had significant effect ($p < 0.05$) on Ca, Mg, Na, P, Zn, Mn and Cu. Growth stage had significant effect ($p < 0.05$) on Ca, Mg, K, P, Mn and Fe. The Ca, Mg, K, P, Mn, and Fe content decreased ($p > 0.05$) with growth stages. Cultivar PAN harvested pre-anthesis had significantly highest ($p < 0.05$) Mg.

Significant cultivar ^{x} growth stage interaction was observed on all mineral parameters. Cultivar inclusion had significant effect on Ca, Mg, Na, P, Zn, Mn and Cu. Cultivar PAN harvested preanthesis had higher Mg, K and Cu. Cultivar 4LF harvested at post-anthesis had significantly higher Fe than others. Stage inclusion in the mineral composition significantly affected minerals except for Zn and Cu.

The calcium in the current study (10.9-14.1 g/kg) is higher than the daily requirements for lactating cow (3.1 g/kg), dry cow (1.8 g/kg) and growing calves (5.0 g/kg) (NRC, 1996). Potassium of the current study is significantly high (20.9- 25.9 g/kg), six times higher than the daily requirements for lactating cow (2.1 g/kg), dry cow (1.6 g/kg) and growing calves (2.6 g/kg) (NRC, 1996). Sodium content of the current study is lower (4.1 g/kg) than the sodium required by lactating cow (6.0 g/kg), dry cow (6.0 g/kg) and growing calves (7.0 g/kg) (NRC, 1996). For micro-minerals, Zinc, Manganese and Iron levels in the current study are more than double of the daily requirements for lactating cows, dry cows and growing calves. Copper of the current study is lower than the daily requirement for lactating cow (100 mg/kg), dry cow (100 mg/kg) and growing calves (100 mg/kg) (NRC, 1996). Macro-minerals Ca, Mg, Na, P and micro-mineral Cu was decreasing as the plant matures. Although the Calcium, Magnesium and Phosphorus contents were decreasing with growth stages, it was high enough to meet the daily requirement for lactating cow, dry cow and growing calves. The opposite was the case for Sodium and Copper as they were lower than the daily requirements for lactating cows, dry cows and growing calves.

3.4. Conclusion

It can be concluded that the interaction of cultivar and growth stage influence the nutrient and mineral composition. As the plant develops to maturity, the CP, NDF and ADF increases. However minerals decrease as the plant matures. Although the nutritional composition changed with advancing maturity, the quality remained high throughout the growth stages.

CHAPTER 4: *IN SACCO* RUMINAL DRY MATTER AND PROTEIN DEGRADABILITY OF SELECTED FORAGE SOYA BEAN CULTIVARS HARVESTED AT DIFFERENT GROWTH STAGES

Abstract

Ruminal degradation of both dry matter and protein are important in determining the nutritional value of forages intended to be supplementary protein sources for ruminants on low quality forages. The objectives of the study were to determine the rumen degradability of dry matter and crude protein of three tri-folate soya bean cultivars harvested at different growth stages. Soya beans were planted in a shaded house in 63 * 25L pots which were randomly arranged in a 3 (cultivars; locally denoted 4-LF, PAN and TGX)) by 3 (stage of harvest; pre-anthesis, anthesis, post-anthesis) factorial design. Approximately 5 g samples were incubated in duplicate for 0, 6, 12, 24, 48, 72, 96 and 120 hour periods in the rumen of three Bonsmara steers. The residues were then analyzed for residual dry matter and nitrogen. Cultivar TGX harvested at pre-anthesis had significantly higher ($p < 0.05$) calculated at k=0.08 than others. Higher ($p<0.05$) rate constant 'c' was recorded for cultivar PAN at anthesis stage and cultivar 4LF at post-anthesis.There was no significant ($p > 0.05$) cultivar X growth stage for the soluble fraction 'a', potential degradable fraction 'b', 'a+b', effective degradability at 'k=0.02' and 'k=0.08'. The cultivar had no effect ($p > 0.05$) on all parameters.Cultivar TGX harvested at post-anthesis had significantly higher ($p < 0.05$) 'a' than the others. Cultivar 4LF harvested at anthesis had significantly lower ($p>0.05$) 'c' than others. Except for 'a' and k=0.08, growth stage inclusion had no effect on crude protein degradability.

Keywords: dry matter, crude protein, disappearance, cultivar

4.1 Introduction

Leguminous forage is of good quality as it has low cell wall and high protein, factors which promote higher consumption compared to other types of forage (Dwain *et al*, 1999). During winter, some plants are killed by winter frost, and the remaining natural crops and grass available for animals are usually fibrous. Therefore these crops and grass would not have enough nutrients required by the animal.

Knowledge of feed degradation in the rumen is important because the rate of degradation predicts the feed quality (Rambau *et al*, 2017). Evaluation of ruminal degradation provides a basis for formulating rations to meet the protein requirements of livestock (Ørskov, 2000).Ruminal degradability is influenced by many factors, mainly by the stage of maturity, forage species and preservation method (Elizalde *et al.*, 1999).

The *in sacco* technique is the technique most commonly used to estimate ruminal degradation of dietary protein (Seker and Can, 2012).

The study was conducted to determine the ruminal degradation of soya beans harvested at three different growth stages

4.2. Materials and method

4.2.1. Experimental site

The trial was carried out at the University of Venda Experimental farm and Animal Science Nutrition Laboratory (22.9761°S, 30.4465° E), Limpopo, South Africa, as described in chapter 3.

4.2.3. Ethical consideration

Ethical clearance for the study was granted by the Ethics Committee of the University of Venda (Project no: SARDF/19/ANS/03/1504)

4.2.4. Animals and management

Three Bonsmara steers fitted with rumen cannula of 8 cm internal diameter (ANKOM-flexible cattle purchased from Bar Diamond Inc., USA) were used to determine dry matter (DM) and nitrogen (N) degradability of selected soya bean cultivars. The animals were placed in a pen with clean water available during the whole experiment. The animals were fed with commercial complete cattle finisher diet *ad libitum*.

Table 4.1Chemical composition of commercial complete cattle finisher diet that was used in this study.

	g/kg
Composition	120
Protein (min)	
Calcium (max)	8
Phosphorus (min)	3
Moisture (max)	120
Fiber (max)	200
Fat (min):	25
Urea (max):	1.25
% Derived from Urea	29.9%
	mg/kg
Monensin NA	30
Zinc Bacitracin	50

Supplied by Driehoek feeds (Vaal water, Waterberg, Limpopo, South Africa)

4.2.6. Experimental design and rumen incubation

Samples were prepared from forage harvested as per experimental described in Chapter 3, which were replicated three times per treatment combination. The nylon bag technique of Ørskov and McDonald (1979) was used .Approximately 5g samples were weighed in labeled nylon bags (external dimension: 6 × 12 cm, pore size of 46 µm). For effective suspension in the rumen, the nylon bags were tied using plastic bands to a flexible vinyl plastic tube resistant to rumen micro organism, approximately 40 cm long and of 6 mm outer diameter. The nylon bags were then incubated in the rumen of 3 cannulated steers in duplicate each of 0, 6, 12, 24, 48, 72, 96 and 120 hours. The zero hour bags (control) was washed without incubation in the rumen.

At the end of incubation, the bags were removed from each animal and rinsed in cold tap water until clear. Washed bags were oven-dried at 60°C for 48 h, before weighing and chemical analyses. The bags were allowed to cool down in the desiccators when they were removed from the oven after drying them and weighed to determine the DM disappearance. The final residues were subsequently ground through a 1 mm sieve and analyzed for degradability in duplicates. The bags were inserted in the rumen at 07:30 h before the morning feeding time.

4.2.7. Mathematical calculations

The nutrient degradation was calculated by the difference between the amount in the control sample and degraded residues and expressed as percentages. The kinetics of degradation of DM and protein over time was described using the mathematical model of Ørskov and McDonald (1979), in which data was fitted the Neway "Fitcurve" Excel software version 6 (Chen, 1997), where;

$$P= a + b\,(1 - e^{-c\,(t-Lt)})$$

Where P is ruminal nutrient disappearance at time t,
a is the soluble fraction (%),
b is the potentially degradable fraction (%),
c is the rate of degradation of the b fraction (%/h) and Lt is the lag phase (h).

Potential degradability (PD) was estimated as (a+b), and effective degradability (ED) was calculated using rumen fractional outflow rates (k) of 0.02, 0.05 and 0.08 per hour according to Ørskov and McDonald (1979):

$$\mathrm{ED} = \mathrm{a} + \frac{\mathrm{bc}}{(\mathrm{k} + \mathrm{c})}$$

Where k is the ruminal outflow rate 0.02, 0.05 and 0.08, which describe low, medium and high feeding levels respectively.

4.2.8 Statistical analysis

All parameters were subjected to ANOVA using GLM procedures of MINITAB software version 17 (2014) based on the statistical model:

$Y_{ijkl} = \mu + S_i + G_j + (SG)_{ij} + \varepsilon_{ijkl}$

Where Y_{ijk} is the observation on the i^{th} cultivar, j^{th} growth stage μ
= Overall mean
S_i = Effect of i^{th}cultivar
G_j= Effect of j^{th} growth stage
ε_{ijkl}= Random error

A significant difference between the treatment means was compared using the Tukey's test at ($P < 0.05$).

4.3 Results

4.3.1 *In sacco* dry matter degradability

Degradability constants and calculated effective degradability at three passage rates for dry matter and disappearance of soya beans cultivars (4LF, TGX and PAN) harvested at preanthesis, anthesis and post-anthesis growth stages are presented in Table 4.2.There was significant ($p < 0.05$) cultivar ^{X} growth stage interaction on fraction 'a', 'b', 'a+b', 'k=0.02' and 'k=0.05' with no effect on 'c' and 'k=0.08'. Cultivar had significant ($p < 0.05$) effect on fraction 'a', 'b', 'a+b' and no ($p > 0.05$) effect on effective degradability. Growth stage had no ($p > 0.05$) effect on fraction 'a' and 'c'. Fraction 'b', 'a+b', 'k=0.02', 'k=0.05' and 'k=0.08' are decreasing with growth stages. Fraction 'c' was constant for all the cultivars and growth stages. Effective degradability decreases as the outflow rate increases.

4.3.2 *In sacco* crude protein degradability

Degradability constants and calculated effective degradability at three passage rates for crude protein and disappearance of soya beans cultivars (4LF, TGX and PAN) harvested at different growth stages is presented in Table 4.3. There was significant ($p < 0.05$) cultivar ^{X} growth stage interaction on fraction 'a', 'k=0.02', 'k=0.05' and 'k=0.08' with no effect on 'c', 'b' and 'a+b'. Cultivar had significant ($p < 0.05$) effect on fraction 'a', 'k=0.02', 'k=0.05' and 'k=0.08'. Growth stage had significant ($p < 0.05$) effect on fraction 'a', 'k=0.02' and 'k=0.05'. Cultivar TGX harvested postanthesis had significantly highest ($p < 0.05$) fraction ‘a’. Fraction ‘b’ and ‘a+b’ decreased with growth stages. Fraction ‘a’, ‘c’, ‘k=0.02’, ‘k=0.05’ and ‘k=0.08 increased from pre-anthesis to anthesis, then decreased from anthesis to post-anthesis.

Table 4.2:Degradability constants (a, b and c)and calculated effective degradability at three passage rates for dry matter and disappearance of soya beans cultivars (4LF, TGX and PAN) harvested at pre-anthesis, anthesis and post-anthesis growth stages.

			a	b	a+b	c	K=0.02	K=0.05	K=0.08
Pre-anthesis	4LF	6	114.4^{a}	402.0^{a}	516.4^{a}	0.4	384.7^{a}	297.7^{a}	250.3
	TGX	6	122.1^{a}	403.2^{a}	525.4^{a}	0.4	388.5^{a}	299.0^{a}	244.7
	PAN	6	114.1^{a}	435.7^{a}	549.8^{a}	0.4	389.7^{a}	292.9^{a}	246.6
Anthesis	4LF	6	112.7^{a}	392.1^{a}	504.8^{b}	0.4	378.1^{a}	289.0^{a}	245.1
	TGX	6	132.0^{a}	410.2^{a}	542.2^{a}	0.4	370.0^{a}	283.1^{a}	240.4
	PAN	6	97.4^{c}	382.7^{b}	480.2^{c}	0.4	369.7^{a}	286.8^{a}	241.9
Post-anthesis	4LF	6	116.1^{a}	365.4^{c}	481.5^{c}	0.4	366.4^{b}	291.1^{a}	242.6
	TGX	6	133.4^{a}	399.2^{a}	532.6^{a}	0.4	361.1^{c}	284.2^{a}	234.6
	PAN	6	102.6^{b}	393.2^{a}	495.8^{c}	0.5	363.9^{c}	281.7^{b}	239.6
SEM			1.68	1.55	1.62	0.081	1.53	1.13	0.93
Cultivar mean									
	4LF	18	114.4^{b}	386.5^{b}	500.9^{b}	0.4	376.4	292.6	246.0
	TGX	18	129.1^{a}	404.2^{a}	533.4^{a}	0.4	373.2	288.8	241.1
	PAN	18	104.7^{c}	403.9^{a}	508.6^{b}	0.4	374.4	287.1	242.7
SEM			0.84	0.78	0.81	0.041	0.77	0.57	0.47
Stage means									
Pre-anthesis		18	116.8	413.6^{a}	530.5^{a}	0.4	387.6^{a}	296.5^{a}	247.2^{a}
Anthesis		18	114.0	395.0^{b}	509.1^{b}	0.4	372.6^{b}	286.3^{b}	243.7^{a}
Post-anthesis		18	117.3	385.9^{b}	503.3^{b}	0.4	363.8^{c}	285.7^{b}	238.9^{b}
SEM			0.95	0.99	1.00	0.063	0.89	0.78	0.80
Significance									
Cultivar			**	*	*	NS	NS	NS	NS
Stage			NS	*	*	NS	**	*	*
cultivar×stage			**	**	**	NS	*	*	NS

**: *P* <0.01; *: *P* <0.05; (ns) non-significant: *P* >0.05. ^{abc}For different effects, means for the different parameters with different superscripts differ significantly at *P* <0.05. *a*: soluble fraction, *b*: insoluble but potentially degradable fraction, *a* + *b*: Potential degradability, *c*: degradation rate constant (h_{-1}), ED: effective degradability, *K*: rumen outflow rate (h_{-1}), N: Number of Replications, g/kg: grams per kilograms and SEM: Standard Error Mean.

Table 4.3:Degradability constants (a, b and c) and calculated effective degradability at three passage rates for crude protein and disappearance of soya beans cultivars harvested at different growth stages.

			a	b	a+b	c	K=0.02	K=0.05	K=0.08
Pre-anthesis	4LF	6	169.6^{b}	226.5	386.2	0.5_{a}	207.8^{a}	188.4^{b}	182.2^{b}
	TGX	6	174.8^{b}	38.2	217.5	0.9_{a}	205.5^{a}	196.4^{a}	192.8^{a}
	PAN	6	163.9^{b}	57.4	221.4	0.3_{b}	199.1^{b}	186.3^{b}	180.4^{b}
Anthesis	4LF	6	168.3^{b}	57.6	226.0	0.4_{b}	204.7^{a}	191.8^{a}	185.7^{b}
	TGX	6	193.1^{a}	88.8	281.9	0.2_{b}	209.4^{a}	202.9^{a}	196.7^{a}
	PAN	6	166.7^{b}	49.1	215.8	1.6_{a}	206.2^{a}	197.4^{a}	191.9^{a}
Post-anthesis	4LF	6	169.2^{b}	55.2	224.4	0.5_{a}	207.6^{a}	192.5^{a}	181.1^{b}
	TGX	6	190.2^{a}	58.4	248.6	0.5_{a}	205.0^{a}	199.6^{a}	190.3^{a}
	PAN	6	168.4^{b}	55.3	220.0	0.6_{a}	201.5^{a}	190.9^{a}	184.3^{b}
SEM			0.91	1.84	1.36	0.029	0.75	0.63	0.56
<u>Cultivar mean</u>									
	4LF	18	169.0^{b}	113.1	278.8	0.5	206.7^{a}	190.9^{b}	183.0^{b}
	TGX	18	186.0^{a}	61.8	249.3	0.5	206.6^{a}	199.6^{a}	193.2^{a}
	PAN	18	166.3^{b}	53.9	219.1	0.8	202.2^{b}	191.5^{b}	185.5^{b}
SEM			0.46	0.92	0.68	0.015	0.38	0.32	0.28
<u>Stage means</u>									
Pre-anthesis		18	169.4^{b}	107.3	275.0	0.5	204.1	190.4^{b}	185.1^{b}
Anthesis		18	176.0^{a}	65.2	241.3	0.7	206.8	197.4^{a}	191.4^{a}
Post-anthesis		18	175.9^{a}	56.3	231.0	0.5	204.7	194.3^{a}	185.2^{b}
SEM			0.72	1.03	0.83	0.03	0.65	0.51	0.44
Significance									
Cultivar			*	NS	NS	NS	*	*	*
Stage			**	NS	NS	NS	NS	*	*
cultivar×stage			**	NS	NS	**	*	*	**

**: *P* <0.01; *: *P* <0.05; (ns) non-significant: *P* >0.05. $_{abc}$Column means with different superscripts differ significantly at *P* <0.05. *a*: soluble fraction, *b*: insoluble but potentially degradable fraction, *a* +

b: Potential degradability, *c*: degradation rate constant (h_{-1}), ED: effective degradability, *K*: rumen outflow rate (h_{-1}), N: Number of Replications, g/kg: grams per kilograms and SEM: Standard Error Mean.

4.4. DISCUSSION

There was significant ($p< 0.05$) cultivar ^{X} growth stage interaction on fraction ‘a’, ‘b’, ‘a+b’, ‘c’, ‘k=0.02’ and ‘k=0.05’ with no effect on k=0.08. Cultivar had significant ($p< 0.05$) effect on degradability and no ($p> 0.05$) effect on effective degradability. Growth stage had no ($p> 0.05$) effect on fraction ‘a’ and ‘c’. Fraction ‘b’, ‘a+b’, ‘k=0.02’, ‘k=0.05’ and ‘k=0.08’ are decreasing with growth stages. Fraction ‘c’ was constant for all the cultivars and growth stages. Effective degradability decreases as the outflow rate increases

Higher degradation rate constant ‘c’ was recorded for cultivar PAN at anthesis stage and cultivar 4LF at post-anthesis. Cultivar TGX harvested at pre-anthesis had significantly higher k=0.08 than others. Interaction of cultivar and growth stage had no effect on soluble fraction ‘a’, ‘b’, ‘a+b’, effective degradability ‘k=0.02 and k=0.05. Growth stage inclusion significantly affect ($p< 0.05$) dry matter degradation rate constant for ‘c’ and k=0.08. Mustafa and Seguin (2003) reported higher soluble fraction ‘a’ 450 g/kg for silage soya bean for dry matter and lower 373 g/kg potential degradable DM fraction. However the current study reported lower soluble fraction ‘a’ and higher potential degradability fraction ‘b’ value than that reported by Mustafa and Seguin (2003). The low potential degradable DM (373 g/kg) reported by Mustafa and Seguin (2003) is similar to that of cultivar 4LF at post-anthesis growth stage.

Leguminous species have lower levels of condensed tannins (Rolando, 1999). Protein degradability is decreased by tannins which complex with feed protein and have the capability to reduce the activities of rumen microbes and to interact with proteins and carbohydrates (Salawu *et al.,* 1997). Plant cell walls are the major source of dietary fibre for animals. Polysaccharides in cell walls cannot be degraded by mammalian enzymes. Instead, animals depend on microbial fermentation, and ruminants are especially well-adapted for using plant fibre for energy.

The fraction 'a' represents that there is a component that is being degraded rapidly and/or a soluble component. Einar *et al.,* (2008) had higher level of soluble fraction ‘a’ (338 g/kg) on DMD compared to the soluble fraction ‘a’ in the current study (118.5 g/kg), this means that the findings in the current study was slowly degraded compared to that of Einar *et al*., (2008). However, their finding of potential degradable fraction ‘b’ (395 g/kg) was comparable to that of the current study at post-anthesis growth stage (391 g/kg). Mustafa and Senguin, (2003) also recorded a higher level of soluble fraction ‘a’ (450 g/kg) compared to that of the current study. But the potential degradable fraction ‘b’ was lower (373 g/kg) than that of the current study. Similar trends were observed for CPD when comparing the findings of the current study with that of Einar *et al.,* (2008) and Mustafa and Senguin, (2003). This may be due to the silage soya beans used by both Einar *et al.,* (2008) and Mustafa and Senguin, (2003), which resulted in an increased soluble fraction 'a' with similar findings observed for other soluble fractions of silage soya bean and soya bean.

Dry matter disappearance for soluble fractions increased significantly over time with a decrease in effective degradability. As the period of incubation increases, more ruminal microbes were able to penetrate the nylon bags and speed up the degradation process resulting in fewer samples that was not utilized. Dry matter disappearance from different soya bean cultivars in this study was above 500 g/kg after 48hrs incubation. Ørskov and Mc'Donald (1987) suggested that degradability values after 48hrs of incubation mean a high rate of degradability. This is because degradability after 48hrs is regarded as equal to digestibility. The soluble DM contents indicate the potential to be good sources of more nutrients for microbial growth.

4.4.1. Crude protein degradability

There was no significant effect in cultivar and growth stage interaction except for potential degradable fraction 'b', 'a+b', and k=0.02. Cultivar had no effect on crude protein degradability except for 'a' and k=0.08. Cultivar TGX harvested at post-anthesis had significantly higher 'a' than the others. Cultivar 4LF harvested at anthesis had significantly lower 'c' than others. Except for 'a' and k=0.08, growth stage inclusion had no effect on crude protein degradability. Mustafa and Seguin (2003) reported higher soluble fraction 'a' 594 g/kg for silage soya bean for crude protein and high crude protein degradability 320 g/kg. The current study reported lower CP soluble fraction 'a' however it reported similar potential degradability with cultivar 4LF and TGX at preanthesis growth stage when compared to that of Mustafa and Seguin (2003).

Cultivar 4LF had lower levels of soluble fraction 'c' at anthesis growth stage. Einar *et al.,* (2008) had higher level of soluble fraction 'a' 601 g/kg on CPD compared to the soluble fraction 'a' in the current study (180.2 g/kg). However, their finding of soluble fraction 'b' (304 g/kg) was lower than that of cultivar 4LF and TGX of the current study at pre-anthesis growth stage (330 g/kg). Mustafa and Senguin, (2003) also recorded a higher level of soluble fraction 'a' (594 g/kg) compared to that of the current study. But the soluble fraction 'b' was lower (320 g/kg) than that of the current study. Overall the high soluble fraction in the study of [Einar *et al.,* (2008) Mustafa and Senguin (2003)] had higher soluble fraction 'a' for both dry matter and crude protein degradability. This may be attributed to the silage, silage quality of the Soya bean that was used. Small particles are digested at faster rates than large particles because they have more surface area exposed per volume of tissue hence the forage passage rate was high.

4.4.2. Dry matter and crude protein degradability kinetics

The fraction 'a' represents that there is a component that is being degraded rapidly and/or a soluble component. There was no difference in soluble fraction ‘a’ of CP. No difference was observed in fraction ‘b’ of CP. Effective degradability (ED) of CP decreased with an increase in the outflow rate. Mupangwa *et al.* (1997) observed ED and DM to decrease as the outflow rate increased. Effective degradability of CP calculated at 2, 5 and 8% outflow rates from the rumen showed that soya bean cultivar TGX had high levels than cultivar 4LF and PAN. The post-anthesis stage had high levels calculated at 2, 5 and 8% outflow rate.

Effective degradability (ED) of DM decreased with an increase in the outflow rate. Effective degradability (ED) of DM calculated at 2, 5 and 8% outflow rates from the rumen showed that soya bean cultivar TGX had higher levels (373.9-400.3 g/kg) at 2% but cultivar TGX had high levels (297.5 g/kg and 247.1 g/kg) at 5 and 8%. The pre-anthesis stage had high levels calculated at 2, 5 and 8% outflow rates.

High soluble CP components have been degraded infraction 'a' for both cultivars. For slowly degradable fraction ‘b’, the values of soya bean cultivar TGX and PAN were comparable (414.4 g/kg – 410.4 g/kg) and higher than that of cultivar 4LF (388.8 g/kg).

Variation in degradation parameters of the leguminous species may be due to variation in chemical composition. These variations in PD of DM and CP in the rumen have been reported as a result of variations in fiber content levels (Gusha*et al.,* 2013) or due to other factors such as maturity and cultivar.

4.5. CONCLUSION

In sacco rumen degradability of dry matter was not affected by cultivar. Based on the findings, pre-anthesis growth stage is ideal for all the cultivars as high values of ‘b’, ‘a+b’ ‘k=0.02’ and ‘k=0.05’. In sacco rumen degradability of crude protein was affected by both cultivar and growth stage.Cultivar 4LF had 0% rate of degradation ‘c’ at anthesis stage. From the findings, the preanthesis growth stage is ideal for all the cultivars as it has higher ‘b’ and ‘a+b’ values.

CHAPTER 5: DETERMINATION OF *IN VITRO* DIGESTIBILITY OF SOYA BEAN CULTIVAR 4LF, TGX AND PAN HARVESTED AT DIFFERENT GROWTH STAGES

Abstract

Not all protein in feed is digestible. This can be due to the anti-nutritional factors present in the feeds. Only the digestible protein contributes to the metabolize protein, the true protein that is absorbed post-ruminally, from which amino acids can be assimilated. The absorption of true protein in the small intestine is dependent on the flow and digestibility of microbial and rumenun degradable dietary protein. The objectives of the study were to determine the *in vitro (ileal)* digestibility of dry matter (DM) and crude protein of rumen digestion of three tri-folate soya bean cultivars harvested at different growth stages. Soya beans were planted in a shaded house in 63 * 25L pots which were randomly arranged in a 3 (cultivars; locally denoted 4-LF, PAN and TGX)) by 3 (stage of harvest; pre-anthesis, anthesis, post-anthesis) factorial design, Forage samples were incubated in duplicate for 24 and48 hour periods in the rumen, from which residues were analyzed for DM and nitrogen (N), *In vitro enzymatic* DM and CP digestibility of the rumen digesta were determined by sequential digestion in pepsin (abomasal) and pancreatin (small intestine) solutions. Cultivar TGX harvested at post-anthesis had significantly higher ($p < 0.05$) crude protein degradability at 24 hours than others. Growth stage had no effect ($p > 0.05$) on digestibility except for dry matter degradability at 48 hours. There was no ($p > 0.05$) significant difference of the cultivar and growth stage on degradability and IVCPD at 24 and 48 hours. Rumen crude protein degradability at 24 hours increased with growth stage. High dry matter degradability was observed with low *in vitro* dry matter digestibility at 24 and 48 hours of incubation. In conclusion different cultivar harvested at different growth stages had no effect on DM and CP digestibility.

Keywords: dry matter, crude protein, degradability, *in vitro* digestibility, *4LF, TGX, PAN*, Pepsin-HCL solution.

5.1. Introduction

Different methods are used to estimate DM and CP digestibility of the feed. These methods involve buffers, chemical solvent, rumen fluid and enzymes that are either commercially available or extracted from rumen contents (Ruba *et al.*, 2008). A three-step procedure (Tilley and Terry,

1963) estimated post ruminal digestion which closely simulates physiological conditions in the animal. This is achieved by estimating the nutrient digestibility in abomasum and intestinal digestion using pepsin and pancreatin, respectively, from ruminal degradability residues (Gargallo *et al*., 2006).

In vitro digestion techniques using rumen fluid as inoculum have been proven to be useful in assessing the relative digestibility of different feedstuffs (Minson, 1990). The main chemical component of feeds that determines the rate of digestion is neutral detergent fiber (NDF), which is a measure of cell wall content. Legume contains less cell wall and is consumed in quantities about 20% greater than grasses (Forbes, 1986) this means that legume is highly digestible compared to grass. In vitro digestibility is essential as it shows how much of the protein is digested and absorbed by the body, it is also considered as the second step of feed evaluation (Mahmoud *et al*., 2017).Protein content and dry matter in the animal feed is the main key to determine the quality of forage.

The experiment aimed to estimate the post-ruminal digestion of Crude protein and dry matter in different varieties of soya bean cultivar harvested different stages of growth.

5.2. Materials and method

5.2.1. Experimental site

The study was conducted at the University of Venda (22.9761º S, 30.4465º E) in Thohoyandou, as described in chapter 3. The use of animals, as well as protocols and procedures in this study, were approved by the Ethical and Higher Degree Committees of The University of Venda.

5.2.2. Bags specification and sample preparation

Samples used in the current experiment were prepared as described in Chapter 3.

5.2.4. Experimental design for post-ruminal digestibility

Samples used in the current experiment were prepared as described in Chapter 3 with the time interval of 24 and 48 hours. Approximately 5 g of ground sample of soya bean cultivar 4LF, TGX and PAN were weighed and placed into a labeled nylon bags (6 × 12 cm, pore size of 41 μm). The nylon bags were attached using elastic bands, to flexible vinyl plastic tubes, approximately 40 cm long and of 6 mm outer diameter. The flexible vinyl plastic tubes were tied with 10 cm ropes of different colors to help identify them according to hours and then secured to a rubber stopper. Triplicate bags per species treatment per incubation time per animal per growth stage were inserted into the rumen and withdrawn at 24 and 48 incubation hours. The bags were inserted in the rumen at 07:30 before the morning feeding time. After each incubation time, the bags were removed from the rumen, washed in running tap water without squeezing, till runoff was clear, then finally washed with deionized water and dried at 60^oC for 48 hours.

5.2.5. Determination of post-ruminal digestion

Pepsin + pancreatic digestion procedure

Rumen un-degraded residues (RUR) were removed from the bags manually. The RURs were composited by species treatment, incubation hours and the steers, and subsequently ground to pass through a 2 mm sieve.

The experimental design was completely randomized, with 3 bags for each of 3 cultivars (4LF, TGX, and PAN) * 3 growth stage. A total of 9 bags for each cultivar containing 1g of RURs of each soya bean sample was introduced into incubation bottle which contained 750ml of a 0.1 N HCl solution adjusted to pH 1.9 with 0.125g of pepsin per sample (P-7000; Sigma) and were incubated for 1 hour with constant horizontal movement at 39°C. After incubation, the bags were rinsed with tap water till runoff is clear, then finally rinsed with deionized water and introduced into the incubation bottles containing 750ml of phosphate buffer (0.5 mol ℓ^{-1} KH2PO$_4$ buffer adjusted to pH 7.75), containing 0.375g of pancreatin (P-7545; Sigma) for each sample. 10.05g of thymol to prevent bacterial growth was added. Bags were incubated for 24 hours with constant horizontal movement at 39°C. After incubation, bags were rinsed with tap water until the runoff is clear, then finally rinsed with deionized water and dried at 60°C for 24 hours. After drying, the residuals in the filter bags were analyzed for DM and N.

5.2.6. Statistical analysis

All parameters were subjected to ANOVA using GLM procedures of MINITAB software version 17 (2014) based on the statistical model:

$Y_{ijkl} = \mu + S_i + G_j + (SG)_{ij} + \varepsilon_{ijkl}$

Where Y_{ijk} is the observation on the i^{th} cultivar, j^{th} growth stage µ

= Overall mean

S_i = Effect of i^{th} cultivar

G_j= Effect of j^{th} growth stage

ε_{ijkl}= Random error

A significant difference between the treatment means was compared using the Tukey's test at ($P < 0.05$).

5.3. Results

5.3.1. *In vitro* dry matter digestibility

The ruminal degradability and *in vitro* digestibility data of DM of soya bean cultivars (4LF, TGX and PAN) harvested at different (pre-anthesis, anthesis and post-anthesis) growth stages in nylon bags are summarised in Table 5.1.There was no significant ($p > 0.05$) interaction of cultivar and growth stage on all parameters. Cultivar had no significant effect ($p > 0.05$) on dry matter degradability. Cultivar TGX harvested post-anthesis had significantly highest ($p < 0.05$) rumen crude protein degradabiity at 24 hours. Growth stage had no effect ($p > 0.05$) on digestibility except for rumen dry matter degradability at 48 hours.

Table 5.1:Dry matter disappearance (g/kg) after 24 and 48-hour incubation in the rumen and subsequent*in vitro* digestibility of soya bean cultivars harvested at different growth stages.

	24 h	48 h	$IVDMD_{24}$	$IVDMD_{48}$

Pre-anthesis	4LF	9	325.1	293.8	664.3	732.8
	TGX	9	332.7	278.6	773.7	825.4
	PAN	9	334.6	284.5	734.5	855.0
Anthesis	4LF	9	327.7	287.1	757.5	840.2
	TGX	9	324.9	290.9	713.1	708.7
	PAN	9	328.2	287.6	753.4	781.8
Post-anthesis	4LF	9	326.0	309.2	780.9	840.2
	TGX	9	346.9	302.8	844.7	841.0
	PAN	9	330.9	305.7	768.5	844.0
SEM			2.40	2.31	3.93	1.82
Cultivar mean						
	4LF	27	326.3	296.7	734.2	807.1
	TGX	27	334.8	290.8	874.7	791.7
	PAN	27	331.2	292.6	752.1	826.9
SEM			1.20	1.16	1.97	0.91
Stage means						
Pre-anthesis		27	330.8	295.0	761.6	826.1
Anthesis		27	326.9	285.7	852.1	812.7
Post-anthesis		27	334.6	298.7	747.4	786.9
SEM			1.51	1.32	1.99	0.97
Significance						
Cultivar			NS	NS	NS	NS
Stage			NS	NS	NS	NS
Cultivar×stage			NS	NS	NS	NS

**: *P* <0.01; *: *P* <0.05; (ns) non-significant: *P* >0.05. $_{ab}$????????Column means with different superscripts differ significantly at *P* <0.05. N: Number of observations, IVDMD$_{24}$: *In vitro* dry matter digestibility after 24 hours of rumen incubation, IVDMD$_{48}$: *In vitro* dry matter digestibility after 48 hours of rumen incubation and SEM: Standard Error Mean.

5.3.2. *In vitro* crude protein digestibility

The ruminal and *in vitro* digestibility of CP of soya bean cultivars (4LF, TGX and PAN) harvested at different (pre-anthesis, anthesis and post-anthesis) growth stages in nylon bags are summarised in Table 5.2. There was no significant ($p > 0.05$) cultivar X growth stage interaction for crude protein degradability at 48 hours, IVCPD at 24 and 48 hours. Significant effect was observed on crude protein degradation at 24 hourscaused by cultivar TGX at pre-anthesis growth stage.

Table 5.2: Crude protein disappearance (g/kg) after 24 and 48-hour incubation in the rumen and subsequent *in vitro* digestibility of soya bean cultivars harvested at different growth stages.

			24 h	48 h	IVCPD24	IVCPD48
Pre-anthesis	4LF	6	183.6	206.1	878.3	625.8
	TGX	6	192.3	187.3	596.7	311.9
	PAN	6	190.9	187.1	459.0	503.0
Anthesis	4LF	6	191.7	220.6	673.7	571.0
	TGX	6	199.2	204.8	632.6	501.7
	PAN	6	202.7	201.7	759.5	411.0
Post-anthesis	4LF	6	190.8	221.8	637.5	211.1
	TGX	6	207.8	215.2	578.1	494.4
	PAN	6	205.5	213.5	489.0	535.0
SEM			2.70	2.81	33.5	47.7
Cultivar mean						
	4LF	18	188.7	216.1	729.8	469.3
	TGX	18	199.7	200.8	602.5	137.2
	PAN	18	199.7	200.8	569.2	483.0
SEM			1.35	1.41	16.75	23.85
Stage means						
Pre-anthesis		18	195.0	212.9	460.3	586.7
Anthesis		18	192.7	195.3	504.5	494.5
Post-anthesis		18	200.4	209.5	424.3	413.5
SEM			1.44	1.47	18.30	24.06
Significance						
Cultivar			NS	NS	NS	NS
Stage			NS	NS	NS	NS
Cultivar×stage			NS	NS	NS	NS

**: $P < 0.01$; *: $P < 0.05$; (ns) non-significant: $P > 0.05$. $_{ab}$ Column means with different superscripts differ significantly at $P < 0.05$. N: Number of observations, IVCPD$_{24}$: *In vitro* crude protein digestibility after 24 hours of rumen incubation, IVCPD$_{48}$: *In vitro* crude protein digestibility after 48 hours of rumen incubation and SEM: Standard Error Mean.

5.4. Discussion

5.4.1. *In vitro* dry matter digestibility

There was no significant effect in interaction of cultivar and growth stage except for dry matter degradability at 48 hours. Cultivar TGX harvested at post-anthesis had significantly higher crude protein degradabilty at 24 hours than others. Growth stage had no effect on digestibility except for rumen dry matter degradability at 48 hours. Heitholt *et al.,* (2004) reported *in vitro* dry matter of 719 g/kg – 757 g/kg which is similar to the findings of the current study. Similar results 750 g/kg was also reported by Acikgoz *et al.,* (1998), however he observed a decrease with growth stages. Current study reported an increase in dry matter digestibility with growth stage. Not much information was available for *in vitro* DM for soya bean.

Plant cell walls are the major source of dietary fiber for animals (Dwayne *et al*, 1997). Legumes have lower fiber content compared to grass and other forage therefore they are more digestible (Dwayne *et al*, 1997). As the plant matures the lignin content increases. Cultivar PAN was of a dwarf family, which may attribute to a high level of DM.

5.4.2. *In vitro* crude protein digestibility

There was no significant effect in interaction of cultivar and growth stage except for crude protein degradability at 24 hours. Crude protein degradability at 24 hours increased with growth stages. Stage had no effect on all parameters except for crude protein degradability at 24 hours. Seiter *et al.,* (2004) reported digestibility of 606 g/kg which is similar to that of the current study for cultivar 4LF at pre-anthesis stage. Cultivar TGX had high level of crude protein degradability at 24 hours while 4LF had high levels on crude protein degradability at 48 hours and IVCPD at 24 hours. Crude protein degradability increased with each growth stage while IVCPD is not affected. Comparable results of crude protein degradability at 24 hours (188.9 g/kg) in the current study at pre-anthesis growth stage with that of Sollenberger *et al.,* (2003) (180 g/kg) was observed. Mislevy *et al.,* (2005) reported crude protein degradability of 11.4 and 189 g/kg. The finding of 189 g/kg was similar to the CPD at 24 hours in the current study. Sieter *et al.,* (2004) reported lower crude protein degradability levels (820 g/kg – 133 g/kg) when compared to that of the current study (188.9 g/kg -216.8 g/kg). IVCPD observed by Sieter *et al.,* (2004) was 606 g/kg which is comparable to that of the current study at 24 hours for cultivar TGX.

Peiretti *et al.,* (2017) recorded IVCPD values of (876.5 g/kg) at pre-anthesis growth stage which was comparable to that observed at pre-anthesis growth stage for cultivar 4LF (878.3 g/kg).

Peiretti *et al.,* (2017) concluded that advancing maturity stage significantly alters digestibility of soya bean. This is because legumes have lower fibre content compared to grass and other forage therefore they are more digestible and as the plant matures the lignin content increases (Dwayne *et al*, 1997).Blade *et al*., (1993) reported that the stages of maturity affect both degradability and digestion.

5.5. Conclusion

The cultivar X growth stage interaction affected the 48 hour DM disappearance and, subsequently the residue *in vitro* digestibility. *In vitro* dry matter and crude protein was not affected by cultivar and growth stage. Dry matter degradability was influenced by cultivar 4LF at anthesis stage. Crude protein degradability was influenced by cultivar TGX at pre-anthesis stage. The effect on degradability was caused by cultivar 4LF at anthesis stage, with higher level recorded by cultivar 4LF at post-anthesis stage. Therefore for degradability dry matter, cultivar 4LF should be used at post-anthesis growth stage. The effect on crude protein was caused by cultivar 4LF at anthesis stage however it had high value at post anthesis stage. based on the findings, cultivar 4LF should be used at post-anthesis stage.

CHAPTER 6: GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

6.1. General discussion

The study's specific objectives were to determine the ideal harvest stages of tri-foliate cultivars *(4-LF, PAN, and TGX)* of Soya beans in terms of the following nutritional parameters; The chemical composition dry matter (DM), Ash, crude protein (CP), Neutral detergent fiber (NDF) Acid detergent fiber (ADF), macro and micro minerals. *In sacco* rumen degradability of dry matter and protein. Different growth stages were used to improve the quality of forage as it is influenced by the plant's stage of maturity. Growth stage improve some nutrients (such as CP) and reduce others, however, increase in growth stage increased the crude protein which is the most important and valuable nutrient. Researchers have been using soya bean to conduct research but not many were available on degradability and digestibility. It has been reported that different soya bean cultivars have comparative nutrient composition (Devine and Harley, 1998). Therefore, in the present study, it was hypothesized that different cultivar and growth stages would not improve chemical composition, DM and CP ruminal degradability and *in vitro* digestibility of soya bean.

The high CP levels, up to post-flowering stages in the legume samples indicate that the plants were still growing. The CP content in all three soya bean cultivars was above the recommended minimum requirements for lactation (120 g/kg DM) and growth (113 g/kg DM) in ruminants (NRC, 1981). The CP content for all soya bean cultivar ranged from 180 to 214 g/kg which were still enough to meet both lactation and growth requirements of ruminant livestock (NRC, 1981). The observed protein content of all the cultivars in the study were comparable to the values obtained in the study of Hintz *et al,* (1992) and cultivars; Corsoy, Pella and Williams from the study of Devine and Harley (1998).The concentrations of CP of soya beans in the current study are high enough for these cultivars to be used as protein supplements to mature natural grasses, which are frequently deficient in protein (Kristine *et al.,* 2013). High protein content with growth stage may be correlated with the continuous supply of nitrogen available from rhizobial fixation. With minimal variation in the three soya bean cultivars, rhizobial nitrogen fixation was similar for all cultivars and were exposed to the same environment. The differences in the protein content of the soya bean may be related to the differences of the cultivars.

Zinc is important to wound healing and a sense of taste and smell. The zinc required by sheep is 20 mg/kg dry matter for growing animals. The zinc found in this study for both cultivars was above 60 g/kg which is enough for the maintenance of sheep and cattle. Manganese requirement by sheep per day is 20 mg/kg per day dry matter. The manganese found in the three different soya bean cultivar was 72 mg/kg and above. This is more than enough for the maintenance of the animal body.

Blade *et al.* (1993) found that the potentially degradable protein did not vary between forage legumes but decreased with maturity. Stages of maturity affect both degradability and digestion. This is because as the crop grows, nutrients increases or decreases depending on the growth stage. Ørskov and McDonald (1979) suggested that higher outflow rates result in less feed being degraded. Higher rates of ruminal NDF degradability have been reported for legumes compared with grasses (Mustafa *et al*, 2000). Varga and Hoover (1983) also indicated that NDF of legumes is degraded at a faster rate than grass NDF. The high protein content and fragility of legume cell walls, especially that of young vegetative material, results in high DM and N degradation at early stages of growth (Baloyi *et al*, 2008).

6.2. Conclusion

Overall the study indicates that different cultivars were not effecting the nutrient composition and dry matter degradability of the soya bean. Growth stage influences chemical composition, degradability and digestibility. In vitro digestibility was not affected at different hours. Cultivar PAN had high nutrient content at pre-anthesis stage, but cultivar 4LF had high digestibility at postanthesis. For degradability, higher levels were recorded at pre-anthesis stage.

6.3. Recommendations

It is recommended that soya bean cultivar 4LF, TGX, and PAN can be used as a form of potential feed resources mainly as protein supplements to ruminants fed on low-quality basal forages especially during dry season. Therefore:

i. There is a need to encourage shift from using soya bean meal to forage soya beanas protein source in dry season.

Further research is necessary to study the following:

Degradability and digestibility of soya bean plants.

More study is needed with the same cultivar in the current study so that people will be able to compare different data.

More study with the cultivar in the current study but at different environmental condition and different soil composition.

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APPENDIX

Appendix 1: Analysis of variance for chemical composition (g/kg DM) for soya bean cultivar 4LF, TGX, and PAN harvested at: pre-anthesis, anthesis and post-anthesis growth stages.

Source	Df	DM	Moisture	Ash	Fat	ADF	NDF	Protein
Treatment	8	0.398301**	0.399235**	1.05617**	0.444100**	83.3700**	64.8195**	2.15672**

Error	9	0.000611	0.000617	0.00054	0.000111	0.0011	0.0549	0.00005

**: P < 0.01; *: P < 0.05. df: Degree of Freedom, DM: Dry Matter, CP: Crude Protein, NDF: Neutral Detergent Fibre, ADF: Acid Detergent Fibre

Appendix 2: Analysis of variance for macro- (g/kg DM) and micro- (mg/kg) for soya bean cultivar 4LF, TGX, and PAN harvested at: pre-anthesis, anthesis and post-anthesis growth stages.

Source	D	Ca	mg	K	Na	P	Zn	Mn	Cu	Fe f
Treatment	8	0.050256**	0.027618**	0.277543**	0.000176**	0.002112**	6.6389**	189.139**	1.8750*	27267.8**
Error	9	0.000339	0.000033	0.008033	0.000033	0.000039	0.2778	0.444	0.3889	720.8

**: P < 0.01; *: P < 0.05. df: Degree of Freedom, Ca: Calcium, Mg: Magnesium, K: Potassium, Na: Sodium, P: Phosphorus, Zn: Zinc, Cu: Copper, Mn: Manganese and Fe: Iron

Appendix 3:*insacco* dry matter disappearance (%) of soya beans cultivar 4LF, TGX and PAN harvested at pre-anthesis, anthesis and post-anthesis growth stages.

Source	Df	a	b	C	a + b	K1	K2	K3
Cultivar	2	9.0945**	6.1500*	0.000106*	17.273**	0.1558	0.4756	0.37404
Stage	2	0.1926	11.9483**	0.000006	12.336**	8.6930**	2.2289**	1.02917*
Cultivar × stage	4	1.0387*	5.7746**	0.000031	11.025**	0.2872	0.1939	0.08419
Error	9	0.2585	0.9669	0.000017	1.077	0.2684	0.1908	0.21393

**: P < 0.01; NS: P > 0.05. ab within a section in column, means with different superscripts are significantly different (P < 0.05). (SEM) Standard error Mean; (NS) not significant; DM: Dry matter; ED: Effective degradability

Appendix 4: *in sacco* crude protein disappearance (%) of soya beans cultivar

Source	Df	a	b	C	a + b	K1	K2	K3
Cultivar	2	6.8241**	61.94	0.002006	53.58	0.39165**	1.43407**	1.71711**
Stage	2	0.8581**	44.68	0.000872	31.83	0.11527	0.73905**	0.78557**
Cultivar stagex	4	0.5920*	80.78	0.005847	80.90	0.16012*	0.09309	0.11271
Error	9	0.1209	33.88	0.000883**	28.64	0.04166	0.09294	0.06087

**: P < 0.01; NS: P > 0.05. ab within a section in column, means with different superscripts are significantly different (P < 0.05). (SEM) Standard error Mean; (NS) not significant; DM: Dry matter; ED: Effective degradability

Appendix 5: Analysis of variance for dry matter disappearance (g/kg) after 24 and 48 hourincubation in the rumen and then in vitro digestibility of soya beans cultivar 4LF, TGX and PAN harvested at pre-anthesis, anthesis and post-anthesis growth stages.

Source	Df	DMD	IVDM	CPD	IVCP
Cultivar	2	0.000369	0.01266	0.1852	2.6605**
Stage	2	0.000936	0.01356	4.4180	0.5416
Cultivar × stage	4	0.003153	0.03067	1.7191	0.3450
Error	9	0.065588	0.01638	1.6436	0.4923

**: P < 0.01; df: Degree of Freedom, DMD24: Dry matter degradability at 24 hours incubation; DMD48: Dry matter degradability at 48 hours incubation; IVDMD24: In vitro dry matter digestibility after 24 hours of rumen incubation and IVDMD48: In vitro dry matter digestibility after 48 hours of rumen incubation.