بسم الله الرحمن الرحيم

Sudan University of Science and Technology
College of Graduate Studies

Evaluation of Growth, Yield, Nutritive Value and Antibacterial Activity of *Clitoria ternatea* Forage

تقويم النمو والإنتاجية والقيمة الغذائية والنشاط البكتيري المضاد لعلف الكلايتوريا

A Thesis submitted in Fulfillment of the Requirements for the Degree of Doctorate of Philosophy in the Science and Technology of Animal Production in Forage Crops

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الإستهلال

قال تعالى:

﴿ ٱلَّذِى جَعَلَ لَكُمُ ٱلْأَرْضَ مَهْدًا وَسَلَكَ لَكُمْ فِيهَا سُبُلًا وَأَنزَلَ مِنَ ٱلسَّمَآءِ مَآءً فَأَخْرَجْنَا بِهِۦٓ

أَزْوَٰجًا مِّن نَّبَاتٍ شَتَّىٰ ﴿٥٣﴾ كُلُوا۟ وَٱرْعَوْا۟ أَنْعَٰمَكُمْ ۗ إِنَّ فِى ذَٰلِكَ لَءَايَٰتٍ لِّأُو۟لِى ٱلنُّهَىٰ ﴿٥٤﴾ ﴾

سورة **طه** الايات (53-54)

وقال تعالى:

﴿ أَوَلَمْ يَرَوْا۟ أَنَّا نَسُوقُ ٱلْمَآءَ إِلَى ٱلْأَرْضِ ٱلْجُرُزِ فَنُخْرِجُ بِهِۦ زَرْعًا تَأْكُلُ مِنْهُ أَنْعَٰمُهُمْ

وَأَنفُسُهُمْ ۖ أَفَلَا يُبْصِرُونَ ﴿٢٧﴾ ﴾

صدق الله العظيم سورة **السجدة** الاية (27) .

Dedication

To my parents who supported me with care, hope and continuous help to achieve this research

To my dear brothers and sisters

To my respectable teachers

To my colleagues

Wesal Abbas Eltaib

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First I want to thank Allah who supported me with health, patience and strength to complete this study.

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Abstract:

This study included three trials on *Clitoria ternatea*; namely: fodder trial, feeding trial and antibacterial activity trial.

First trial (fodder trial): This trial was conducted at the demonstration farm, College of the Agricultural Studies (Shambat), Sudan University of Science and Technology, during the period: 25/11/2018- 30/06/2019; to evaluate growth, yield and chemical composition of *Clitoria ternatea* forage as affected by poultry manure and eggshell fertilizers. The trial was carried out in randomized complete block design of four treatments and five replicates. The treatments consisted of: T1: no soil fertilizer used, T2: 5000kg/ha poultry manure, T3: 4600 kg/ha poultry manure+ 400kg /ha eggshell, and T4: 4200 kg/ha poultry manure +800kg/ha, eggshell. Plant height (cm), stem diameter (cm), number of leaves per plant, leaf/ stem ratio, fresh and dry yield (tons/ha) were measured. Samples of *C. ternatea* (leaves and stems) were taken over three harvests to analyze of chemical composition by using near-infrared reflectance spectroscopy (NIRS). The results showed that the poultry manure with eggshell at 4200 kg/ha poultry manure +800kg/ha eggshell was more effective in increasing plant height, stem diameter, number of leaves per plant, leaf/ stem ratio and shoot yield of *C. ternatea*, than control and poultry manure alone. While the above mentioned fertilizers had a little effect on leaves and stems chemical composition.

Second trial (feeding trial): The study was conducted at research farm, KUKU Campus, Sudan University of Science and Technology; during the period 16/10/2019- 2/1/2020; to evaluate the effect of feeding *C. ternatea* hay versus alfalfa hay on growth, carcass characteristics and meat quality of growing lambs. Twenty four (24) lambs (male) of Sudanese desert sheep were used, with average weight 18.82 kg and 4 months old. All lambs were treated with the necessary medication, ear tagged, weighed and divided randomly into four groups (six lambs

of each group). The feed was provided *adlibitum* in the form of total diet; with hay: concentrate ratio of 40:60. Group (A), hay composed of 100% clitoria hay; group (B), hay composed of 50% clitoria hay+50% alfalfa hay; group (C), hay composed of 75% clitoria hay+25% alfalfa hay. While for group (D) hay composed of 100% alfalfa hay. The total length of trial period was 77 days (14 days adaptation period and the 63 day for data collection). The results showed that the daily dry matter intake was (1.06±0.10), (1.10±0.15), (1.12±0.06) and (1.08±0.08) kg for groups A, B, C and D, respectively; and the difference was not significant. Daily weight gain was highest for group B (236.1±12.20 g) and lowest for group D (177.5±6.54 g), and the difference was highly significant ($P \leq 0.01$). Also FCR was best for group B (4.70±0.60) and lower for group D (6.10±0.49) (DM intake (kg) ÷ weight gain (kg), and the difference was highly significant ($P \leq 0.01$). The carcass yield was highest in group B ($p \leq 0.01$); while the highest dressing percentage was recorded by group A. Meat chemical composition had no significant differences between groups.

Third trial (antibacterial activity trial): This trial was conducted to investigate the effectiveness of methanol extracts of *C. ternatea* leaves against gram positive (*Staphylococcus aureus* and *Streptococcus agalactia*) and gram negative bacteria (*Escherichia coli* and *Salmonella typhi*). The antibacterial activity was evaluated according to the agar well diffusion and disc diffusion methods. The results showed a highly significant difference at ($p \leq 0.01$) between different tested bacteria in zones of inhibition by agar well diffusion method. The highest range of inhibition zone was recorded by *Staphylococcus* (2.63±0.78cm) and the lowest was recorded by *Salmonella typhi* (1.27±0.25cm). The result showed that the disc method had a highly significant difference at ($p \leq 0.01$), between different tested bacteria in zones of inhibition, in the first (10^{-1}) and second (10^{-2}) dilutions. *Staphylococcus aureus* recorded the highest range of inhibition zones (0.90± 0.1 cm) and (0.05±0.01 cm) in the first (10^{-1}) and second (10^{-2}) dilutions, respectively. While the results showed no inhibition zones for all tested bacteria in the third and fourth dilutions.

مستخلص الدراسة:

تم إجراء ثلاث تجارب على علف الكلايتوريا، شملت التجارب: تجربة إنتاج علف الكلايتوريا، تجربة إستخدام الكلايتوريا في التغذية و تجربة النشاط المضاد للبكتريا لأوراق الكلايتوريا.

اولاً: تجربة إنتاج الكلايتوريا كعلف:

أجريت هذه الدراسة بالمزرعة التجريبية بكلية الدراسات الزراعية (شمبات)، جامعة السودان للعلوم والتكنولوجيا خلال الفترة من2018/11/25 الى 2019/6/30م، لدراسة تأثير التسميد بروث الدواجن وقشر البيض على النمو، الإنتاجية والتركيب الكيميائي لعلف الكلايتوريا. أستعمل تصميم القطع العشوائية الكاملة بخمس مكررات. شملت المعاملات: معاملة1 (دون استخدام سماد)، معاملة 2 (روث دواجن 5000 كجم للهكتار)، معاملة3 (4600 كجم روث دواجن+ 400 كجم قشر بيض للهكتار) ومعاملة 4 (4200 كجم روث دواجن + 800 كجم قشر بيض للهكتار). تم قياس إرتفاع النبات (سم)، محيط الساق (سم)، عدد الأوراق، نسبة الأوراق للسيقان، الاوزان الرطبة والجافة للمجموع الخضري (طن/ هكتار). كذلك أخذت عينات الكلايتوريا (اوراق وسيقان) خلال الثلاث قطعات لتقدير التركيب الكيميائي للكلايتوريا بإستخدام جهاز مطياف الاشعة تحت الحمراء القريبة. أظهرت النتائج أن إستخدام روث الدواجن وقشر البيض معاً بمعدل 4200 كجم روث دواجن + 800 كجم قشر البيض للهكتار؛ كان ذا تأثير في زيادة إرتفاع النبات، محيط الساق، عدد الأوراق، نسبة الأوراق للسيقان، الوزن الرطب والجاف للمجموع الخضري للكلايتوريا مقارنة بعدم إستخدام السماد وباستخدام روث الدواجن لوحده. بينما أظهرت النتائج وجود تأثير طفيف للتسميد على التركيب الكيميائي لأوراق وسيقان الكلايتوريا.

ثانياً: تجربة التغذية:

تم إجراء هذه التجربة بحظيرة الأبحاث، مجمع كوكو- جامعة السودان للعلوم والتكنولوجيا؛ في الفترة من 16/ 2019/10 الى 2020/1/2م؛ لدراسة أثر إستخدام دريس الكلايتوريا بالمقارنة مع دريس البرسيم الحجازي في التغذية علي نمو وخصائص جسد الذبيح ونوعية اللحم للحملان النامية. تم إستخدام 24 حملاً (ذكور) من الضأن الصحراوي السوداني بمتوسط وزن 18.82 كجم وعمر حوالي 4 شهور. تم إجراء كل المعالجات الصحية الضرورية للحملان. ثم تم تركيب علامات الأذن البلاستيكية المرقمة للتمييز، ثم وزنت وقسمت الحملان عشوائياً لأربع مجموعات (6 حملان لكل مجموعة). أستخدم النظام الحر في التغذية بإستخدام تركيب العليقة الكاملة المحتويه على 60:40 دريس الى مركز. للمجموعة (أ) تألف الدريس من: 100% دريس كلايتوريا، المجموعة (ب) تألف الدريس من خليط دريس (50% دريس كلايتوريا+ 50% دريس برسيم)، المجموعة (ج) تألف الدريس من خليط دريس (75%

دريس كلايتوريا+ 25% دريس برسيم)، اما المجموعة (د) تألف الدريس من دريس البرسيم100%. كان طول الفترة الكلية للتجربة 77 يوماً، (14 يوماً فترة تعود و 63 يوماً لجمع البيانات). أظهرت النتائج أن معدل الإستهلاك اليومي كان: (1.06± 0.10)، (1.10± 0.15)، (1.12± 0.06) و (1.08± 0.08) كجم مادة جافة للمجموعات (أ)، (ب)، (ج) و (د): على التوالي مع عدم وجود فروق معنوية ($p>0.05$). سجلت المجموعة (ب) أعلى معدل نمو يومي (236.1 ±12.20 جرام)، بينما سجلت المجموعة (د) ادنى معدل نمو يومي (177.5±6.54 جرام)، مع وجود فروق معنوية عالية بين المجموعات ($p\leq0.01$). كما أوضحت نتائج معدل التحويل الغذائي وجود فروق معنوية عالية ($p\leq0.01$)؛ حيث سجلت المجموعة (ب) أفضل معدل تحويل غذائي (4.7±0.60) بينما سجلت المجموعة (د) ادنى معدل تحويل غذائي (6.10 ± 0.49) ،(المادة الجافة الماكولة (كجم) ÷ زيادة الوزنية (كجم). كذلك أظهرت النتائج أن كمية جسد الذبيح كانت أعلى في المجموعة (ب). أما نسبة التصافي فقد كانت أعلى في المجموعة (أ). كذلك اوضحت النتائج عدم وجود فرق معنوي بين المجموعات في التركيب الكيميائي للحم

ثالثاً: تجربة النشاط المضاد للبكتريا:

أجريت هذه الدراسة لمعرفة الفعالية المضادة للبكتريا للمستخلص الميثانولي لأوراق الكلايتوريا ضد البكتريات موجبة (البكتريا العنقودية الذهبية والبكتريا العقدية) وسالبة (بكتريا الايكولاي والسالمونيلا) الجرام إعتماداً على طريقة الإنتشار بالحفر وطريقة الإنتشار بالأقراص.

أوضحت النتائج وجود فروق معنوية عالية ($p\leq0.01$) بين البكتريات المختبرة بطريقة الإنتشار بالحفر؛ حيث سجلت العنقودية الذهبية أعلى مساحة تثبيط (2.63± 0.78) سم بينما سجلت السامونيلا ادنى مساحة تثبيط (1.27± 0.25) سم. أوضحت نتائج طريقة الإنتشار بالأقراص وجود فروق معنوية عالية بين البكتريات المختبرة ($p\leq 0.01$) في التخفيف الأول (10^{-1}) و الثاني(10^{-2})؛ حيث سجلت العنقودية الذهبية أكبر مساحة تثبيط حول الأقراص (0.9 ±0.1) و (0.05±0.01) سم للتخفيف الأول والثاني على التوالي. بينما لاتوجد مساحات للتثبيط للبكتريات المختلفة عند التخفيف الثالث (10^{-3}) والرابع (10^{-4}).

LIST OF CONTENTS

List of tables

List of appendices

List of abbreviations

ADF	Acid Detergent Fiber
ANOVA	Analysis of Variance
Ca	Calcium
CF	Crude Fiber
cm	Centimeter
C: N	Carbon to Nitrogen ratio
CP	Crude Protein
C.t	*Clitoria ternatea*
DDM	Digestible dry Matter
DM	Dry Matter
DMI	Dry Matter Intake
EE	Ether Extract
et al	And Others
FAO	Food and Agriculture Organization
FCR	Feed conversion ratio
g	Gram
G+ve	Gram positive
G-ve	Gram negative
ha	Hectare
Kg	Kilogram
ME	Metabolizable Energy
Mg	Milligram
MPA	Multiple Purpose Analyzer
N	Nitrogen
NDF	Neutral Detergent Fiber
NFE	Nitrogen Free Extract
NIRS	Near Infrared Reflectance Spectroscopy
ppm	Parts Per Million
RFV	Relative feed value
Wt	Weight

CHAPTER ONE

Introduction

CHAPTER ONE

1. Introduction:

Sudan is mostly an arid country, within different ecological zones that include: the desert in the extreme north followed by the low rainfall savanna woodland, the high rain savanna woodland in the south, and the mountain zones, (Appendix1), (FAO, 2015). In addition, Sudan has a big livestock wealth according to FAO, (2021); In 2020, the livestock population was estimated at about 109.9 million head, including about 31.8 million cattle, 41 million sheep, 32.2 million goats and 4.9 million camels; but the livestock is suffering scarcity, due to the competition between the human food and animal feed. In this respect, the lack of forage especially leguminous crops during the summer season where there is a severe shortage of fodders of protein sources for ruminant animals. *Clitoria ternatea* L. is a summer forage crop which can be cultivated to solve this problem. It is grown in Sudan in an area of about 105,000 feddans. The average yield ranges between7 to 12 tons/fed fresh forage (3-5 tons/fed dry matter), (Abdala, 2009). And it is high nutritive value, with a higher leaf nitrogen percentage and lower acid digestible fiber than most other tropical legumes **(**Conway and Doughton, 2005). This plant is mainly used as forage and it is well adapted to various climates (Gomez and Kalamani, 2003). In addition, this crop is originally selected as a cover crop and widely planted as an ornamental on fence rows. It is adapted to a wide range of soils from sandy to deep loams (Morsy and Awadalla, 2017). *C.ternatea* is good for short and medium term pastures, protein banks, and excellent for hay making (Hernández and Sánchez, 2014).

Use of *C. ternatea* in sheep feeding provides a positive effect on wool growth and live weight gain (Schlink, 1998). This plant pharmacologically is an anxiolytic,

anti inflammatory, analgesic, anti microbial and anti carcinogenic (Lijon *et al*., 2017). It also can be used to discover bioactive products that may serve as leads in the development of new pharmaceuticals in food preservation as well as natural plant based medicine (Kamilla *et al*., 2009).

In Sudan production, uses and researches of *Clitoria ternatea* are relatively limited. In addition the traditional smallholder livestock and farmer owners in Sudan are not aware of most of its benefits and cultivation practices; therefore more studies are needed.

The Objectives of this study were:-

1- To study the effect of poultry manure and eggshell fertilizers on growth, yield and chemical composition of *Clitoria ternatea*.
2- To evaluate the effect of feeding *Clitoria ternatea* hay versus alfalfa hay on growth, carcass characteristics and meat quality of growing lambs.
3- To test the antibacterial activity of *Clitoria ternatea* leaves against Gram positive (*Staphylococcus aureus* and *Streptococcus agalactia)* and Gram-negative bacteria (*Escherichia coli* and *Salmonella typhi)*.

CHAPTER TWO

Literature Review

CHAPTER TWO

2. Literature Review:

2.1: *Clitoria ternatea* forage:

The shape of flowers of the clitoria plant is a reflection of genus name (Singh *et al*., 2017). Whereas the name of the species ("Ternatea"), comes from Ternate, an eastern Indonesian island (Oguis *et al*., 2019). *Clitoria* comprises 60 species distributed mostly within the tropical belt with some species found in temperate areas, (Shahnas, and Akhila, 2014). The most frequently reported species is *Clitoria ternatea* L, which is mainly used as forage as it is a highly palatable for livestock; also it had various medicinal usages, (Dhanraj *et al*., 2018).

2.1.1: Synonyms:

Butterfly pea (Australia); Blue-pea, Cordofan-pea and honte (French); blaue Klitorie (German); Fula criqua and Clitoriaazul (Portugese); (Singh *et al*., 2017), Azulejo, bandera, Conchitis, Papito, Zapatico de la reina, Conchita azul, Campanilla, Lupita, pito de parra, Bejuco de conchitas, Zapotillo and Choroque (Spanish); Cunha (Brazil); Pokindang (Philippines); Zapatillo de la reina (El Salvador); Kordofan pea (Sudan) (Singh *et al*., 2017); Nagar hedi (Kannada) (Pendbhaje *et al*, 2011); and Mavi Kelebek Sarmaşığı (Turkish) (Singh *et al*., 2017).

2.1.2: Taxonomic classification:

Kingdom: Plantae; Division: Angiosperms; Class: Eudicots; Order: Fabales; Family: Fabaceae; Genus: *Clitoria;* Species: *C. ternatea*. (Dhale, 2017).

2.1.3: Origin and distribution:

Butterfly pea has its origins in Central and South America, (Conway and Doughton, 2005); although it is now widely distributed throughout the semiarid and sub humid tropics, including Central and South America, the Caribbean, parts of Africa, Asia and Australia, (Conway and Doughton, 2005), (Appendix 2). In Africa found in Angola, Benin, Burundi, Cabinda, Cameroon, Cape Verde Is, Djibouti, Ethiopia, Gabon, Chad, Ghana, Guinea, Ivory Coast, Kenya, Mali, Mozambique, Malawi, Nigeria, Sao Tome, Senegal, Somalia, Sierra Leone, (Neelamma *et al.*, 2016), South Africa, Sudan, Tanzania, The Gambia, Togo, Uganda, Zaire, Zambia, Zimbabwe, Indian Ocean: Mauritius, (Neelamma *et al.*, 2016).

2.1.4: Morphological description:

It is herbaceous perennial legume; stem is fine twining, sparsely pubescent, and sub erect at base (Hernández and Sánchez, 2014). Leaves pinnate with 5 to 9 leaflets; the leaflets elliptic, ovate or nearly orbicular, 1.5-5 cm long, 0.3-3 cm wide, (Dhale, 2017), with apex acute or rounded, often notched, and base cuneate or rounded, both surfaces sparsely appressed pubescent; the petioles 1.5-3 cm long (Dhale, 2017), (Appendix 3). Flowers are blue or white petals are unequal, style bearded below the stigma. Pod are linear and compressed with 5-7 cm long, flat with 6 to 10 seeds, in each pod. Seeds are black in color (Pendbhaje *et al*, 2011), (Appendix 4). Number of seeds/kg: 23,000 (Hernández and Sánchez, 2014). The root system of *Clitoria ternatea* consists of a fairly stout tap root (Chauhan *et al*., 2012); and may grow to more than 2 meters deep (Dhanraj *et al*., 2018) with few branches and many slender lateral roots (Chauhan *et al*., 2012).

2.1.5: Ecology:

2.1.5.1: Soil requirements:

Butterfly pea grows on a wide range of soil types (Conway and Collins, 2005[a]), (from sands to heavy clays) of moderate fertility but is extremely well adapted to heavy clay alkaline soils (Pendbhaje *et al.*, 2011).

2.1.5.2: Water:

It requires summer rainfall of 500 mm over 3 months but grows best between 700-1,500 mm (Hernández and Sánchez, 2014). While, More and Hake (2019), reported that it requires approximately 400 mm of rainfall; but also performs well under irrigation areas. *C.ternatea* grows from drier areas like Kordofan in the Sudan (Morsy and Awadalla, 2017); to the fairly drought tolerant in Zambia. Due to the nature of *C. ternatea,* it cannot tolerate prolonged inundation or water logging but can tolerate short term flooding (More and Hake, 2019).

2.1.5.3: Sun light:

It is normally grown in full sunlight; but can also grow under light shade in rubber and coconut plantations (Cook *et al*., 2005; FAO, 2012; Hernández and Sánchez, 2014; Neelamma *et al*, 2016; More and Hake, 2019). Whereas Souza *et al*. (2017) reported that a high level of shading increased the leaf length and survival of butterfly pea, while it decreased the mass of the forage produced.

2.1.5.4: Temperature:

Clitoria ternatea is a summer growing legume and most productive on deep, fertile soils when temperatures are warm, (Conway and Collins, 2005[a]). It needs moderate temperature under 25 ºC; but not suited to locations with frequent or severe frosts (More and Hake, 2019). It stands up well in hot summer temperatures and having low frost tolerance (More and Hake, 2019).

2.1.6: Reproductive behavior:

Flowers can develop in 4-6 weeks after sowing and continue to flower when temperature and moisture are adequate (Hernández and Sánchez, 2014), Flowering can occur throughout the year, given sufficient soil moisture and frost free conditions (Hernández and Sánchez, 2014); while Nulik *et al*. (2013), reported that *Clitoria ternatea* flowers between 1 to 3 months after establishment, whenever adequate growing conditions occur.

In addition, Butterfly pea is self pollinated (Chen *et al*., 2018), with partial out crossing (Cook *et al*., 2005; Hernández and Sánchez, 2014). For seed production hand harvest was economical, but can yield 700 kg/ha by mechanical harvesting methods (direct-heading) (Neelamma *et al*., 2016). *Clitoria ternatea,* can produce useful seed crops without fertilizers (Nulik *et al*., 2013).

2.1.7: Environmental impact:

Its roots fix nitrogen (Oguis *et al*., 2019) with the help of specialized bacteria on its roots and convert it into a form that can be used by growing plants (Nulik, *et al*., 2013). This plant is also used to improve soil quality (Dighe *et al*., 2009). *C. ternatea* grows horizontally, makes tightly covered (Afrianto *et al*., 2020).

2.1.8: Palatability by livestock:

Butterfly pea is a highly palatable forage legume (Neelamma *et al.,* 2016 and Morsy and Awadalla, 2017); generally preferred by livestock over other legumes (Gomez and Kalamani, 2003 and Mukherjee *et al.*, 2008).

2.2: Cultural practices:

Germination and establishment of *C. ternatea* is most favorable when the temperature is range from 24 to32 C^{o}, (McDonald, 2002 and Conway, 2005[a]). The best results are achieved by planting into soil moisture 2-6 (cm), in narrow rows 15-50 (cm) apart, (Neelamma *et al.*, 2016); at about 2-4 kg/ha for long term pastures and about 6 kg/ha for short term pastures to achieve plant densities of 5-10 (plants/m^{2}) (Neelamma *et al.*, 2016). Perennial legumes such as *Clitoria ternatea* should be cut at 5–10 (cm) height above the ground to produces a high quality ration, and allows vigorous re growth, (Hernández and Sánchez, 2014). It exhibits excellent re growth after cutting or grazing during short period and produce high yields, (Gomez and Kalamani, 2003) (Appendix 5). In addition Conway, (2005[b]), reported Butterfly pea can flower within six weeks of planting and under suitable growing conditions, and can be graze within 12 weeks of planting. The suitable cutting interval is 56 days for best dry matter and protein yield, (Hernández and Sánchez, 2014).

2.3: Fertilizers application:

Fertilizers are essential nutrient supplements, which can enhance the productivity and growth of plants (Purnomo *et al.*, 2017). Fertilizers are classified into two types; inorganic and organic (Homenauth, 2013). The inorganic (Mineral) fertilizer is a substance are in the form of inorganic salts obtained by extraction and (or) by physical and (or) chemical industrial processes, (Homenauth, 2013). Organic fertilizer (Poultry manure), contains all the essential nutrients required for crops production (Ritz and Merka, 2013), and its value as an organic fertilizer and a source of plants nutrients (Ritz and Merka, 2013). Organic fertilizers for example using of eggshell powder, as a stabilizing material for improving soil properties (Amu *et al.*, 2005). In addition, Lambert and Litherland, (2000), reported that the

fertilizer has only small direct effects on feed quality, the nitrogen fertilization having greatest effect through lifting protein concentration. Fertilization with phosphorus, potassium, or other nutrients that increase yield; may actually slightly reduce forage quality when growth is fast, (Ball *et al*., 2001). Fertilizers is not normally used when *Clitoria ternatea* is sown on suitable soil, but phosphorous and Sulphate may be required on infertile soils, (Hernández and Sánchez, 2014; Neelamma *et al.*, 2016; Singh *et al*., 2017). Alderete-chaez *et al*., (2011), showed that *C.ternatea* is able to increase the levels of nitrogen, phosphorus and potassium in the soil during the period of growth. Abusuwar (2017) reported that poultry manure led to an increase of the fresh and dry matter in *Clitoria ternatea* compared with the control (no fertilizer). Ahmed and Elfeel (2012) reported that under the arid land poor soils, addition of fertilizers significantly increased forage productivity and quality and minerals uptake, especially addition of NPK improved crop productivity and quality of *C.ternatea.* Abusuwar (2017) reported that higher CP in *C. ternate* was obtained by the poultry manure treatment compared to inorganic (NPK) fertilizer.

2.3.1: Poultry manure as fertilizer:

Organic fertilizers (manures) are derived from the wastes of plants and animals (Homenauth, 2013). Litter from poultry, cows, sheep, etc., are commonly used for fertilizers, (Homenauth, 2013). Abusuwar (2017), reported that the composted organic fertilizers (poultry and cow manures) are more effective in increasing nodulation, productivity and improving forage quality of *C. ternatea* than inorganic fertilizer (NPK) under adverse conditions of salinity in arid lands. Carpici (2011), reported that nitrogen fertilizer, tends to increase dry matter yield. In addition, Abusuwar, (2017) reported that planting of *C. ternatea* without fertilizer resulted in higher CF content (31.0%) compared to (28.0%) (29.0%) in poultry manures and inorganic (NPK) fertilizers, respectively.

2.3.2: Eggshell as fertilizer:

Country hen eggshell calcium is best natural source of calcium and it is about 90% absorbable. Also it contains trace amounts of other micro elements (Radha, and Karthikeyan, 2019). Calcium plays an important biochemical functions and activating various enzymatic systems, thus contributing to the proper development of plants (El Habbasha and Ibrahim, 2015).

Calcium involved in the formation of lecithin, and phospholipid which is important in the plant cell membrane, and in the permeability of these membranes (Rodríguez, 1992). Nikose (2015) reported that eggshell along with other bio waste (animal and plant waste), resulted a remarkable growth in the plant. Cell wall strength and thickness are increased by calcium addition (Easterwood, 2002). In addition, Kris (2010), reported the calcium, is cell wall component of plant, and calcium deficiency causes weakness and instability of the plant. In addition the fresh and dry weight of legume increased with increasing concentration of hen eggshell (Radha,and Karthikeyan, 2019). In addition, the clover plant when grown on eggshells fertilized soil grew 10 mm larger than the plants without eggshells (King'ori, 2011). The leaves number and leaf area of cowpea plant increases with increasing concentration of hen eggshell in soil, (Radha and Karthikeyan, 2019). Calcium compared to other nutrients increases plant height and dry matter (Nelson and Niedziela, 1998). Calcium acts in the mitotic cell division in the growth of meristem is and the absorption of nitrate (Rodríguez, 1992).

Havlin *et al*. (2005); reported that calcium is important in nitrogen metabolism and protein formation by enhancing NO_3 uptake. And it is also important in translocation of carbohydrates and other nutrients (Havlin *et al*., 2005). Also calcium is an element associated with the transport of nitrogen (N) and interaction with phosphorus (P) and potassium (K) (El Habbasha and Ibrahim, 2015).

2.4: Compatibility with other species:

It has been grown successfully with elephant grass (*Pennisetum purpureum*), (Trivedi, 2002; Hernández and Sánchez, 2014; Neelamma *et al.*, 2016), forage sorghums (*Sorghum bicolor*), and millets, as well as *Panicum maximum* (Hernández and Sánchez, 2014). Also planted with pangola (*Digitaria eriantha*), *Andropogon gayanus*, *Dichanthium aristatum. Cenchrus ciliarus,* and *Chloris gayana* (Hernández and Sánchez, 2014).

It increases soil fertility and enhances subsequent crop yields (maize, sorghum and wheat) when grown as green manure or ley pasture (Morsy and Awadalla, 2017). Elfeel *et al*. (2013) showed that the *leucaena* and *clitoria* mixed cropping system positively ameliorated the soil properties.

2.5: Growth performance of *Clitoria ternatea*:

The plant height of *C. ternatea* was 22.9±7.9 cm at 120 days after planting (Ratnawaty *et al*., 2013). Whereas the plant height (cm) of first cut of *Clitoria ternatea* when cut at 80 days as influenced by sowing dates (April- June) was between 84.16 cm and 89.85cm, (Morsy and Awadalla, 2017). The number of leaves per plant was between 61.33 and 66.22, (Morsy and Awadalla, 2017). While Leaf: Stem ratio was between 1.59 and 1.78, (Morsy and Awadalla, 2017); (Abreu *et al*., 2014) reported that the leaf: stem mass ratios at 35, 50, 70, and 90 days, were 3.11, 3.10, 2.30, and 1.37, respectively. While Macedo *et al*. (2015), reported that leaf: stem ratio of clitoria was 0.94. Whereas Barro and Ribeiro (1983) reported that the ratio after hay making had values of 1.26, 0.87, 0.73, and 0.97 during 42, 50, 70, and 84 days, respectively.

2.6: *Clitoria ternatea* forage yield:

The climate and soil environment are prime determinants of the yield and quality of forages (Asia-Pacific Network, 2005). The yield of *C. ternatea* forage was 25-29 ton (DM/ha), with seed production of 2.2 ton (DM/ha) per harvest at 42 day of cutting interval (Sutedi, 2013).

Forage productivity of *Clitoria ternatea* vigorously depends on environmental factors such as climatic conditions, the timing of outset cutting (Mahfouz *et al*., 2019), cutting intervals (Jingura *et al*., 2001; Njarui *et al*., 2004; and Ogedegbe *et al*., 2011) and the frequency and stubble height of cutting, planting date, availability of water and plant density, etc (Mahfouz *et al*., 2019). (Jingura *et al*., 2001; Njarui *et al*., 2004; and Ogedegbe *et al*., 2011), reported the application of fertilizer effect on DM production of legumes.

Dry matter yield varies from 1.1 to 3.3 t/ha in first year under rained condition; while under irrigated condition it yields around 13.3 tones (dry matter per hectare), (Trivedi, 2002). In addition clitoria and phillipesara were cultivated during the summer season under Egyptian conditions, were produced 17-24.9 tons (per ha) green fodder of first and second cuts, (Abdelhamid and Gabr, 1993). Also Macedo *et al*. (2015) reported the dry matter production of *Clitoria* was 9.80 t/ha; over 240–260 days. While Hernández and Sánchez (2014) reported the yield of *C.ternatea* was up to 15 tons (DM/ha/year) and 4,200 kg (DM/ha) at 4 month. But in dry land conditions in the sub-humid tropics, it normally produces 2-6 (t/ha/year) dry matter.

2.7: Chemical composition of *Clitoria ternatea:*

Many factors affect on forage quality; the most important factors are forage species, stage of maturity at harvest, and storage methods; while the secondary factors are, temperatures during forage growth, soil fertility, fertilization and variety (Ball *et al*., 2001). In addition Adjei and Fianu (1985) recorded that the cut interval generally had little effect on stem nitrogen free extract (NFE).

Legume leaf has higher nutritive value than grass leaf; also leaf is higher quality than stem (Lambert and Litherland, 2000). In addition the harvesting age affected the majority of the chemical constituents and gas kinetic parameters related to the stems (Abreu *et al*., 2014). The leaves of this legume were the least affected part by the aging (Abreu *et al*., 2014). Kalamani and Gomez (2001) reported that nutritive quality values of *Clitoria ternatea* declines less with age.

The C.P. content of *C.ternatea* ranges from 10.5 to 25.5% on dry matter basis (Trivedi, 2002). In addition the level of crude protein and crude fiber in the leaves were 20-22 and 21-29% respectively; and the total plant protein ranges between 14 to 20%; dry matter digestibility levels vary between 60-75%, (ANG, 2012). Elfeel *et al*. (2013) reported that levels of crude protein contents in clitoria leaves ranged from 25.6% to 26.4%, the ash ranged from 10.1% to 10.8, and the fiber varied from 11.9% to 14.7. In addition, Kalamani and Gomez, (2001), reported that percentages of crude protein and crude fiber in the leaves were 21.5% and 21.5-29%, respectively.

According to Hernández and Sánchez (2014), the digestibility up to 80% with nitrogen concentrations of 3.0% for leaf, and 1.5% for whole plant, also leaf had low acid detergent fiber (ADF, 20%).

C. ternatea (butterfly pea) can grow in poor soils and contains high crude protein as well (19%), (Cook *et al*., 2005). In addition, Hartutik *et al.* (2012) reported that the ash, crude protein (CP), crude fiber, crude fat, neutral detergent fiber, acid detergent fiber, hemicellulose, cellulose and lignin were 7.56%, 18.38%, 32.99%, 1.75%, 51.42%, 37.33%, 14.09%, 25.03%,11.74% respectively.

The crude protein of *Clitoria ternatea* in the first and second cuts, (at rate 0.0 Kg fed^{-1} phosphorus fertilizer), were 20.37 and 17.82%, respectively, while the crude fiber was 24.64 and 25.77%, respectively (Morsy and Awadalla, 2017). The protein contents in clitoria leaves ranged from 25.6% to 26.4%, the ash ranged from 10.1% to 10.8, whereas the fiber varied from 11.9% to 14.7 (Elfeel *et al*., 2013).

Macedo *et al*. (2015), reported that at 47 days of age, average concentrations of crude protein; calcium and phosphorus in *Clitoria ternatea* were 22.1%, 1.17% and 0.41%, respectively. Macedo-Barragán *et al*. (2011), reported that the average ADF content of clitoria hay was 35.74 ± 5.06% with minimum and maximum values of 29.06 and 47.07% respectively.

Ratnawaty *et al*. (2013) reported that the nutrients content of *C.ternatea* legume harvested on 120 days after planting the DM (%) , Ash% , CP% , CF% , EE%, NDF (% DM), ADF (% DM), Cell (% NDF), and Hemi-cell. (% NDF) ,were 91.13%, 6.16%, 18.16% , 38.03%, 2.43%, 59.22%, 42.63%, 31.17%, and 16.59% ,respectively.

2.8: *Clitoria ternatea* as hay:

Industry acceptance of butterfly pea hay is increasing as availability and grower experience increases, (Conway and Collins, 2005[b]). Its hay does not contain any compounds likely to cause toxicity or palatability problems (Conway and Collins, 2005[b]). Good quality hay can be made from *Clitoria ternatea* as it was well accepted by livestock (ANG, 2012). DM, ash, ether extract, CP and NFE of C.*ternatea* hay were, 89.04%, 8.92, 4.24, 23.06 and 34.84%, respectively (on DM basis), (Barro and Ribeiro, 1983).

2.9: *Clitoria ternatea* and other forage legumes:

Butterfly pea is faster and less risky to establish than leucaena (Conway *et al*., 2001; Gomez and Kalamani, 2003). Also it's easy to establish, including on heavy clays and surface crusting soils, palatable and high nutritional value, good for fertility restoration; also high forage and seed production (Neelamma *et al*., 2016); in addition Hall, (1985) reported that *Clitoria ternatea* had persisted for 14 years and spread under heavy dry seasons in North West Queensland. Some soils well suited to butterfly pea, such as shallow open downs soils, may be too shallow for leucaena (Conway and Doughton, 2005 and Cook *et al.*, 2005). In Mexico, during the rainy season, the highest dry matter production was recorded for *Clitoria* and *lablab* (9.80 and 8.93 t/ha, respectively, over 240–260 days), while mucuna produced 5.5 t DM/ha in 120 days. Leaf production in *Clitoria* (4.73 t/ha) exceeded that in lablab (3.23 t/ha) and mucu-na (2.69 t/ha), while leaf: stem ratio was 0.94 for Clitoria, 1.0 for mucuna and 0.58 for lablab (Macedo *et al.*, 2015). In addition *C. ternatea* yields forage with a potential nutritive value comparable to the traditionally cultivated forage legume crops (e.g., alfalfa or clover) (Abreu *et al*., 2014).

2.10: Advantages of *C. ternatea* over lucerne (*Medicago sativa* L.):-

A soil pH between 6.5 and 8.0 is optimum forage production for Alfalfa and it tends to be sensitive to soil acidity (ESGPIP, 2008); while *C.ternatea* grows in various types of soil pH of 5.5-8.9, (Sutedi, 2013 and Chen *et al*., 2018). Butterfly pea is a tropical, warm climate species, whereas lucerne is a temperate, cool climate species (Cullen and Hill 2006). In addition it is not easy to consistently grow good quality in north Queensland or the northern parts of central Queensland. Butterfly pea can produce good quality hay in these areas (Conway and Collins, 2005[b]). *C. ternatea* has also better potential nutritive value than alfalfa and clover (Abreu *et al*., 2014). Lucerne when feeding during early morning dew on, may lead to the problem of bloat, due to foaming, hemicelluloses, higher quantity of soluble leaf cytoplasmic proteins, pectins and saponins, (ANG, 2012).

C. ternatea has thin stem and large leaves, nil bloat and non toxic, (Gomez and Kalamani, 2003; Avalos *et al*., 2004 and Cook *et al*., 2005). In addition, the first and second cuts of *C. ternatea* (hay), during the summer season under Egyptian conditions have better acid and neutral detergent fiber, acid detergent lignin cellulose, crude protein and fiber, and hemicellulose than alfalfa hay (Abdelhamid and Gabr, 1993).

Clitoria ternatea intake by ruminants, enhance animal performance in spite of a greater consumption of cell walls, but does not differ in dry matter, crude protein NDF intake and net energy when compared with alfalfa hay (Avalos *et al*., 2004). Also the milk production was similar when feeding alfalfa hay or *Clitoria* hay to Brown Swiss cows in tropical conditions. It is possible to include 50% of *Clitoria* hay in feed supplements without drop in milk production, with a 30% saving in feed supplement cost (Avalos *et al*., 2004).

2.11: Uses/Applications of *Clitoria ternatea*:

Butterfly pea (*Clitoria ternatea*), is a multipurpose forage legume. It provides bioactive compounds use for medicinal, and it is also an ornamental plant and cover crop, (Gomez and Kalamani, 2003).

This plant can be used as fodder, green manure, food and widely planted in gardens and fences for the beauty of its flowers ((Hernández and Sánchez, 2014). It is used as hay as well as for grazing. It should be grazed lightly and in rotations to preserve the pasture for longer period, (Trivedi, 2002). In tropical Asia including India, the purple flowers are used for many medical purposes (Karel *et al*, 2018). Many countries have used it for human consumption, ornamental purposes and animal production (Conway and Doughton, 2005).

The flower extract consists of antioxidant activity and protective effect, (Karel *et al*, 2018). It has been commonly used as anti stress, anti depressant, anti inflammatory treatments and as anti microbial, (Karel *et al.*, 2018).

2.11.1: Green manure:

The application of green manure is one of the alternatives in order to reduce the negative effects of some applications in intensive agriculture practices on the soil and the environment, (Ramazan *et al*., 2019).

Clitoria ternatea can be used as a green manure (FAO, 2012 and Cook *et al*., 2005); for optimum yield as a green manure crop, use of seeding rate of 12 (kg/ha). As a component of grass-legume pastures, can also be planted behind a blade plough or using a "crocodile seeder "(Neelamma *et al.*, 2016).

2.11.2: Food and beverage use:

Butterfly Pea is a blue flower which contains a lot of anthocyanin. And the anthocyanins act as potential sources for eco-friendly natural food dyes (Oguis *et al*., 2019 and Thanh *et al*., 2020). Furthermore, *C. ternatea* was used for coloring goat milk yogurt (Dewi *et al*., 2019). In Malay a cooking, an aqueous extract is used to colour glutinous rice (Darsini and Shamshad, 2015). Whereas the young shoots, leaves, flowers and tender pods of *Clitoria* are eaten as vegetable in Kerala and Philippines, (Swati, 2019). The antioxidation ability of the total phenolic compounds of butterfly pea is benefit for preserving flavor and color and for preventing vitamin destruction in processed foods (Chen *et al*., 2018). Also Chan *et al*. (2017) reported that the flower of *Clitoria ternatea* with deep blue color is generally used as a natural food colorant. In Southeastern Asia (Indonesia and Malaysia) and Madagascar, these flowers are often used as a food dye or dipped in batter and deep fried in form of fritters (Karel *et al*., 2018). It can also color several traditional foods such as: *putu, onde-onde, bandang, , cendol*, candy, *getuk lindri*, sticky rice *tapai, barangko*, and others (Saati *et al*., 2018; Angriani 2019; Febrianti 2019; Permana 2019; Palimbong and Pariama 2020; and Afrianto *et al*., 2020).

2.11.3: Ornamental values:

C. ternatea widely grows in the warm climatic conditions as an ornamental plant, it requires very little care while cultivation, (Singh *et al*., 2017). It has attractive flowers, is a very valued plant for garden lovers as an important ornamental crop due to its attractive nature, (Singh *et al*., 2017). The attractive color of these flowers makes their way to garden and home as well as ornamental crop adding value to it (Karel *et al*., 2018).

2.11.4: Uses of *C. ternatea* as forage in animal feeding:-

High quality forages such as leguminous fodders have been found to provide adequate dry season supplementation, and improve the productivity of ruminants (Amole *et al.,* 2013). Forage legume is an important group of forage plants, containing high nutritive value; and one of the legume plants which are a potential ruminant feed is *Clitoria ternatea* (Sutedi, 2013). *C. ternatea* is a high-quality fodder for cattle and goats (Nulik *et al*., 2013); and constitutes an important and economic animal feed source with capacity to improve diets and ration quality for ruminants, (Avalos *et al*., 2004).

Clitoria ternatea had similar lactation performance to Gliricidia as protein supplements to maize stover basal diet (Juma *et al.*, 2006[a]). Farmers can use Clitoria and Mucuna as protein supplements at 2 (kg) dry matter for increased milk production, (Juma *et al.*, 2006[a]). *Clitoria ternatea* escalates diets and rational quality for ruminants due to its nutritional contents (Gomez and Kalamani , 2003; Avalos *et al*., 2004; Juma *et al*., 2006[b]; and Shamnad, 2019).

Feeding leaf meal of *Clitoria ternatea* based diets to goat kids should be encouraged among farmers in order to improve ruminant performance (Swati and Varsha, 2014). In addition Nulik *et al*., 2013, reported that the goats fed on *Clitoria ternatea* and *Centrosema pascuorum* for 2 months (November to January) had live weight gains of 33 and 58 g/head/day, respectively.

The average daily weight gain of cattle grazing on mixture of *Brachiaria mutica* grass and *C. ternatea* was 680 g/day (Sutedi, 2013). Live weight gains of 0.7-1.3 kg (head/day) was recorded for steers grazing on pure clitoria pastures in central Queensland, Australia, (Neelamma *et al.,* 2016).

2.11.4.1: Effect of *Clitoria ternatea* on sheep performance:

Butterfly pea is a leguminous fodder, and it is a good source of livestock feeding, because the thin stem and large leaves, non toxic nature which makes it highly palatable by livestock (Karel *et al*, 2018). The animals' feed preferences are influenced by feed availability, plant structure, animal's nutrient deficiencies (e.g. salt) and appetite, (Mutwedu *et al*., 2020) and different species of animals prefer different types of feed (Mutwedu *et al*., 2020).

Sutedi (2013) reported that this plant can be fed to ruminant as fresh forage or hay without any negative effect on growth performance of animal. Castillo-Lopeza and Domínguez- Ordóñezb (2019); reported the factors that influence microbial protein synthesis in the rumen are the rumen pH, dietary fat, availability of carbohydrates, degradable protein in rumen, and feed intake. *C.ternatea* has high nutritive value; it was a better solution to poor quality ruminant diets (Gomez and Kalamani, 2003). In growing male lambs weight gain was between 120 and 160 g/day; while was 100 to 129 g/day in females when fed with *Clitoria ternatea*, (Avalos *et al*., 2004). The Sheep fed with fresh, chopped *Clitoria* forage, the dry matter intake was 79 g/kg $W0.^{75}$; and dry matter digestibility was 53% (Barros *et al*., 1991). In addition sheep was fed with clitoria based concentrate average daily gain was 152-160 g per day, similar to that obtained with a poultry manure/rice bran concentrate but more profitable (Perez *et al*., 1993). The rams when fed with Guinea grass/*Clitoria* hay and *Chloris gayana* hay; the DMI were 919 g/day; and 669 g/day respectively; while DM digestibility was 65.2% and 55.51% respectively (Sandoval *et al*., 2009). In addition Schlink (1998) reported that the Verano and *Clitoria ternatea* hays fed as a supplement to a spear grass diet produced similar live weight and wool growth.

2.11.4.1.1: Lamb carcass yield and dressing percentage:

Dressing percentage is generally unrelated to lean meat yield, so a high dressing percentage not necessarily corresponds to a high carcass yield (Jacob and Calnan, 2018). The results that obtained by Rajkumar *et al*., (2014), showed that the increase in slaughter weight resulted in increased empty body weight and carcass yield; but did not influenced dressing percentage. Osman and EL-Shafei (1967) reported that the warm dressing percentage in Sudan desert sheep ranged from 38.7 to 54.3 percentage. The dressing percentage (hot carcass/ slaughter weight), were 44.46±1.78, 44.57±0.79 and 46.20±0.81 when lambs slaughtered at (26.07±1.23kg), (32.88±0.37kg) and (35.76±1.30kg) respectively, (Rajkumar *et al*., 2014); while the dressing percentage (hot/ empty weight) were (54.15±1.41), (54.23±0.77) and (55.43±0.92) when lambs slaughtered at (26.07±1.23kg), (32.88±0.37kg) and (35.76±1.30kg) respectively, (Rajkumar *et al*., 2014).

2.11.4.1.2: Lamb meat chemical composition:

The composition of meat was 75% of water, 19% of protein, 3.5 % of soluble non protein, 2.5% of lipid (Lawrie, 1979), and inorganic component such as phosphorus, potassium, sodium, magnesium and trace element comprise 0.65% of fresh meat, vitamin in minute quantities (Lawrie, 1979). *Longismass dorsi* muscle composition in grazing Nellore Brown ram Lambs were 74.14%, 18.05%, 2.08% and 4.48 for moisture, protein, fat and ash respectively (Jalajakshi *et al*., 2016). In addition, Omer and Ekhlas (2018) reported that the chemical composition of meat contained (75.6±0.45) moisture, (1.02±0.02) ash, (1.20±0.09) ether extract; (20.6±0.23) crude protein. Lamb meat chemical composition (%) was 74.02±0.18 moisture, 19.08±0.10 protein, 4.97±0.28 fat and 1.12±0.01 ash when lambs were slaughtered at (32.88±0.37kg), (Rajkumar *et al*., 2014). In addition De Brito *et al*. (2016), reported no sensory or other meat quality trait differences were found between lambs fed on different forage types.

2.11.5: Medicinal uses:

2.11.5.1: Traditional uses:

The flowers of *Clitoria ternatea* are used worldwide as ornamental and used as a food colorant, (Jeyaraj *et al*., 2021). In addition it has traditional usages in treatment of some incurable diseases such as, neurological disorder, hyperglycemia, urinary disorder, goiter, respiratory disorders and cancer (Lijon *et al*., 2017).

Butterfly pea (*Clitoria ternatea*), is widely used in traditional Indian systems of medicine as a brain tonic and is believed to promote memory and intelligence, (Gomez and Kalamani, 2003). Traditional medicines derived from medicinal plants are used by about 60% of the world's population (Darsini and Shamshad, 2015). Herbal formulations are preferred due to lesser side effects and low cost (Darsini and Shamshad, 2015).

The traditional practice of eating the flowers of *Clitoria ternatea* and drinking their infusion as herbal tea in some of the Asian countries (Zakaria *et al*., 2018); is believed to promote a younger skin complexion and protective against skin aging (Zakaria *et al*., 2018). Its seeds roasted and powdered are given in doses of 30-60 grains in cases of ascites and enlargement of abdominal viscera (Chauhan *et al*., 2012). The roots of *C. ternatea* have bitter taste with purgative, laxative and diuretic properties; generally used for the treatment of indigestion, constipation, arthritis and eye ailments and fever (Mukherjee *et al*., 2008). In addition the root part was used for the treatment of skin diseases, enlargement of the abdominal viscera, and sore throat (karel *et al*., 2018). Also seeds and leaves were usually used as a brain tonic and to promote cognitive skills, (karel *et al*., 2018).

2.11.5.2: Pharmacological uses:

Clitoria ternatea has been evaluated for its medicinal properties and shows promising effects as having antidiabetic, antioxidant and hepatoprotective activities, (Zingare *et al*., 2013). The presence of multi active secondary metabolites which was present in the leaves and seeds extracts of the *Clitoria ternatea*, had made the plant a very useful medicinal plant (Chakraborty *et al*., 2017).

2.11.5.2.1: Effect on respiratory system:

Butterfly pea has potential to be used as pharmaceutical. The flavonoid quercetin has been shown to minimizing upper respiratory infections in humans, (Morris, 2009). It is used in common cold, cough, asthma as it acts as an expectorant and minimizes the irritation of respiratory organs, (Neelamma *et al.*, 2016).

2.11.5.2.2: Effect on digestive system:

It is an antiemetic, antidypsetic mild laxative and cholagogue. Therefore it is used in emesis, dyspepsia, constipation jaundice and piles. It is used in healing ulcers of pylorus duodenum etc (Pendbhaje *et al*., 2011). The antiulcer potential of aqueous and ethanolic extracts of *Clitoria ternatea* were evaluated in different experimentally induced ulcer models in rats (Rai *et al*., 2015). Ethanolic extract (200 and 400 mg (per kg) and aqueous extract 200 and 400 mg (per kg) of whole plant were examined in pylorus ligation and indomethacin induced gastric ulcer in rats; the results showed, the high dose of alcoholic extract recorded significant antiulcer activity in pylorus ligation and indomethacin induced ulceration (Rai *et al*., 2015).

2.11.5.2.3: Effect on urinary system:

The seeds of clitoria were used in swollen joints, and for urinary problems crushed seeds are taken with cold or boiled water (karel *et al*., 2018). In addition Uma *et al*., 2009 reported the crude methanol extract of *Clitoria ternatea* blue flowers was used against urinary pathogens.

2.11.5.2.4: Effect on circulatory system:

Being haemostatic and blood purifier, it is benefit in haemorrhagic disorders and vatarakta. Hot infusion of dhamasa is given to prevent small pox (Neelamma *et al.*, 2016).

2.11.5.2.5: Immunomodulatory effects:

The plant extracts have immunomodulatory effects that strengthen the immune system (Gupta *et al*., 2010). Kamilla *et al*. (2014) they reported that the methanolic extract of *C. ternatea* was effective against aspergillosis in immunocompromised mice comparable to that of amphotericin. In addition Anarthe *et al*., (2017), reported that the methanolic extract of *Clitoria ternatea* showed significant immune stimulating activity with specific and non specific mechanism, due to the presence of prominent amount of flavonoids and phenols.

2.11.5.2.6: Effect on central nervous system:

Ethanol extract of the root of *Clitoria ternatea* showed significant neuropharmacological activity (Gupta *et al.*, 2010). The seeds and leaves of *Clitoria ternatea* widely used as brain tonic and believed to promote memory and intelligence, (More and Hake, 2019). In addition, Shahnas, and Akhila (2014) reported that the aqueous extract of *Clitoria ternatea* can be used against

Alzheimer's disease. The isolated compound may act as a lead compound for identifying new derivatives which could be used for improving memory, (Shahnas, and Akhila, 2014). Parvathi and Ravishankar (2013) reported that ethanolic root extract of *Clitoria ternatea* has significant anti depressant activity. In addition *C. ternatea* root extracts increase rat brain acetylcholine content and acetyl choline esterase activity in a similar fashion to the standard cerebro drug pyritinol (Taranalli and cheeramkuzhy, 2000).

2.11.5.2.7: Antioxidant effect:

The aqueous extracts of *Clitoria ternatea* flower were shown to have stronger antioxidant activity when compared with ethanol extracts (Kamkaen and Wilkinson, 2009). Methanol extract of white flowered variety of *Clitoria ternatea* showed higher antioxidant activity as compared to blue flowered variety of *Clitoria ternatea* (Zingare *et al*., 2013). *C. ternatea* has antioxidant activity that prevents lipid peroxidation in erythrocytes, protein oxidation, and free radical-induced hemolysis, (Phrueksanan *et al*., 2014).

2.11.5.2.8: Anti-inflammatory effect:

Leaf and flower extracts of *C. ternatea* have been identified as having inflammatory activity (Suganya *et al*., 2014; Singh *et al*., 2018). *Clitoria ternatea* roots methanol extract when given by oral route to rats was found to inhibit both the rat paw oedema caused by carrageenin and vascular permeability induced by acetic acid in rats (Pendbhaje *et al*., 2011). The anti inflammatory, analgesic studies of petroleum ether extract (60-80c) from the flowers of *Clitoria ternatea*, (kumar and Ishwar, 2012); and the results showed that it exhibited significant anti inflammatory activity at both the dose level 200 and 400 mg/kg (body weight) ($P<0.01$), (kumar and Ishwar, 2012).

2.11.5.2.9: Antibacterial effect:

The crude extract from seeds of *C. ternatea* showed strong antimicrobial activity, (Pendbhaje *et al*., 2011). Ethyl ether and alcoholic extracts of leaves of *Clitoria ternatea* had positive reactions against bacterial and fungal (Candida albicans) pathogen, (Kapoor and Purohit, 2013). Lakna (2017) reported that the gram positive bacteria are more susceptible to antibiotics, due to the lack of an outer membrane; whereas gram negative bacteria have an outer membrane, less susceptible to antibiotics.

Mhaskar *et al*. (2010) reported that crude extract from seeds of *Clitoria ternatea* showed maximum zone of inhibition against *E. coli* (22±0.5 mm) at 0.75 mg concentration. And the callus extract showed maximum zones of inhibition against *S. typhi* (16±2mm); while the lowest zones were recorded against *E. coli* and *S. aureus* with values 12±1 mm and 12±0.9 mm, respectively, (Mhaskar *et al*., 2010).

The flower extract consists of antioxidant activity and protective effect (Karel *et al*., 2018). It has been commonly used as anti stress, anti depressant, anti microbial, and in anti inflammatory treatments, (Karel *et al*., 2018). All parts of *Clitoria ternatea* contain peptides called cliotides that have potent as anti microbial properties against *Escherichia coli* (Nguyen *et al*., 2011).

Ponnusamy *et al*. (2010), reported that ethyl acetate extracts of *C. ternatea* showed the widest zone of inhibition against *A. formicans* (18 mm), *A. hydrophilia* (19 mm), *B. subtilis* (19 mm) and *P. aeruginosa* (21 mm), next to that ethanol extract of *C. ternatea* showed highest inhibition zones for A. *formicans* (18 mm) and *E. coli* (14 mm), followed by Acetone extract showed maximum zone of inhibition for *S. agalactiae* (19 mm), and *K. pneumonia* (17 mm). In addition Darsini and Shamshad (2015) reported that the organic solvent (Ethanol, Methanol, Hexane) and water extract from the whole plant of *Clitoria ternatea* were tested against,

Salmonella typhimurium, *Proteus vulagaris*, *Shigella dysenteriae*, Darsini and Shamshad (2015); and the results showed prominent antibacterial activity against the tested microbial pathogens. Parekh and Chanda, (2006) and Lakna, (2017), who reported that the gram positive bacteria are more susceptible towards plant extracts when compared to gram negative bacteria. The cell wall in gram positive bacteria is a single layer, whereas the gram negative cell wall is multilayered structure, (Yao and Moellering, 1995 and Lakna, 2017). In addition Uwimbabazi., *et al* (2015), and Pahune *et al*., (2013), reported the bactericidal activity increased with the increase of the extract concentration.

CHAPTER THREE

Materials and Methods

CHAPTER THREE

3. Materials and Methods:

3.1. Trial (1): Fodder trial:-

3.1.1: Experimental location and climatic data:

This study was conducted at the demonstration farm of the College of Agricultural Studies, Sudan University of Science and Technology at Shambat Khartoum North, during the period: 25/11/2018 - 30/06/2019 to evaluate growth, yield and chemical composition of *Clitoria ternatea* forage as affected by poultry manure with eggshell fertilizers. The latitude of the location is 15º 40´ N and longitude is 32º 32´ E. The information about the weather during the growth of *Clitoria ternatea* plants was obtained from the Ministry of Irrigation and Water Resources Meteorological Authority, Khartoum, Sudan, (Appendix 6).

3.1.2: Plant material:

The seeds of *Clitoria ternatea* cultivar were bought from local market.

3.1.3: Land preparation:

The land was ploughed by a disc plough (40 cm), harrowed twice by a disc harrow, leveled by a leveler and ridged up by a ridge 70 cm apart.

3.1.4: Experimental design:

The trial was done in randomized block design of four treatments and five plots as replication of each treatment; plot size was 3×4 m^2.

3.1.5: Planting:

Sowing date of *Clitoria ternatea* was on 25/11/2018 at the rate of 12.5 kg/ ha. The seeds were sown on top of the shoulder of the ridge (2.5 cm depth) at a distance of 70 cm between rows and 20 cm between holes. Five seeds were planted in each hole. Thousand grain weight (T.G.W) was 50 grams and germination % was 85.

3.1.6: Treatments:

The treatments consisted of:-

(1) Treatment1: no fertilizer (control).

(2) Treatment2: 5000kg/ha poultry manure.

(3) Treatment3: 4600 kg/ha poultry manure+ 400kg /ha eggshell.

(4) Treatment4: 4200 kg/ha poultry manure +800kg/ha eggshell.

The eggshell was collected from Bahri Halawany, then washed, and shade dried; then grinded, (Appendix 7).The fertilizers were broadcasted once before the sowing of seeds. A representative soil sample (0-20), (20-40) and (40-60 cm) were taken before sowing to determine some physical and chemical properties (Table 1). The soil type was determined by using soil texture triangle (Appendix 8).

Table (1): Mechanical and chemical properties of the experimental soil site:-

Depth (cm)	pH paste	ECe dS/m	Soluble Cations Meql/L			SAR	Soluble anions Meql/L		
			Na	K	Ca+Mg		CO_3	HCO_3	CL
0-20	7.9	1.09	7.4	0.8	2.7	6	0.0	0.2	0.1
20-40	7.9	1.38	5.7	1.3	6.8	3	0.0	0.2	0.1
40-60	7.9	1.26	9.2	0.3	3.2	7	0.0	0.2	0.1

Depth (cm)	N %	P (ppm)	O.C %	Ex.Ch. Cations Meql/L			CEC Meql/100g	ESP	$CaCO^3$ %	Particle size distribution (%)		
				Na	K	Ca+Mg				Sand	Silt	Clay
0-20	0.001	3	0.001	5.3	0.4	32.3	38	14	13	41	20	39
20-40	0.001	4	0.001	5.8	0.4	36.8	43	14	14	33	22	45
40-60	0.001	3	0.001	5.1	0.4	34.5	40	13	14	34	23	43

3.1.7: Irrigation:

The first irrigation was immediately after sowing of seeds and the frequency of irrigation was every 12-15 days, depending on temperature, relative humidity and soil moisture conditions. Manual weeding by a hand implement "Nagama" was done, five weeks after planting. And then weeding was done whenever necessary.

3.1.8: Weed control:

The weeds which appeared in the experiment included:

1. Moleita (Conchus cornutus (*L.*).
2. Nageel (*Cynodon dactylon* (*L.*).
3. Rabaa (*Trianthema pentandra* (*L.).*
4. Sakaran (*Datura stramonium (L.).*
5. Seida (*Cyperus rotundus* (*L.*).
6. Um gelagil (*Aristolochia bracteolata*).

3.1.9: Measurements of growth parameters:-

3.1.9.1: Plant height (cm):

Twenty plants were randomly measured at (15, 30, 45, 60 days) from the base of the main stem to the tip of the panicle using a meter tape; average plant height was calculated.

3.1.9.2: Stem diameter (cm):

From the plant above stem diameter (cm) was determined by measuring on the center of the plants above, average was determined.

3.1.9.3: Number of leaves:

It was counted from the above plants and the average was determined.

3.1.10: Leaf to stem ratio:

Leaves were separated from stem and branches of the above ground plants. Both leaves and stems were oven dried at 105º for 24 hours and then weighed to calculated leaf/stem ratio.

3.1.11: Total yield per hectare:

Three cuts were harvested throughout the year with cut interval of 56 days; the first cut was at 60 days after sowing. The fresh and dry yield (ton/ hectare) were measured at the end of each harvest.

3.1.11.1: Fresh yield (t/ha):

All plants from each plot were cut at 10 cm height above the ground; then weighed to measure fresh weight, and the yield was transformed into ton per hectare.

3.1.11.2: Dry yield (t/ha):

The sample was taken from each plot, then oven dried at 105º (for 24 hours) to obtain dry yields, and then the weight was transformed into ton per hectare.

3.1.12: Chemical composition of leaves and stems (%):

Samples of *Clitoria ternatea* (leaves and stems) were taken from each three cuts and analyzed for dry Matter (DM), crude Protein (CP), Crude fiber (CF), ether extract (EE) and ash by using near-infrared reflectance spectroscopy (NIRS). NFE was calculated by equation: NFE%= DM- (CP%+CF%+EE%+ Ash %).

3.1.12.1: Near infrared reflectance spectroscopy (NIRS) analysis:

For NIRS analyses, 10-15g of dried samples were evaluated by using near infrared reflectance spectroscopy (NIRS); the reflectance mode using a BRUKER, MPA model (Bruker, OPUS software version 7.2), (Appendix 9).

3.1.13: Statistical Analysis:

Data collected were presented as mean± standard deviation and were analyzed using SPSS (Version 17.0) (2008) computer software program as one way analysis of variance (ANOVA), treatment means were separated by the least significant difference (LSD) method.

3.2. Trial (2): Feeding trial:-

3.2.1: Site of the study:

The study was conducted during the period from 16/10/2019 to 2/1/2020 at the research farm of the colleges campus (Veterinary Medicine; Animal Production Science and Technology) (KUKU complex), Sudan University of Science and Technology.

3.2.3: Experimental animals:

Twenty four (24) lambs (male) of Sudanese desert sheep were used in this experiment, with average weights 18.82± 1.67 kg and aged 4 months. All animals were treated with the necessary medication against external and internal parasites. The animals were ear tagged, weighed and divided into four groups (six lambs per group as replicates).

3.2.4: Experimental design:

A completely randomized design (CRD) of four treatments and six lambs (replications) per treatment were used.

3.2.5: Housing:

The lambs were housed in a farm with (12 meter length, 5 meter width and 2.5 meter height), all sides of farm were closed by sexcebanda and the roof was from zink. The farm was provided with 24 individual cages; (12 cages in each side of farm), each cage was 1.5 meter length, 1meter width and 1.2 meter height. There was a spacing in the middle (2 meter) along the farm. All cages were provided with individual feeders and drinkers (Appendix 10). The trial period was 77 days, the first 14 days for adaptation of animals for diet and 63 days for data collection. The general lambs' health was daily checked every morning throughout the period of the experiment; (Appendix 11).

3.2.6: Feed and feeding:

The feed was provided ad libitum in the form of total diet; with hay: concentrate ratio of 40:60 (Table 2). The diets were offered once a day, at 9:00 am. The hay of clitoria and alfalfa were dried under shade before grinding (Appendixes 12) and (Appendixes 13).

Hay and hay mixtures:

1- Group (A): hay composed of 100% *Clitoria ternatea*.

2- Group (B): hay composed of 50% *Clitoria ternatea* +50% alfalfa.

3- Group(C): hay composed of 75% *Clitoria ternatea* + 25% alfalfa.

4- Group (D): hay composed of 100% alfalfa.

Table (2): Ingredients of experimental diets (%):-

Ingredients (%)	Ration A	Ration B	Ration C	Ration D
Sorghum	38.00	38.00	38.00	38.00
Wheat bran	7.00	7.00	7.00	7.00
Groundnut cake	4.00	4.00	4.00	4.00
Molasses	9.00	9.00	9.00	9.00
***Clitoria ternatea* hay meal**	40	-	-	-
Hay mixture (50%*Clitoria*+50%Alfala)	-	40	-	-
Hay mixture (75%*Clitoria*+25%Alfalfa)	-	-	40	-
Alfalfa hay meal	-	-	-	40
Salt	1.00	1.00	1.00	1.00
Lime stone	1.00	1.00	1.00	1.00
Total	100%	100%	100%	100%

3.2.7: Feed samples and chemical composition:

Feed samples (*Clitoria ternatea* hay, alfalfa hay and concentrates) were collected weekly and chemically analyzed according to (A.O.A.C, 1990), to determine the percentages of DM, CP, CF, EE and Ash. Whereas the NFE was calculated by using the following equation: NFE= 100-(CP%+CF%+EE%+Ash %), (Table 3).

Table (3): Chemical composition of complete mixed rations used in trial:

	Chemical composition						
	DM%	**CP%**	**CF%**	**EE%**	**Ash%**	**NFE%**	***ME**
Ration A	90.99	15.44	19.37	2.38	7.64	46.16	10.02
Ration B	90.72	15.21	18.95	2.26	7.84	46.46	9.98
Ration C	90.86	15.33	19.16	2.32	7.74	46.31	10.00
Ration D	90.44	14.97	18.53	2.13	8.04	46.77	9.93

* ME was calculated according to the equation given by MAFF (1975) as follows: ME (MJ/Kg DM) = 0.012 CP +0.031 EE +0.005 CF + 0.014 NFE

All lambs had free access to mineral blocks which were available throughout the experimental period, (Table 4).

Table (4): Chemical composition of the mineral block (weighs 5 kg):-

Composition	**Values**
Vitamin A	175000 IU
Vitamin D3	35000 IU
Vitamin E	200 Mg
phosphorus	3000 Mg
Calcium	5000 Mg
Magnesium	500 Mg
Iodine	60 Mg
Manganese	500 Mg
Cobalt	60 Mg
Zinc	250 Mg
Iron	1500 Mg
Copper	500 Mg
Selenium	25 Mg
Sodium	37 %

Source: Company of Alnajm Aldahabi for Salt Cubes (2019).

3.2.8: Lambs performance:

3.2.8.1: Daily feed intake (kg):

The feed intake of each lamb per group was recorded daily, weighed individually as difference between amount offered and the refusals, the dry matter values from rations were used to calculate the dry matter intake.

3.2.8.2: Live body weight (kg):

The initial live body weight was recorded for each lamb at the beginning of the trial, and then the lambs were weighed individually every week at 8 am.

3.2.8.3: Daily weight gain (gram):

Daily live weight gain is a weight gain during the study divided by the length period of the study. Total body weight gain was recorded during the whole trial, and the average daily gain was computed.

3.2.8.4: Feed conversion ratio (FCR):

The feed conversion ratio (FCR) was calculated according to the equation:-

Average daily dry matter intake (kg) ÷ Average daily weight gain (kg).

3.2.9: Slaughter procedure and slaughter data:

A total of 12 lambs from the four groups (three from each group); were selected randomly and then destined for slaughter. Lambs offered water but not feed for 12 hours before slaughter. The lambs were weighed before slaughter, at the abattoir of the Animal Production Researches (Kuku); then bleed for 10-15 minutes and

skinned; then evisceration was performed. The alimentary tract was removed, weighed and then after clearing the contents weighed again to obtain the empty body weight. The internal offal's (heart, liver, spleen, lung and trachea, diaphragm, pancreas, genital organs, omental fat and mesenteric fat), were carefully removed and weighed. The kidneys and their fat were left intact in carcass. Then carcasses were weighed hot and after chilling for 24 hours at 4°C.

3.2.9.1: Dressing percentage (%):

Dressing percentage is the weight of a carcass, expressed as a percentage of the live weight of the animal (Jacob and Calnan, 2018).

The Dressing % was calculated according to the following equations:

1- Dressing % = (Hot carcass weight/ slaughter weight) × 100
2- Dressing % = (Hot carcass weight/ empty body weight)× 100
3- Dressing % = (Cold carcass weight/ slaughter weight) × 100
4- Dressing % = (Cold carcass weight/empty body weight)×100

3.2.9.2: Meat samples:

Samples were taken from *L. dorsi* muscle, after 24 hours postmortem for chemical analysis and quality parameter.

3.2.9.3: Meat chemical composition:

Determination of moisture, protein, fat (ether extract) and ash percentages of meat samples were performed according to AOAC (1990) methods.

3.2.9.3.1: Determination of moisture (%):

Five grams of sample dried overnight in drying oven at 100°C. The dried sample was cooled in desiccators and weighed. The moisture percentage was calculated as follows:

$$\textbf{Moisture (\%)} = \frac{\text{(Weight of sample before drying – Weight of dried sample)}}{\text{Weight of sample before drying}} \times 100$$

3.2.9.3.2: Determination of protein (%):

Kjeldahl method was used to determine nitrogen percentage. One gram of sample was digested in Kjeldahl flask by adding one gram of catalysts and 25ml conc. H_2SO_4, and heated for 3 hours. The digested samples were cooled, and then 100ml of distilled water was added to each flask. Twenty five ml of 2% boric acid pipetted into 250 ml conical flask and two drops of methyl red indicator solution added; and into the decomposition chamber of the distillation apparatus was added 12 ml of 40% NaOH solution. Five ml of digested sample solution introduced, into a kjeldahl flask. The condenser tip of the distillation apparatus then dipped into the boric acid contained in the conical flask. The ammonium in the sample solution distilled into the boric acid until it changed completely into blue. Then the distillate was titrated with 0.1 N HCL solution. The formula used for calculation of crude protein was as follows:-

$$\text{Crude protein (\%)} = \frac{T\,(N \times 14 \times VF) \times 6.25}{\text{Weight of sample} \times 1000} \times 100$$

Where:

T: Titre value
N: Normality of HCL.
14: Each ml of HCL is equivalent to 14 mg nitrogen.
VF: Total volume of digest
1000: To convert from mg to g.
Crude protein %: Nitrogen %×6.25

3.2.9.3.3: Determination of fat (%):

Fat was determined by ether extraction .Two grams of sample were taken into Soxhlet apparatus. Then the sample was subjected to continuous extraction with ether for 6 hours. The fat and residual solvent allowed drying in an oven at 100c for 30 minutes; to complete evaporate the solvent. The fat was cooled and weighed for ether extraction percentage. The formula used for calculation of crude fat was as follows:-

$$\text{Fat (\%)} = \frac{\text{Fat weight}}{\text{Sample weight}} \times 100$$

3.2.9.3.4: Determination of ash (%):

Five grams of sample weighed into previously dried and known weight of crucible; then placed inside a muffle furnace at 105°c. The temperature was increased gradually till it reached 600°c for 3 hours. Then the sample cooled into desiccators and weighed. The ash percentage was calculated as:

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

3.2.10: Statistical Analysis:-

Data collected were presented as mean± standard deviation and were analyzed using SPSS (Version 17.0) (2008) computer software program as one way analysis of variance (ANOVA), treatment means were separated by the least significant difference (LSD) method.

3.3. Trial (3): Antibacterial activity trial:-

3.3.1: Antibacterial activity analysis of *Clitoria ternatea* leaves:

3.3.1.1: Plant material:

Clitoria ternatea plants were collected from the farm of College of the Agricultural Studies, Department of Agronomy; Sudan University of Science and Technology at Shambat, Khartoum North; Sudan.

3.3.1.2: Preparation of leaves extract:

The dried leaves of plant were powdered into fine particles using an electrical blender. The fine powder was stored in air tight containers. Fifty grams of leaf material was soaked in 250 ml methanol for 24 hours then put on rotary shaker (220 rpm) for 24 hours, (Appendix 14), then filtered using standard filter paper. The filtrate was transferred into vials and allowed to evaporate until completely dry and left in the refrigerator at (4º C).

3.3.1.3: Test of microorganisms:

The Gram-positive and Gram-negative bacteria were used for antibacterial activities studies: Gram-positive bacteria included *Staphylococcus aureus* and

Streptococcus agalactia; Gram-negative bacteria included *Escherichia coli* and *Salmonella typhi*. The test organisms were supplied by Microbiology lab, College's campus of Veterinary medicine and Animal Production Science and Technology; Sudan University of Science and Technology.

3.3.1.4: Agar Well Diffusion Method:

The antimicrobial test was performed by following agar well diffusion method (Perez *et al.*, 1990); with some modifications by using Mueller Hinton Agar No. 2 medium. Muller Hinton agar was prepared as per the instructions by the manufacturer, once the media solidified. The media was punched with 3 mm diameter hole, and then the plates were inoculated with bacterial species with size of 10^6 colony-forming units (CFU)/ml of bacteria were spread with an L-shaped glass rod; then the well was filled with extract. Inhibition zones around the wells were measured after 24 hours of incubation at 37°C by measuring the diameter of the inhibition zones (cm) (Mean ±SD).

3.3.1.5: Disc-Diffusion Method:

The leaves extract of plant were subjected to a serial dilution using sterile distilled water as a diluents. 1g from crude extract was added into a tube containing 9 ml of sterile distilled water, from this tube, a serial dilution was done and covered a dilution range of 10^{-1} to 10^{-4}. This helped to determine the minimum inhibitory concentration (MIC) of extract on each strain (Uwimbabazi *et al*., 2015). Filter paper discs were prepared (3mm diameter); then sterilized and soaked in different concentrations of extract, and then they were aseptically placed over the media with specific bacteria. The plates were incubated in an upright position at 37 °C for 24 hours. The diameters of inhibition zones were measured in cm (Uwimbabazi *et al*., 2015).

3.3.2: Statistical Analysis:

Data collected were presented as mean± standard deviation and were analyzed using SPSS (version 17.0) (2008) computer software program as one way analysis of variance (ANOVA), treatment means were separated by the least significant difference (LSD) method.

CHAPTER FOUR

Results

CHAPTER FOUR

4. Results:

4.1: Trial (1): Fodder trial:-

4.1.1: Measurements of growth parameters:-

4.1.1.1: Plant height (cm):

The statistical analysis showed highly significant differences at ($p \leq 0.01$) in the plant height. The highest mean of plant height was obtained by treatment 4 with values (7.30±0.45), (12.30±0.73), (19.36±1.40) and (49.44±1.72) cm for day 15, 30, 45 and 60 respectively (Table 5); while control (treatment 1) obtained the lowest plant height with values (4.60±0.89), (7.44±0.65), (13.52±0.98) and (25.76±1.13) cm for day 15, 30, 45 and 60 respectively, (Table 5).

4.1.1.2: Stem diameter (cm):

The results showed no significant differences between treatments on 15, 30 and 60 days; while they exposed highly significant differences at ($p \leq 0.01$) among different treatments in 45 day of plant age; whereas the highest stem diameter was recorded by treatment4 (0.72±0.04 cm), but the lowest stem diameter was obtained by treatment1 (0.50±0.10 cm), (Table 5).

4.1.1.3: Number of leaves per plant:

The results showed no significant differences between treatments in number of leaves on 15 and 30 days; whereas highly significant differences at ($p \leq 0.01$), between treatments were obtained on 45 and 60 days in number of leaves. The highest number of leaves was obtained by treatment4 with values (20.80±0.84) and (28.20±1.30) for days 45 and 60, respectively, (Table 5).

Table (5): Effect of poultry manure and eggshell fertilizers on plant height; stem diameter and leaves number of *C. ternatea*:-

Trait	Period	T1	T2	T3	T4	Sig
Plant height (cm)	15 days	4.60 ± 0.89^{d}	5.80 ± 0.84^{bc}	6.70 ± 0.84^{abc}	7.30 ± 0.45^{ab}	**
	30 days	7.44 ± 0.65^{bcd}	8.24 ± 0.87^{bcd}	8.20 ± 0.67^{bcd}	12.30 ± 0.73^{a}	**
	45 days	13.52 ± 0.98^{cd}	14.72 ± 1.40^{cd}	18.38 ± 1.10^{ab}	19.36 ± 1.40^{ab}	**
	60 days	25.76 ± 1.13^{d}	36.52 ± 2.63^{c}	47.62 ± 1.71^{ab}	49.44 ± 1.72^{ab}	**
Stem diameter (cm)	15 days	0.24±0.06	0.32±0.13	0.26±0.05	0.34±0.05	Ns
	30 days	0.46±0.05	0.50±0.10	0.60±0.12	0.58±0.08	Ns
	45 days	0.50 ± 0.10^{d}	0.60 ± 0.07^{bc}	0.68 ± 0.04^{abc}	0.72 ± 0.04^{a}	**
	60 days	0.76±0.15	0.68±0.08	0.74±0.05	0.76±0.05	Ns
No. of leaves/ plant	15 days	5.20±0.45	5.80±0.44	5.60±0.55	5.40±0.55	Ns
	30 days	8.20±1.30	8.60±0.89	9.40±0.89	7.80±0.84	Ns
	45 days	14.80 ± 1.30^{bcd}	15.34 ± 0.85^{bcd}	15.6 ± 1.67^{bcd}	20.80 ± 0.84^{a}	**
	60 days	24.00 ± 1.00^{d}	27.80 ± 0.76^{abc}	27.80 ± 1.48^{abc}	28.20 ± 1.30^{abc}	**

T1: Treatment1, T2: Treatment2, T3: Treatment3, T4: Treatment4, No: Number. Treatment1: no fertilizer used (control). Treatment2: 5000kg/ha poultry manure. Treatment3: 4600 kg/ha poultry manure+ 400kg /ha eggshell. Treatment4: 4200 kg/ha poultry manure +800kg/ha eggshell.

a, b ,c, d means the mean with different superscripts within a row are significantly different.

NS= No significant difference.

** = highly significant differences ($p\leq0.01$).

4.1.2: Leaf to stem ratio:

The results showed that the treatments of fertilizers had highly significant effect on leaf to stem ratio of *Clitoria ternatea at* ($p\leq0.01$). Treatment 4 recorded the highest Leaf to stem ratio (3.86± 0.50); compared with other treatments leaf/stem ratio ranged between (2.08± 0.64) to (2.60±0.04), (Table 6).

Table (6): Effect of poultry manure and eggshell fertilizers on leaf: stem ratio of *C. ternatea*:-

Treatment	Leaf: Stem ratio
T1	2.08 ± 0.64^{b}
T2	2.42 ± 0.43^{b}
T3	2.60 ± 0.04^{b}
T4	3.86 ± 0.50^{a}
Sig	**

Means with different superscripts within a column are significantly different.

** = highly significant differences ($p\leq 0.01$).

4.1.3: Total yield per hectare:

4.1.3.1: Fresh yield (t/ha):

The results showed that fresh yield was highly significantly affected by the fertilizers applied, ($p\leq 0.01$). Highest fresh yield was obtained by treatment 4 over the three harvests, with yield (0.95±0.15), (6.53±0.95) and (11.03±2.11) tons/hectare during first, second and third cuts respectively (Table 7).

Table (7): Effect of poultry manure and eggshell fertilizers on fresh yield (Ton/hectare) of *C. ternatea*:-

Treatment	First cut	Second cut	Third cut	Total yield
T1	$0.58\pm.08^{cd}$	3.76 ± 0.47^{cd}	5.23 ± 0.44^{d}	9.56 ± 0.66^{d}
T2	0.69 ± 0.16^{cd}	5.63 ± 0.84^{ab}	7.24 ± 1.11^{bc}	13.56 ± 1.50^{bc}
T3	0.86 ± 0.07^{ab}	4.3 ± 0.3^{cd}	$8.02\pm.73^{bc}$	13.19 ± 1.02^{bc}
T4	0.95 ± 0.15^{ab}	6.53 ± 0.95^{ab}	11.03 ± 2.11^{a}	18.5 ± 3.12^{a}
Sig	**	**	**	**

Means with different superscripts within a column are significantly different.

** =highly significant differences ($p\leq 0.01$).

4.1.3.2: Dry yield (t/ha):

Similar to fresh yield, dry yield was highly significantly affected by the fertilizer type applied, ($p \leq 0.01$), also treatment4 had the highest dry yield production with yield (0.25±0.039), (1.76±0.18) and (4.64±1.28) tons/hectare during first, second and third cuts respectively (Table 8).

Table (8): Effect of poultry manure and eggshell fertilizers on dry yield (Ton/hectare) of *C. ternatea*:-

Treatment	First cut	Second cut	Third cut	Total yield
T1	0.15 ± 0.022^{cd}	1.20 ± 0.18^{cd}	2.05 ± 0.1^{bcd}	3.40 ± 0.18^{bcd}
T2	0.18 ± 0.041^{bcd}	1.73 ± 0.32^{ab}	2.44 ± 0.25^{bcd}	4.36 ± 0.41^{bcd}
T3	0.22 ± 0.019^{abc}	1.17 ± 0.07^{cd}	2.68 ± 0.23^{bcd}	4.06 ± 0.28^{bcd}
T4	0.25 ± 0.039^{ab}	1.76 ± 0.18^{ab}	4.64 ± 1.28^{a}	6.65 ± 1.46^{a}
Sig	**	**	**	**

Means with different superscripts within a column are significantly different.

** = highly significant differences ($p \leq 0.01$).

4.1.4: Chemical composition of leaves and stems (%):-

4.1.4.1: Chemical composition of leaves (%):-

4.1.4.1.1: Dry matter (%):

The DM percentage in leaves was not significantly different with fertilizer types applied in first and second cuts ($p > 0.05$); while it was highly significantly different among treatments at ($p \leq 0.01$) in the third cut. The highest DM percentage was recorded by treatment 2 (90.56±0.26); but the lowest DM percentage was recorded by treatment 1 (89.12±0.50), (Table 9).

4.1.4.1.2: Crude protein (%):

The crude protein showed no significant differences between treatments in first and second cuts ($p>0.05$); whereas the results had significant differences among treatments ($p\leq0.05$) in crude protein percentage in the third cut; the treatment 2 was obtained the highest protein percentage (33.67±0.58); while the lowest was recorded by treatment1 (29.63±0.84) (Table 9).

4.1.4.1.3: Crude fiber (%):

There were no significant differences in leaves among treatments ($p>0.05$) in crude fiber during the first, second and third cuts (Table 9).

4.1.4.1.4: Ether extract (%):

No significant difference between treatments ($p>0.05$) in EE on first, second and third cuts, (Table 9).

4.1.4.1.5: Ash (%):

The result showed no significant difference between treatments ($p>0.05$) in ash percentage in the first and third cuts; while there were significant differences in second cut; the highest ash percentage in leaves in second cut was recorded by treatment1 (7.55±0.30); compared with other treatments. Ash percentage ranged between (6.30±0.24) and (6.77±0.35), (Table 9).

4.1.4.1.6: Nitrogen Free Extract (%):

No significant differences between treatments ($p>0.05$) in NFE percentage during the first, second and third cuts (Table 9).

Table (9): Effect of poultry manure and eggshell fertilizers on Chemical composition of *Clitoria ternatea* leaves:-

%	Cuts	Treatments				Sig
		T1	T2	T3	T4	
DM	First cut	93.48±0.20	92.98±0.24	93.53±0.21	93.56±0.36	NS
	Second cut	91.43±0.29	91.59±0.77	91.44±0.21	91.55±0.20	NS
	Third cut	89.12 ± 0.50^{cd}	90.56 ± 0.26^{ab}	90.42 ± 0.28^{abc}	89.78 ± 0.32^{bcd}	**
CP	First cut	22.25±1.89	23.44±2.06	24.33±3.72	27.73±4.57	NS
	Second cut	35.55±1.22	34.71±1.09	35.40±1.01	34.07±2.94	NS
	Third cut	29.63 ± 0.84^{cd}	33.67 ± 0.58^{ab}	32.60 ± 0.67^{ab}	29.64 ± 2.35^{cd}	*
CF	First cut	15.56±0.71	13.10±0.85	14.63±2.04	15.08±0.75	NS
	Second cut	18.23±1.61	18.67±1.67	16.85±1.00	19.08±0.41	NS
	Third cut	16.25±1.37	18.58±0.84	19.11±1.38	17.88±1.49	NS
EE	First cut	3.24±0.21	3.07±0.11	3.32±0.12	3.17±0.12	NS
	Second cut	3.72±0.21	3.87±0.21	3.92±0.34	4.05±0.37	NS
	Third cut	5.08±0.37	4.39±0.16	4.32±0.13	5.84±1.00	Ns
Ash	First cut	8.32±0.78	8.06±0.08	8.35±0.53	8.23±0.15	NS
	Second cut	7.55 ± 0.30^{a}	6.56 ± 0.36^{bcd}	6.30 ± 0.24^{bcd}	6.77 ± 0.35^{bcd}	**
	Third cut	7.89±0.34	7.43±0.62	7.73±0.87	7.89±0.75	NS
NFE	First cut	44.10±1.57	45.32±1.55	42.90±5.85	39.35±4.30	NS
	Second cut	26.37±2.78	27.77±2.18	28.97±2.25	27.58±2.12	NS
	Third cut	30.27±0.63	26.49±1.85	26.66±2.06	28.53±3.18	NS

Means with different superscripts within a row are significantly different.

NS = No significant difference.

* = Significant differences ($p \leq 0.05$).

** = highly significant differences ($p \leq 0.01$).

4.1.4.2: Chemical composition of stem:-

4.1.4.2.1: Dry matter (%):

The DM percentage was significantly different with the different fertilizer types applied during first and third cuts ($p \leq 0.05$); while no significant differences among treatments at ($p > 0.05$) in second cut; treatment 1 recorded the highest DM percentage (97.13±0.24) in first cut; but treatment 4 recorded the highest DM percentage of stem during second and third cuts with values (96.65±0.41) and (94.80±0.16), respectively, (Table 10).

4.1.4.2.2: Crude protein (%):

The crude protein showed highly significant differences between treatments at ($p \leq 0.01$), in the first cut, the highest protein percentage was recorded by treatment4 (11.19±0.44) while the lowest was recorded by treatment1 (7.29±0.87), (Table 10). While no significant differences were observed among treatments in crude protein percentage in second and third cuts ($p > 0.05$); but treatment4 recorded higher protein percentages in stem with values (12.17±1.79) and (10.58±1.23) for second and third cuts, respectively, (Table 10).

4.1.4.2. 3: Crude fiber (%):

The result showed highly significant differences in stem among treatments in crude fiber during the first cut at ($p \leq 0.01$), the highest crude fiber percentage was recorded by treatment1 (37.77±0.18), while the lowest was recorded by treatment 4 (33.70±0.32), (Table 10). No significant differences in stem among treatments in crude fiber during the second and third cuts ($p > 0.05$).

4.1.4.2.4: Ether extracts (%):

The obtained results showed no significant difference ($p>0.05$) in EE between treatments on first and second cuts; but significant differences were at ($p\leq0.05$) among treatments on third cut, and the highest EE percentage recorded by treatment 4 (2.40±0.30) (Table 10).

4.1.4.2.5: Ash (%):

The result showed highly significant differences between treatments at ($p\leq0.01$), in ash percentage on first cut; while no significant differences ($p>0.05$) on second and third cuts. The highest ash percentage was obtained by treatment 4 (7.95±0.31), (8.78±1.19) and (7.40±0.35) in first, second and third cuts, respectively, (Table 10).

4.1.4.2. 6: Nitrogen Free Extract (%):

Also the result showed no significant differences between treatments ($p>0.05$) in NFE percentage during the first, second and third cuts, (Table 10).

Table (10): Effect of poultry manure and eggshell fertilizers on Chemical composition of *Clitoria ternatea* stems:-

%	Cuts	Treatments				Sig
		T1	T2	T3	T4	
DM	First cut	97.13±0.24^{ab}	96.74±0.02^{cd}	97.10±0.13^{ab}	96.81±0.11^{cd}	*
	Second cut	96.38±0.35	96.43±0.41	96.12±0.46	96.65±0.41	NS
	Third cut	94.56±0.16^{bcd}	94.42±0.04^{cd}	94.73±0.05^{abc}	94.80±0.16^{ab}	*
CP	First cut	7.29±0.87^{bcd}	7.81±0.53^{bcd}	8.40±0.59^{bcd}	11.19±0.44^{a}	**
	Second cut	9.25±0.25	11.31±1.96	10.11±0.88	12.17±1.79	NS
	Third cut	10.12±1.13	8.99±0.45	9.35±1.08	10.58±1.23	NS
CF	First cut	37.77±0.18^{a}	35.72±1.18^{bc}	36.15±1.02^{bc}	33.70±0.32^{d}	**
	Second cut	41.35±1.32	39.94±1.15	39.80±1.18	39.67±1.30	NS
	Third cut	40.04±0.85	41.73±0.54	41.09±1.00	40.51±1.85	NS
EE	First cut	2.20±0.16	2.20±0.07	2.30±0.07	2.17±0.14	NS
	Second cut	1.97±0.06	1.76±0.30	1.79±0.21	1.80±0.12	NS
	Third cut	2.10±0.28^{abcd}	1.72±0.15^{bcd}	1.87±0.17^{bcd}	2.40±0.30^{ab}	*
Ash	First cut	6.82±0.37^{bcd}	6.81±0.08^{bcd}	7.04±0.22^{bcd}	7.95±0.31^{a}	**
	Second cut	7.78±0.10	8.50±0.68	7.99±0.21	8.78±1.19	NS
	Third cut	7.31±0.36	7.30±0.08	7.30±0.23	7.40±0.35	NS
NFE	First cut	43.05±1.27	44.19±0.58	43.21±1.16	41.80±0.51	NS
	Second cut	36.03±0.65	34.91±1.58	36.44±1.55	34.24±1.40	NS
	Third cut	34.99±1.03	34.68±0.99	35.12±1.71	33.91±0.16	NS

Means with different superscripts within row are significantly different.

NS = No significant difference.

* = Significant differences ($p \leq 0.05$).

** = highly significant differences ($p \leq 0.01$).

4.2: Trial (2): Feeding trial:-

4.2.1: Daily dry matter intake (kg):

The daily dry matter intake was no significant difference ($p>0.05$) between experimental groups. The daily dry matter intake (kg) were (1.06±0.10), (1.10±0.15), (1.12±0.06) and (1.08±0.08) for group A, B, C and D respectively (Table 11).

4.2.2: Daily weight gain (gram):

As shown in table (11), the results exposed highly significant differences ($p\leq 0.01$) among experimental groups in daily weight gain ; group B was recorded the highest daily live weight gain (236.1±12.20 g), compared with (197.70±9.93), (206.6±4.6) and (177.5±6.54) for group A,C and D respectively .

4.2.3: Feed conversion ratio:

Feed conversion ratio (FCR) among the treatment groups given in table (11) was highly significant differences between four groups at ($p\leq 0.01$). Group B was recorded the best FCR (4.70±0.60) compared with group A (5.36±0.55), group C (5.43±0.31) and group D (6.10±0.49).

Table (11): Growth performance of lambs in experimental groups:-

Item	**Group (A)**	**Group (B)**	**Group (C)**	**Group (D)**	**Sig**
No	6	6	6	6	-
period (days)	63	63	63	63	-
Initial wt (kg)	19.03±1.00	18.75±1.21	19.02±0.94	19.12±1.4	-
Final wt (kg)	31.49 ± 1.46^{bcd}	33.62 ± 1.60^{ab}	32.03 ± 1.08^{abc}	30.30 ± 1.5^{cb}	**
Total wt gain (kg)	12.46 ± 0.63^{bc}	14.87 ± 0.77^{a}	13.02 ± 0.29^{bc}	11.19 ± 0.41^{d}	**
Daily wt gain (g)	197.7 ± 9.93^{bc}	236.1 ± 12.20^{a}	206.6 ± 4.60^{bc}	177.5 ± 6.54^{d}	**
Total feed intake(kg)(as fed)	73.36±7.22	76.51±10.47	77.80±4.23	75.38±5.61	NS
Total D.M. intake (kg)	66.75±6.57	69.41±9.50	70.69±3.84	68.17±5.08	NS
Daily feed intake(kg) (as fed)	1.16±0.11	1.21±0.17	1.24±0.07	1.20±0.09	NS
Daily dry matter intake (kg)	1.06±0.10	1.10±0.15	1.12±0.06	1.08±0.08	NS
FCR	5.36 ± 0.55^{bc}	4.70 ± 0.60^{d}	5.43 ± 0.31^{bc}	6.10 ± 0.49^{a}	**

Wt= weight No = Number of animals

FCR= Feed Conversion Ratio

Means with different superscripts within a row are significantly different.

NS= No significant differences

** = highly significant differences ($p\leq0.01$).

4.2.4: Slaughter data:

4.2.4.1: Carcass yield (kg):

Means of the carcass yield were highly significant differences ($p \leq 0.01$) between experimental lambs. The results were summarized in table (12). The slaughters weight were (30.67±1.15), (32.00±0.50), (31.87±0.55) and (29.40±0.79) for group A, B, C and D respectively. Although the empty body weight (EBW) was higher in group B (28.24±0.32kg) and lowest in group D (24.97±1.10 Kg). Also the hot carcass weight was higher in group B (14.83±0.29); compared with (14.43±0.51), (14.23±0.25) and (13.00±0.50) for group A, C and D respectively. The highest cold carcasses weight was recorded by group B (14.37±0.15) compared with (13.97±0.47), (13.73±0.25) and (12.50±0.50) for group A, B, C and D, respectively, (Table 12).

4.2.4.2: Dressing percentage (%):

Dressing percentage was highest in group A compared with other groups under study; and the percentages were (47.07±0.53), (53.65±0.54), (45.55±0.39) and (51.92±0.58) when calculated as (Hot carcass wt/ slaughter wt) ×100, (Hot carcass wt/empty body wt)× 100, (Cold carcass wt/ slaughter wt)× 100 and (Cold carcass wt/ empty body wt)×100 respectively (Table 12);but the lowest Dressing percentage was recorded by group D with values (44.21±0.70), (52.07±0.88), (42.51±0.71) and(50.06±0.84) when calculated as: (Hot carcass wt/ slaughter wt) ×100, (Hot carcass wt/empty body wt)× 100, (Cold carcass wt/ slaughter wt)× 100 and (Cold carcass wt / empty body wt)×100 respectively, (Table 12).

Table (12): Carcass characteristics of slaughtered lambs in experimental groups:-

Item	Group (A)	Group (B)	Group (C)	Group (D)	Sig
Slaughter wt (kg)	30.67 ± 1.15^{abcd}	32.00 ± 0.50^{abc}	31.87 ± 0.55^{abc}	29.40 ± 0.79^{cd}	*
Hot carcass wt (kg)	14.43 ± 0.51^{abc}	14.83 ± 0.29^{abc}	14.23 ± 0.25^{abc}	13.00 ± 0.50^{d}	**
Cold carcass wt (kg)	13.97 ± 0.47^{abc}	14.37 ± 0.15^{abc}	13.73 ± 0.25^{abc}	12.50 ± 0.50^{d}	**
Empty body wt (kg)	26.90 ± 0.78^{bc}	28.24 ± 0.32^{ab}	27.17 ± 0.32^{abc}	24.97 ± 1.10^{d}	**
Dressing %					
Hot carcass wt/ slaughter wt (%)	47.07 ± 0.53^{ab}	$46.35\pm.46^{ab}$	44.70 ± 0.39^{cd}	44.21 ± 0.70^{cd}	**
Hot carcass wt / empty body wt (%)	53.65±0.54	52.53±0.44	52.39±0.59	52.07±0.88	Ns
Cold carcass wt/ slaughter wt (%)	45.55 ± 0.39^{ab}	44.90 ± 0.53^{ab}	43.10 ± 0.39^{cd}	42.51 ± 0.71^{cd}	**
Cold carcass wt / empty body wt (%)	51.92 ± 0.58^{ab}	50.88 ± 0.13^{abcd}	50.55 ± 0.59^{bcd}	50.06 ± 0.84^{bcd}	*

Means with different superscripts within a row are significantly different.

NS= No significant difference

*= Significant differences ($p\leq0.05$).

** = highly significant differences ($p\leq0.01$).

4.2.4.3: Meat chemical composition (%):

Data presented in table (13) showed the proximate analysis of the longismus dorsi muscle of the slaughtered lambs fed on different levels of *Clitoria ternatea* hay, The values of moisture, protein, fat and ash percentage of the meat obtained from experimental lambs were similar and no significant differences ($p>0.05$) were found .

Table (13): Meat chemical composition of slaughtered lambs in experimental groups:-

composition%	Group (A)	Group (B)	Group (C)	Group (D)	Sig
Moisture	73.84±0.09	73.62±0.30	73.41±0.66	74.24±0.59	NS
Protein	20.83±0.04	21.00±0.18	20.99±0.37	20.63±0.25	NS
Fat	1.86±0.04	1.98±0.12	2.06±0.26	1.81±0.11	NS
Ash	1.13±0.01	1.13±0.03	1.15±0.05	1.06±0.08	NS

NS= No significant difference.

4.3: Trial (3): Antibacterial activity:

The result of antibacterial assay showed that the methanolic extract of *Clitoria ternatea* leaves had antibacterial activity against tested bacteria. The highest activity was recorded against G+ve bacteria compared with G-ve bacteria.

4.3.1: Agar Well Diffusion Method:-

The result showed highly significant differences at ($p \leq 0.01$), between tested bacteria in zones of inhibition. The highest range of inhibition zones recorded by *Staphylococcus* (2.63± 0.78 cm) followed by *Streptococcus agalactia* (1.60± 0.36 cm), *Escherichia coli* (1.30± 0.20 cm) and *Salmonella typhi* (1.27± 0.25 cm) (Table 14).

Table (14): Antibacterial activity of leaves extract of *Clitoria ternatea* by well diffusion method:-

Microorganisms	Zone of inhibition (cm)
Stapylococcus aureus	2.63 ± 0.78^{a}
Streptococcus	1.60 ± 0.36^{b}
E. coli	1.30 ± 0.20^{b}
Salmonella typhi	1.27 ± 0.25^{b}
Sig	**

Means with different superscripts within a column are significantly different.

** = highly significant differences ($p \leq 0.01$).

4.3.2: Disc-Diffusion Method:-

The result showed highly significant difference at ($p \leq 0.01$), between tested bacteria in zones of inhibition, in first and second dilutions; while no significant difference ($p > 0.05$), between treatments in third and fourth dilutions**.** The highest range of inhibition zones in first dilution, obtained by *Staphylococcus aureus* (0.90± 0.1cm), followed by *Streptococcus Agalactia* (0.40±0.17cm), *Escherichia coli* (0.40±0.1), and *Salmonella typhi* (0.23±0.06 cm) (Table 15). Also in the second dilution *Staphylococcus aureus* recorded the highest inhibition zone (0.05±0.01cm), compared with no inhibition for the others. No inhibition zones of all tested bacteria in dilutions third, fourth (Table 15).

Table (15): Antibacterial activity of diluted leaves extract of *Clitoria ternatea* by disc-diffusion method:-

Dilution of leaf extract	**Zone of Inhibition (cm) (Mean ± SD)**				
	Staphylococcus aureus	***Streptococcus agalactia***	***Escherichia coli***	***Salmonella typhi***	**Sig**
10^{-1}	0.90 ± 0.10^{a}	0.40 ± 0.17^{b}	0.40 ± 0.10^{b}	0.23 ± 0.06^{b}	**
10^{-2}	0.05±0.01	Growth	Growth	Growth	**
10^{-3}	Growth	Growth	Growth	Growth	NS
10^{-4}	Growth	Growth	Growth	Growth	NS

Means with different superscripts in the row are significantly different.

NS= No significant difference.

** = highly significant differences ($p \leq 0.01$).

CHAPTER FIVE

Discussion

CHAPTER FIVE

5. Discussion:

5.1: Trial (1): Fodder trial:-

5.1.1: Measurements of growth parameters:-

5.1.1.1: Plant height:

The highest mean of plant height was obtained by treatment4; while control (treatment1) obtained the lowest plant height during days 15, 30, 45 and 60 respectively. This may be due to that the eggshell is source of calcium; in the present study the treatment T4 contain highest level of eggshell powder; this agreed with Kris (2010), who reported the calcium is cell wall component of plant and calcium deficiency, causes weakness and instability of the plant. The present study is compatible with the findings of Nikose (2015) who reported that eggshell along with bio-waste when used in potted plants resulted in a remarkable growth in the plant. Also matched with, Nelson and Niedziela, (1998); who reported that calcium compared to other nutrients increases plant height. Also agreed with King'ori, (2011) who reported that when clover plant was grown on eggshell fertilized soil grew 10 mm greater than the plants without eggshell.

5.1.1.2: Stem diameter (cm):

The results showed that the increased stem diameter with eggshell addition; this may be attributed to the fact that the eggshell is source of calcium and this element is cell wall component of plant. This result compatible with findings of Kris (2010) who reported that eggshell as source of calcium and calcium is cell wall component of plant. Also this result matched with Nikose, (2015), who reported that eggshell along with bio-waste when used in potted plants resulted in a remarkable growth in the plant

5.1.1.3: Number of leaves:

No significant differences between treatments in number of leaves per plant, on 15 and 30 days, whereas a highly significant difference between treatments were found on 45 and 60 days of plant ages. Treatment 4 recorded the highest value of number of leaves per plant during 45 and 60 days of plant age. This result agreed with Radha and Karthikeyan (2019) who reported the leaves number of cowpea plant increases with increasing concentration of hen eggshell in soil.

5.1.2: Leaf to stem ratio:

Treatment 4 recorded the highest leaf to stem ratio (3.86± 0.50), compared with other treatments leaf to stem ratio was ranged between (2.08± 0.64) and (2.60± 0.04); this may be due to increase addition of eggshell in T4 compared with other treatments, caused increase leaf to stem ratio due to increase leaves number and leaf area. This agreed with Radha and Karthikeyan (2019) who reported the leaves number and leaf area of cowpea plant increases with increasing concentration of hen eggshell in soil. This result resembled to the result that reported by (Abreu *et al*., 2014) who recorded that leaf to stem ratios at 35, 50, 70, and 90 days, were 3.11, 3.10, 2.30, and 1.37, respectively, and higher than the results obtained by Barro and Ribeiro (1983); who reported that the ratio after haymaking had values of 1.26, 0.87, 0.73, and 0.97 during 42, 50, 70, and 84 days, respectively; this differ maybe due to loss of leaves during hay making.

5.1.3: Total yield per hectare:

5.1.3.1: Fresh yield (t/ha):

The fresh yield results showed significantly different among the different fertilizers applied. Higher fresh yield was obtained by treatment4 over the three harvests, during first, second and third cuts respectively; this result agreed with Radha and Karthikeyan (2019) who reported that the fresh weight of legume increased with increasing concentration of hen eggshell. The enhanced growth and increased fresh weight may be due to the effect of calcium on plant cell division and absorbed nitrates; this matched with Rodríguez, (1992) who reported that calcium acts in the mitotic cell division in the growth of meristems and the absorption of nitrates.

5.1.3.2: Dry yield (t/ha):

Similar to fresh yield, dry yield differed and was significantly affected by the fertilizer type applied, also treatment4 had the highest dry yield during first, second and third cuts; this agreed with Nelson and Niedziela (1998); they recorded that calcium compared to other nutrients increases plant dry matter. Also this result agreed with Radha and Karthikeyan (2019), who reported that the and dry weight of legume increased with increasing concentration of hen eggshell

5.1.4: Chemical composition of leaves and stems:

5.1.4.1: Chemical Composition of *Clitoria ternatea* Leaves:-

5.1.4.1.1: Dry matter:

The DM percentage in leaves showed no significant differences with the different fertilizer types applied in first and second cuts, while significant differences were found at ($p \leq 0.01$) among treatments in the third cut; the highest DM percentage in third cut was recorded by treatment2; but the lowest DM percentage in leaves was recorded by treatment1. This may be due to higher amount of nitrogen in poultry

manure fertilizer which increased dry matter in plant; this agreed with Carpici, (2011), who reported that N tends to increase DM yield. Also matched with, Abusuwar (2017) who reported that Poultry manure led to an increase in the dry matter in *Clitoria ternatea* compared with the control.

5.1.4.1.2: Crude protein:

The crude protein showed no significant differences between treatments in first and second cuts; whereas the results showed significant differences at ($p \leq 0.05$), among treatments in crude protein percentage in the third cut. The highest protein percentage in third cut was obtained by treatment2; while the lowest was recorded by treatment1; this result was higher than that recorded by Elfeel *et al*. (2013); who recorded that levels of protein contents in *Clitoria* leaves ranged from 25.6% to 26.4%; this difference might be due to grown the crop under arid dry saline soils; while in the present study the crop was grown under clay loam fertile soil. This agreed with Abusuwar (2017) who reported that higher CP in *C. ternatea* was obtained by the poultry manure treatment compared to inorganic (NPK) fertilizers. Also this agreed with Lambert and Litherland (2000) who reported that the nitrogen fertilization having greatest effect through lifting protein concentration.

5.1.4.1.3: Crude fiber:

There were no significant differences in leaves among treatments in crude fiber percentages during the first, second and third cuts. This agreed with Abreu *et al*. (2014); who recorded that leaves of this legume were the least affected part by the aging process. Also this result agreed with Lambert and Litherland, (2000) who reported that fertilizer has only small direct effects on feed quality. Also this result comparable with Ball *et al*.,(2001) who recorded that fertilization with phosphorus (P), potassium (K), or other nutrients, that increase yield may actually slightly reduce forage quality when growth is rapid.

5.1.4.1.4: Ether extract:

No significant differences between treatments in EE percentage, in Leaves during first; second and third cuts at ($p \leq 0.05$). This result agreed with Lambert and Litherland, (2000) who reported that fertilizer has only small direct effects on feed quality. Also this result comparable with Ball *et al*.,(2001) who recorded that fertilization with phosphorus (P), potassium (K), or other nutrients that increase yield, may actually slightly reduce forage quality when growth is fast.

5.1.4.1.5: Ash:

The result showed no significant difference between treatments in ash percentage during, first and third cut; while the result showed significant differences in second cut at ($p \leq 0.01$). The highest ash percentage in leaves in second cut was recorded by treatment1 (7.55±0.30); compared with other treatments the ash percentage ranged between, (6.30±0.24) and (6.77±0.35). This result agreed with Lambert and Litherland, (2000) who reported that fertilizer has only small direct effects on feed quality. Also this result comparable with Ball *et al*.,(2001) who recorded that fertilization with phosphorus (P), potassium (K), or other nutrients that increase yield, may actually slightly reduce forage quality when growth is fast.

5.1.4.1.6: Nitrogen Free Extract (NFE):

No significant differences between treatments in NFE percentage during the first, second and third cuts. This result agreed with Lambert and Litherland, (2000) who reported that fertilizer has only small direct effects on feed quality. Also this result comparable with Ball *et al*., (2001) who recorded that fertilization with phosphorus (P), potassium (K), or other nutrients that increase yield, may actually slightly reduce forage quality when growth is fast.

5.1.4.2: Chemical Composition of *Clitoria ternatea* stems:-

5.1.4.2.1: Dry matter:

The DM percentage significantly differed with the different fertilizer types applied during first and third cuts at ($p \leq 0.05$); while no significant differences among treatments at ($p \leq 0.05$) in the second cut; whereas treatment1 recorded the highest DM percentage in first cut; but treatment4 recorded the highest DM percentage of stem in second and third cuts. This may be due to increased deposition of calcium in plant tissues, that can increase DM yield; this agreed with Nelson and Niedziela (1998), who recorded the Ca when was compared to other nutrients increases plant dry matter. Also agreed with Radha,and Karthikeyan, (2019), who reported that dry weight of legume increased with increasing concentration of hen eggshell.

5.1.4.2.2: Crude protein:

The crude protein showed significant differences between treatments in the first cut at ($p \leq 0.01$), but there were no significant differences among treatments in crude protein percentage during second and third cuts; while treatment4 recorded the highest protein percentages in stem. This may be due to Ca has an effect on nitrogen transport, and effect on protein content in plant. This agreed with El Habbasha and Ibrahim (2015) who reported that calcium is an element associated with the transport of nitrogen and interaction with potassium (K) and phosphorus (P). Also this result agreed with Havlin *et al*. (2005) who reported that calcium is important in nitrogen metabolism and protein formation, by enhancing NO_3 uptake.

5.1.4.2.3: Crude fiber:

The result showed significant differences at ($p \leq 0.01$), in crude fiber of stem between treatments in the first cut; while no significant differences among treatments in crude fiber in stem observed during the second and third cuts ($p > 0.05$). The highest crude fiber percentage was recorded by treatment1 during

first and second cut. This result agreed with Abusuwar, (2017) who showed that planting of *C. ternatea* without fertilizer caused higher CF content, compared with poultry manures and inorganic (NPK) fertilizers, in an arid saline environment of western Saudi Arabia.

5.1.4.2.4: Ether extract:

The results showed no significant differences between treatments in EE during first and second cuts. But significant differences were found at ($p \leq 0.05$), among treatments in third cut, with highest EE percentage in third cut was recorded by treatment4. This may be due to increased absorption of calcium by plant; the calcium element introduce in the formation of lecithin, and phospholipid. This agreed with (Rodríguez, 1992); who reported that calcium is involved in the formation of lecithin, and phospholipid, which is important in plant cell membrane and in the permeability of these membranes.

5.1.4.2.5: Ash:

The result showed significant differences between treatments at ($p \leq 0.01$) in ash percentage in first cut; while no significant differences in second and third cuts; whereas the highest ash percentage was obtained by treatment4 during first, second and third cuts. This may be due to increased eggshell addition caused plant uptake of minerals; this agreed with Radha, and Karthikeyan, (2019), who reported the country hen's eggshell, contains calcium and trace amounts of other micro elements and it is best natural source of calcium and it is about 90% absorbable.

5.1.4.2.6: Nitrogen Free Extract (NFE):

The result showed no significant differences between treatments in NFE during the first; second and third cuts. This agreed with Adjei and Fianu (1985); recorded the cut interval, generally, had little effect on stem NFE.

5.2: Trial (2): Feeding trial:-

5.2.1: Daily dry matter intake (kg):

The DM intake had no significant difference among different groups (P>0.05). This is result compatible with Sutedi (2013), who reported *C. ternatea* can be fed to ruminants as fresh forage or hay without any negative effect on growth performance of animal. Also this result agreed with Avalos *et al*. (2004), they reported that the clitoria intake by ruminants improves animal performance; but not differ in dry matter intake when compared with alfalfa hay.

5.2.2: Daily weight gain (g):

The results exposed significant differences ($p \leq 0.01$) among different groups in daily live weight gain. Group B had recorded the highest daily live weight gain (236.1±12.20 g), compared with (197.70±9.93), (206.6±4.6) and (177.5±6.54) for group A, C and D respectively. This result compatible with Schlink, (1998) who reported the use of *C. ternatea* in sheep feeding provides a positive effect on live weight gain.

5.2.3: Feed conversion ratio:

The feed conversion ratio (FCR), showed highly significant differences among groups ($P \leq 0.01$). Group B recorded the best FCR (4.70±0.60) compared with other groups, this may be due to optimum rumen environment enhanced by equal percentage of legume hays compared by higher FCR when used each hay separately; like in group A and D. This was in line Castillo-Lopeza and Domínguez- Ordóñezb (2019); who reported the factors that influence microbial protein synthesis in the rumen are the rumen pH, dietary fat, availability of, degradable protein in rumen, feed intake and carbohydrates. Group D recorded the lower FCR compared with other groups; this result was matching with Abreu *et al*. (2014), who recorded that *C. ternatea* has better potential nutritive value than alfalfa and clover.

5.2.4: Slaughter data:

5.2.4.1: Carcass yield (kg):

The hot carcass weight was higher in group B (14.83±0.29); compared with (14.43±0.51), (14.23±0.25) and (13.00±0.50) for groups A, C and D, respectively. Similar to hot carcass weight the highest cold carcasses weight was in group B (14.37±0.15) compared with (13.97±0.47), (13.73±0.25) and (12.50±0.50) for groups A, C and D, respectively. This result might be due to higher slaughtered weight in group B compared with other groups under study; this matched with Rajkumar *et al*., (2014); who reported the results showed the increase in slaughter weight resulted in increased empty body weight and carcass yield.

5.2.4.2: Dressing percentage (%):

Dressing percentage was the highest in group A compared with other groups under study. This result matched with Jacob and Calnan, (2018), they recorded a high dressing percentage not necessarily correspond to a high carcass yield. This result is in line with the range of sheep dressing percentage, according to Osman and EI-Shafie (1967), they reported that the warm dressing percentage in Sudan desert sheep ranged between 38.7 and 54.3 percentage.

5.2.4.3: Meat Chemical composition (%):

The results showed the proximate analysis (moisture, protein, fat and ash percentages) of the *longismus dorsi* muscle of slaughtered lambs was similar and no significant differences (P>0.05) among the different groups were observed. This result agreed with De Brito *et al*. (2016), who reported no sensory or other meat quality trait differences were found between lambs fed on different forage types.

5.2.4.3.1: Moisture (%):

The Moisture percentages ranged from (73.41±0.66) to (74.24±0.59). This result was approximately similar to Lawrie, (1979), and Omer and Ekhlas, (2018) whose recorded moisture percentages in meat were 75% and 75.6±0.45, respectively; while agreed with Jalajakshi *et al*., (2016) (74.14%) and Rajkumar *et al*., (2014) (74.02±0.18).

5.2.4.3.2: Crude protein (%):

Protein content ranged between (20.63±0.25) and (21.00±0.18); this result was higher than that of Lawrie, (1979), Jalajakshi *et al*., (2016) (18.05%) and Rajkumar *et al*., (2014) (19.08±0.10); but similar to the finding of Omer and Ekhlas (2018) (20.6±0.23).

5.2.4.3.3: Crude fat (%):

Fat values ranged between (1.81±0.11) and 2.06±0.26); this result was higher than that recorded by Omer and Ekhlas, (2018) (1.20±0.09); but was lower than Lawrie, (1979) (2.5%) and Rajkumar *et al*., (2014) (4.97±0.28); and nearly similar to results recorded by Jalajakshi *et al*., (2016) who found that the fat % was 2.08%.

5.2.4.3.4: Ash (%):

Ash percentage ranged between 1.06±0.08 and 1.15±0.05; this result was lower than that of Jalajakshi *et al*., (2016) (4.48%); and similar to that obtained by Omer and Ekhlas, (2018) (1.02±0.02) and Rajkumar *et al*., (2014) (1.12±0.01).

5.3: Trial (3): Antibacterial activity trial:-

In this study gram positive bacterial strains were more susceptible to the extract when compared to gram negative bacteria; this agreed with (Parekh and Chanda, 2006) and Lakna, (2017). This may be due to the fact that these two groups differ in their structure of the cell wall components. This agreed with Lakna (2017) and Yao and Moellering, (1995), who reported the cell wall in Gram positive bacteria, is of a single layer, whereas the gram negative cell wall is multilayered structure.

5.3.1: Well Diffusion Method:

Clitoria ternatea leaves methanol extract showed antibacterial activity against Staphylococcus, *Streptococcus agalactia*, *Escherichia coli* and *Salmonella typhi*; this matched with Kapoor and Purohit, (2013); Darsini and Shamshad, (2015) who reported that *Clitoria ternatea* had antibacterial activity.

5.3.2: Disc-Diffusion Method:

The results showed that the inhibition zones decreased with increase dilutions of extract; this may be due to low concentration of extract content; this agreed with Uwimbabazi *et al*. (2015) and Pahune *et al*., (2013), who reported that the bactericidal activity increased with the increase of the extract concentration.

Conclusion:

From the results obtained, the poultry manure at the rate of 4200 kg/ha +800kg/ha eggshell were better in increasing plant height, stem diameter, number of leaves per plant, leaf/ stem ratio and shoot (fresh and dry yields) of *C. ternatea* than control (no fertilizer used) and poultry manure alone under Shambat- Sudan conditions. While the results showed that fertilizers had a little effect on leaves and stems chemical composition.

The study concluded that the lambs group fed on diet contained hay mixture (50% *Clitoria* +50% alfalfa hay), recorded best results in weight gain, feed conversion ratio and carcass yield when compared with other groups. While the lambs fed with diet contained pure *Clitoria ternatea* hay, recorded the highest dressing percentage. Meat moisture, crude protein, fat and ash percentages were not significantly different between groups of lambs ($p>0.05$). From the results above, *Clitoria ternatea* can be used as mixture with alfalfa and had positive effect on lamb performance. While *Clitoria ternatea* can be used as alternative for alfalfa without any negative effect on lambs' feed intake, weight gain, carcass yield, dressing percentage and meat quality.

The methanol extract of *Clitoria ternatea* leaves had highest antibacterial activity against G+ve compared with G-ve. By well diffusion method the highest range of inhibition zone was recorded by *Staphylococcus* (2.63± 0.78 cm) and the lowest was recorded by *Salmonella typhi* (1.27± 0.25 cm). By disc method the result showed that the effectiveness of the extracts was dependent on the concentration used, thus the increase of extract concentration increased the inhibition zone.

Recommendations:

1- Poultry manure and eggshell as fertilizers are recommended to reduce the cultivation cost and globe pollution.
2- Further studies should be carried out to evaluate the growth, yield and quality of *Clitoria ternatea* forage under different environments in Sudan.
3- Using of *Clitoria ternatea* as alternative fodder to alfalfa for sheep during drought season.
4- Further studies should carry out to determine the affect of supplementation of *Clitoria ternatea* hay in diets on performance of other ruminants'.
5- Further studies on the isolation and identification of active substances from the methanol extracts of *C. ternatea* to disclose compounds with better value for food preservation as well as natural plant based medicine.

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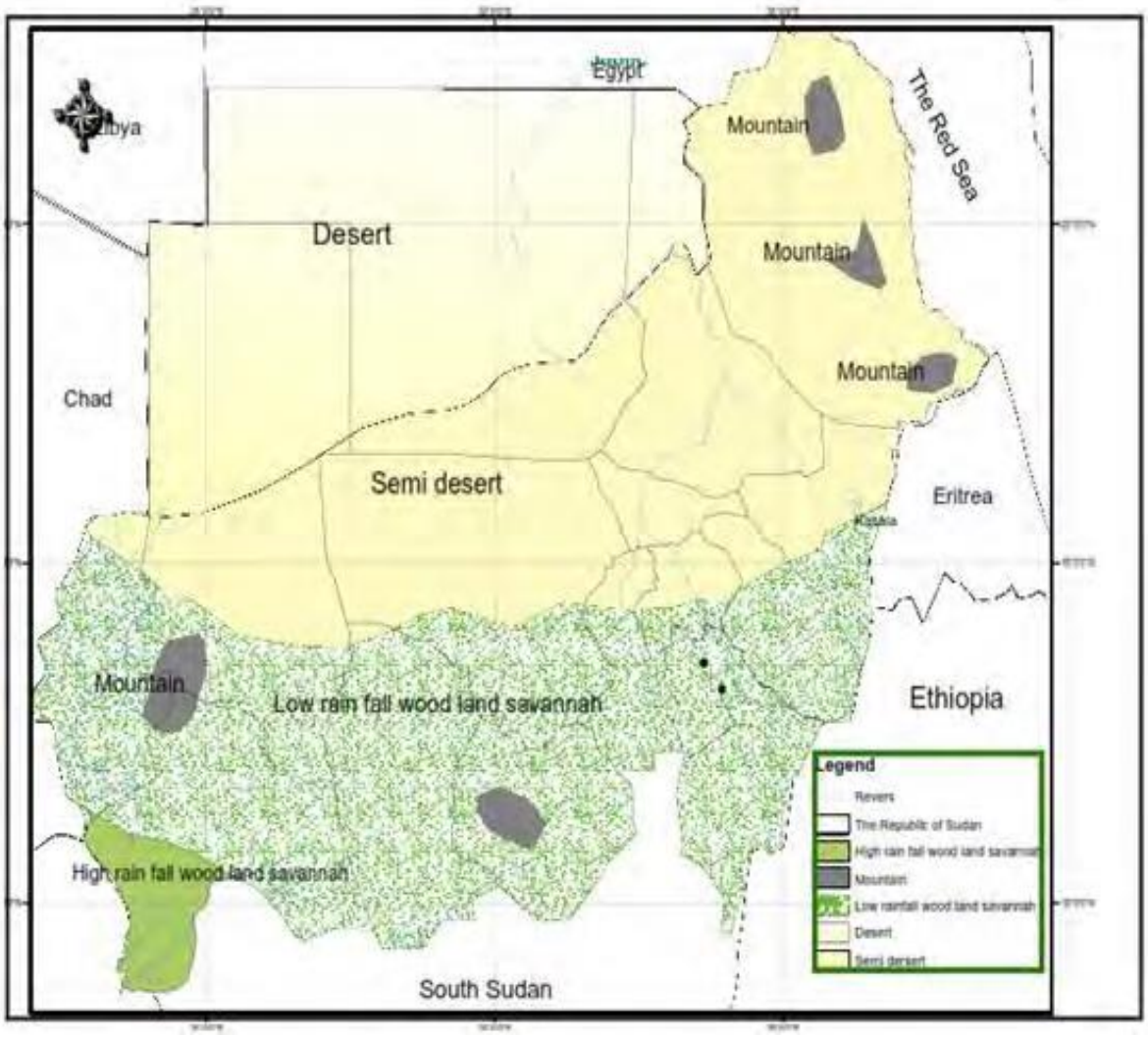
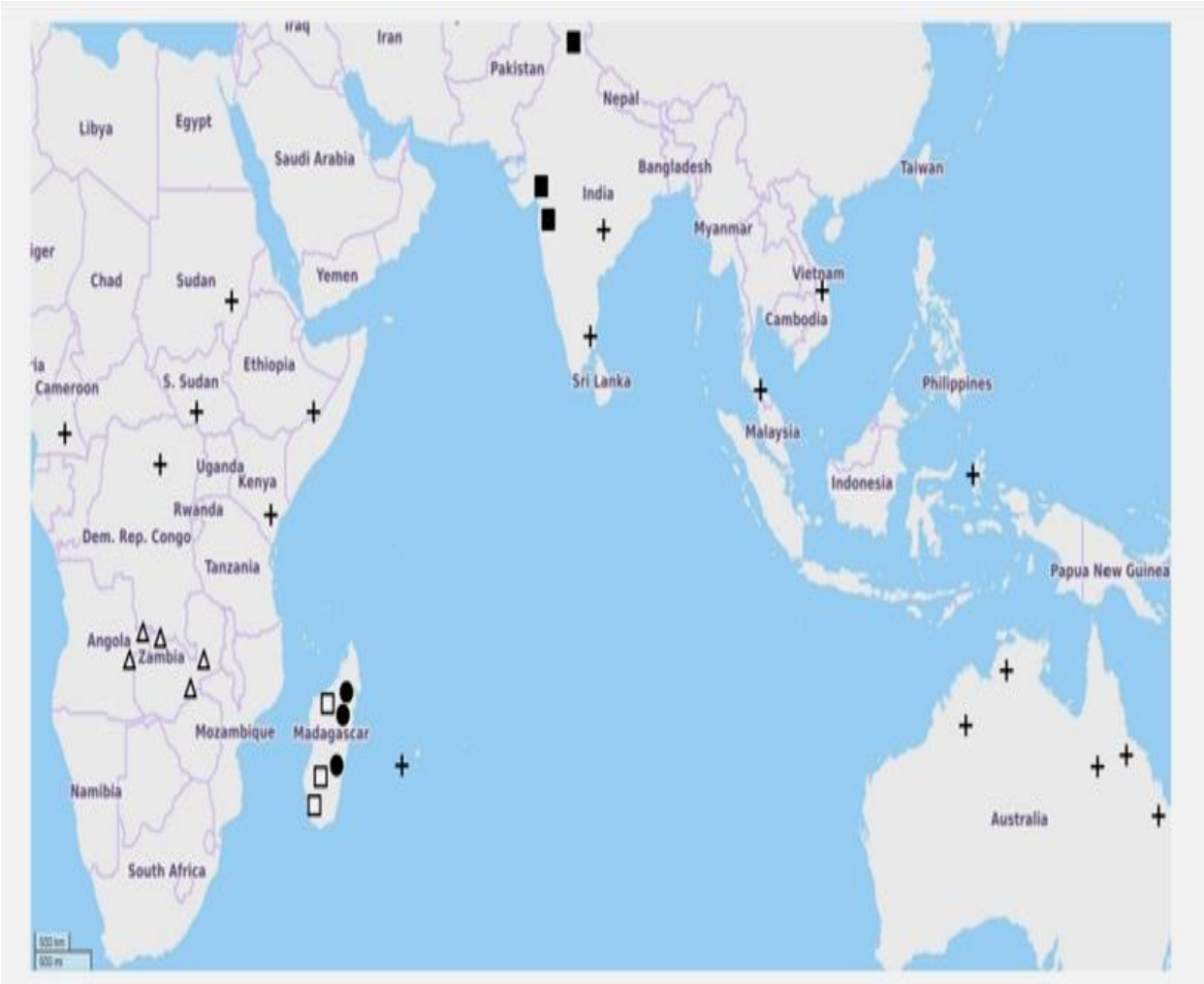
Appendices

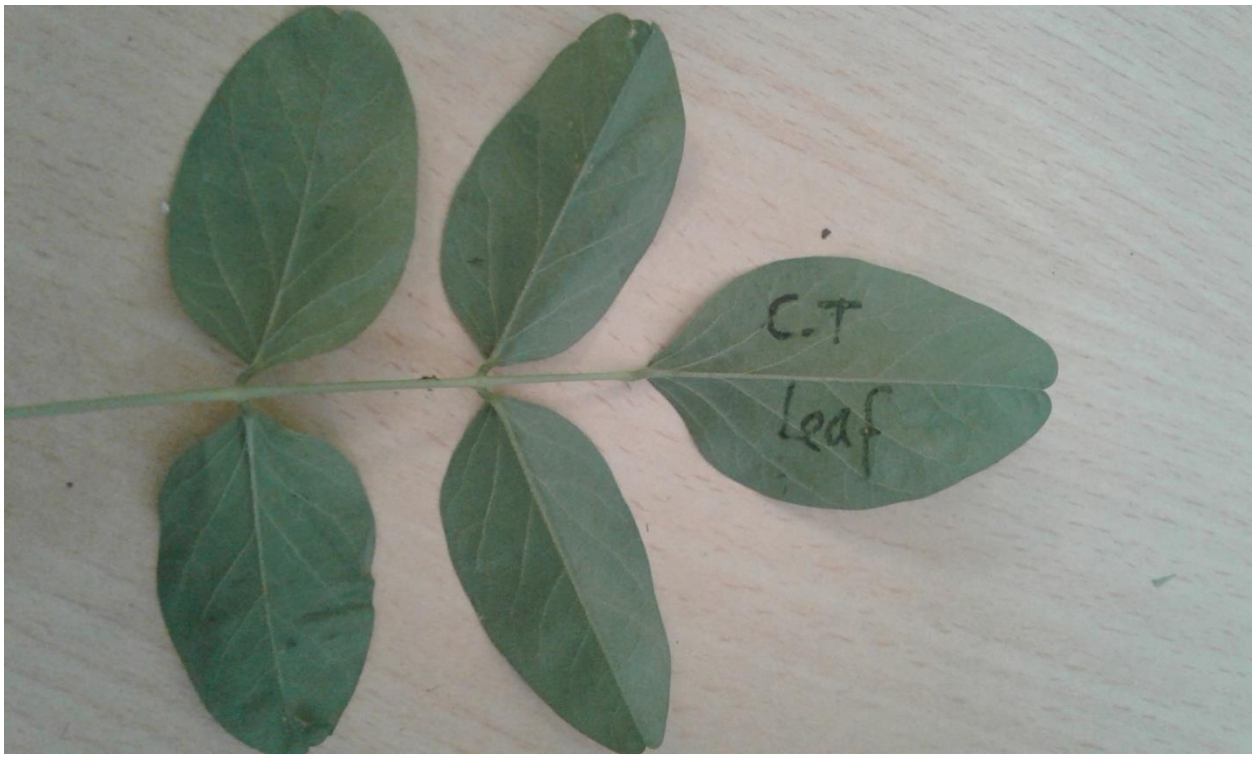


Appendix (1): Ecological classification of vegetation covers of the republic of Sudan. Source: FAO, 2015



Appendix (2): Distribution of Clitoria subgenus, Clitoria species. Points of occurrence are approximate. Map data from Openstreetmap.org. Symbols represent:

■ C. biflora, □ C. heterophylla, Δ.C. kaessneri, ●C. lasciva, + C. ternatea. Source: Oguis *et al*, 2019.



Appendix (3)*: Clitoria ternatea* pinnate compound Leaf

-a-

-b-.

Appendix (4): a- Seeds of *Clitoria ternatea* and b-Morphology of *Clitoria ternatea* plant.

Appendix (5): *Clitoria ternatea* re growth after cutting.

2018												
	Jan	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
Maximum Temperature(Cº)	29.4	36.7	38.0	39.0	41.4	39.8	38.1	35.9	37.5	39.1	26.0	30.4
Minimum Temperature(Cº)	14.1	18.7	21.0	20.4	25.9	26.9	26.3	25.5	25.3	24.6	17.8	16.9
Relative Humidity (%)	30	24	19	16	23	34	45	54	47	32	24	28
2019												
	Jan	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
Maximum Temperature(Cº)	32.8	33.3	34.8	39.7	42.3	39.8	39.8	35.3	38.9	37.0	35.6	30.7
Minimum Temperature(Cº)	15.7	16.1	16.9	20.6	23.8	24.6	26.5	24.8	26.2	23.2	17.6	15.1
Relative Humidity (%)	26	24	18	15	20	36	38	58	43	43	25	28

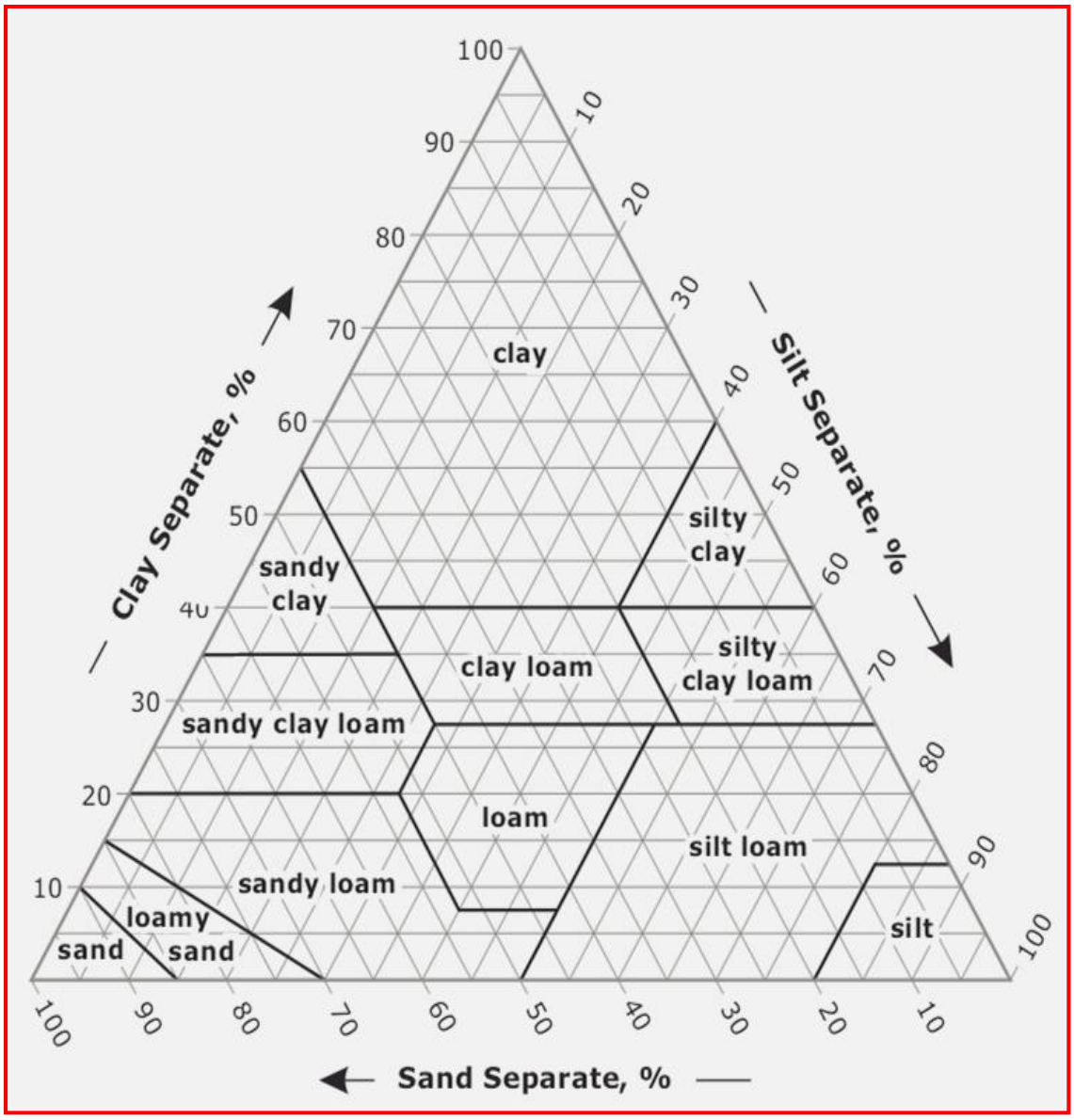
Appendix (6): Monthly accumulative air temperature (Cº) and relative humidity (%) of the experimental area during 2018 and 2019.

Source: The Ministry Of Irrigation and Water Resources Meteorological Authority, Khartoum. Sudan (2018-2019)

Eggshell drying under the shade

Harsh meal of eggshell Egg-shell meal

Appendix (7): Eggshell processing as fertilizer



Appendix (8): A soil textural triangle used to determine soil textural class from the percentages of sand, silt and clay in the experimental soil.

Soil texture triangle from USDA, NRCS 2012.

Appendix (9): NIRs, used in chemical analysis of fodder

Appendix (10): Cages with individual feeders and drinkers

Appendix (11): Checks of lamb’s health (every morning)

Appendix (12): *Clitoria ternatea* hay drying under the shade.

Appendix (13): Alfalfa hay drying under the shade

Appendix (14): Rotary Shaker apparatus.