Effects of three commercial flukicidal products on the growth of beef cows in Mpumalanga

By

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DECLARATION

I, Somwe Delphin Lukamba, declare that the dissertation:" **Effects of three commercial flukicidal products on the growth of beef cattle** "which I hereby submit for the degree Master of Agricultural Science: Production Management at the university of Pretoria is my own work and that all sources used or quoted have been indicated with a full reference and acknowledgements. This dissertation has not previously been submitted by me for a degree at this or any other tertiary institution.

SOMWE DELPHIN LUKAMBA DATE

DEDICATION

At the moment I think about you who the destiny has taken away from my affection, I am sadden, eyes full of tears, poor late son Aaron Ebondo Somwe, late young brother Mutwale Ebondo, late uncle Marius Kitenge, late grandparents Petro Ngoie Mbele, Musumba, Celestin Elembe Muledi and Pemba Kilolo receive from the bottom of my heart and in the deep of the vault beneath the ground the expression of my thanks, appreciation, admiration and gratitude, I will never stop to mourn, to pay you tribute and to feel the pain for your memory, your departure in the world of rest.

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ABSTRACT

The effects of three commercial flukicidal products on the growth and body mass change of beef cows and heifers were investigated due to the concerns of farmers that these substances may have adverse effects on the performance of extensive beef cattle. Body mass of 108 adult non-pregnant Bonsmara cows of about four years of age and weighing between 400-600kg, as well as 126 two years old Beefmaster heifers weighing between 121kg-300kg were studied after oral administration of Flukazole C® (Virbac, Registration nº. G3533), FluxacurNF® (MSD Animal Health, Registration nº. G3202), or topical application of Sovereign pour- on® (MSD Animal Health, Registration nº. 3831) over a six month period during which most internal parasite challenges were expected. Cows and heifers from these groups with sheltering Fasciola spp infection were stratified according to their weights and randomly assigned to four treatment groups namely: Group 1 comprising unmedicated animals (controls), Group 2 included animals treated with Flukazole C orally at 1ml/10kg body mass; Group 3 included animals treated orally at 1ml/10kg body mass with Fluxacur and Group 4 was animals treated topically with Sovereign pour on at the dose of 1ml/10kg body mass. Weights of animals were recorded monthly, faecal and blood samples were collected twice during the trial namely at days 35 and 118 of the trial. Pregnancy status was also determined at day 57. No differences were observed in body condition scores, or body mass between treatment groups. Differences were observed in body mass change between treatment groups during the experimental period. The results show that there was no significant effect of treatment per se on the growth of either heifers or cows in this specific experiment.

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LIST OF ACRONYMS

ADG	Average Daily Gain
AAVP	American Association of Veterinary Parasitologists
AHI	Animal Health Ireland
AHVLA	Animal Health Veterinary Laboratory Agency
BCS	Body Condition Score
BZs	Benzimidazoles
B.W	Body weight
C	Control
Cm	Centimeter
ELISA	Enzyme-Linked Immunosorbent Assay
Epg	Eggs per gram
F	Fluxacur
FC	Flukazole C
g	Gram
GIN	Gastro-intestinal Nematodes
GLP	Good Laboratory Practices
GMP	Good Management Protocols
HR	Human Readable
ID	Identification
IMB	Irish Medical Board
KZN	Kwazulu Natal

Km	Kilometer
l	Liter
m	Meter
ml.	Milliliter
MRU	Malelane Research Unit
MSD	Merck Sharp & Dohme
RH	Relative humidity
RIM	Research Inventory and Management
RPO	Red meat Producers Organization
S	Sovereign Pour-on
SANAS	South African National Accreditation System
TCBZ	Triclabendazole
USD	United State Dollars
W H O	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Background

Developing countries are confronted by multiple problems related to lack of animal proteins or lack of sound benefits from available livestock. The rich potential of livestock is not efficiently exploited due to high prevalences of diseases, bad or none existent agricultural policies, several constraints including lack of adequate nutrition or unbalanced diet and traditional management (Bekele *et al*., 1992; Daynes & Graber, 1974).

Cattle constitute a major source of animal proteins and provide a large portion of the meat that is consumed by human beings. To produce animal products one needs to master the intrinsical and extrinsical factors of domestic animals. Furthermore, proteins from cattle are also an important source of income for many communities even a significant source of foreign currencies from exports for nations worldwide (Randolph, *et al*.2007; Bekele *et al*, 1992; Daynes & Graber, 1974).

Ruminant populations are one of the most important assets possessed by many parts of the tropics. This sector in Nigeria for example is capable of generating an annual revenue of up to U.S. $2 billion and provides valuable animal protein for human consumption and occupation for over one million families engaged in livestock trade. Even in some countries such as Botswana (Carmichael, 1972), the economy and livelihood of people are dependent to a large extent upon ruminant populations. Proteins from cattle are of great value either at slaughter or at milking. The knowledge of different parameters of cattle contributes to good husbandry management.

Fasciolosis is a worldwide major parasitic disease affecting livestock e.g. sheep, goats, cattle, buffalo as well as other domestic ruminants and human. It is caused either by *Fasciola hepatica* in temperate regions or by *Fasciola gigantica* in tropical regions which have di-heteroxenous life cycles (Spithill, 1999). Estimated infections in human are greater than 17 million across 61countries, and a further 180 million people are at risk of being infected (Gulsen *et al*., 2006; Mas-coma, 2005; Rim *et al*., 1994; Hopkins, 1992).

Some previous studies on the subject of liver fluke infection of cattle give typical figures of infection rates in sub Saharan countries: Chad 62%; Central African Republic 45%; Cameroon 45%; Ethiopia 30-90% (Megard, 1976); Malawi, Zambia and Zimbabwe 50-70% (Le Roux, 1957) and Uganda 53.7% (Ogambo, 1972). Previous studies in live animals have suggested that the prevalence of *fasciolosis* infections in Tanzania ranges from 17.8% in small scale dairy farms to 94% in traditional cattle farms (Keyyu *et al.*, 2005 & 2006; Swai *et al.*, 2006). The study conducted by Keyyu *et al.* (2006) reported up to 100% liver condemnation rates in some slaughter slabs in Iringa region in Tanzania caused by liver flukes in cattle. These figures give a clear understanding and perception into the enormous losses that may result from considerable depression on weight gains (Sewell, 1966) and milk yields as well as some deaths, whole carcass condemnation (Fabiyi & Adeleye, 1982) and whole or partial liver condemnation. The same may be said in respect of losses in many Asian countries such as Indonesia (Soesetya, 1975; Edney & Muchlis 1962) and Bangladesh (Rahman & Rahman, 1972).

The treatment of liver fluke infection is the most reliable control method. Wolstenholme *et al*., (2004) reported that the frequency and the spread of liver fluke had increased, at the same time accessible control methods on the market were failing and losing their efficacy (Fairweather, 2005).

The South African liver fluke drugs market offers a multitude of current classes of products such as BZs, Halogenated salicylanalides and the combination of the above and other antihelmintics. A pour-on flukicidal, object of our study has been introduced in the South African market. This product Sovereign pour- on is a combination of Triclabendazole and Ivermectin. Its efficacy against liver fluke, round worms and blue ticks on cattle has been proven internationally in Australia and locally in South Africa. This study is undertaken to evaluate and compare the gain in term of body mass in weaned heifers and cows following treatment with three flukicidal remedies including the Sovereign pour-on.

From years ago till now the disease is receiving much attention because of its economic importance. Liver fluke as well as other parasites have long been subjected to scientific researches. New approaches are needed to control liver fluke infection both in term of good practices in farming management and treatment. However, one of the main challenges when deciding on a parasite-treatment strategy in a herd is to determine whether the application of an anthelmintic will produce an economic return to compensate the cost of the treatment (Rew, 1999).

The parasitism is associated with various clinical signs which are: weight loss and loss of body condition, retarded growth, anaemia, hypoproteinaemia, vomiting and threatening obstruction of vital body organs. Mortality can occur if the infection is untreated. Animals are infected either by ingesting contaminated food or water or by transmission of Arthropod vectors (Elsheika & Khan, 2011).

The control of the disease is a challenge and requires an integrated approach on:

- Animals (e.g. by increasing animal resistance through improvement of hygienic and alimentary conditions)

- Detection and destruction of parasites.

- Limiting of infection and re-infestation.

- Selecting animals that are resistant to the effect of the parasite and thereby increasing the genetic prevalence of such cattle (sheep or goat) in a herd or flock.

According to Reinecke (1983) and Elsheika and Khan (2011) this approach demands some multiple actions for an effective control:

1. Drugs action including the choice and the correct use of the anthelmintic, the behavior of animals, the time of treatment pertaining to the life cycle of the parasite and the season.

2. Actions on the pasture in term of improvement, choice of grazing time, the grazing rotation in term of pasture and animals.

3. Actions on feeding boxes and water supply points.

4. Actions on the farm management system.

The pharmaceutical industry has made spectacular progress regarding both internal and external parasiticides. Several combination products containing nematocidal and flukicidal have been evaluated. Ivermectin has been associated to Abamectin as an oral (Stevenson *et al.*, 2002), or a pour- on combination (Sargent *et al.* 2009). These combinations are justified by the benefits of treating simultaneously both nematodes and liver flukes (Loyacano *et al.*, 2002).

1.2 Problem statement

The most critical determinator of beef farm profitability is net income per hectare farm land which is mainly influenced by annual beef mass produced per hectare. On the eastern Highveld of Mpumalnga and KZN bush veld of South-Africa innumerable diseases are affecting livestock. Among them parasitic infestations are taking the lion share. One of the most important parasitic diseases and erosive factors that negatively affect this parameter is a liver fluke (*F. gigantica* and *F. hepatica*) infestation in all classes of cattle. Other factors that can affect live mass are a merciless weather, poor pasture conditions and lack of dietary supplementation. These factors will worsen the effects of a liver fluke infestation. A rumour was spread in the field by certain veterinary pharmaceutical companies that the pour-on flukicidal does not work against liver fluke infestation in the field while the pour-on product has been well tried and tested in Australia and South Africa.

A different evaluation was considered i.e. a field study wherein cows or heifers in a beef herd were selected to see if there was a significant effect in relation to weight gain after the treatment with different products in the field.

Knowing that the field evaluation could never be as accurate as the registration trials, an attempt was however made to evaluate the above outcome.

1.3 Aim of the study

This study aims at investigating the body mass change or growth in cows and weaned heifers after preventative and curative treatment with three commercial flukicidal products. The literature review discloses a general view of Fasciolosis. However, for a good assessment of the effects of these products on the growth of beef cattle and how appropriate they could be, an understanding of liver fluke infection, economic and financial losses caused by the disease, grazing behavior of cattle and the benefits of liver fluke treatment are important.

1.4 Relevance of the study

Considering the studies done by Loyacano *et al.*,(2002) regarding the effect of gastrointestinal nematodes and liver fluke on weight gain and reproductive performances of beef heifers, Elitok *et al.,* (2006) on the field trial on comparative efficacy of four fasciolicides against natural liver fluke infection in cattle; Sargent *et al*.,(2009) about the seasonal differences in the efficacy of pour-on formulations of Triclabendazole and Ivermectin or Abamectin against late immature liver fluke (*F. hepatica*) in cattle as well as other studies regarding the benefits of the use of antihelmintics, knowing the fact that locally the investigation on body mass gain and growth of beef cattle following the treatment with a topical flukicidal product and flukicidal drenches has not been well documented. Moreover, the effects of Liver fluke on production parameters and the impact of flukicidal use have been and still are an important area of research. For these reasons, this study needed to be carried out.

1.5 Ethical Considerations

The ethics committee which is composed of members from SPCA, veterinary pharmaceutical industry, veterinary academics werev consulted for the approval to conduct the trial. Also the cattle were handled humanely as set out by the South African Red Meat Producers Organization (RPO) guidelines for cattle handling which is the routine at this particular farm used for the trial.

CHAPTER 2

LITERATURE REVIEW OF FASCIOLOSIS

Fasciolosis is a worldwide zoonotic disease. Its incidence is reported to be increasing in certain regions of the world and creating a serious public health concern in many regions of the world (WHO, 1995).

Infection by members of the genus Fasciola (*F. hepatica* and *F. gigantica),* commonly known as liver flukes, may be responsible for morbidity and mortality in most mammal species, but are of particular importance in sheep and cattle to livestock producers (Dalton,1999). The two species of the greatest veterinary importance are *F. hepatica* and *F. gigantica* and amphibious snails are their intermediate hosts. Adult parasites are found in the bile ducts and the immature flukes in the liver parenchyma of infected final hosts. Clinical disease is usually characterized by weight loss, anaemia and hypoproteinaemia.

F. hepatica, a liver fluke, is a causative agent of fascioliasis in mammals (sheep, cattle, goat, ox and other ruminants, pig, hare, rabbit, beaver, elephant, dog, cat and kangaroo). In the unusual hosts, such as man and the horse, the fluke may be found in the lungs, under skin or other locations. The parasite *F. hepatica* is a hermaphroditic trematode, which is particularly common in sheep and cattle. Transmission of *F. hepatica* is dependent on the presence of its lymnae snail intermediate hosts. Following ingestion of metacercaria by the hosts, the juvenile worms burrow through the host gut walls and migrate to the liver, where they cause extensive damages before moving into the bile ducts. Finally, the parasites pass through the bile duct walls and develop into mature forms that live in the microenvironment of the bile ducts. This worm causes important economic losses due mainly to liver damage and reduced production of meat and milk (Oldemir, 2006).

2.1. Specie Description

As stated by Soulsby (1982) and Dunn (1978) the taxonomic classification of the organisms incriminated in fasciolosis is presented below as follows:

1. Phylum: - Platyhelminthes,
2. Class: - Trematoda,

3. Sub- class: -Digenea,
4. Super Family: -Fasciolidea,
5. Genus: -Fasciola,
6. Species: - *Fasciola hepatica* Linnaeus and *Fasciola gigantica*

Fasciola gigantica is the common liver fluke of livestock found in tropical and warmer regions of Africa including South Africa and Asia where it causes severe disease in livestock (Kaufmann, 1996; Merck veterinary manual, 1991; Boray, 1985). *F. gigantica* causes big losses in livestock in India, Pakistan, Indonesia, Indochina, the Philippines; the near east such as the southern states of the Soviet Union and Turkey (Boray, 1985). Its size varies from 24 to75 mm in length and up to 12 mm in breadth. The body has a flatten shape more elongate than leaf-shaped; anterior end cone shaped and large; sloping shoulders; posterior end rounded. Both species have oval, operculated golden brown eggs different in size (Thienpont *et al*, 1985).

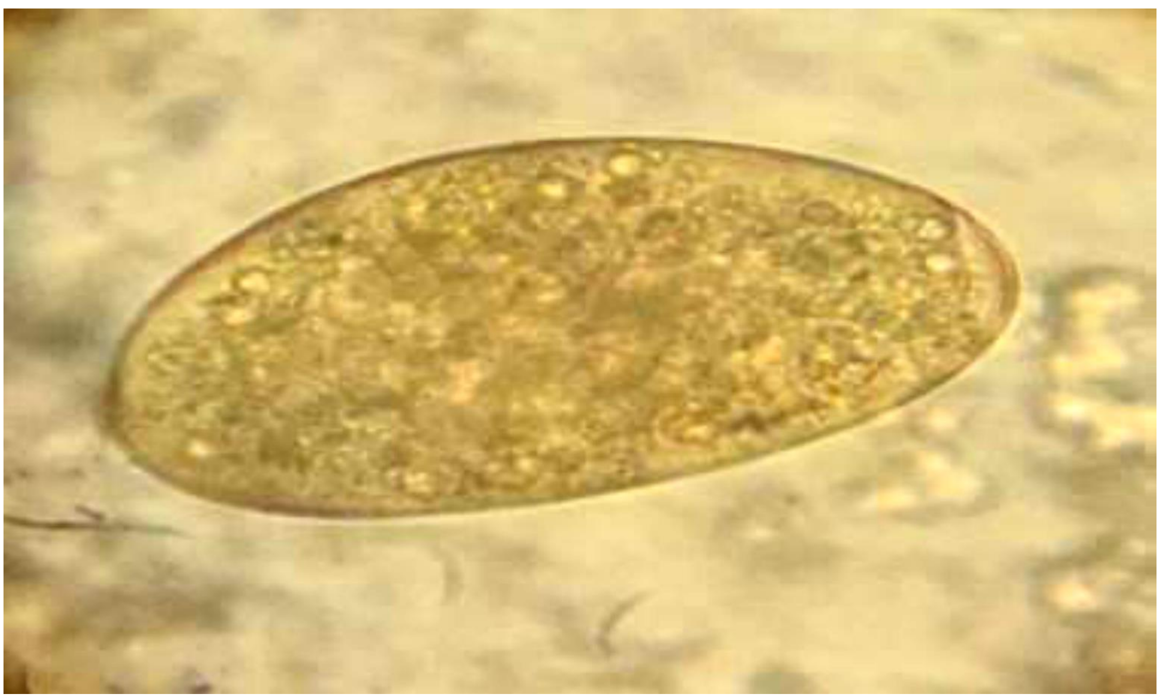


Figure 2.1 Egg of *F. gigantica* (from Thienpont *et al*, 1985)

F. hepatica has a worldwide distribution. It is the most common cause of liver fluke disease in temperate areas of the world and high altitude regions in east and South Africa except Africa .The disease occurs especially in cattle and sheep causing important economic infection and loss

(Kaufmann, 1996; Merck veterinary manual, 1991). His predilection site is the liver. The mature parasite is flat, leaf-shaped, and grey-brown in color, approximately 2-5cm in length and 1cm width. The young fluke is around 1-2 mm at the time of entry into the liver. The anterior end is conical and marked off by broad shoulders from the body. Microscopically the tegument is covered by backwardly projecting spines. Both oral and ventral sucker can be seen (Elsheikha & Khan, 2011;Taylor *et al*., 2007).The egg is large (130-145 umx70-90 um), nearly elliptic with similar poles and symmetrical, markedly barrel shaped side-walls, granular, yellowish brown contents filling the whole egg(fertilized egg surrounded by a great mass of yolk cells); no blastomeres, operculated (Thienpont *et al*, 1985).

Figure 2.2 Egg of *F. hepatica* (Borgsteede, 2011)

F. hepatica *F. gigantica*

Figure 2.3 Adult stage of Fasciola *spp* (from Astrat, 2004).

2.2 Intermediate Hosts

Fasciola genus has snails of the genus Lymnaea as the intermediate hosts. The ecology of the snail intermediate host plays a major role in determining the epidemiology of the disease. The Lymnaea species are the most important in the transmission of *F. hepatica*. They include: *L. truncatula*, widespread in Europe, Asia, Africa and North America; *L. bulimoides* in North America; *L. tomentosa* in Australia. Other species, which have been incriminated in the transmission of *F. hepatica*, include *L. viator* and *L. diaphena* (South America), *L. columnella* (USA, Australia, Central America and New Zealand) and *L. humilis* (North America) (Soulsby, 1982; Dunn, 1978). *L. truncatula* (Fig. 2.2) is the most common intermediate host for *F. hepatica* in different part of the world (Njau *et. al*., 1989) and in Ethiopia (Graber, 1974). It is an amphibious or mud-dwelling snail

which prefers moist temperature conditions (15-22ºC) though it appears that variants found in the tropics have adaptation to higher temperatures mostly in the lowlands areas and can breed and survive at 26ºC with sufficient moisture. The most important intermediate hosts of *F. gigantica are L. natalensis and L. auricularia* (Urquhart, 1996; Soulsby, 1982; Dunn, 1978). *L. natalensis* (Fig.

2.2) is the recognized intermediate host for *F. gigantica* (Yilma & Malone, 1998). Other species serving as secondary hosts to this species are *L. rufescens* and *L. acuminate* (Indo-Pakistan) and *L. rubiginosa* (Malaysia).

In South Africa the intermediate hosts of Fasciola *spp.* are: *L. nataliensis* carrying *F. gigantica*, *L. truncatula* for *F. hepatica* and *L. Columnella* whose parasitology is not known in South Africa but is widely thought to be susceptible to both Fasciola s*pp* (Quayle et al, 2010).

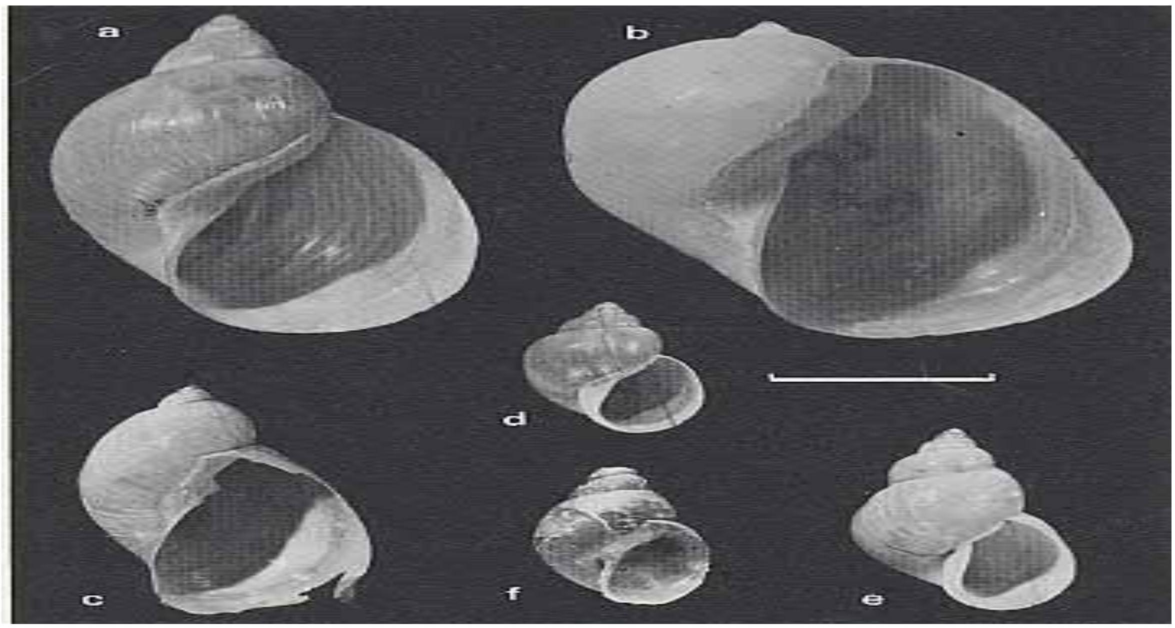


Figure2. 4 *Lymnaea spp*: a and b *L. natalensis*; c *L. columnella*; d, e and f *L. truncatula* (from Astrat, 2004)

2.3 Parasite distribution and habitats of the snail Lymnaea truncatula, intermediate host of the Liver Fluke *F. hepatica*, in South Africa

A study conducted by De Kock *et al.(*2002) regarding the distribution of the snail *L. truncatula* in South Africa showed a large extent reflecting a discontinuity of the distribution excluding Lesotho, some parts of Mpumalanga, Gauteng and North West provinces of the country. Different type of water body including swamps, muddy substratum and slow flowing-water areas were used for water collection and Lymnea presence determination. About 42% of water originated from swamps, 45.8% from slow flowing-water areas and 62.5% were collected from muddy substratum. 86.3% of samples were recovered from habitats having a mean annual air temperature of 10-20ºC, more than 69% from localities with a mean annual rainfall of 600-900 mm. Data analysis indicated that water body and temperature are playing a decisive role toward the determination of *L. Truncatula* presence in a given location (De Kock *et al*., 2002). Considering a temperature index calculated for all mollusc species, *L. Truncatula* was ranked second among 53 species due to its affinity for low temperatures.

Lymnaeid snails are the first intermediate hosts for several Fasciola *spp*. The main snail host for the liver fluke, *F. gigantica*, is *L. natalensis* which is widely distributed in Africa (Brown, 1994). Another study by Van Eeden *et al*.,(1965) indicated that this species also has a wide distribution range in South Africa that extends into the Western Cape (as far as the Knysna district), Kwa-Zulu Natal and the eastern Mpumalanga provinces. Surveys executed over the past 2 years in farm dams and other water bodies around Pretoria showed *L. natalensis* to be the most abundant freshwater snail in this area.

In conformity with the ecological requirements of their respective snail invertebrate hosts, the two fluke species appear to have different altitudinal distributions in South Africa and seem to largely exist in areas that are geographically isolated. Generally *F. gigantica* is associated with lower lying land while *F. hepatica* is customarily found above an altitude of approximately 800 m. A similar situation extends over sub-Saharan Africa but the disease is nowhere common in people.

Information on the distribution of veterinary fasciolosis in South Africa consists mainly of case reports or outbreaks in isolated places and mostly in artificial habitats such as drinking troughs. Neitz (1965) recorded *F. hepatica* infection in horses from Eastern Cape and Alves *et al*., (1988) cited records from Kokstad, KwaZulu-Natal, condemnations of equine meat at Johannesburg municipal abattoir and reported an another case from Gauteng. Goats from low-lying marshy ground in Limpopo and KwaZulu-Natal provinces have been reported infected by *F. gigantica* (Varta & Krecek, 2002). Evidence from Limpopo province suggests that sometimes antelopes such as Impala and Kudu that are making use of natural water bodies (not specified) can serve as reservoir hosts for *F. gigantica* infection in domestic animals. From a distributional point of view however, the metacercarial cyst is the 'weak link' in the *F. hepatica* life-cycle. This is due to the cyst susceptibility to desiccation and high temperatures, i.e. RH < 70% and temperatures above 25°C, particularly the former although they survive longer at lower than at higher temperatures (Chen & Mott, 1990; Kendall, 1965; Ollerenshaw & Rowlands, 1959). The only area in South Africa satisfying these cut-offs for *F. hepatica* is the central Drakensberg mountain range in Lesotho and the northern part of the eastern Cape (Schulze, 1997) including the area that have been reported by

Prinsloo and Van Eeden (1973) having high incidences of *fasciolosis* in sheep. The only other areas where the mean daily spring/summer RH exceeds 70% are the coastal strip of Eastern Cape and Kwa Zulu-Natal and lowvelds of Mpumalanga and Limpopo, but mean daily temperatures are above 25°C here. The fact that *Fasciola* parasites are not common and their distributions are more restricted than those of their invertebrate hosts is explained and reflected probably by the insufficiency of data on these parasites in South Africa.

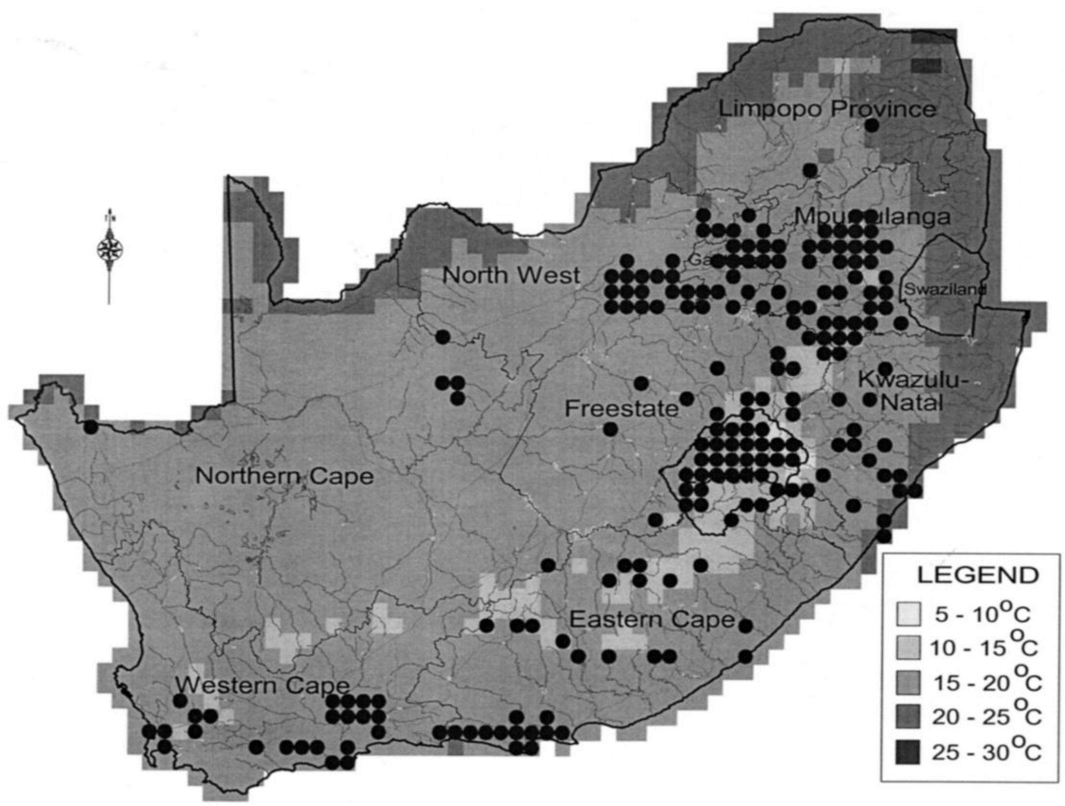


Figure2. 5 The geographical distribution of *L. truncatula* in 1/16- degree square loci and mean annual air temperature in South Africa

Table 2.1 Monthly reports on Liver fluke infestation in livestock in December 2012 and January 2013 respectively from various provinces of the country done by veterinarians in the indicated areas

Province	**Mpumalanga**	**KwaZulu Natal**	**Western Cape**
Area	Middleburg	Camperdown	George
Vets reporting the disease	Dr.Fourie, Dr.Barkhuizen	Dr Anthony van Tonder	Dr. Strydom, Dr.Truter, Dr. pettifer
Level of importance	2	1	2
Specie Affected	B	B	B and O

Province	**Mpumalanga**	**Free State**	**KwaZulu Natal**	**Eastern Cape**	**Western Cape**
Area	Lydenburg, Volksrust	Frankfort, Villiers	Mooi River, Underburg	Aliwal North, Humansdorp	Caledon, George.
Vets reporting the disease	Dr.Gustav.T Dr Andre .V	Drs.Dries.L, Hattingh, Hauptfleisch	Drs.Fowler, Hartley& Mallett	Drs.Casper.T., Van Niekerk, Jansen van vuuren,Louw	Drs.Ian Herbs, Strydom, Truter & Pettifer
Level of importance	Lyden: 3 Volks: 1	Frank: 2 Villiers:1	2	1	Caledon:1, Gearge:3
Specie affected	Lyden: Band O Volks:O	B	B	Aliwal:O Humansdorp :B	Caledon:O George:Band O

Level of importance scale: 1= one case

2 = more than one case but less than ten

3 =more than ten cases

Specie affected: B = bovine

O = ovine

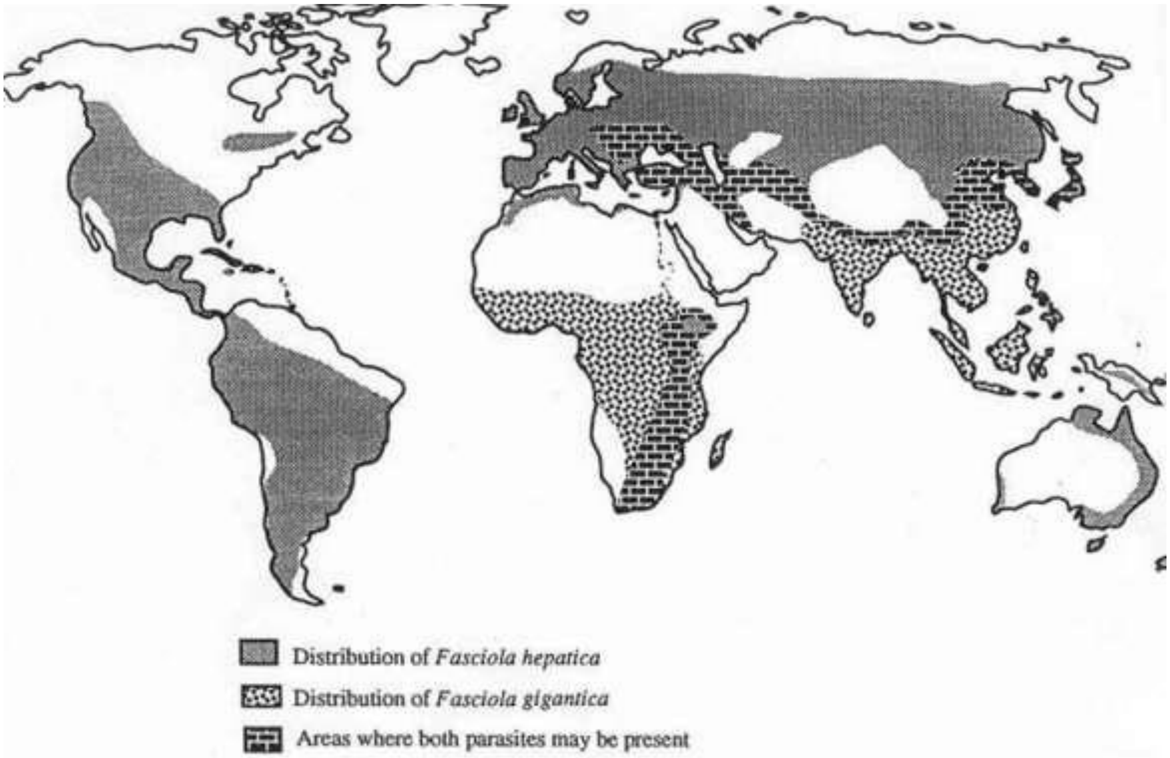


Figure2. 6 The worldwide distribution of fasciola species (from Torgeson &Claxton, 1999)

2.4 Pathogenesis, Lesions and Symptoms

The disease evolves in sub-acute, acute and chronic phases depending on the localisation of parasites (Reinecke, 1983).The Merck veterinary manual (1991) states that cattle are more resistant to the disease and *fasciolosis* turns to be symptomless. Cattle are more resistant to fluke infection than sheep (Boray, 1999). *F. gigantica* determines mainly the chronic form of the disease which seems to be important than the acute form. The most characteristic symptom of sub-acute and chronic *fasciolosis* is anaemia. Symptoms are usually associated with ascites, proliferating hepatic fibrosis followed by the dystrophic calcification of the bile ducts, hyperplastic cholangitis, oedema (bottle jaw), digestive disturbances (constipation, diarrhoea) and gradual cachexia, reduced milk production, unthriftiness (Kaufman, 1996; Merck veterinary manual, 1991; Boray, 1985; Reinecke 1983). The course of the disease is generally determined by the number of metacercariae ingested over a short period of time (Merck veterinary manual, 1991). The larger size of the fluke determines the severity of the pathological and the clinical disease due to *F.gigantica* (Boray, 1985).

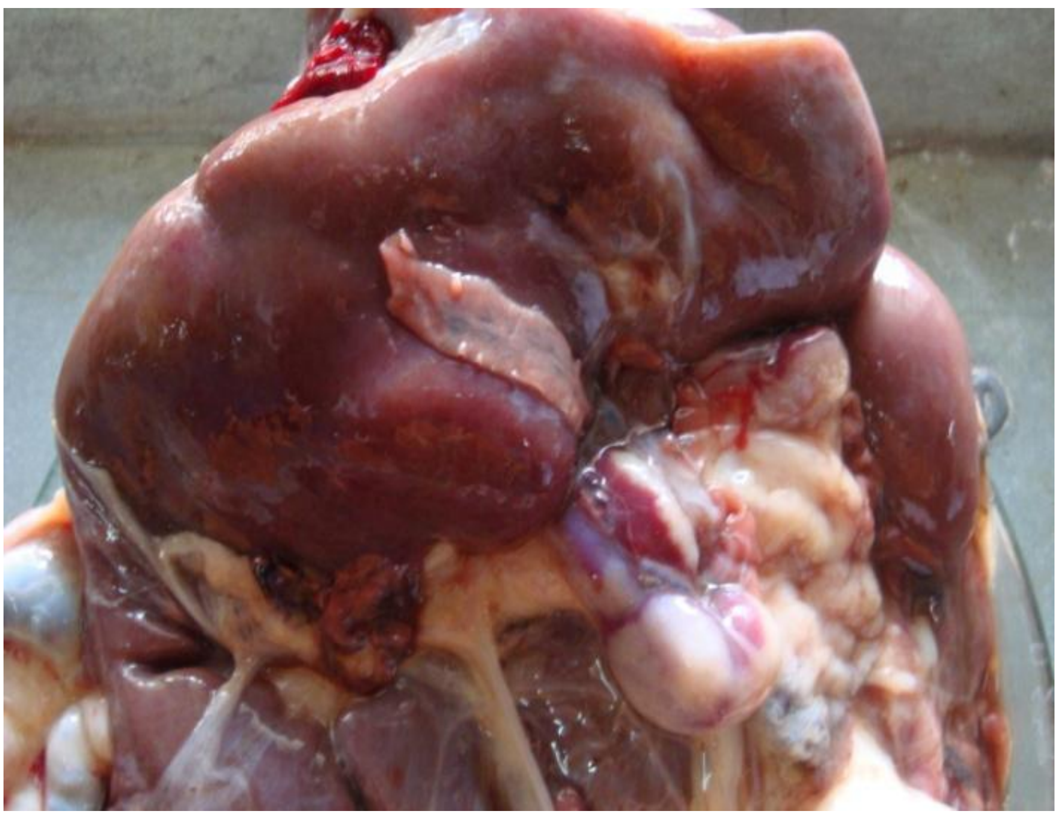


Figure 2.7 Example of a cattle liver naturally infected with liver fluke (from Affeza *et al*, 2013)

2.5 Diagnosis of fasciolosis

The classical diagnosis of Fascioliasis relies on examination of liver and finding of adult parasite or presence of its eggs through coprological examination (Kakar *et al*. 2011). Recently, a method based on detection of a *F. hepatica*-specific copro-antigen has been developed and commercialized (Mezo *et al*., 2004). Currently on the market an ELISA (enzyme linked immuno-sorbent assay) test exists which detects the presence and levels of antibodies against liver fluke. This test measures such levels which indicate wether flukes are presently detected or have in the previous 4 - 6 months been in a liver. The antibodies take about 2 -3 weeks to reach detectable levels and recede after approximately 6 months after the last active liver fluke presence. At necropsy or at slaughter adult flukes are found in the bile ducts, immature stages may be squeezed or teased from the cut surface. The nature of the liver is also diagnostic. Clinical signs of the disease can also be used to diagnose *Fasciolosis* (Kaufmann, 1996; Merck veterinary manual, 1991; Reneicke, 1983)

2.6 Differential diagnosis of Fasciolosis

The Paramphistome egg is often larger than that of Fasciola *spp* and has distinct embryonic cells, a transparent shell and a distinct operculum. A small knob is frequently found at the posterior pole. The paramphistomum egg contains blastomeres; it is pale grey to greenish.

2.7 Host immunity

It has been demonstrated that over time, cattle may build up a partially protective immune response to *F. hepatica.* The degree of parasite establishment and the pathologic impact of the infection is determined by interaction of such factors as age of the host, innate resistance of the host, previous exposure of the host, and present level of parasite exposure. Older cattle with previous exposure are said to have a greater resistance to infection than do young parasite-naive calves. Moreover, flukes are progressively rejected in order to have the great majority of flukes acquired during the major transmission period of 1 year lost before the same time the following year. Due to the fact that acquired immunity is only partially protective, still, cattle can be reinfected each year (Kaplan, 2001).

2.8 An overview on antihelmintics commonly used for Fasciolosis treatment.

Approaches to the control of fasciolosis in ruminants are compared for developed countries, and for developing countries with particular reference to regions growing irrigated rice. In developed countries liver flukes of ruminants are currently controlled by strategic and tactical drenching programs, which, for the most part, are based on knowledge of the life cycles of the parasites, their intermediate hosts and the epidemiology of the disease. In developing countries it is usual to accept the damage caused by fasciolosis (Dumag, *et al*., 1979).

As regards fasciolicides, Carbon tetrachloride, Hexachloroethane and Hexachlorophene were used extensively in the past. In South Africa the use of Carbone tetrachloride and Hexachloroethane were recommended as a curative measure (Le roux, 1957). For some years now and currently, Nitroxynil, Mentichlophalon, Trichopholan and Rafoxanide are in use (Fabiyi, 1987).The above drugs are either banned or have an extended withdrawal period in lactating cow producing milk for human consumption (Taylor *et al*., 2007).

To date, control has relied heavily on the use of drugs and Triclabendazole (TCBZ) in particular, because of its high activity against all stages of fluke in the final host (McConville *et al.*, 2009 Boray *et al*., 1983). However, reports of reduced drug activity (possibly indicative of drug resistance), along with genuine cases of Triclabendazole (TCBZ) resistance, have risen in recent years (Fairweather, 2009). This is a worrying scenario and highlights the need and breakthrough for alternative drugs.

Examples of useful drugs in control of Liver fluke:

1. Triclabendazole*: active against late immature and mature stages.
2. Closantel*: active against immature and mature stage of flukes.
3. Clorsulon*: against mature stage only.
4. Oxyclozanide: against late immature stage of flukes.
5. Nitroxynil*: against late immature and mature stages of fluke.
6. Rafoxanide*: against immature and mature stages.
7. Albendazole: against mature stage only.

The Irish medical board (IMB) in March 2010 ruled that “Veterinary medicines containing these substances should not be used in dairy animals intended for milk production, including pregnant heifers intended for milk production for human consumption.” According to Ireland Animal Health this ruling may probably change (AHI, 2011).

2.9 Different drug combinations

Several combination products containing nematocidal and flukicidal compounds are available on the market and have been evaluated, and these data indicate that a combination or the combined use of such compounds does not affect the efficacy of the flukicide. Clorsulon has been combined with Ivermectin as an injectable formulation (Hutchinson *et al.*, 2009; Ibarra-Velarde *et al.*, 2001), Closantel has been combined with Ivermectin as an injectable (Borgsteede *et al.*, 2008) and a pour-on formulation. Triclabendazole (TCBZ) has also been combined with levamisole, Ivermectin and Abamectin as an oral (Stevenson *et al.*, 2002) or pour-on combination (Sargent *et al.*, 2009). A combination flucacide and nematocide formulation (Nitromec1) containing Nitroxynil (340 g/l), Clorsulon (67 g/l) and Ivermectin (6.7 g/l) was developed to provide a non-Triclabendazole flucacide to maximise the efficacy against early immature stages of liver fluke through the reported synergism of Nitroxynil and Clorsulon (Fairweather &Boray, 1999; Boray, 1997) and to provide concurrent nematode control with Ivermectin. These combinations are justified by the benefits to treat simultaneously for nematodes and *F. hepatica* (Loyacano *et al.*, 2002).

The South African market is crowded with 18 products. The current classes of liver fluke remedies for cattle sold in South Africa are:

1. Benzimidazoles e.g. Triclabendazole, Ricobendazole
2. Halogenated salicylanalides. E.g. Closantel, Rafoxanide.
3. Combinations of the above with other antihelmintics e.g. Abamectin & Triclabendazole, Oxfendazole & Triclabendazole.

The current study is about Fluxacur (Triclabendazole & Abamectin), Flukazole C (Triclabendazole & Oxfendazole) and Sovereign Pour- on (Triclabendazole & Ivermectin).

Table 2.2 some trade names of/in South Africa for liver fluke treatment in cattle (adapted from Van wyk 1978)

		F. hepatica			
Compound	**Trade name(RSA)**	**Dose(mg/kg)**	**4weeks**	**6weeks**	**Adult**
Closantel	Seponver	2,5	x	x	A
Nitroxynil	Trodax	10	-	-	HE

Oxyclozanide	ICI Liver fluke remedy	10	-	-	HE
Rafoxanide	Ranide, injectable	2,5	X	A	A

HE=Highly Effective=80-100% effective

Figure 2.8 Products used for the trial: Sovereign pour on, Flukazole C, Fluxacur

2.10 Life cycle

The life cycle of *Fasciola spp* consists of five different phases which are:

1. The freeing of eggs from the snail to the outside environment and their subsequent development.
2. The hatching of miracidia and their search for the penetration of intermediate host, development and multiplication of the parasites inside the snail.
3. Emergence of the cercariae from the snail and their encystment.
4. The ingestion of the infective metacercariae by the final hosts.
5. Development to adult worms (Dalton, 1999; Reinecke, 1983).

The completion of the life cycle of the liver fluke *F. hepatica* is closely dependent on climatic conditions. Factors such as the survival and development rate of the fluke eggs, availability and distribution of the snail intermediate host *L. truncatula*, rate of development of infection within the snails and survival on pasture of the metacercaria larvae are closely linked to the temperature and humidity of the environment (Hanna, 2003; Dalton, 1999).

The timing and intensity of the metacercarial peak on pasture in late summer and autumn in any year are also closely related to the climatic conditions prevailing in the preceding six to nine months. The minimum temperature for development of *F. hepatica* eggs is about 10°C. The rate of development ranges from 80 days at this temperature to 10 days at 25°C (Ross & Mc Kay, 1929 reported in: Dalton, 1999). Thus, eggs shed onto pasture in winter and spring usually begin to develop in early April, accelerating as the mean day/night temperature rises, with large numbers of miracidium larvae hatching in late May. Snails also emerge from hibernation and begin breeding when the temperature exceeds 10°C, so the availability of the new generation of juvenile snails in late spring approximately coincides with hatching of the miracidia. This April rise of eggs hatching with miracidia being released is relevant to the northern hemisphere. In the southern hemisphere this pattern is the opposite. In the south there are large numbers of cercaria being shed from the snail to contaminate the grass and herbage suronding these moist areas. These cercaria become encysted metacercaria on the foliage.

Fluke egg development, survival of miracidia and successful location of snails by the free-swimming larvae are very dependent on the presence of adequate moisture, as indeed is the emergence, feeding, breeding and distribution of the amphibious snail hosts. At 15°C, larval development of *F. hepatica* within the snail is completed in 80 days, decreasing to 30 days at 25°C (Rowcliffe & Ollerenshaw, 1960 In: Dalton1999) while *F. gigantica*'s development period span is 11 weeks (Reinecke, 1983).

2.10.1 Part of the life cycle of Fasciola hepatica outside the host

Fluke eggs released by the adult worms get to the intestine through the bile duct and leave the host through the faeces. The eggs' development depend on the environmental moisture (Borgsteede, 2011; Dalton, 1999).The developmental rate is influenced by the temperature. The first larval stage and the miracidium development require a minimum time of 10 days at 26ºC and 6 weeks at 15ºC. Below 10ºC, no development is possible (Dalton, 1999).The opening of the operculum of the egg releases the miracidium. A snail host is needed by the miracidium within few hours following the hatching (Reinecke, 1983). Through the action of proteolytic enzymes, the miracidium penetrates the snail and migrates to the hepato-pancreas as a young sporocyst which grows into redia and probably into a daughter redia (Borgsteede, 2011). Many of the adult fluke's characteristics are found in the cercariae which have a tail. Released from the snail, the cercariae migrate and stick around vegetation. The tail disappears from the cercariae and they become metacercariae by forming a cyst. This is the infective stage for the mammalian final host (Affroze *et a*l, 2013; Boray, 2007). The passage from miracidium stage to metacercariae needs a minimum of 5 weeks. The Snails can shed cercariae for a couple of weeks. In periods lacking humidity, the release of cercariae can stop and can be continued when the conditions are wet again. Metacercariae can survive for long periods of up to almost 1 year. Moisture is influencing effectively their survival. They can survive a few degrees below 0ºC. Liver fluke is able to overwinter (Borgsteede, 2011; Hanna, 2003).

2.10.2 Part of the life cycle of Fasciola hepatica within the host

Digestive enzymes from the abomasum and duodenum destroy the outer cyst wall of metacercariae being ingested and the young fluke escapes actively from the cyst. The young fluke gets to the peritoneal cavity under the influence of proteolytic enzymes after its penetration into the small intestine wall.

Most of the young flukes are found in the liver within 3 to 4 days. Scarcely brains, lungs or any other organs may be reached (ectopic flukes). In pregnant cows, the foetus is vulnerable to intrauterine infection that can lead to abortion. However, a time period of six weeks is needed for flukes to get to the bile ducts after invading the liver through the parenchyma (Bergsteede, 2011). Flukes are hermaphroditic (with male and female genital organs) and proterandric (male sexual maturity reached first).

2.10.3 Comparison of Fasciola hepatica and Fasciola gigantica

According to Bergsteede (2011), the life cycles of both species are very similar. The intermediate hosts of *F. gigantica* are more aquatic than those of *F. hepatica*. *F. gigantica* development stage within the snail is slower as well as the migration process of *F. gigantica* inside the final host, resulting in a prepatent period of about 13–16 weeks. Adult flukes are larger (maximum 7.5 cm long and 1.2 cm wide) and of different shape compared to *F. hepatica*. Eggs are larger (maximum 0.19 mm long and 0.1 mm wide).

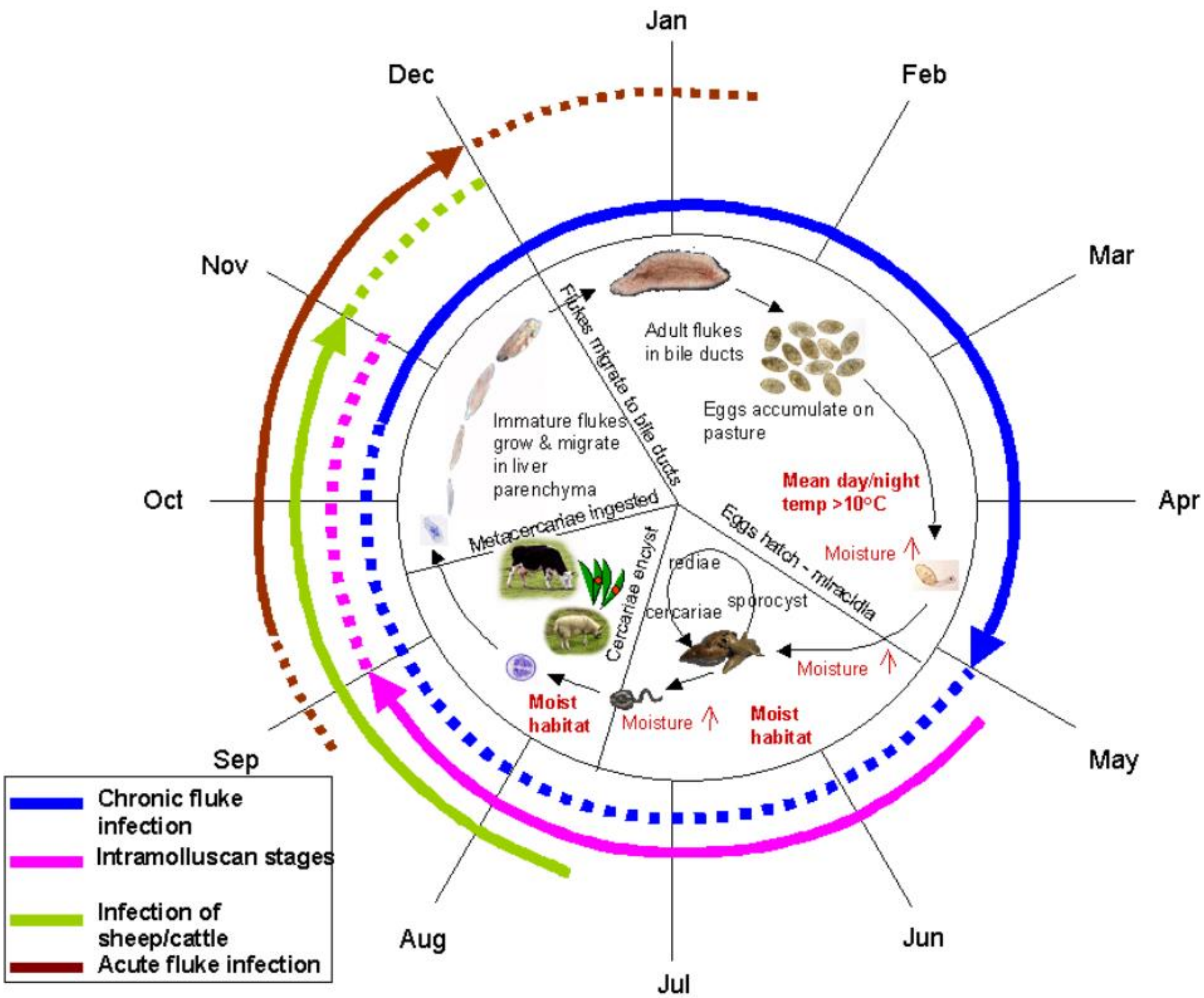


Figure 2.9 Life cycle *of F. hepatica* (from Hanna, 2003)

2.11 Control methods for *Fasciolosis*

Drugs are the most reliable control mean of Liver fluke that is why in order to reduce risks of losses due to acute fluke infection, it is necessary to dose at 4 to 6 week intervals until December using flukicidal drugs that are active against the most immature stages. In moderate risk fluky areas, sheep should be dosed at 10-week intervals from September to late January (Hanna, 2003). As a preventative measure in high-risk fluke areas, it is advisable to dose all sheep at 10-week intervals from April to November, with an additional treatment in January to remove chronic infections. Animals that ingest lower numbers of metacercariae usually survive the acute phase of infection, during which immature flukes migrate through the liver parenchyma. Once the flukes become established in the major bile ducts, they mature and begin producing eggs 8-10 weeks after the initial infection. The eggs pass into the intestine with the bile. Provided that there is not a subsequent invasion of young flukes,

the liver parenchymal tissue regenerates and recovers function, albeit with substantial fibrous scarring. It is these animals, harboring chronic infections of mature fluke that provide the source of fluke eggs for contamination of the pasture during the winter and spring months, and therefore represent the main contributors to disease in the next year. In any management programme for control of fascioliasis, it is essential to eliminate mature flukes from overwintered stock by dosing several times during the winter months or before lambing; using a flukicide that is active against the adult worms. Cattle, unlike sheep, develop a degree of natural resistance to second and subsequent infections by incoming juvenile flukes. Worm survival is poorer in cattle as compared to sheep, and the rate of maturation is slower. As a result of these differences, cattle rarely suffer from acute fasciolosis. However, chronic infections are common in over-wintered stock, necessitating one or more treatments during the housing period with flukicide active against the adult worms, in order to reduce the risk of pasture contamination after turn out.

The control of fasciolosis in South Africa appears to be limited to ad hoc chemotherapy of the terminal hosts (mostly livestock in South Africa), targeting the adult fluke. Furthermore, no available creditable literature recording methods of control targeting the snail hosts of this disease *(L. nataliensis or L. truncatula*) could be found. Control of these snail species, as part of a co-ordinated, integrated control programme for liver fluke disease, is not operational in South Africa (Quayle *et al.*, 2010).

2.12 Other control measures

Besides the use of anthelmintic drugs to eliminate flukes from livestock (sheep, cattle, horse and goat) the likelihood of infection on individual farms may be reduced by measures targeted at the molluscan intermediate host which is the snail. Drainage of potential snail habitats is consistent with environmentally sensitive programs of land improvement, and can have long term benefits for parasite control (AHVLA, 2012). But permanent destruction of snail habitats may be expensive and ecologically sensitive, or controversial, especially in widespread habitats. When snail habitats are small and localised fencing of such areas, or annual treatment with molluscicides, may be more feasible (AHVLA, 2012).Their efficacy is reduced in extensive and well-established snail habitats where the population can be augmented by immigration from surrounding areas of untreated land. Pasture management can also be used in various seasonal, and minimal or maximal rotational systems, with separate or mixed grazing by sheep and cattle (Boray, 1999).

2.12.1 Plants and their effects on animal nutrition

a) Effect of different forage plants on parasitism

Traditionally, around the world several plants are recognized as anti-parasitic plants because they contain chemical substances with anthelmintic effects on parasites affecting agricultural crops (Krueger *et al*., 2009) or animal parasites (De Jesús *et al.*, 2010; Galicia *et al*., 2008; López et al., 2008). It has been established that certain forage plants have properties to combat parasites (Hutchings, 2003; Adewunmi *et al*, 2001; Baker *et al*, 2001; Deharo *et al*, 2001; Waller *et al*, 2001; Guarrera, 1999). The antiparasitic properties of plants are resulting from plant secondary metabolites helping the plant to resist herbivory (Hatching, 2003). According to Athanasiadou *et al.* (2000) the mode of action of the compound in the plant is a direct anthelmintic effect.

There are a couple of plants that are effective against liver fluke in ruminants.
Cabret (1986) recommends the use of turpentine that is extracted from pine and various other conifers. The turpentine should be distilled and the spirits that are produced should be added to castor oil. Turpentine spirits can also be mixed with comestible linseed oil, but should be used with caution and given exactly as prescribed since turpentine can cause spasmodic closure of the mouth if it enters the respiratory tract. Common juniper is another conifer that is effective against liver fluke (Duval, 2003).

The South African plant *Curtisia dentata* commonly used for generations by rural communities as a drug treating a number of sicknesses caused by bacteria and fungi in either human being or animals (Shai *et a*l., 2008; Dold & Cocks, 2001) and against animal parasites(Shai *et al*., 2009).

b) Inclusion of browse and tall forages

When exposed to multiple species pasture including browse, grass and forbs, cattle prefer grazing. Under tree forage are believed to be contaminated by parasites due to the shrubs and forbs tree shades providing more moisture which is a favorable condition for larvae survival. Parasite larvae are concentrated under the pasture canopy (Karki, 2010). Including browse and tall forage will push cattle to a browsing behavior and keep their head up, therefore minimizes and reduces parasite infections (Karki, 2010).

2.12.2 Grazing habits of cattle

Grazing livestock have their herbage intake rate and bite depth affected by Sward height and density. Bite depth on cattle herbage intake is said to be a relatively constant proportion of sward height; its range varies from 34% to 48% of the ungrazed sward (Ungar & Ravid, 1999; Brereton & Mc Gilloway, 1996; Laca *et al*., 1992). This information taken in account cattle grazing swards at 6 to 8 cm could be taking only the top 2 to 3 cm off the top of the sward. The grazing habits of animals have an effect on the occurrence, the transmission and the control of the disease. Cattle are more often known as grazers of wet marshy areas favored by the fluke snail, so the eggs are shed in a suitable environment. Cattle and sheep are often refraining from grazing next to the faecal materials.

2.12.3 Pasture management and animal nutrition

a) Pasture and Hay field, Crop field and pasture rotation system

Pasture land can be tilled and serve as crop production field while crop field can be developed into pasture. The parasite burden is considerably lowered by the conversion and ploughing operations which is killing parasite eggs and larvae. In addition, if there are available different portions of hay; the land used plan can be changed to reduce parasite concentration (Karki, 2010).

b) Pasture improvement for better nutrition

To resist parasite infection, livestock needs to be well nourished and have good immunity. Pasture development requires knowledge and skill to make it productive all the year. It needs to include the combination of warm-season and cool season forage species (legumes, grass and forbs). The quality of forage is increased by inclusion of legumes in the grazing system (Karki *et al*, 2009) and eventually the quality of animal diet. Legumes are known to have a higher nitrogen concentration and lower fibre concentration than most grass species. They are respectively related to protein concentration and forage digestibility. Animal feed quality requires high nitrogen and low fibre content.

c) Lowering the stocking rate

Overstocking is a three-edged sword as it leads to high parasite load, unsustainable pasture because of damages to pasture plants and soil deterioration, and poor animal performance which remains low because of low forage availability or unavailability, high parasite incidence as well as environmental stress. The availability of forage for grazing animals should determine the stocking rate. The lower the stocking rate the lower the parasite incidence will be. Prolonged, excessively high stocking rates will result in a loss in body condition score, lower calving rates, and poor herd health (Thorne & Matthew, 2007).

d) Alternate and mixed grazing systems

Alternate or mixed grazing of sheep and cattle should be considered as strategies to manage and control internal parasites. Sequential grazing with sheep following cattle has been found to be effective in lowering parasite infection in sheep comparatively to systems involving sheep only (Marley *et al*, 2006). Waller (1997) stated that alternate grazing between cattle and sheep can yield goods results in parasite control for both species especially under temperate regions, this success being achieved from very scarce pasture change.

Every parasitic worm species have a specific host and grazing cattle and sheep in alternate years will decrease worm challenge even though *Nematodirus* can affect both cattle and sheep. Still not all studies have seen a benefit of the system. Moss *et al* (1998) conducted a three year trial in New Zealand to investigate and compare the impact of alternating sheep and cattle grazing and different pasture species on parasitism of lambs. The results showed a decrease of larval numbers on pasture in cattle side but the parasite load in lambs was not reduced. Another study showed the exact opposite of the previous study with lower faecal egg counts and population in alternately grazed with cattle at 6 months intervals. Based on speculation Moss *et a*l (1998) alleged lambs developed an increased immunity response to the greater larval availability in absence of cattle. The 4 year alternate grazing study carried out by Bairden *et al* (1995) demonstrated advantageous parasite control in calves up to the second grazing season, but the end of study revealed the similarity in parasite burdens between previous calves and those on the set stocked cattle only practice. Regulating the stocking densities will help decrease larval intake and infection in young vulnerable animals where there is no clean grazing.

e) Mixed-species grazing implementation

Cattle and goats do not have in common most of gastrointestinal parasites with major pathogenic importance. Therefore, they complement each other by lowering the parasitic larva of the other species (Miller, 2004;

Urquhart *et al*., 1988).When co-grazed; parasite larvae of another species have their effect reduced by each species thus minimize the probability of infection. If getting them along is not feasible or preferred, each species can graze alternatively the pasture. However, the risk of parasite problem in calves being much less than in goats, young calves need more attention and care to prevent them from *H.contortus* infection. Besides parasite control benefits, the mixed species grazing offers other benefits such as higher forage utilization which can be maximized and fight weed problem by making use of each species forage preferences(Abaye *et al.*,2008; Coffey, 2001). Cattle prefer to graze grasses while goats select from a wide range of grasses, brush and weeds, and browse. Many weed species found on the pasture grazed by cattle alone can be minimized by introducing goats into the grazing system. Also, quantity of meat produced per unit of pasture may be increased in mixed-species grazing than when either species are grazed alone because of higher forage utilization, reduction in weed problem, and minimization of the gastrointestinal parasitic problems.

f) Applying control grazing in case of multi-species pasture

Under continuous grazing system, animals are left on the whole pasture throughout the grazing season (Karki & Gurung, 2009). Animals select and graze most palatable plants and plant parts first and less palatable later on. When there are multiple species in a pasture, few species may be more palatable than others. So, most palatable species will be grazed repeatedly as long as animals can get them. Moreover, overgrazing of the palatable species cause its low availability or extinction in the uncontrolled grazing. Therefore, pasture having multiple species should be managed applying controlled or rotational grazing. Control grazing can be practiced by adopting rotational grazing system or some other specialized grazing systems.

2.12.4 Grazing management systems

The infection rate is an important factor that influences the worm load of grazing livestock. It indicates the number of infective larvae ingested daily from a contaminated pasture (Barger, 1999).If grazing livestock daily forage intake is considered to be relatively constant, with that the infection rate can be known by an approximate calculation of the number of larvae relative to the dry matter. The infection rate may be affected also by plant species and the choice of grazing system.

2.12.5 Soil health maintenance to minimize parasite eggs and larvae

A good quality soil is populated with different types of organism such as dung beetles, earthworms and nematode trapping fungi. It has been demonstrated that parasite eggs and larvae are killed by earthworms by ingestion or by pushing them far below the ground surface into burrows. In the same way faeces is scattered by dung beetles allowing it to desiccate and reducing the moisture content. In addition the beetles ingest or carry the faeces down to underground burrows, reducing the amount of parasite in the pasture. Nematode trapping fungi are known to trap soil nematodes including parasite nematode larvae and eat them as their food. Emptied soil environment will be harmful to these beneficial organisms.

2.12.6 Positioning of waterers and feeders high enough to avoid contamination with faeces

Knowing the fact that parasite eggs are shed into faeces to contaminate the pasture, waterers and feeders should be situated at a relatively high and distance above the ground depending on the height of animals to avoid water and feeds contamination and pollution by faecal materials. Separate waterers and feeders can be provided to young kids using a creep.

2.12.7 Biological control

Figure 2.10 Identifying when and where snails are present in the grazing system is essential for the design of a liver fluke control program. Removal of snails by ducks limits liver fluke transmission

Suhardono *et al*. (2006) investigated on the possibility of successful biological control of fasciolosis by echinostome flukes. Larval echinostomes were found to be able to displace aggressively other larval flukes from their snail hosts and parasitic castration of snails by larval echinostomes (Estuningsih, 1991; Lie, 1973). Although this knowledge, previous workers were unable to work out an adapted way to apply this strategy in the field. Faeces from five to ten ducks naturally infected with *E. revolotum* was mixed with bovine for rice field fertilization purposes, or by building the duck or chicken pen over the effluent drain from a cattle pen before it entered an adjacent field. Simultaneous application of the mixed faeces is needed at the same place in the rice field for a maximum competition between *F. gigantica* and *E. revolotum* miracidia for snails. This strategy was found effective in eliminating metacercariae from rice fields in proximity to cattle pens or villages that would then constitute the greatest potential source of infection for livestock. This work faced resistance in some other areas as option for liver fluke control needed due to the concurrent infection of duck lands with schistosomes the cercariae of which cause dermatitis by penetrating the skin of rice-field workers.

Collection and storage of cattle and buffalo faeces in a trench reduces the spread of fasciolosis. Fasciola eggs leave the infected host or sick animal in the faeces. After storage for two months the Fasciola eggs will have succumbed to high temperatures prevailing in the trench. The trench should be located away from run-off water, above the water table and away from animal access.
Size of trench:

1. Length: 3.50 m
2. Width: 2.50 m
3. Depth: 1.50 m

Roof and fence for the trench:
The trench requires proper roof and fence to protect dung from rain and sun light which will cause it to lose value as a fertilizer. In addition, the trench should be fenced for human and animal safety and to prevent loss of dung. The roof is made of local materials. Duration of dung storage
Dung is stored in the trench for two months before using it as a fertilizer. During this period, Fasciola eggs in the faecal will die at temperatures of around 60 C in the dung mass.

2.13 Epidemiology of *Fasciola spp*

Although fasciolosis occurs in a variety of domestic and wild animals in South Africa, little is known of its epidemiology. Humans are in fact accidental hosts, but stool analyses show that human infections do occur in the wetter grazing and stock-raising areas of Limpopo, Mpumalanga and KwaZulu-Natal provinces. These are uncommon; the possibility exists that eggs found in human stools may have come from eating infected raw liver rather than from patent infections (Quayle *et a*l., 2010).
Pastures contaminated with parasites eggs serve as reservoir and provide a good environment for the hatching of eggs to larvae which undergo few development stages and become infective. Most cases of acute fasciolosis result from ingestion of metacercariae in late summer from eggs hatching in the same year from May to July. These eggs were shed during the winter and spring by adult worms inhabiting the bile ducts of chronically-infected untreated sheep and cattle that survived infection in the previous year. However, some metacercariae do survive on pasture over winter, particularly if the conditions are mild. In addition, immature fluke infections in snails cease development when the intermediate host enters hibernation deep in the mud at the onset of winter. The development

resumes when the snails reactivate in April, and may increase the number of metacercariae on the late spring/early summer pasture. Under favorable conditions infective larvae move to the tip of herbage and enter into grazing animal's gastro intestinal tract together with the forages eaten by these animals. This is the case of parasites requiring only one host to complete their life cycle (direct life cycle) like most of the roundworms of veterinary importance (Urquhart *et al.*, 1988). Parasites that require more than one intermediate hosts to complete their life cycle such as Liver fluke have their eggs, larvae and intermediate host provided with good environmental conditions for their survival and development. So sheep and cattle may begin to acquire low levels of fluke infection from the beginning of the grazing season due to these overwintering larvae. While such infections rarely cause significant clinical signs, the damage to the liver may have implications for the metabolism of anthelmintic drugs later in the season (Hanna, 2003).

A sound understanding of the relationship between the epidemiology of the disease and its life cycle is vitally important for the development of effective control strategies for liver fluke.

The spread of diseases is said to be brought by chronically infected domestic animals in contaminating the pastures with parasite eggs such as liver fluke eggs; this pasture contamination occurs often in environment with particular favorable climatic conditions and suitable intermediate hosts.

Figure 2.11Cattle grazing poor wet pasture in a typical snail habitat – a slowly running stream fed by springs (Boray, 2007, Australia)

Figure 2.12 Liver fluke and Roundworms infected cow pastures. A vlei section flowing from the left neighboring farms into Goedverwacht farm

Figure 2.13 End of the rain season, March 2012, water levels already receding in the vlei

Figure 2.14 Typical snail and flukey area in Goedverwacht farm

The vlei areas consist of different sizes and shapes of water pools which all mostly dry up in the winter months. Thus these are grazed at some stage by the cattle. These liver fluke and roundworms infested pastures were grazed by the cow herd from May – June 2012 during their exposure.

2.13.1 Metacercariae on the herbages

A study done in Bangladesh by Affroze *et al.*, (2012) regarding the risk factors and gross pathology of bovine liver fluke infection at Netrokona district in Bangladesh revealed the presence of a large number of metacercariae was found on Jonlydal/Futki (Hygroryza aristata) one of the herbages found in the study at about one inch below from the tip of the leaf and about three inches above the water surface on the leaf and stem of Hygroryza aristata (Affroze *et al*, 2013)

Figure 2.15 Fasciola metacercariae on the stem of herbage

Figure 2.16 Metacercariae encysted on grass blades. One single miracidium hatching from a fluke egg can produce up to 4000 infective cysts (metacercariae). Actively swimming cercariae released from the snail attach to substrates, especially vegetation (Boray, 2007)

2.13.2 Factors influencing the survival and development of parasites (Round worms)

Parasites do not survive, grow, or develop in any environment. They require a minimum of favorable environmental conditions for their survival, growth and development such as moisture and heat. Wet and warm (65ºF–85ºF; larvae survival and development is arrested beyond this temperature range) environment is favorable for the survival and development of parasite larvae in the pasture environment (Miller, 2004). Parasite larvae remain close to the ground up to a level where there is enough moisture for their survival. A continuous film of moisture on the herbage also serves as a medium for the travel of larvae from faecal mass to the surrounding foliage. When there is a lot of moisture, larvae travel towards the tip of the forage to be ingested by grazing animals. When it is dry, larvae go back close to the ground surface and remain there until environment becomes favorable for them to travel up the forage again. Where moisture is not limiting, temperature is seen to have a greater influence over migration (Stromberg, 1997).

Silangwa and Todd (1964) found that higher humidity favored larvae migration toward foliage, with significantly more larvae moving at 95% compared with 56% relative humidity. Larvae were also likely to climb wetted leaf blades. The ability of larvae to climb herbage was affected by adverse low temperatures with significant more larvae climbing at 27C than 4C. Generally, the moisture available under the forage canopy is adequate for larvae survival. Normally; the density of larvae remains high within 12-24 inch from the faeces and 2-3 inches up the forage plants. However, parasite larvae can reach beyond 2-3-inch height on the forage plant when it is warm and wet. Therefore, it is recommended that goats should not be allowed to graze forages lower than 5 inches. Moreover, parasite density remains high in certain areas of pasture where faecal material is accumulated such as around water source, under the tree shade (during hot days), and around feeding areas. Also, parasite larvae population will usually be high in overstocked pasture than in normally stocked or under stocked pastures.

Liver fluke prevalence will be high where there are water bodies (swamp areas) and snails. After reviewing several studies O'Connor et al (2006) reported differences between nematodes species on the rate of departing from the faecal material. Van Dijk *et al* (2009) reported that the longevity of L3 larvae is related to temperature and humidity but that these two parameters alone cannot fully account for the observed abundance on pasture, with a number of studies noting that the decline in L3 populations at pasture are more rapid than predicted. Additional factors could include predation, loss to the soil, temperature fluctuations and sunlight.

2.13.3 Distribution of parasite larvae on pasture

According to Sutherland and Scott (2010) the ability of the parasites to establish and the exposure to the infective stages are the two major factors responsible for over dispersion of parasites. The amount of light, temperature and moisture at different height layers within the sward depend on factors related to it and influence larvae distribution. Typically shorter sward will be drier and hotter than tall swards of the same density and thickly crowded sward will be cooler and moister than scattered swards (Soil Association, 2000).

A study done by Moss and Bray established that a large number of larvae were recovered from denser swards. According to Crofton (1948) who investigated on climatic conditions in herbage, there was a considerable difference between the temperatures within the sward and the air, having a general gradient of the temperature between the upper and the lower parts of the herbage. Besides, he proved that the base of the sward was very humid no matter the lowness of atmospheric humidity. Referring to ultra violet irradiation, a similar vertical gradient would be expected to exist

(Van Dijk *et.al,* 2009). This same study suggested that the constant movement of larvae on and off herbage might be explained by the exposure to UV, secondly the reason why 90% of larvae are generally found at the lower part of the sward, furthermore the reason why the leaf shape appears to influence the height to which larvae migrate. The authors established that the optimum distance for larvae horizontal migration is determined by an energy related trade off and there is likely to be a further trade off determining vertical migratory behavior: that of being probably being ingested by climbing higher versus being exposed to higher UV irradiations intensity, higher temperatures and lower moisture at higher position.

2.13.3.1 Horizontal distribution of L3 larvae on pasture

The effect of successive harvests of grazable herbage next to faecal deposition on the population dynamics of L3 larvae was demonstrated by Boom and Sheath (2008). They stated studies (which included Gruner and Sauve, 1982) deepened the subject of development and migratory behavior of L3 larvae from cattle faecal pats. The observation from the majority of these studies is that from the faecal pats, few larvae migrate further than 30cm, even after a time relatively long and significant rainfall. Boom and Sheath harvested herbage four times in this trial (22-248 days) in three zones (0-20cm, 20-35cm and 35-45cm from the centre of the faecal pat) from around the faecal pats. They noticed that L3 remained combined into a body close to the faecal pats; they emerged from even after two successive harvests and significant rainfall. The effect of repeated grazing events by non-infective

stock was simulated by these successive harvests. There was a drop of less than 3% in L3 presence on herbage of the original population after two grazing events.

Factors affecting rain splash dispersal of infective larvae from cow pats has been described by Gronvold *et al* (1989). Very few L3 larvae were scattered due simulated rainfall on dry pats in contrast to pre-watered pats where L3 larvae were stimulated to be exposed to the stroke of rain drops. More than ninety percent of larvae were passively transported by splash droplets rather than active migration under controlled laboratory conditions. Tufts of rejected grass are frequently surrounding and covering faecal pats on pasture. Horizontal movement of splash droplets will be restricted by the abovementioned situation causing the fall of larvae a few cm from the pat. L3 larvae were discovered up to 90cm away from the pats where splash droplets could be in free movement. *Cooperia* and *Ostertagia spp* L3 larvae distribution was investigated around faecal pats regarding calves' grazing behavior in another research done by Gruner and Sauve (1982); they referred also to the L3 larvae distribution with the age of the faecal pat. The findings suggest that the move from the faecal pat to the surrounding grass is progressive and takes 6-8 weeks. (Gruner & Sauve, 1982)

2.13.3.2 Vertical distribution of L3 larvae on pasture

Crofton (1948) used three different plant species: Festuca (fescsue), Clover and Carex nigris (common sedge) to assess the number of L3 larvae (*Ttrichostrongylus retotaeformis)*. On edge (25cm in height) larvae were found to be distributed and limited to the base 5cm, whilst clover leaves and stems were evenly vertically covered. The plant height influenced larvae distribution on Festuca: 90% of the larvae were found in the lower 7,5cm (and 50% below 3,8cm) on grass 13cm high whilst on grass 7,5cm high 90% 0f larvae were found below 2,5cm. Besides, this study investigated on larval distribution in the soil 'mat' and herbage which was measured throughout the year and revealed highly seasonal differences. Most of larvae were found on the foliage and many others in the soil than the 'mat' in the summer months(June to August).More larvae have been seen in the 'mat' in the spring and autumn and fewer in the soil or on the foliage whilst in winter larvae were found to be concentrated in the 'mat' only. The laboratory experiments carried out under controlled environmental conditions by Silangwa and Todd (1964).They observed the L3 (Trichostrongylid) larvae ability to move vertically on the herbage. Their findings showed that even under favorable conditions there was a very small proportion (2-3%) of larvae applied to the soil actually climbed the foliage.

The majority of larvae 59% were found in the bottom 2, 5cm, 27% up to 5cm, 10% to 7,5cm, 3% to 10 cm and 1% above this, supporting the findings of Crofton. The vertical distribution of L3 larvae on grass or clover and in soil at a controlled temperature and humidity was investigated over 4 days by Callinan and Westrcott (1986). Most of L3 larvae were found on the soil with very few, 25 being recovered from herbage. From the few that move onto the herbage, 68% were recovered at 0-2cm and only 4% over 6 cm. According to this study there was no significant difference between the number of larvae found on grass and on clover.

The above studies have proven that more larvae have a general disposition to find themselves on the base of the sward, but this situation may not translate to more per kilogram of dry matter. A report from Moss and Vlassof (1993) showed the ryegrass/white clover swards densities (larvae dry matter) to be lowering the 0-25 mm zone and grew at the similar levels in the 26-75 mm, 76-125 mm and > 125mm zones over this height. Some plant species such as Chicory were found to have lower larval densities over 75 mm from the base than below this height. Assuming that daily dry matter intakes are similar on both swards, herbage with higher larval density will result in high larval intake by livestock than herbage with lower densities corroborating Niezen *et al.*,(1998) findings commenting on high larval densities being found on the top stratum of clover, particularly on ryegrass and common bent (brown top), in this situation grazing livestock will be vulnerable to high levels of parasitism and infectivity than those in lower larval density grazing fields.

2.14 Economical and financial losses due to Fasciolosis

2.14.1 General view

In cattle, fasciolosis generally is subclinical, but is considered to produce marked economic effects (Torgerson & Claxton, 1999). However, until now surprisingly few studies have been conducted to estimate the effect of Fasciolosis on productivity (Vercruyssea & Claerebout, 2001) and most of these studies were improperly controlled (Dargie, 1987).

Worldwide livestock are experiencing severe economic losses of productivity caused by parasites. Agriculture industry is suffering economic loss evaluated at over 42.5 million Rand annually due to liver fluke infection in domestic animals in Australia (Spithill *et al.*, 1999; Boray, 1997). Economic losses of several million dollars per year have been reported due to fasciolosis in terms of mortality, liver condemnations at slaughter houses, poor weight gain, infertility, reduction in traction power of oxen, and low calf weight at birth (Ngategize *et al.*, 1993; Njau *et al.*, 1988). However, data on production losses caused by *F. gigantica* in cattle are usually only concerned with liver condemnations in slaughtered stock (Anon, 1986; Losos, 1986; Bitakaramire, 1968). Production losses in young growing cattle are particularly important since they are more vulnerable than adult cattle. It has been established that there is an acquired resistance in older cattle, both to reinfection and in rejection of an existing infection (Hammond & Sewell, 1990)

Table 2.3 some estimated costs of liver fluke to the South African cattle industry

Cost	Per annum cost	Once off cost
Preventative drenching	R15-30 per animal	
Reduced Milk productivity	R500 per infected animal	
Liver Condemnation		R300 per infected animal

The cost portion attributable to liver fluke is thus difficult to separate from that associated with other parasites. Aside from the basic estimates of some of the more obvious costs that are presented above, national, provincial or local infection statistics are needed to assess meaningfully the economic impact of liver fluke disease. None of these data are available (Quayle *et al*, 2010)

2.14.2 Figures of financial losses due to Fasciolosis in different countries

The financial loss is a monetary value that is used as an index of the true value of particular resources to the society (Morris &Meek, 1980). The following lines are showing losses of revenue from animal production due to liver fluke infection in some countries including South Africa.

2.14.2.1 Figures of financial losses due to Fasciolosis in different countries of Africa

Developing countries from southern Africa and Africa in general are confronted by many of the same socio-economic and beef production challenges. Furthermore, cattle are the most important livestock species in Africa. The consequent similarities in climatic and agricultural conditions allows for many areas of similar interest and concern regarding beef cattle production. The challenges discussed in this study relate to low beef production levels particularly due to Liver fluke infection in cattle.

2.14.2.1.1 Figures of financial losses due to Fasciolosis in South Africa

According to a veterinarian, as a rough estimation for South Africa, costs associated with preventative drenching in a commercial livestock setting located in fasciola endemic areas are estimated to be R15.00 per animal per annum for cattle in areas where dosing is carried out once a year (using Triclabendazole which is effective against all mammalian stages of the fluke's development), while in some warmer areas, farmers are required to dose up to three times a year (Using adulticides – drugs effective only against the adult stages of the fluke), pushing costs up to approximately R30.00 per animal (D. Clowes – pers comm.). Milk production is estimated to be reduced by 0.5 ℓ/day, conception and calving rates reduced by 30% and in beef cattle, rates of growth are reportedly reduced by approximately 14% (Chick *et al*., 1980). The condemnation of livers at slaughter is estimated to cost the farmer approximately R300.00 per infected animal, assuming a liver weight of 20 kg and a liver price of R15/kg.

2.14.2.1.2 Figures of financial losses due to Fasciolosis in Zimbabwe

From the previous abattoir studies in Zimbabwe, the condemnation rate of livers due to *F. gigantica* was 46.3% in 1986, with 527 tons of livers either condemned or trimmed (Chambers, 1987) and the loss per head slaughtered amounted to Z$ 3.45. A higher incidence of *F. gigantica* was reported in the higher rainfall areas (70 %) than the drier areas (25 %) (Chambers,1987). Between 1988 and the first quarter of 1990, the condemnation rate ranged from 40.4 % to 43.2 %, with losses due to liver condemnation alone exceeding 71882800 USD annually (Vassilev & Jooste, 1991).

2.14.2.1.3 Figures of financial losses due to Fasciolosis in Tanzania

According to Njombe and Msanga (2009) the livestock population growth 1984 shows 12.5 million cattle, 6.4 million goats,3.1 million sheep and o.3 million pigs while in 2006, there were 18.5 million cattle,13.1 million goats, 3.6 million sheep and 1.2 million pigs. Analysis of primary data (meat inspection) showed that 150 of 469 cattle livers condemned were due to fasciolosis, a relative condemnation rate of 32.0% per month. Based on the current local price of liver, the economic loss per month due to liver condemnation was estimated at Tanzania shillings (TZS) 1,800,000/- (approximately USD1, 500, i.e. R15000), which summed to TZS 21,600,000/- (USD18, 000, i.e. R18000) per annum. The specific cause of liver fasciolosis was *F. gigantica.* These results indicated that *F. gigantica* infection is an important condition that leads to high liver condemnation rates in cattle slaughtered, resulting into high financial loss (Mwabonimana *et al*., 2009).

2.14.2.1.4 Figures of financial losses due to Fasciolosis in Zambia

Estimation of economic losses due to fasciolosis at national or even regional level is currently limited by lack of accurate data on the prevalence of the disease (Phiri *et al*, 2005). Apart from a few reports on this condition in Zambia (Silangwa, 1973; Pandey, 1987; Pandey and Ahmadu, 1998), very little efforts have been made to estimate either the total losses resulting from fasciolosis infection or even partial losses from the condemnation of livers. According to Simwanza (2012) this study made an attempt to estimate at least the partial financial losses due to fasciolosis regarding the whole liver condemnations in Senanga and Shang'ombo districts of Zambia. The results suggest that an average of 11, 848 cattle are slaughtered annually in Senanga and Shang'ombo districts, with a total of 2,406 whole livers being condemned due to liver fluke infection. This implies an annual loss of approximately USD 24,500(i.e. R245000) for the two abovementionned districts. Considering that large portions of Western, Northwestern, Southern, Central, Northern provinces and parts of many other countries in the region are flood plains and therefore equally prone to fasciolosis, these findings indicate that fasciolosis may be causing significant socio-economic losses to the country and to a majority poor rural farmers who depend on livestock as their main source of income and livehood.

2.14.2.1.5 Figures of financial losses due to Fasciolosis in Ethiopia

In Ethiopia the total annual financial losses due to liver condemnation and carcass weight loss were estimated to be 270,211.88 Ethiopian Birr or equivalent to $ 27, 572.64 USD (i.e.R 275,726.4) (Berhe *et al.*, 2009).

Between 1974 and 2003 Ethiopia reportedly experienced about 54 natural disasters, with the worst famine the country has experienced in 1983-1985. The country is not starting from scratch. Up-to-date; it is Africa's leading meat producer. Ethiopia's livestock population is large and significant. According to brightergreen in 2009, the livestock is made up of approximately 40 million cattle, (The sixth largest cattle herd in the world, just behind Argentina), 25 million sheep, 23million goats, and 150,000 camels, as well as tens of millions of poultry. (www.brightergreen.org). Based on this information, it is understandable that the results of the survey on the financial losses incriminating liver fluke could obviously be more than the findings of Berhe, *et al*. in 2009 due to the fact that the research was conducted only on the number of slaughtered animals during the same year, the remaining livestock of the country was not considered for this study but assumption can be made regarding their status as being infected by liver fluke. Furthermore, according to the ministry of agriculture, the very recent livestock census done in 2010 reveals that the Ethiopian livestock population has reached 88 million with 52 million of cattle (MoA, 2010). This data could assist with calculations as to what liver fluke challenge could be present in the livestock population. Thus financial loss regarding liver fluke infection could rise.

2.14.2.1.6 Figures of financial losses due to Fasciolosis in Kenya

Kenya national bureau of statistics stated that in 2009 Kenyan livestock population included a variety of species which are cattle: 11,746,774; Sheep: 1,719,606; Goats: 27,740,153; Camels: 2,971,111; Donkeys: 1,832,519; Pigs: 334,689; Indigenous chickens: 25,756,487; Commercial chickens: 6,071,042; Bee hives: 1,842,496. The Kenyan livestock has goats as its highest animal population followed by indigenous chickens and the cattle population. These figures show that there is a high potential of liver fluke infection due to numbers of susceptible species.

In Kenya the annual national loss has been estimated at between approximately 0.15–0.26 million USD approximately 1,245-2,158 million Rand (Kithuka *et al*., 2002; Wamae *et al.*, 1998; Anon, 1986; Cheruiyot, 1983; Castelino & Preston, 1979). A 10 year retrospective study from 1990-1999 was conducted referring to post-mortem records to determine the prevalence and economic importance of fasciolosis in cattle, goats and sheep. 38 districts in 7 of Kenyan provinces had their abattoirs records examined. Fasciolosis prevalence was calculated. The financial losses caused by condemnation of infected livers were also calculated. Out of 5,421,188 cattle; 1,700,281 sheep and 2,062,828 goats slaughtered, 8% of cattle (427,931 cattle), 3, 6% of sheep (61,955% sheep) and 2, 45% of goats (48,889 goats) were declared liver fluke infected. The economic losses caused by infected liver condemnation from cattle, sheep, and goats was respectively USD 2, 6 million (i.e.R26 million), USD 61,955 million (R619.55 million) and USD 48,889(R488.89 million) (Njeru *et al.*, 2000). These figures show how prevalent fasciolosis is in livestock in Kenya and how the disease is economically important.

Considering the growth of livestock population and the fact that the previous researches have been carried out long ago using available data on that specific time, the national annual loss could evidently rise if any recent trial is conducted with these new figures regarding liver fluke damages.

2.14.2.1.7 Figures of financial losses due to Fasciolosis in Nigeria

Nigerian livestock population is suffering from lack of accurate statistical data which are not even widespread. Different figures have been published by various sources at different times. Still, the Nigerian domestic ruminant population has been assigned a value of 13,9 million cattle which is 60% of the all livestock population, 34,5 million goats; 22 million sheep (both accounting for 35,2% of the ruminants), equine and camels have been valued respectively at 3,6% and 0,6% of the livestock population(RIM, 1992). The cattle herd is a part of Bos indicus and mainly dominated by various types of zebu breeds such as white Fulani (Bunaji), Sokoto Gudaji (Bokoloji) and the

N'dama. The Fulani and the Sokoto Gudaji form approximately and respectively 51% and 12% of the Nigerian cattle population (RIM, 1992). The production losses are particularly high in areas with a network of slow flowing permanent streams, the favorable habitats of the snail vectors. Thus, Fabiyi and Adeleye (1982) estimated an annual loss of over U.S $40 million which is about 400 million Rand on Jos Plateau of northern Nigeria alone. The same is true of Shan and Keyal States in Burma (Griffiths, 1957).

The figures given by Fabiyi and Adeleye for Jos plateau state and by Griffiths for Shan and Keyal states are underestimated compared to the current livestock population of Nigeria. Besides, the trial was conducted just in these few states, other remaining states not being considered. Therefore, if a trial could be conducted taking in account all the livestock population involving all the states in connection of financial losses due to liver fluke infection, the financial losses could eventually go beyond the previous figures.

As after further evidences, it appears that in many African countries there is no clear picture of regular annual animal census, national prevalence of fasciolosis and a regular annual economic loss report due to liver fluke infection. The effects of the disease on the livestock industry can be assessed only by a conduction of regular retrospective studies to determine the prevalence and the economical aspect of liver fluke. Therefore, policy makers, animal health professionals, farmers etc. will be enlightened in respect of the attention the disease deserves in all aspects. Retrospective study reports will reveal with clear understanding whether there has been progress in the control of the disease or in the economic aspect.

2.14.2.2 Figures of financial losses due to Fasciolosis in United States of America

The condemnation of damaged livers at slaughter and the losses in beef production associated with fluke infections are economically significant. Losses due to liver fluke infection at Florida beef industry in United States of America have been estimated at 85 million Rand (i.e.8.5million USD) (Irisk *et al*., 2008).

2.14.2.3 Figures of financial losses due to Fasciolosis in United Kingdom

It is estimated that liver fluke costs the cattle industry of the United Kingdom GBP 23million a year (R400 million) (White, 2005 reported in Quayle *et al*, 2010), and the Australian industry Australian 80 million if (R600 million) (Boray, 1999 in: Molloy& Anderson, 2006).

2.14.2.4 Figures of financial losses due to Fasciolosis in Switzerland

For Switzerland the financial losses were estimated as high as 376 Euro per infected cow (Schweizer *et al.*, 2005).

2.14.2.5 Figures of financial losses due to Fasciolosis in Saudi Arabia

A cross-sectional study was used to determine the prevalence of fasciolosis and liver abscessation in imported slaughtered cattle from January 2009 to December 2011 at Al-Taif, KSA. A total number of 57,593 cattle were slaughtered and 9,414 (16.3%) livers were condemned due to fasciolosis and abscessation effects. Of the slaughtered cattle, a significantly ($p<0.05$) higher prevalence of fasciolosis was recorded (8.6%) than abscessation. Fasciolosis considered the main cause of liver condemnation and was responsible for total liver condemnation in cattle as 52.06%. The economic importance of such infections in terms of lost meat and offal were also estimated as 75000 SR annually (Nabila *et al*, 2012).

2.14.3 Economic effects of liver fluke on animal productions

Like most of parasitic diseases, liver fluke disease brings a cohort of considerable economic losses in the livestock industry. Economics is concerned with allocation of resources. The resources that are affected by the disease are animal productions in term of production of meat, milk, reproduction and fertility, weight gain, draught performance, and wool. Besides there are costs of antihelmintics, drenches (Molina *et al*. 2005). According to Pimentel (2003), no economical assessment has been carried out on the economic impact of liver fluke disease on the livestock industry of South Africa. Literature concerning the costs of this disease in South Africa is thus essentially non-existent, and so for this literature-based report, costs have been speculated on, but not researched beyond that.

In trying to assess the total costs of the liver fluke parasite to the agricultural industry in and of South Africa, economic costs can be associated with the following issues:

1. Animal fatalities,
2. Lost productivity in milking cows,
3. reduced wool production,
4. Delayed conception,
5. Reduced calving/lambing rates,
6. reduced weight gain and loss of condition in slaughter animals,

7. Loss of revenue from condemned livers at slaughter.
8. Loss of weight gain for weaner animals at time of sale

2.14.3.1 Effects of liver fluke on meat (carcass)

According to AAVP (1983) approximately 1.5 million livers are condemned annually in the U.S. due to these parasites. The level of liver condemnation at abattoir meat inspection worldwide varies according to the season and is reported to be as low as 0.26% but in areas where it occurs more commonly the average rate following dry summers is reported to be 5% but can be as high as 10% to 20% following wet summers. In the KZN and East Griqualand areas unofficial reports of condemnations due to liver fluke infections varied from 6.7% to about 40% during the autumn of 2002. The condemnation rate at Cato Ridge Abattoir during September to November 1994 was 24% (unpublished data) and the condemnation rate of livers at the privately owned feedlot abattoir at Wartburg KZN during the autumn of 2002 was 17% (cpdsolutions, 2012)

Meat and milk production decrease are main consequences of liver fluke infection, they express an economic loss. Liver fluke may cause a reduced meat production of up to 20% in cattle and up to 30% in sheep depending on the degree of the infection. According to Animal Health Ireland, losses also occur due to the number of livers condemned in meat plants and up to 50% of livers may be condemned (A.H.I, 2011).

A 3-year database (2005-2008) from an abattoir was retrieved and analyzed. In addition, meat inspection was carried out for one month (July 2008) with focus on fasciola infection and its associated economic loss due to liver condemnation. Results from the retrospective study revealed that 8302 (6.7%) livers out of 123790 examined livers were condemned due to fasciolosis. Analysis of primary data (meat inspection) showed that 150 of 469 cattle livers condemned were due to fasciolosis, a relative condemnation rate of 32.0% per month (Mwabonimana *et al*., 2009).

The study done by Phiri(2006) regarding common conditions leading to cattle carcass and offal condemnations at three abattoirs in the western province of Zambia and their zoonotic implication to consumers reveals that *F. gigantica* infections were the leading cause of condemnations of livers (20.1 %).

2.14.3.2 Effects of liver fluke on lactation

High prevalences of *F. hepatica* infection have been reported in dairy cattle in many countries (McCann *et al.*, 2010; Bennema *et al.*, 2009, 2010;; Mezo *et al*., 2008), very few studies on their effects on milk production have been carried out, and results have not been consistent (Khan *et al.*, 2009, 2010; Oakley *et al.*, 1979). The most likely reason for this could be due to the difficulty of setting up a trial protocol in a commercial dairy herd. The costs involved also have to be carried by some-one and dairy farmers, research institutes or veterinary pharmaceutical companies don't have the capital for this.

Data on the effects of trematodes on milk yield and/or quality are extremely limited and derived mostly from improperly controlled studies; also many of the results have not appeared in the international scientific press. Depression of milk yield ranging from 90-300 kg/ lactation have been associated with *F. hepatica* infections (Randell & Bradley, 1980; Horschner *et al.*, 1970).It has been proven that infection with *Fasciolosis* reduces quantity and quality of milk. For example Ross (1970) found that fasciolosis caused a drop of 14% of milk yield. Infection with liver flukes does not only reduce milk production 0,5litre/day but affects also its composition and quality (Chick *et al.*, 1980).

2.14.3.3 Effects of liver fluke on reproduction and fertility

This is probably the most neglected area of study in relation to the effects of parasites on production, the impact upon age at puberty, and rates of conception, pregnancy, parturition and weaning having been examined in little detail. In cattle, there are indications that *F. hepatica* and *Fasciolicides magna* reduce conception and pregnancy rates (Charlier *et al*.2007; McCauley *et al*., 1983; Malone *et a*l., 1982; Foreyt, 1982; Oakley *et al*., 1979) and studies regarding the effects of liver fluke infection in livestock have demonstrated the occurrence of abortion and stillbirths in association with *fasciolosis* (Sinclair, 1972; Hope Cawdery, 1976), and the last author as well as others (Crossland *et al*., I977), found indications of reduced conception, pregnancy . It has been suggested that fluke infections mainly affect conception and/or establishment of pregnancy.

F. hepatica the common liver fluke, trematode type of worm, is also known by its nuisance in causing lack of stamina leading to reduction in bull sterility (Sundstrom *et al.*, 2000). It has long been reported that infection with liver flukes reduces animal fertility. It was unknown, however, whether this effect was a general consequence of reduced well-being and retarded growth or whether the parasites were somehow affecting hormonal balance. Although few published studies have investigated this issue, mounting evidence suggests that liver flukes do affect sex hormone balance and metabolism. A recent study on prepuberal heifers demonstrated a significant delay of 39 days in the onset of first estrus in fluke-infected animals. Fluke-infected heifers also had significantly higher levels of oestrogen and significantly lower levels of progesterone than did uninfected animals. The cause of this effect is not clear, but evidence suggests that liver flukes somehow alter normal metabolism and/or balance of sex hormones (Kaplan, 2001). Conception and calving rates are reduced by 30% (Chick, 1980).

2.14.3.4 Effects of liver fluke on growth and growth efficiency

In beef cattle, rates of growth are reportedly reduced by approximately 14% (Chick *et al*.1980).
In Europe, a research showed that after being experimentally infected with liver flukes, weaned calves (8 to 9 months old) demonstrated that subclinical infections (averaging 54 flukes per calf) cause a decrease in weight gains by 8% during the first 6 months of infection. Higher levels of infection (average of 140 flukes per calf) reduced weight gain by 29% and led to the appearance of clinical signs in some animals. In another separate study that investigated the effect of fluke infection on feed consumption, growth, and fertility, revealed a retarded growth in heifers infected with subclinical fluke burdens (Kaplan, 2001).

A study done in Belgium on Belgian double muscled bull cattle indicated an average of 23.5kg growth retardation during 75 days of fattening as a loss due to liver fluke disease. The drop in the rate of weight gain in Belgian double-muscled cattle infected with flukes was demonstrated to be significant (Genicot *et al*, 1991).

Liver fluke artificially infected steers at a rate of 1200 metacercariae showed a significant reduction of growth by 14.7% and 14.1% while being respectively grazed at 3.54 beasts/hectare and 4.39 beasts/hectare (Chick *et al*,1980).

2.14.3.5 Effects of liver fluke on draught performance

Work output is said to be reduced by 7–15% due to anaemia resulting from fasciolosis (Roberts *et al.*, 1991). Considering a further indirect reduction of 20% in potential work capacity in animals whose growth has been already restricted by fluke infection, it can be concluded that liver fluke can seriously lower the work potential of both cattle and buffalo. The economic significance of this may, however, be changing rapidly in production systems where hand tractors are replacing animals as sources of draught power. In many systems, however, even if there is a gradual reduction in their use, draught power from large ruminants remains important and still valuable. Indeed, as the prices for fossil fuel increase, draught animal power may remain viable in more isolated and remote areas, particularly with poorer farmers. The cost of the reduced draught capacity caused by *F. gigantica* may be measured as the opportunity cost to a farmer caused by the longer time taken by infected animals to perform a specific task; this amounts to about 27–35% more time with buffaloes according to the conclusions of Roberts and Copeman (unpublished). Further evidence that infection with *F. gigantica* adversely affects draught capacity was collected by Suhardono (2001) in Indonesia.

2.14.4 Beneficial effects of treatment on animal production and performances

One of the main challenges when deciding on parasite- treatment strategy in the herd is to determine whether the application of an anthelmintic or a combination of anthelmintic will produce an economic gain that will pay for the treatment (Rew, 1999), but several field studies have demonstrated that treatment of fluke-infected cattle with an effective flukicide produces a positive cost-benefit result and improved productivity.

2.14.4.1 Effects of liver fluke treatment on growth

Suppression of fluke infections alone significantly increased body condition score (BCS) after the breeding season and increased total weight gain (Loyacano *et al*., 2002).

In South Africa a study were conducted on 300 steers aged 6-8 months artificially infected with *F. gigantica* metacercariae. These steers were ranked in three groups according to their weight and were treated with Triclabendazole 4 and 8 weeks after the infection. Compared to the fourth group of untreated animals, considered as the control group, it was found that the mean average daily weight gain was measured for all the cattle and indicated that cattle in treated groups gained more weight than cattle in the control group, and this was also the case for the calculated mean feed conversion (Steyn *et al*., 2006).
Treatment for fluke infections has also been suggested to improve reproduction in cattle via an increase in growth and improved general body condition (Rickard *et al.*, 1992).Loyacano *et al.* (2001) noted growth performances in calves following parenteral treatment with Doramectin or a combination of Ivermectin and Clorsulon.

Zinsstag *et al.* (1997) reported an increase in live weight in cattle aged 12-24 and 24-36 months by 9, 4% and 17, 5% respectively following two annual treatment with Febendazole. Animals less than

12 months old had 6, 3% higher live weight after being treated twice. The average weight of 3-4years old twice treated were 13, 1% and 8, 2% respectively, compared with their controls.

A study conducted in Philippines over 2 year period time in cattle in an irrigated site and a rain fed cropping areas. Two other areas were considered as control. 468 animals from which 174 were male, all maximally aged six years were the trial animals. They were weighed, drenched with Triclabendazole at the dose rate of 12mg/kg body weight. Results showed that in cattle treated in irrigated and rain fed areas the weight gain was respectively 361g/day and 358g/day. Treatment of fluke infection affected growth rate. Contrastable live weight gain in untreated cattle in the same areas was 67g/day and 343 kg/day in treated areas. Deworming improved also substantially cattle growth, weight and draught power as well as shown in table 2 (Copland & Skerratt, 2008.). Result from a research conducted by Elitok *et al* (2006) regarding a field trial on comparative efficacy of four fasciolocides against natural liver fluke in cattle indicated that the therapeutic suppression of liver fluke infections resulted in significantly increased body weight and body weight gain in replacement of the animals.

2.14.4.2 Effects of liver fluke treatment on reproduction performances

An improved reproductive performance has been reported in first- lactation animals treated with Eprinomectine at calving in New Zealand (McPherson *et al.*, 1999). Although inconsistent, a beneficial anthelmintic treatment effect on reproductive treatment had been pointed out by Hawkins (1993). A study performed in GA, USA, found a higher number of pregnant cows (98%v 75%, P =0, 12) and calved cow (90%v 68%, P=0, 03) for beef cattle treated with Febendazole (Stuedemann *et al*., 1989). Another study with Febendazole (in dual purpose cattle in Gambia) showed an improvement in annual calving risk (52%v44%,P=0,001) in the treated group of animals (Zinsstag *et al.*, 1997). Treatment for liver fluke has also been reported to increase stocker calf gains (Malone *et al*., 1982; Armstrong & Miller, 1980).

In Philippine two years of study were not enough to accurately measure the inter-calving interval due to the fact cows had more than one calf during the study period. However treatment with Triclabendazole in irrigated as well as in rain fed areas yielded an improved inter-calving interval over the untreated ones. The reproductive performance in treated animals knowing the epidemiology and the life cycle of the parasite was essential for the design of an effective liver fluke control program. The great number of animals falling pregnant and many calves being born in treated animals than in untreated ones is an evidence of better reproductive performances (Copland & Skerratt, 2008).

Researchers have determined that treating cattle with anthelmintics reduce the time required to reach breeding weight (Zajac et al., 1991).

2.14.4.3 Effects of liver fluke treatment on milk production

Gross *et al.* (1999) reviewed the results of more than 80 different clinical trials assessing the effect of anthelmintic treatment in dairy cows and its potential effect on milk production, and concluded that overall there was a benefit from such treatment. Various study designs and antihelmintics were applied in the studies assessed, and it was concluded that the median increase in milk production across all the trials was 0.63 kg of milk per day. The majority of trials in which the anthelmintic treatment was applied during the dry period or at calving showed an increase in milk production for treated compared to non-treated cows (Nodtvedt, 2002).

According to Ross (1970), 8% of the 14% loss in milk production due to *Fasciola spp* can be recovered by treatment. The study conducted by Muhammad *et al*. (2009) showed an increase of 0.62 litre (L) per day per animal post-treatment.

2.14.4.4 Effects of liver fluke treatment on draught performances

A survey on Ongole cattle which were treated with a single dose of Triclabendazole administered in July, six weeks after harvest of the second seasonal rice crop in the area revealed that treated animals were used twice as many days as untreated animals for preparing land for planting rice. Untreated animals owners avoided the opportunity cost associated with increased time to prepare their land by hiring animals that had received treatment. Thus, where this hiring option is available, the economic cost associated with reduced work capacity in animals infected with *F. gigantica* may be the cost of hiring replacement animals for land preparation rather than the opportunity cost of a farmer's labor.

2.14.4.5 Environmental effects of liver fluke treatment

Technology introduced for the implementation in this program "fasciolosis in cattle and its control measures", especially the introduction of the biological control measure improves hygiene at household level. The health of the farm family is protected by placing a control on a source of possible infection. An added benefit is that farmers will have manure in greater quantity and it will be of better quality. Farm incomes are accordingly raised and living standards positively impacted (www.aciar.gov.au).

Table 2.4 Observed benefits from the free treatment supplied by the fasciolosis extension program

Activity/condition change	**Rating score N =1**	**F-value (GLM)**
Deworming:		3.89**
Increased size of animals	4.23±0.68	
Increased weight of animals	4.24±0.68	
Improved animal strength for farm work	4.17±0.72	
Increased meat/carcass when slaughtered	3.95±0.76	
Commanded high price when sold	3.98±0.75	
Gave pride to animal owner	3.91±0.75	

** $P < 0.01$

Great extent 3.51–4.50; Some extent 2.51–3.50; Little extent 1.51–2.50;Very little extent 1.00–1.50; No extent <1.00.

In Table 5, data show the range over which the respondents and their animals had been assisted by the free treatment given through the fasciolosis extension program. Findings reveal that farmers in the seven trial villages vary significantly ($P<0.01$). In their perceived benefits from the free treatment (generic name: Rafoxanide). Specifically, the farmers perceived that the free-deworming activity of the program has to some extent increased size, weight and draught power of the animal.

CHAPTER 3

MATERIALS AND METHODS

3.1 Study Area Description

Lekwa is situated in the southern part of the Mpumalanga highveld. Standerton is the major town in the area. Moderate summers and cold winters characterize the climate of the area. The annual rainfall, which falls mainly during summer, varies between 550 and 750 mm. The area of 4 586 km2 is covered by grassland. Mixed farming is practiced in the area. Cattle (Beef and dairy), sheep, poultry, maize, grain sorghum, wheat, sunflower seed and potatoes are produced. Irrigation farming is found next to the Vaal and Waterval Rivers. Coal is mined north of Standerton. Tutuka Power Station is found north of Standerton. The Grootdraai Dam in the Vaal River near Standerton supplies water to the Tutuka Power Station and SASOL.

Distances from Standerton to the major urban areas in Gauteng, Kwa-Zulu-Natal, Mpumalanga and the Free State are as follows:

Johannesburg 170km, Pretoria 187km, Secunda 65km,Ermelo 96km,Volksrust 85km, Durban 480km, Cape Town 1600km,Witbank 40km, Middelburg 153km, Nelspruit 367km, Bloemfontein 450km, Bethlehem 227km, Harrismith 257km, Newcastle140km, Ladysmith 264km

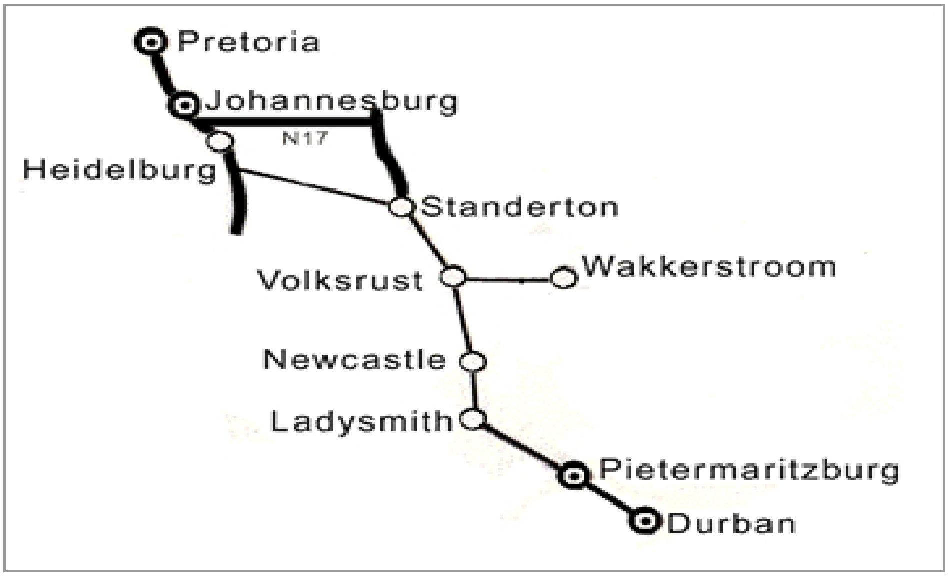


Figure 3.1 Areas Surrounding Standerton

Figure 3.2 Goedverwacht farm in Standerton (S.D Lukamba, March 2012)

3.1.1 Regional Climate

The site is under a moderately cold- temperate climate with thermic continentality. High extremes between maximum summer and minimum winter temperatures are characteristic of the area. Frost and large thermic diurnal differences especially in autumn and spring (Mucina & Rutherford, 2006).

3.1.1.1 Rainfall at Goedverwacht farm in 2012

Standerton is situated in a summer rainfall area receiving a mean average annual rainfall of between 621, 42-752, 36 mm. Standerton has an average monthly rainfall. The Average Monthly Rainfall was obtained from the Standerton weather station, as provided by www.weathersa.com

In the farm area the rainfall is normally 900-950 mm but during this trial season there was only 780 mm. The time the rain was expected and needed the most especially during the mating season from November to January, there was none, it was dry.

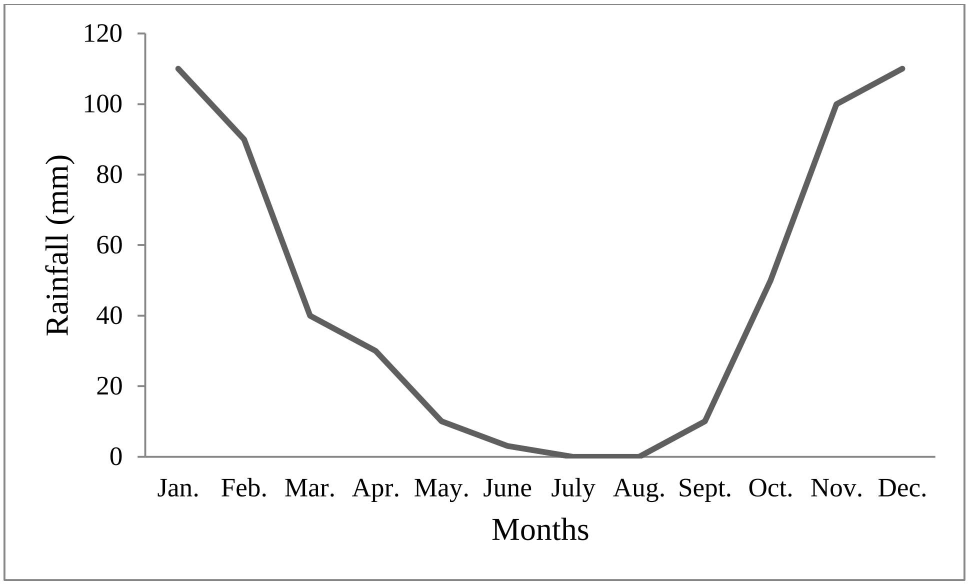


Figure 3.3 Average monthly rainfalls at Standerton

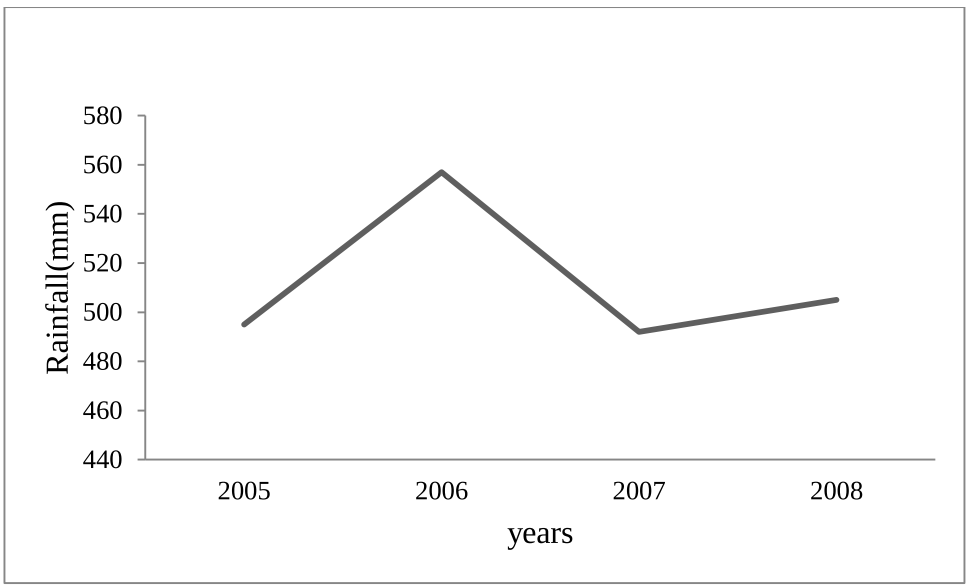


Figure 3.4 Average annual rainfalls at Standerton from 2005 to 2008

3.1.1.2. Temperature at Goedverwacht farm in 2012

According to AGIS comprehensive Atlas (2012) the mean maximum temperature will range annually between 21, 1ºC and 27ºC and the annual mean minimum temperature varies between -1, 9 and 0ºC. Average daily temperatures were obtained from the Standerton weather station

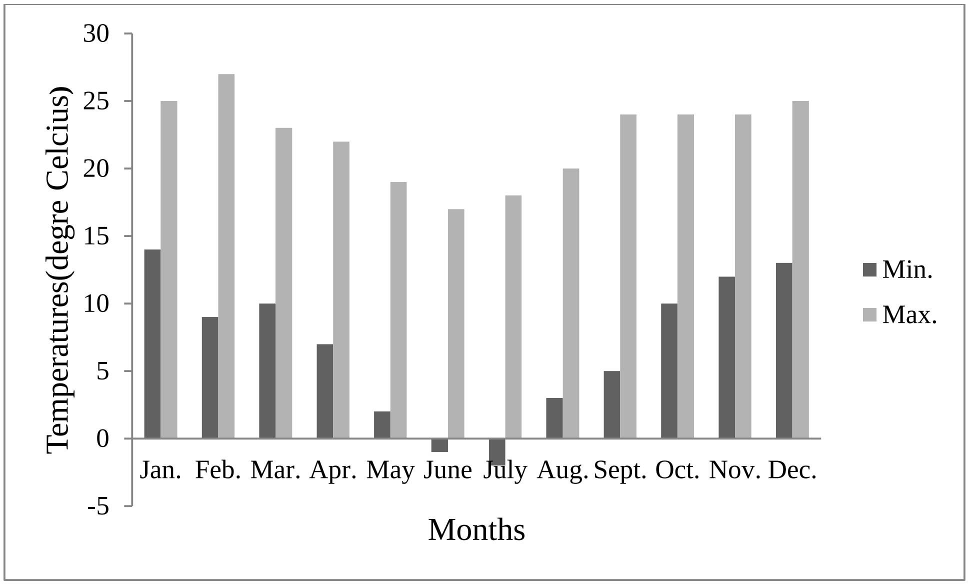


Figure 3.5 Standerton average daily temperatures during the study period in 2012

Min= Minimum; Max=Maximum.

3.1.1.3 Soil and Topography

The study area is located close to Standerton which is situated in the southern part of Mpumalanga. It is a moderate flat to slightly undulating landscape with an altitude varying from 1 500 to 1 700 m altitude.

The predominating soils are very clayey, black vertic or near vertic, mostly of montmorillonitic clays. These are swelling clay soils with high natural fertility. The soils have high swell-shrink potential and are very plastic and sticky. The soils found in the region of the trial site are described as black and red strongly structured clayey soils with a high base status. Top soil in the area ranges in depth from 450 mm to 750 mm and non-calcarious soils with a high natural fertility can be expected. Goedverwacht farm soil varies from nice moderate Avalon soils to Kroonstad and even Wesbank toward the rockier area.

3.1.2 Fauna and Flora at Goedverwacht farm

A variety of vast herd of ungulates such as springbok, black wildebeest and blesbok is found in the native grassland. Birds' populations range from 50 to 380 birds per 100 ha and include a diversity of species.

For the natural fauna in the neighborhood of the farm, farmers themselves have successfully conserved a lot of Steenbok, Grey Duiker, Hare/Rabbit, Porcupine, Redribbok, some Reedbuck and the occasional Fello deer and few present Blesbuck involved in blasted eye infection transmitted by flies around the Blesbuck eyes, laying eggs in cow eyes (it produces worms that may destroy the sight of cattle which is treatable), hatch and bore through the outer part of the eyes and after some metamorphosis it produce animals sight destroying worms which is treatable with a lot of efforts.

The area is exclusively dominated by the red grass Themeda triandra and occurs with a number of other grass species such as Tristachya Leucothrix, Elionorus muticus, Eragrostis racemosa, and Heteropogon Contortus. Some other characteristic species are three-awn-rolling grass like Aristida bipartida, blackseed finger grass Digitaria ternate, large seed setaria, setaria nigrirostis, and S.Incrassata and Panicum coloratum. Other important species are weeping love grass Eragrostis caffra, Brachiaria serrata, Eragrostis plana with feathered Cloris cloris virgata, couch grass Cynodon dactylon and Tassel bristle grass Aristida congesta. The area contains also Dicotyledonous forbs which include Berkheya pinnatifida, flower-in-a-cage Crabbea acaulis, hair flower Chaetacanthus costatus, Salvia repens, Pseudognaphalium luteo-album, Abildgaardiaovata, Anthospermum pumilum, Chamaesyce inaequilatera, Bulbostylis context and Evolvulus Alsinoides (Low & Rabelo,1998).According to the farmer, the farms' wetlands occasionally grazed by animals do not have the best pastures, but during dry periods, he reported that there is a lot of green vegetation and better protein in the area available for livestock.

3.2 Materials and Methods

3.2.1 Experimental animals

Goedverwacht farm in Standerton (Mpumalanga) provided 116 two year Beefmaster weaned heifers weighing 121-300kg and 134 adult dry Bonsmara cows over four years of age, weighing between 400-600kg, were selected for the trial. These animals were all grazed, supplied with winter lick and kept under the same environmental conditions. Blood and faecal samples were also collected for liver fluke confirmation purpose.

3.2.2 Cattle management

The cattle involved were all managed based on the principles of the good management practice (GMP ID ear tags, Intervet branded, distributed by MSD animal health). Cattle were tagged by means of standard tagging pliers for animal identification purpose. Other equipment used include plain serum collection tubes, needles, shoulders, long sleeve collection gloves for faecal sample collection, cooler boxes containing ice bags for samples conservation and expedition, drencher applicators with pipes, a computer for data recording such as animal details (sex, age, weight, breed, colour, amount of drug administered), a Tal-Tec branded scale for animal weighing, stationery, soap and investigational veterinary products. The knowledge of the products and of the life cycle of the parasite were taken into consideration in term of animal treatments, as well as recommendations by the consulting veterinarian.

3.2.2.1 Veterinary products used for liver fluke treatment

Table 3.1 Veterinary Product 1 and description of Sovereign pour-on

Trade name	**Sovereign® Pour-on**
Active ingredients	Triclabendazole: 24%m/v and Ivermectin: 1.5% m/v
Formulation	Pour-on
Date of manufacture	31/12/2010
Expiry Date	31/10/2012
Batch/Lot No	OSOWS0016
Bar Code	8 713184 095862
Storage conditions	Room temperature (15 - 25ºC)
Date received	07/07/2012
Supplier	MSD (Intervet (Pty) Ltd)
Packaging	5L plastic pour-on container
Warnings	Only for external use on cattle
Withdrawal period	
Meat	Do not slaughter animals for human consumption within 39 days of the last treatment
Milk	Do not use in animals producing milk for human consumption. Use only in well-ventilated areas or outdoors. The antiparasitic activity of Ivermectin and Triclabendazole may be impaired if the product is applied to areas of skin with mange scabs or lesions, or with dermatoses or adherent materials, e.g. caked mud or manure. Keep out of reach of children, uninformed persons and animals. Although this remedy has been tested under a large variety of conditions, failure thereof may ensue as a result of a wide range of reasons. If this is suspected, seek veterinary advice and notify the registration holder.

Table 3.2 Parasites Controlled by Sovereign pour-on

	Immature	Adult	Controls against re-infestation for (days)	
Brown stomach worm (*Ostertagia ostertagi*)	*	*	14	
Wireworm (*Haemonchus placei*)	*	*	14	
Cattle bankrupt worm (*Cooperia* spp.)	*	*	14	
Nodular worm (*Oesophagostomum radiatium*)	*	*	21	
Lungworm (*Dictyocaulus viviparus*)	*	*		
Eyeworm (*Thelazia* spp.)				
Fluke age	2 weeks	4weeks	6 weeks	Adult
Liver fluke (*F. hepatica)*	*	*	*	*

* Controls = ≥ 90 % effective

** Aids in control = 60 – 89 % effective

Table 3.3 Dosage: 1 mℓ/10 kg body mass

Body mass(Kg)	**Dose(ml)**	**1l treats (± heads)**
< 100	1 mℓ/10 kg	100 or more
101 – 150	15	66
151 – 200	20	50
201 – 250	25	40
251 – 300	30	33
301 – 350	35	28
351 – 400	40	25
401 – 450	45	22
451 – 500	50	20
501 – 550	55	18
551 – 600	60	16
601 – 650	65	15

Table 3.4 Veterinary Product 2 and description of Fluxacur on roundworms

Trade name	**Fluxacur NF®**
Active ingredients	Triclabendazole: 10% m/v and Abamectin: 0.2% m/v
Formulation	Oral remedy
Date of manufacture	Unknown on container
Expiry Date	09/2012
Batch/Lot No	010/10
Bar Code	7 13184 033772
Storage conditions	Room temperature (15 - 25ºC)
Date received	07/07/2012
Supplier	MSD (Intervet (Pty) Ltd
Packaging	5L plastic pour-on container
Warnings	1. Do not treat lactating cows, sheep and goats producing milk for human consumption, during lactation or within 28 days before the commencement of lactation - dairy cows may be treated during the dry period. 2. Do not slaughter cattle for human consumption within 28 days of last treatment. 3. Do not use in animals younger than 4 months. 4. Immunize all sheep and goats against pulpy kidney before dosing with Fluxacur NF. 5. Do not treat recovering, weak or stressed animals. 6. In case of poisoning immediately consult a doctor and make this information available to him/her. Keep out of reach of children, uninformed persons and animals. Although this remedy has been extensively tested under a large variety of conditions, failure thereof may ensue as a result of a wide range of reasons. If this is suspected, seek veterinary advice and notify the registration holder.

1. Cattle – Endoparasites
2. Gastrointestinal Roundworms

Table 3.5 Worm Species controlled by Fluxacur

Worm Species	Immature	Adult
Wireworm, including inhibited larval stages *(Haemonchus placei)*	*	*
Brown stomach worm *(Ostertagia ostertagi)*	*	*
Cattle bankrupt worm *(Cooperia spp.)*	*	*
Hookworm *(Bunostomum phlebotomum)*	*	*
Nodular worm *(Oesophagostomum radiatum)*	*	*
Eyeworm *(Thelazia rhodesii)*	*	*
False Bruising *(Parafilaria bovicola)*	-	**

Definition: *Controls (≥ 90% effective)

** Aids in control (60-89% effective)

Table 3.6 Description of Fluxacur on Liver fluke

Worm Species	**2 weeks**	**4 weeks**	**6 weeks**	**Adult**
Liver fluke (Fasciola hepatica)	*	*	*	*
	3 weeks	6 weeks	8 weeks	Adult
Giant liver fluke (Fasciola gigantica)	**	**	*	*

Definition: * Controls (≥ 90% effective)

** Aids in control (60-89% effective)

Table 3.7 Veterinary Product 3 and description of Flukazole C

Trade name	**Flukazole C®**
Active Ingredients	Triclabendazole:12 % m/v and Oxfendazole:3.54 % m/v
Formulation	Oral remedy
Date of Manufacture	03/02/2010
Expiry Date	02/2014
Batch/Lot No	U 1131
Bar Code	6 009634 796826
Storage Conditions	Room temperature (15 - 25ºC)
Date received	07/07/2012
Supplier	Virbac RSA (Pty) Ltd
Packaging	5L plastic container

Figure 3.6 Plain serum collection tubes, needles, long sleeve collection gloves for faecal sample collection and shoulders loaded with needles. (Delphin)

Figure 3.7 Tagging pliers, tool box, GMP (Intervet branded) animal ear tags permanently laser marked with traceability and tamper evident features, marked with a 2D bar code (with an identification mark hidden in the code) combined with e.g. a Q01, 164 readable number that can be linked to a central data base by logging on to www.gmptags.co.za (S.D.Lukamba)

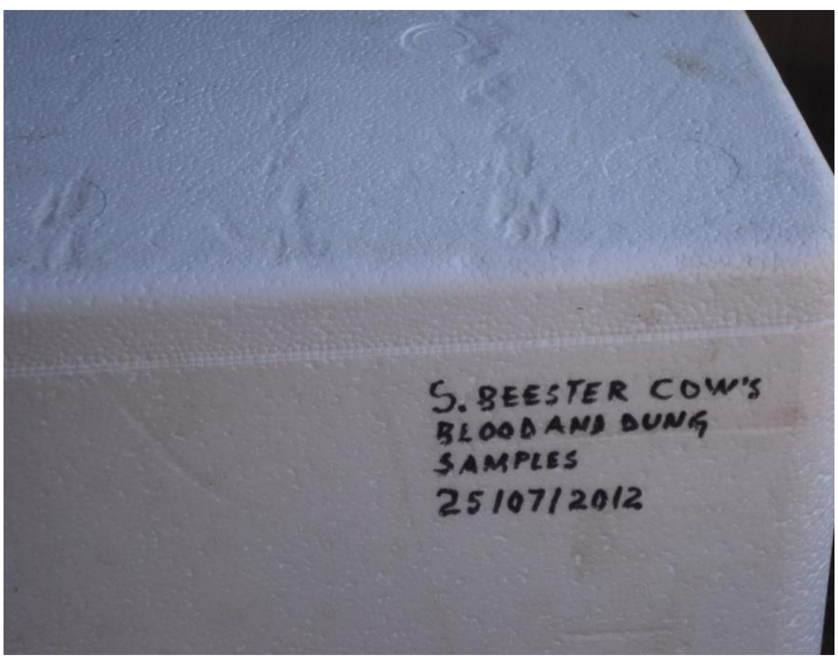


Figure 3.8 Schock-proof clean cooler box containing ice pack for sample Conservation (S.D.Lukamba)

Figure 3.9 Scale and computers for weight capturing. (S.D.Lukamba)

3.2.3 Experimental design

Cows and heifers in their farm groups were ranked from the heaviest to lightest. Animals were numbered 1 to 4 from the heaviest to the lightest down the list and then from 4 to 1 from the heaviest to the lightest and again 1 to 4 etc. until all the animals were numbered in their respective group selection as shown below: 1 2 3 4 4 3 2 1 1 2 3 4 4 3 2 1 1 2 3 4 4 3 2 1 etc. By a selection of 4 straws, each straw from shortest to tallest were drawn. Each straw was representing a product and one straw was for the control group. The straw drawn first was indicating which product is applied to the heaviest (weight group 1) animal, the next straw drawn was indicating that the product were allocated to the next (weight group 2) animal, the third straw drawn was indicating the product to be given to this next (weight group 3) animal, the fourth straw drawn was indicating the product to be given to this next (weight group 4) animal. This was repeated as such until the list with the animals from heaviest to lightest was completed. Weight randomisation was done and all the like numbered animals were identified to be treated with the same product. All the 1's would receive the same product, all the 2's would receive the same product, all the 3's would receive the same product, all the 4's would be left untreated as the control group animals

Management took place under typical farm conditions for each of the farm trials. Both treatment and control groups were managed simultaneously in the same grazing paddock facilities to eliminate the possible effects of different paddocks and feeding management differences. Pregnant cows were separated from other experimental animals to minimise the effect of pregnancy on growth after treatment. The heifers were treated on 24/07/2012 and the cows on 25/07/2012.The allocation of experimental animals is found in addendum (Table 4)

3.2.4 Experimental procedures: Activities

The first operation was the identification of all trial animals using ear tagging pliers and GMP animal ear tags (ID Tags), which were numbered, permanently laser marked available in the set of ten (10). Beside they have traceability and tamper evident features, marked with a 2D bar code (with an identification mark hidden in the code) combined with e.g. a Q01, 164 readable number that can be linked to a central data base by logging on to www.gmptags.co.za.

The very same number Q01, 164 can be connected to the farmers management software and serve as the animal's identification number. Furthermore, this identification system is helping solve problems over ownership disputes, investigation by an authority of law, easy and good management on animals (ex: treatment, dehorning, blood collection, etc) on a daily basis. The laser mark containing the 2D hidden readable bar code can be authenticated against the central database provided that the owner has successfully registered the animals. The animal's full management and medical history could be forwarded to the next owner via the system.

After being tagged, animals were weighed for the first time on empty stomach in the morning before grazing and all details recorded. Cow weight varied from 322-666kg and weaned heifers from 261-394kg. Cow's pregnancy statuses were determined; faecal samples were collected from a heifer replacement and a breeding cow herds on May 2012 for laboratory analysis using long sleeve collection faecal gloves labeled sequentially from 1 to 116(Heifers) and 1x to 134x (cows) in relation of the ear tag‘s number of the animal. The x acting as a suffix to differentiate the heifers from the cow sample just in case that the sample gets mixed in the transport and the laboratory exercise. These numbers were also recorded by pen on the herd report form next to each animal's unique, tamper evident non-re-usable ear tag number e.g. Q01,37152 with test tube and faecal samples numbered 23. Some heifers and cows had an F and a circle next to its number to indicate the absence of faeces in the rectum due to the fact that animals were in the handling race, no need to wait very long for faecal material from each animal. Non pregnant cows without samples have been removed from trial animals. Samples were transported in cooler boxes containing ice-packs to the MSD, Malelane Research Unit laboratory in Mpumalanga.

The laboratory complies with SANAS and general laboratory practice (GLP) evaluations done every 2 years. Tests that were performed were the enzyme-linked immunosorbent assay (ELISA) anti body determination for the presence of liver fluke during a current or previous challenges and a coprological analysis for the presence of liver fluke eggs (egg count: sedimentation) Liver fluke eggs found in the dung sampling would indicate a current infestation with adult egg producing liver flukes.The results showed that heifers were negative both serologically and coprologically for liver fluke, but cows showed some positive results in serology meaning that they were exposed and infected at some stage in their life. In May both the Heifers and cow herds were moved into seperate pastures that were suspected to be infested with liver flukes in order to allow them to be exposed to liver flukes and to become infected.

Figure 3.10 Animal identification by ear tagging (S.D Lukamba)

a) Cow pregnancy status determination

After the identification processes by means of individual identification numbers, the breed, and the pregnancy status determination were recorded for all the cows in April. The breeding days were also determined for the cow herd; it indicates the time period the cows and bulls are running together in the same herd. The start and end breeding dates were entered into the GMP management software system. This means that the cows were mated for 4 months. The pregnancy record % is automatically calculated by the system when the data is entered.

b) Weighing of animals (Empty body mass in the morning)

Figure 3.11 A calibrated, Taltec scale used to record the individual animal weights

Figure 3.12 Taltec scale connected to a board

The cattle were weighed once a month, early in the morning before grazing, for the duration of the trial from March when the trial started, until end of October for heifers and end of August for cows because it was the calving season. There was a big gap between the post-partum and the pre-partum which could affect the results. Cattle were fed *ad libitum* with a standard supplementary winter supplement, the feed were equally accessible to all the trial animals.

c) Cattle ranking in each of the two female groups

Cows and heifers were ranked according to their weights within farm groups. Animals were numbered 1 to 4 from the heaviest to the lightest down the list and then from 4 to 1 from the heaviest to the lightest and again 1 to 4 etc. until all the animals were numbered in their respective group selection as shown under experimental design. All the like numbered animals were allocated to the same treatment or untreated control group. Then the marked straws were drawn at random and allocated to a specific group. This allocation by lucky draw also determined what the group received e.g. a product or left as the untreated control group.

d) Straw drawing

Straws were marked as 1,2,3,4. Each of these represented either the Sovereign pour-on or the Fluxacur NF or the Flucazole C or the untreated CONTROL group.

1. First straw: Start with first bovine group with the product represented by the straw.
2. Second straw: Second bovine bovine group with the product represented by the straw.
3. Third straw: Third bovine bovine group with the product represented by the straw.
4. Fourth straw: Fourth bovine bovine group with the product represented by the straw.
5. Hereafter the heifers are arranged by numerical ear tag arrangement and not by the individual weights or ranking.

   This allows for easy identification of the animals in the crush (race way) when deciding from the control list which remedy was to be administered or if the animal was to be left untreated as a control animal.

e) Faecal collection

Figure 3.13 Faecal sample collection for Fasciola eggs count

A sufficient amount of faecal material was collected rectally by means of clean long sleeve dung collection gloves. After collection of faecal samples the glove was turned inside out to serve as a container. Faecal samples were collected from a heifer replacement and a breeding cow herds on May 2012 for laboratory analysis purposes. The long sleeve collection faecal gloves were used. Faceal samples were labelled sequentially from 1 to 116(Heifers) and 1x to 134x (cows) in relation of the ear tag's number of the animal. The x acting as a suffix to differentiate the heifers from the cow sample just in case that the sample gets mixed in different manipulations during transportation process and the laboratory exercise.

These numbers were also recorded using a pen on the herd report form next to each animal's unique, tamper evident non-re-usable ear tag number e.g. Q01,37321 with test tube and faecal samples numbered 23. Some heifers and cows had an F and a circle next to its number indicating the absence of faeces in the rectum due to the fact that animals were in the handling race, no need to wait very long for faecal material from each animal. Non pregnant cows without samples have been removed from trial animals. After having been carefully tied and correctly labelled with all the necessary data the samples were transported in cooler boxes containing ice-packs to the MSD quarantine fridge for processing, and then sent to Malelane Research Unit laboratory in Mpumalanga for requested analysis.

f) Processing and packaging

Figure 3.14 Processing of faecal sample for overnight delivery

The samples were packaged in a shock-proof cleaned cooler-box. The photograph above shows the cutting off of plastic material restricting the cooling of faecal samples. Because immediate examination was not possible, and some eggs embryonate very quickly, the development continues even outside the host, the cooler box containing faecal sample was then stored at the MSD Animal Health quarantine fridge, hermetically closed and sent to Malelane research Unit (MRU) by overnight courier.

g) Dispatching

Samples were dispatched by overnight post office to post office delivery, most of time sent in at the beginning of the week to avoid week end and holidays. The overnight dispatching and the storage in the fridge intended to minimize favorable conditions for the egg hatching such as heat because summer days can cause the faeces to ferment. Thus, analysis and the results could be corrupted or compromised without the above precautions.

h) Coprological method

Laboratory coprological examination of the bovine samples employed the modified McMaster technique (MAFF, 1986) with a sensitivity of 10 eggs per gram (e.p.g) of faeces.

i) Blood collection

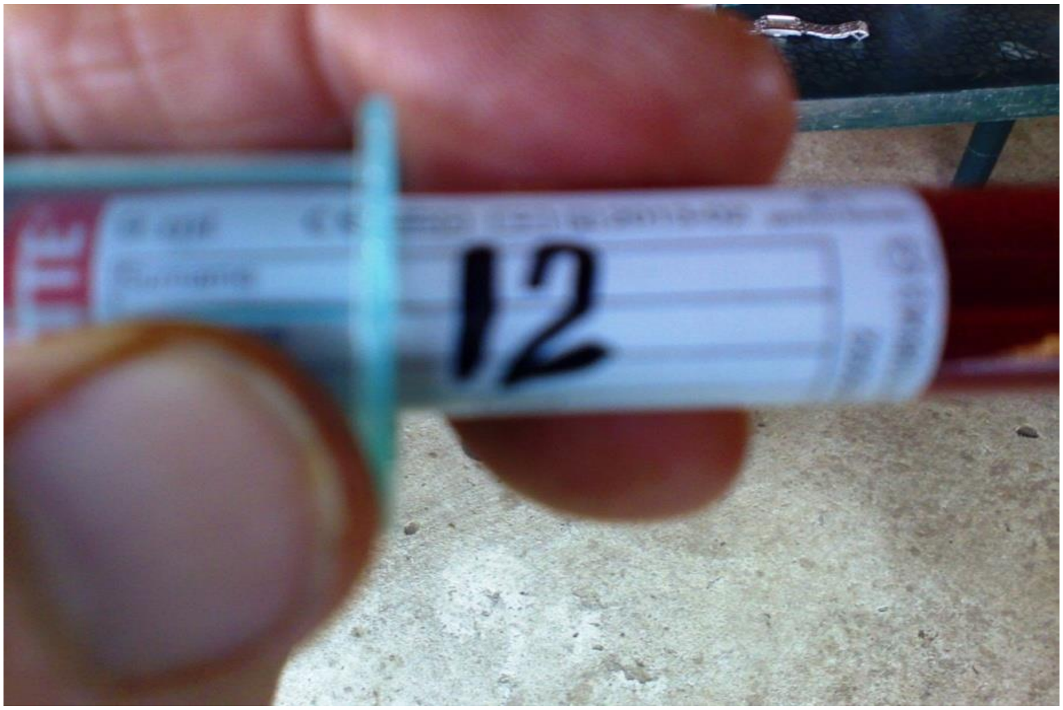


Figure 3.15 Collected Blood Sample

Figure 3.16 Blood Samples

Blood samples were collected from each cow and heifer considered for the trial to determine whether animals were infected with liver fluke or not using marked collection tube vacuttes without anticoagulant for serological test. The samples were collected from a heifer and a breeding cow herds on Wednesday 2nd May2012 (heifers) and Thursday, 3rd May 2012 for the cows. The collected blood samples were sequentially numbered from 1-116 for cows and 1x-134x for heifers. The x acts as a suffix to differentiate the heifer versus cow samples in case the samples accidentally get mixed in the transport and laboratory manipulations. They were kept at MSD Animal health quarantine fridge before the expedition to the laboratory. On the accompanying herd report forms, the number of the test tubes and faecal samples were exactly the same for each animal. Blood sample were sent together with faecal samples to the laboratory. These numbers were also recorded by pen on the herd report forms next to the animal unique tamper evident non-reusable ear tag number.eg QO1, 37325 with test tube and faecal sample numbered 29

j) Body Condition Scoring

The research protocol required the body condition scoring which is a process that needs time especially for big size trial herds like ours, a good sight for an objective appreciation and judgment. The body condition scoring was done the first day of the trial on cows only, it could not be done continuously till end of the trial on the two herds, it was practically impossible. This deviation from the research protocol was due to challenges such as time, number of animals (herd size), slowing of the process and even the subjectivity on scoring. Photos were taken at the monthly weighing sessions to reflect each herd's visual appearnce. The exact weights measured by the calibrated electronic scale were preferred to increase the level of accuracy.

Figure 3.17 The Bonsmara cows showing good breed uniformity and body condition scoring as evident from this picture.

Body condition of heifers on the 9th of March 2012

Figure 3.18 Heifers walking towards a water source

Figure 3.19 Cows in good condition on a lower quality pasture

Body condition of cows on the 29th March 2012

Figure 3.20 Cows and their calves ahead of identification process

Figure 3.21 Calves in a paddock, separated from their dams

Body condition of heifers on the 2nd and 3rd of May 2012

Figure 3.22 Heifers after weighting

Body condition of heifers on the 24th & 25th of July 2012

Figure 3.23 Heifers showing loss of body condition due to a month delay in receiving winter Lick.

Figure 3.24 Six weeks post exposure of cows to potentially nematode and trematode infested pasture. Animals are showing poor coat and skin condition due to winter effect, shortage and delay in winter lick supply.

Figure 3.25 Six weeks post exposure of heifers to potentially nematode and trematode infested pasture. Animals are showing poor coat and skin condition due to winter effect, shortage and delay in winter lick supply.

Four to six weeks post exposure the farmer was concerned about the animals loss of body condition as shown in fig.33&34although winter lick was supplied already in June. It was decided to do a randomized faecal and blood sample collection from 20 animals, on every 5th animal from the cow herd as well as from the heifer herd for laboratory analysis and to evaluate their weights for any signs of severe weight deviations which may have required possible treatment. The selected cows and heifers were tested again as it was still too early to determine fluke egg counts at the four week stage, but the tests were intended more to re-assure the farmer that it was not necessary to deworm the animals at that stage of the trial.

Once the heifers and cows were weighed, the farmer found the weight measurements to be of an acceptable level. The one month delay in winter lick supply had an adverse effect on the animals BCS. In this manner animals required also an extra one month on lick to recover the weight loss due to the delay. Therefore, the treatment did not need to be initiated in any of the animals, which could have resulted in the trial being terminated as per the ethics and trial standards set out.

The weight loss incurred was due to the decrease in nutritional value of the pastures in the beginning of winter period (winter effect) and a month delay in winter lick supply (May-June) due to strike actions occurring at Voermol feeds the animal feed company's manufacturing plant.

Figure 3.26 Cows in good condition due to their pregnancy status

Body condition of heifers on the 30th & 31st of October 2012

Figure 3.27 Heifers at the end of trial, good condition (summer effect) with a bull among them

Figure3.28 Calf identification during calving season, tagged with the same tag numbering system as the dams

Figure 3.29 Cows on a pasture in the beginning of summer season. Animals are showing an improved body condition (summer effect)

3.2.5 Results of serological tests from cow and heifer herds (Elisa)

The results of ELISA test for liver fluke antibody determination is found in table appendix (table 6). According to the results, out of 246 animal samples tested, 81 were positive (32%), 145 tested negative (68%). Out of 114 samples from heifers, 91 or 79% were positive, and 10 samples of the

132 from the cows tested positive (7,5%). The results indicate that heifers had an exposure previously or recently to liver fluke at some stage. Only a few cows were liver fluke infected.

3.2.6 Animal treatment with the prescribed products

All animals belonging to group 1 were treated with Sovereign pour on, animals from group 2 were treated with Flukazole C, Fluxacur was administered to animals from group 3 and group 4 animals was a control group. Below are lists showing cows and heifers treatments. The treatments were done respectively on the 24th and 25th July 2012. The calculated treatment dosage or product amount was rounded to the highest full number. In the administration or application process there is always a waste of a certain amount of product due to mistakes, lack of restraining and sometimes difficult animals. Low dosage will not yield better results. The rounding of the calculated treatment dosage to the highest full number intended not to overdose but to compensate any loss or waste that occurred during administration process. This was also done to compensate for the lack of dosing gun accuracy with administration of decimal quantities.

For Example:

23.4 ml – rounded off to 25 ml

47.1 ml - rounded off to 50 ml etc.

3.2.3.1 Treatment application method

Figure 3.30 Sovereign pour-on container suspended higher and ready for application on animals

Figure 3.31 Sovereign pour on application method.

The way of applying Sovereign pour on can influence the efficacy of the product. Sovereign should be applied in a short, thick area in a band along the top line from the middle of the back to the tail head to ensure minimum evaporation and maximum absorption. The product contains alcohol which evaporates easily. Compliance to the manufacturer mode of use is needed for reducing the evaporation and increasing the absorption of the product.

Figure 3.32 Oral drench application

Figure 3.33 The two oral drenches in containers

Figure 3.34 Two containers filled with Fluxacur (F) and Flukazole C (FC) connected to pipes and applicators ready for use

Containers are suspended at a high level for a better flow of product and easy application. The containers are filled regularly if there is any substantial decrease of drug quantity.

Figure 3.35 Restraining of animals and oral dosing

Any animal manipulations require a restraining for safety and security purposes. Besides, the restraining helps gain time. After being restrained the applicator is introduced in the animal's mouth for a better and easy administration of the product. The lack of restraining will lead to more waste of product, and risk of injuries to animals and man. At the end of the trial all control animals were treated as well.

3.2.7 Observations, analytical procedures and contingencies

The following observations were recorded by farm management:

1. Identification and sorting date
2. Initial weighing early in the morning on empty stomach before grazing
3. Monthly live mass early in the morning before grazing
4. General comments regarding any obvious visual appearance (body condition score) of the animals
5. Blood sample in plain red stopper tube for ELISA test from each animal for liver fluke infestation
6. Faecal sample from each animal for Fasciola egg count
7. Breed / type
8. Pregnancy status of each cow
9. Origin, from the group on the farm
10. Any disease insult and treatment thereof
11. Live mass at end of trial period
12. Body condition score at the onset and end of the trial that was discarded according to the management circumstances

All deviations from this protocol were reported to farm management who reported to investigators. Such deviations had been recorded and taken into account during statistical analysis.

The body condition scoring was not performed as required by the research protocol due to different challenges on the fields and especially the farmer availability, the subjectivity and the slowing of the process due to lack of time and the big size of the trial herds.

3.2.8 Statistical analysis

The effect of treatment on the growth: the start weight, weight difference March-August and July – August for cows; the start weight, weight difference March-October, July-August for heifers and the growth of cows and heifers (treatment x growth) were analysed using general linear model (GLM) of SAS (2013) with SStype (3) method. The relationship between type of treatments and other variables were analyzed using Post Hoc test with Bonferroni method, multiple comparisons of descriptive statistics at a significance level of p 5%($P<0.05$). Test of normality such as Kolmogorov-Smirnov and Shapiro-Wilk were performed for the evaluation of the effect of treatment on heifers' growth. Chi-square test and standard deviations were calculated to assess the significance of the effect of treatment on various parameters with level of significance considered to be $P<0.05$. Correlations were done using Proc correlation procedures. Statistics were based on cases with no missing data for any variable listed.

CHAPTER 4

RESULTS

4.1. Effects of flukicidal treatments on mass gain

4.1.1. Effects of flukicidal treatments on mass gain of cows

Summary statistics of live weight gain of Bonsmara cows treated with three commercial flukicidal products are presented in Table 1.

The weights of cows were stable from March to April of 2012, but a continual and progressive decrease in weights was noted from April until August of the experimental period. Statistical analysis revealed that the average weights of cows in different flukicidal treatment groups did not differ significantly. Nevertheless, the average weight of cows in the treatment group FC was lower in March, April, May and June but a slight increase was observed in July and August. The average weight of cows was numerically lower in August compared with weights recorded in March, April, May and June. The average weight of cows in August was higher compared with the weights in July. The standard deviations of weights of cows were not stable throughout the study. Lower standard deviations of weights were observed in the treatment group F for March, April, May, June and July. Still the standard deviation of weights was high in August which is the cold and windy part of winter in this region. The lowest standard deviation was observed in the treatment group FC in August (±44.92Kg). The standard deviations remained lower in the treatment groups F, FC, and S, but group C had a high standard deviation, although the difference was not significant. (P value was greater than 0.05)

Table 4.1 Summary statistics of live weight of Bonsmara cows during the experimental period from March to August 2012 (Pooled results)

TREATMENTS		LIVE WEIGHT Means ±SD (kg)
March	**C**	471.70±63.54
	F	467.25±51.13
	FC	458.48±53.66
	S	470.74±55.42
April	**C**	471.70±63.54
	F	467.25±51.1
	FC	458.48±53.66
	S	470.74±55.42
May	**C**	450.25±60.13
	F	448.77±46.78
	FC	433.88±52.81
	S	450.55±54.95
June	**C**	445.59±63.97
	F	440.25±49.00
	FC	437.37±55.39
	S	440.74±53.44
July	**C**	433.25±57.45
	F	431.70±51.51
	FC	431.96±52.34
	S	431.96±52.92
August	**C**	439.03±54.29
	F	429.74±48.25
	FC	439.03±44.92
	S	431.81±45.30

C= Control; F= Fluxacur; FC= Flukazole C; S= Sovereign;

4.1.2 Effects of flukicidal treatments on mass gain of heifers

Table 4.2 indicates averages of pooled results (weights recorded in different months pooled), the standard deviation and the level of significance of the effects of treatments on live weights of heifers. The average weights were stable from March to April 2012. A slight increase was observed in May. From May to September there was a decrease in live weight of heifers. The standard deviation of weights of heifers was uniform from March to June. Nevertheless, it fluctuated from July to October. The highest standard deviation for weight of heifers during the trial was recorded in August in treatment group F (±36.77Kg), although the P-values were high (P>0.05) indicating no significant differences in weights from the beginning until the end of the experimental period. A significant difference was noted between March (P=0.031) and April (P=0.031) for the treatment group FC. In June the live weights of heifers in treatment group C differed (P=0.022) from those in treatment group F (P=0.047); in August, the weights of heifers in treatment group C (P=0.035) differed from those in treatment group F (P=0.009) and the treatment group FC (P=0.003). In September, the weights of heifers differed between treatment group C (P=0.11), treatment group F (P=0.91), treatment group FC (P=0.004) and treatment group S (P=0.81). In October the weights of heifers differed significantly between treatment group C (P=0.030) and treatment group FC (P=0.009).

Table 4.2 Summary statistics of live mass of Beefmaster heifers during the experimental period from March to October 2012 (Pooled results)

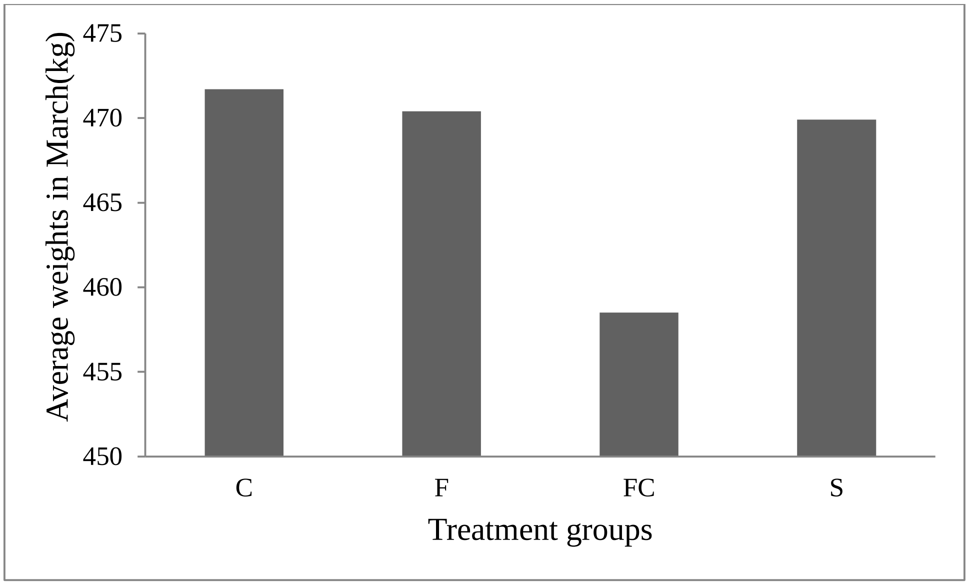
TREATMENTS		LIVE WEIGHT Means ±SD(kg)	P
March	**C**	302.74 ±26.35[a]	.075
	F	304.26±24.73[a]	.526
	FC	303.06±28.39[b]	.031
	S	304.25±22.11[a]	.671
April	**C**	302.74±26.35[a]	.075
	F	304.26±24.73[a]	.526
	FC	303.06±28.39[b]	.031
	S	304.25±22.11[a]	.671
May	**C**	302.29±24.89[a]	.127
	F	306.70±23.99[a]	.592
	FC	306.45±29.05[a]	.075
	S	304.11±22.44[a]	.904
June	**C**	286.03±26.05[b]	.022
	F	289.46±27.71[b]	.047
	FC	282.93±26.73[a]	.296
	S	291.55±21.34[a]	.999
July	**C**	282.32±27.31[a]	.287
	F	283.36±24.00[a]	.865
	FC	279.67±21.55[a]	.816
	S	282.81±22.13[a]	.769
August	**C**	286.87±28.11[b]	.035
	F	290.33±36.77[a]	.009
	FC	287.58±28.45[b]	.003
	S	286.77±25.21[a]	.435
Sept.	**C**	278.00±25.28[a]	.117
	F	281.23±23.57[a]	.918
	FC	280.87±25.02[b]	.004
	S	282.33±22.95[a]	.813
Oct.	**C**	310.70±27.15[b]	.030
	F	308.86±23.60[a]	.231
	FC	311.06±27.35[a]	.009
	S	316.37±29.81[a]	.151

C= Control; F= Fluxacur; FC= Flukazole C; S= Sovereign; p=p value

4.2 Data on live weight changes, growth from March to July and the effects of Flukicidal treatment on live weights and growth of cattle from August to October

4.2.1 Data on the live weight changes of cows from March to July

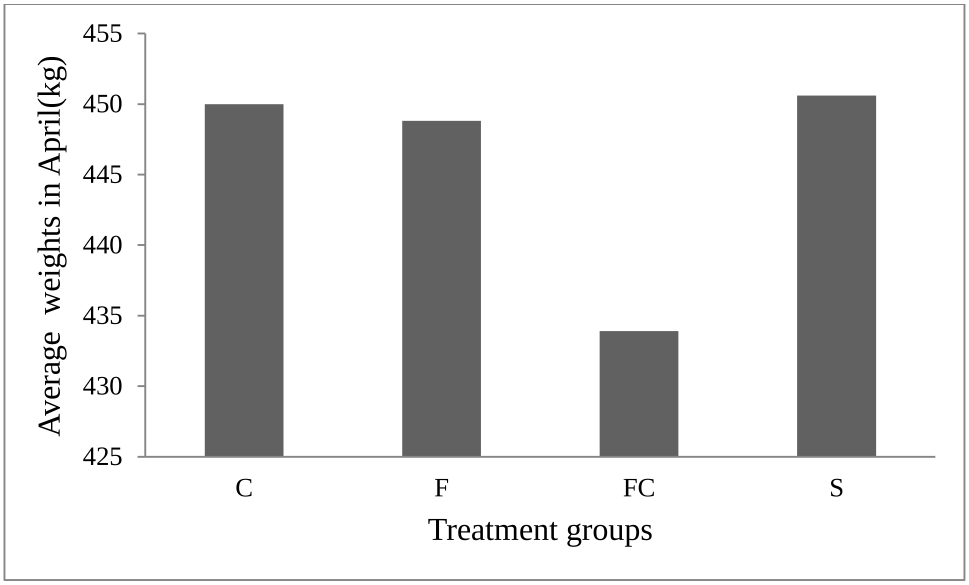
The data on live weights of cows are presented in Graphs 4.1 to 4.5, for each month of the trial from March to July and the effects of Flukicidal treatment on the live weights of cows in August are presented in Graph 4.6 and the combined data on live weights and the effects of Flukicidal treatments on live weights of cows are summarized in Graph 4.7



Graph 4.1 The live weight changes of cows in March.

C= Control; F= Fluxacur; FC= Flukazole; S= Sovereign

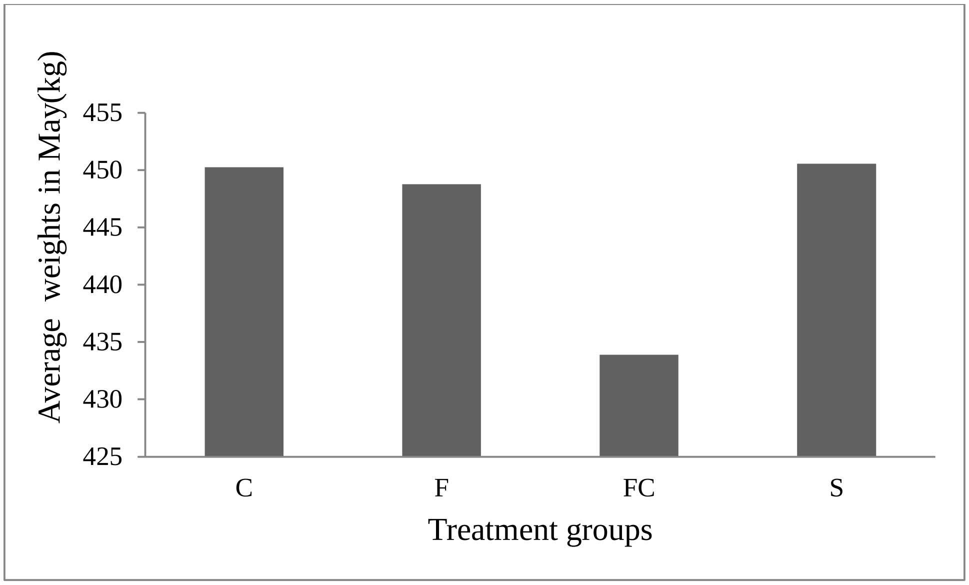
In March cows from all treatment groups were in good condition with a starting weight of 450kg an above as shown in the Graph 4.1. Cows from the control and Flukazole treatment groups were closer to 500kg; Cows assigned to treatment groups Flukazole C and Sovereign pour-on were weighing above 450kg.



Graph 4.2 The live weight changes of cows in April

C= Control; F= Fluxacur; FC= Flukazole; S= Sovereign

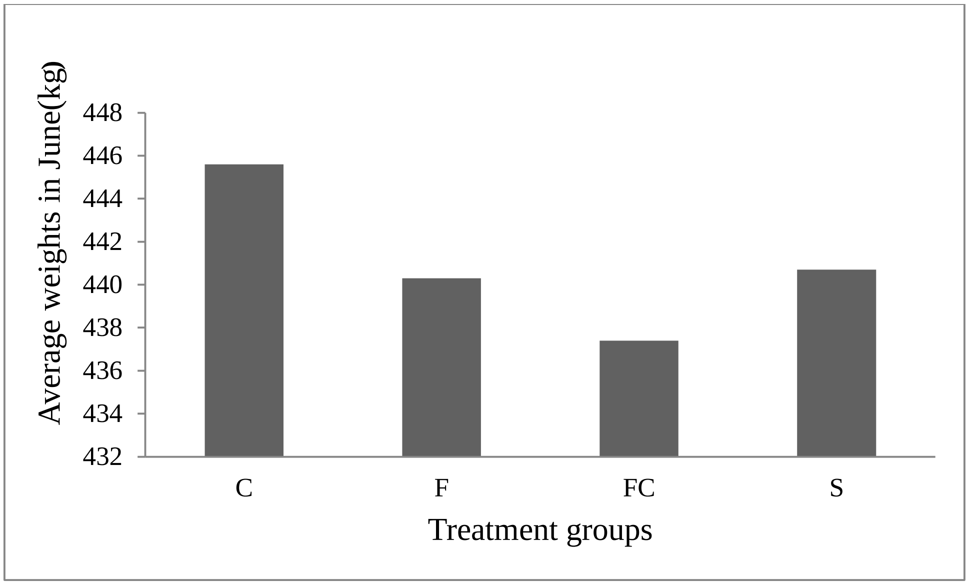
Graph 4.2 shows the same tendency as the previous month (March), cows maintained their live weights changes. No change in live weights has been observed from March to April.



Graph 4.3 The live weight changes of cows in May

C= Control; F= Fluxacur; FC= Flukazole; S= Sovereign

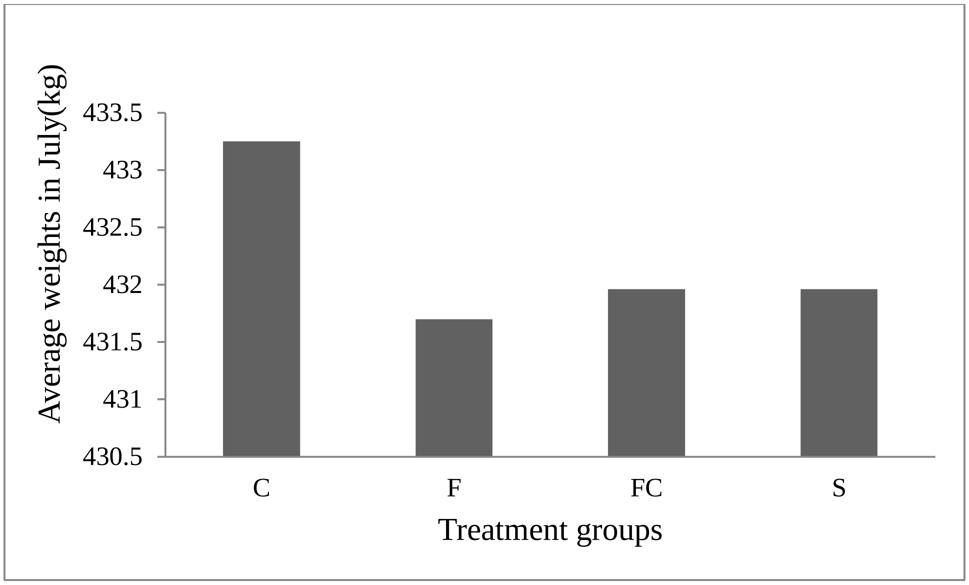
Graph 4.3 shows the weight fluctuation in different treatment groups. Cows from all different treatment groups showed a severe decrease of weights compare to those from April. Respective weight losses were: -21.45kg for cows from control group, -18.48kg for cows from treatment group F, -25.60kg for cows from treatment group FC, and -0.14kg for those from treatment group S. The highest weight loss was noted in cows from treatment group FC and the lowest weight loss was observed in cows from treatment group S which seemed to maintain their weight.



Graph 4.4 The live weight changes of cows in June

C= Control; F= Fluxacur; FC= Flukazole; S= Sovereign

Graph 4.4 indicates the status of weights of cows in June, two months after running short of supplements. The weights decreased respectively in treatment groups C (-4.66kg), F (-8.52kg), and S (-9.81kg). A slight increase was observed in treatment group FC (+3.49kg).

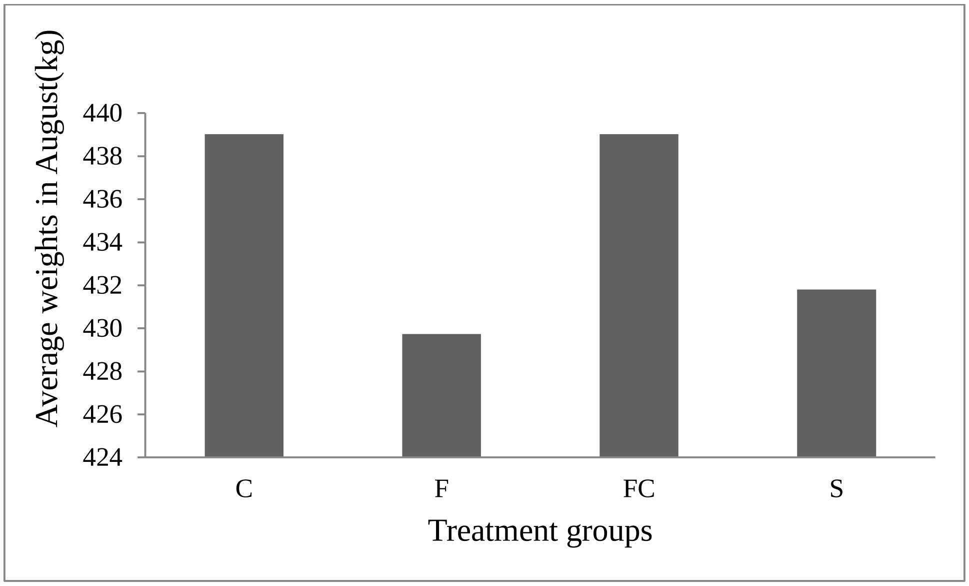


Graph 4.5 The live weight changes of cows in July

C= Control; F= Fluxacur; FC= Flukazole; S= Sovereign

Graph 4.5 indicates the decrease in weights of cows in July. All treatment groups displayed a weight loss. Cows from group C showed a weight loss of -12.34kg, while those from treatment group F had lost -8.55kg, cows belonging to the treatment group FC had lost -5.41kg and those from treatment group S had shown a loss of -8.78kg. However, the results show that cows from treatment groups F and S lost almost the same weight. Cows from the treatment group C showed the highest weight loss (-12.34kg).

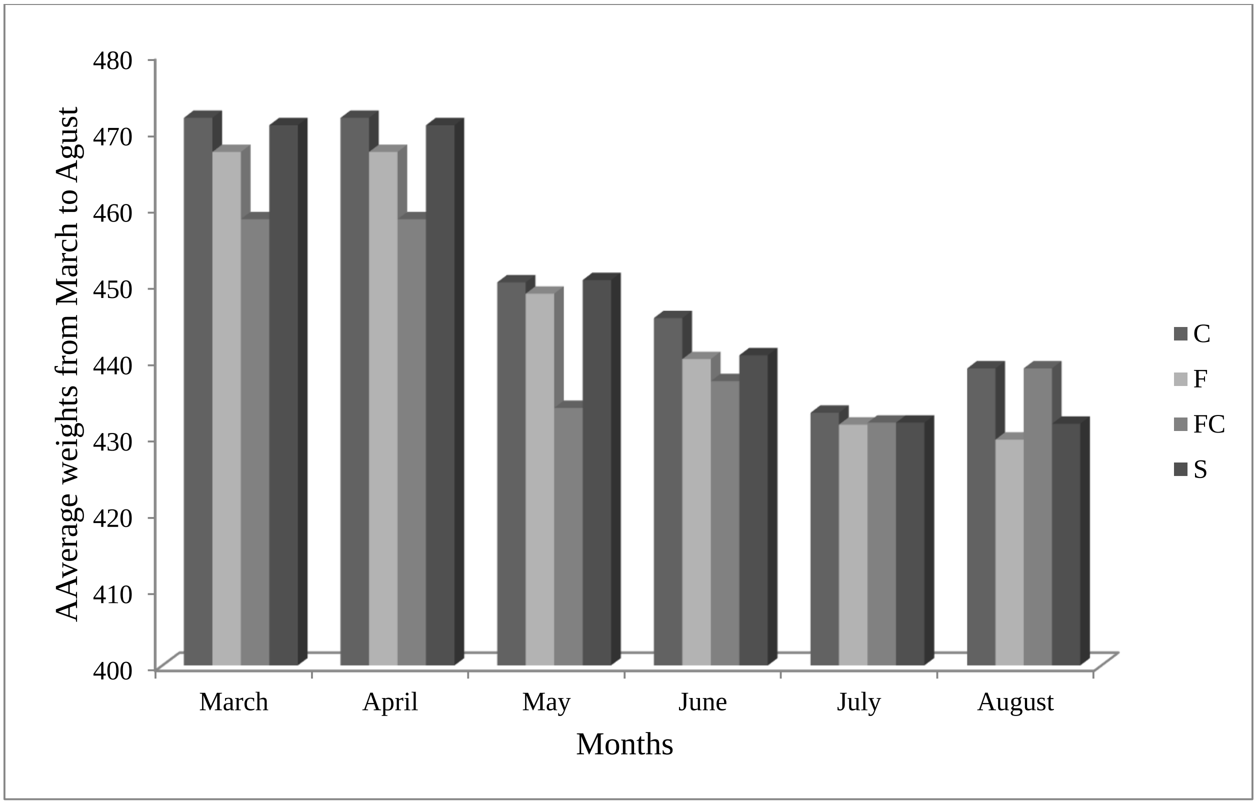
4.2.2 The effects of Flukicidal treatment on the live weight changes of cows in August.



Graph 4.6 Effects of flukicidal treatments on the live weight changes of cows in August

C= Control; F= Fluxacur; FC= Flukazole; S= Sovereign

A weight increase was noted in August compared with July, especially cows from the treatment groups C (5.78kg) and FC (8.07kg). Cows from treatment groups F and S showed respectively a decrease of 1.96kg and -0.15kg.

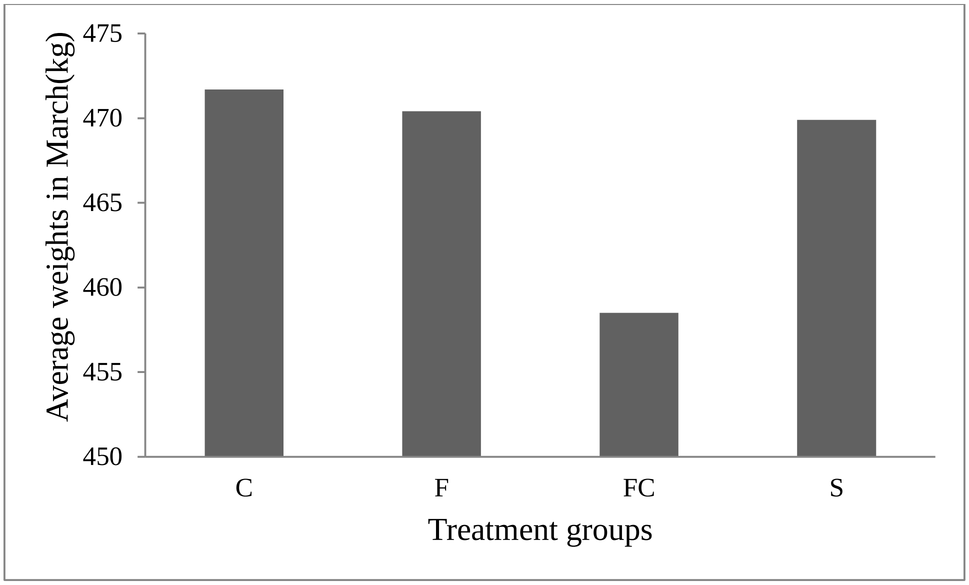


Graph 4.7 Combined data of live weights from March to July and the effects of flukicidal treatments on the live weight changes of cows in August

C= Control; F= Fluxacur; FC= Flukazole; S= Sovereign

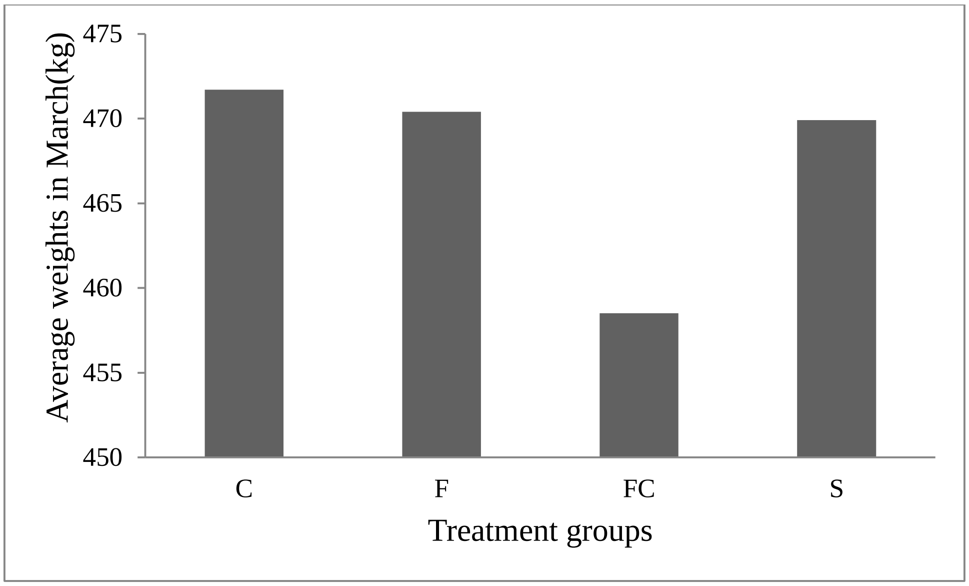
4.2.3 Data on the growth of cows from March to July

The data on the growth of cows over time are presented in the graphs 4.8 to 4.12, for each treatment group from March to July and the effect of the Flukicidal treatment on the growth of cows in August are presented in Graph 4.13 and the combined data of the growth and effects of Flukicidal treatments on the growth of cows are found in Graph 4.14.



Graph 4.8 The growth of cows in March

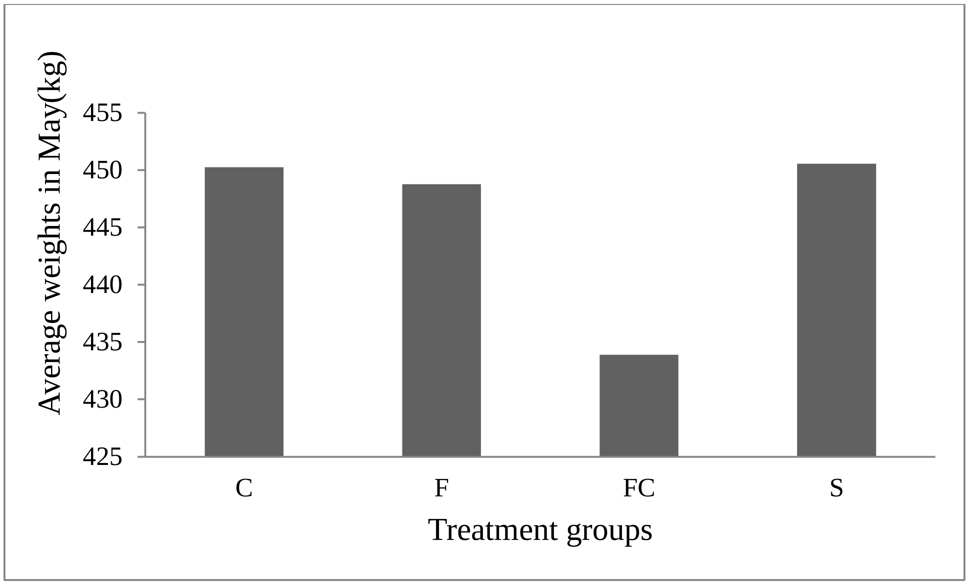
C= Control; F= Fluxacur; FC= Flukazole; S= Sovereign



Graph 4.9 The growth of cows in April

C= Control; F= Fluxacur; FC= Flukazole; S= Sovereign.

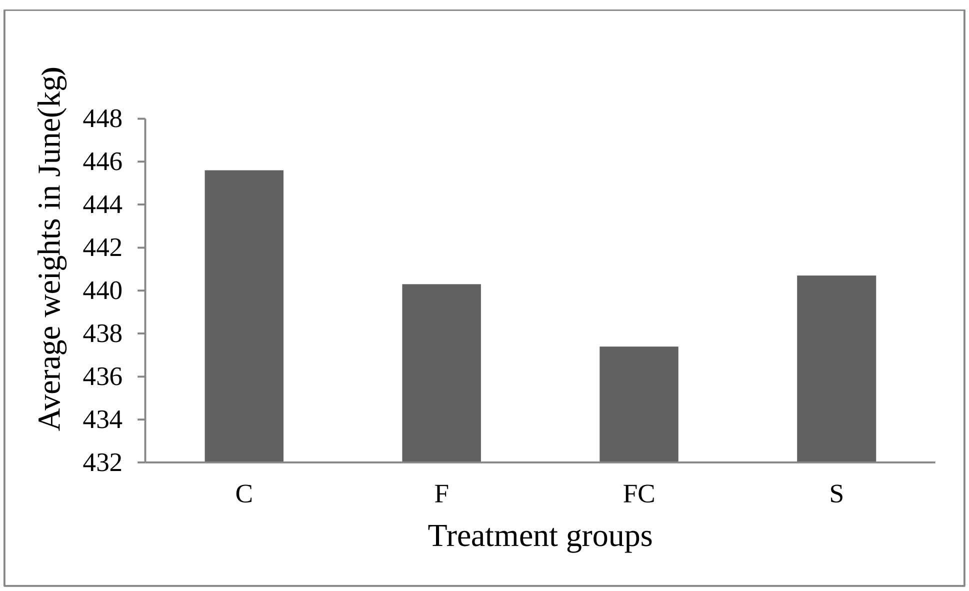
In April cattle tend to maintain their weights.



Graph 4.10 The growth of cows in May

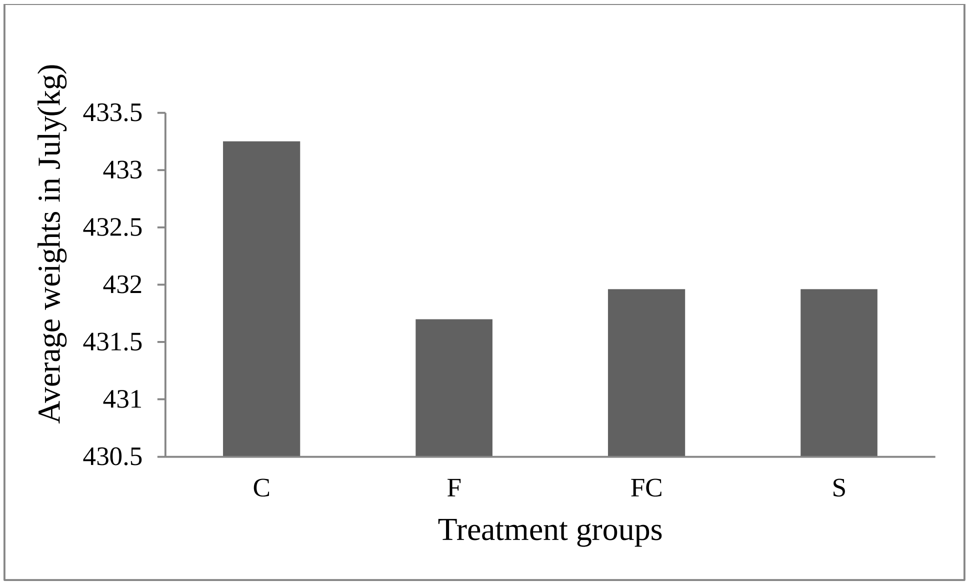
C= Control; F= Fluxacur; FC= Flukazole; S= Sovereign

The trend shows a very deep growth depression in cows from FC treatment group, but those from Sovereign pour-on treatment group had the most beneficial effect on growth compared to cows from the three other treatment groups.



Graph 4.11 The growth of cows in June

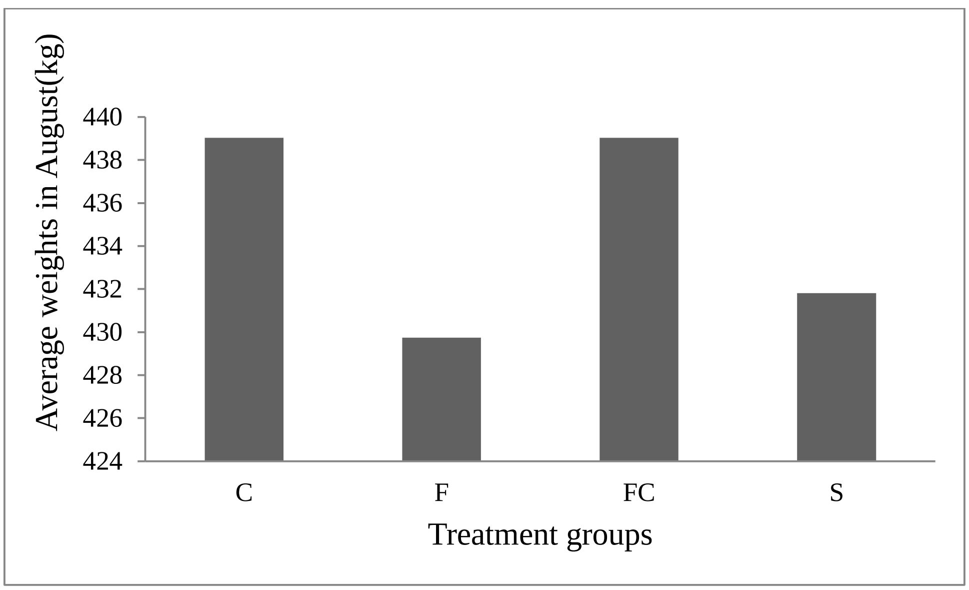
C= Control; F= Fluxacur; FC= Flukazole; S= Sovereign



Graph 4.12 The growth of cows in July

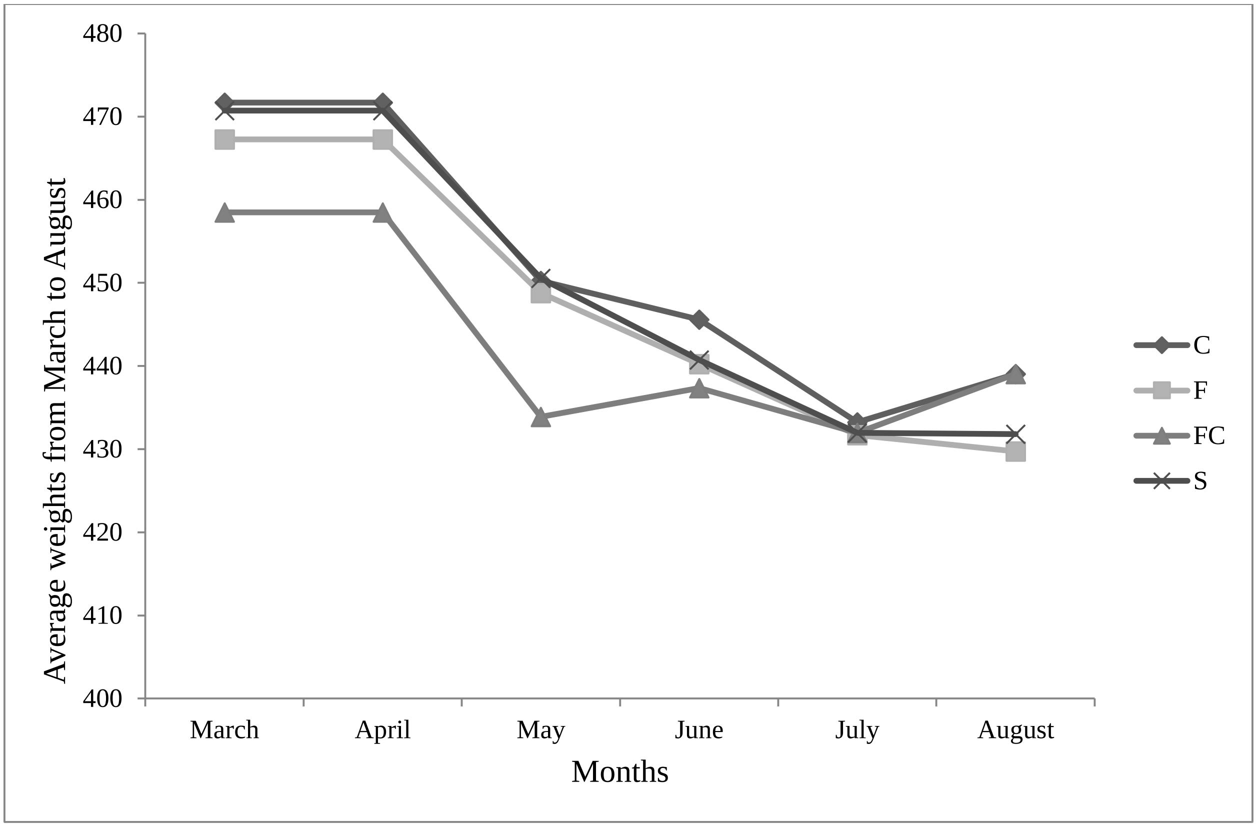
C= Control; F= Fluxacur; FC= Flukazole; S= Sovereign

4.2.4 The effects of flukicidal treatments on the growth of cows in August



Graph 4.13 Effects of flukicidal treatments on the growth of cows in August

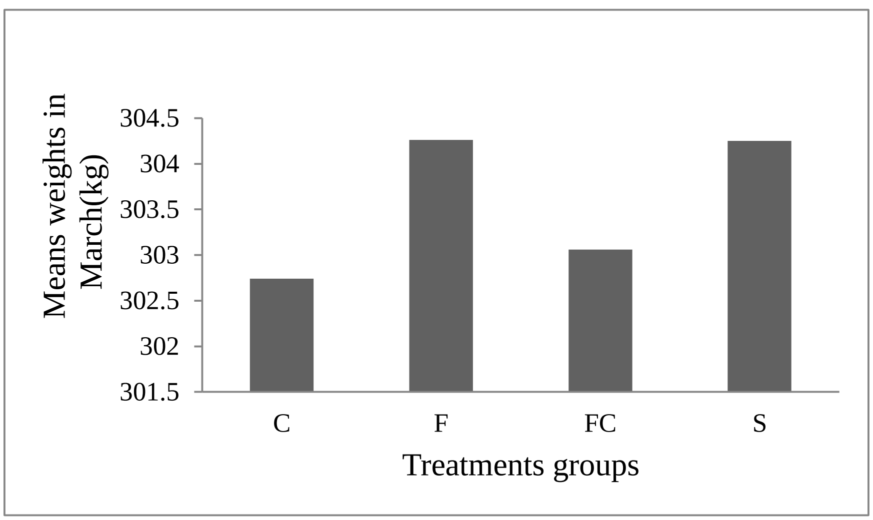
C= Control; F= Fluxacur; FC= Flukazole; S= Sovereign



Graph 4.14 The combined data of the growth of cows from March to July and the effects of Flukicidal treatment on the growth of cows in August

C= Control; F= Fluxacur; FC= Flukazole; S= Sovereign

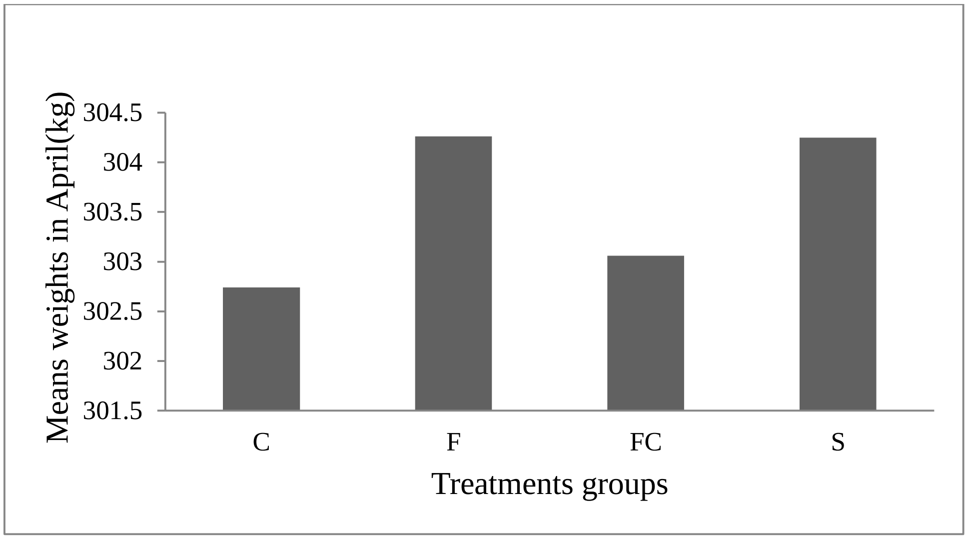
4.3 Data on the live weight changes of heifers from March to July



Graph 4.15 The live weights of heifers in March

C= Control; F= Fluxacur; FC= Flukazole; S= Sovereign

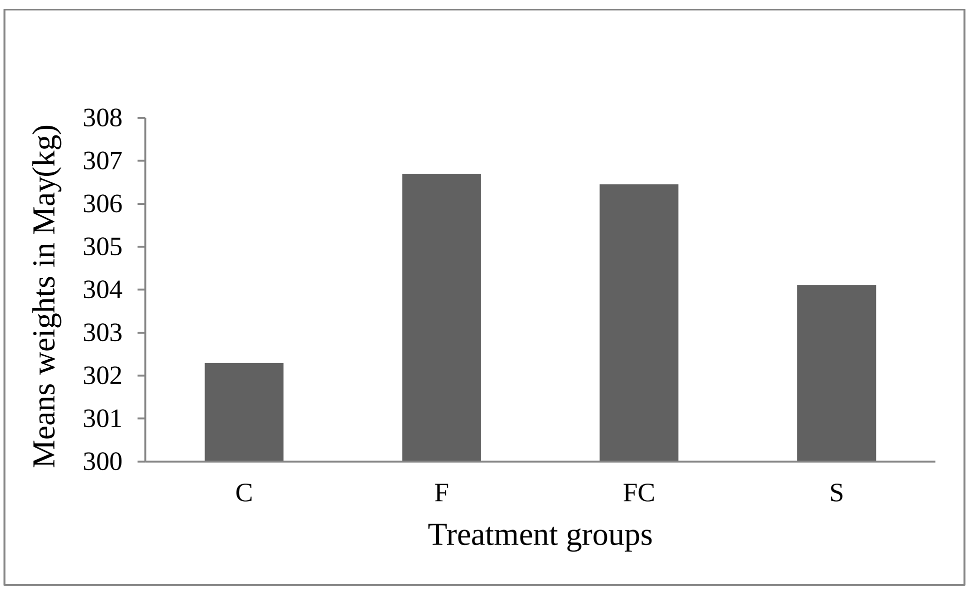
During this first experimental month, the average weights of heifers ranged from 302 to 304kg. Heifers from treatment groups F and S had the same average weights, but heifers from group C had lower average weights compared to those from treatment groups F, FC and S.



Graph 4.16 The live weights of heifers in April

C= Control; F= Fluxacur; FC= Flukazole; S= Sovereign

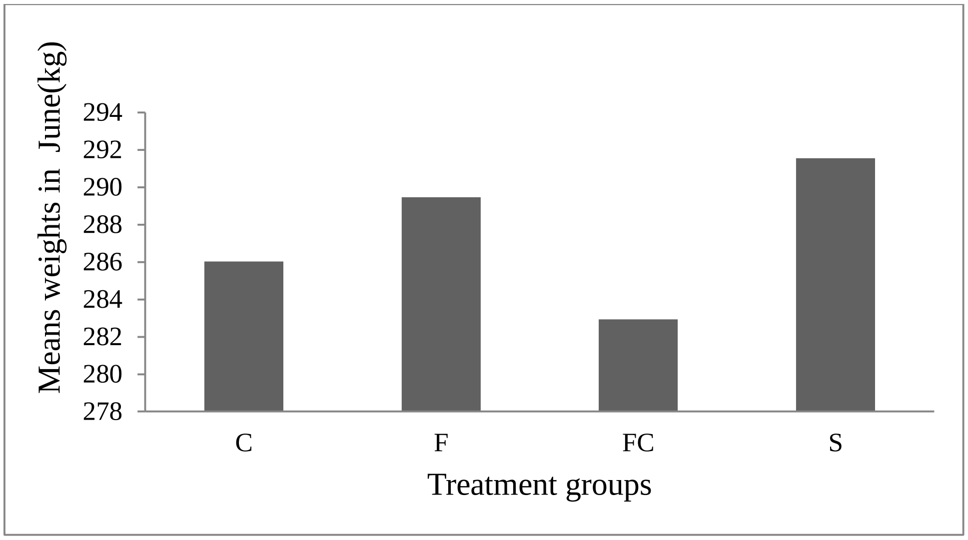
Heifers from all the four treatment groups maintained their average weights. No change has been noted and the weights remained stable.



Graph 4.17 The live weights of heifers in May

C= Control; F= Fluxacur; FC= Flukazole; S= Sovereign

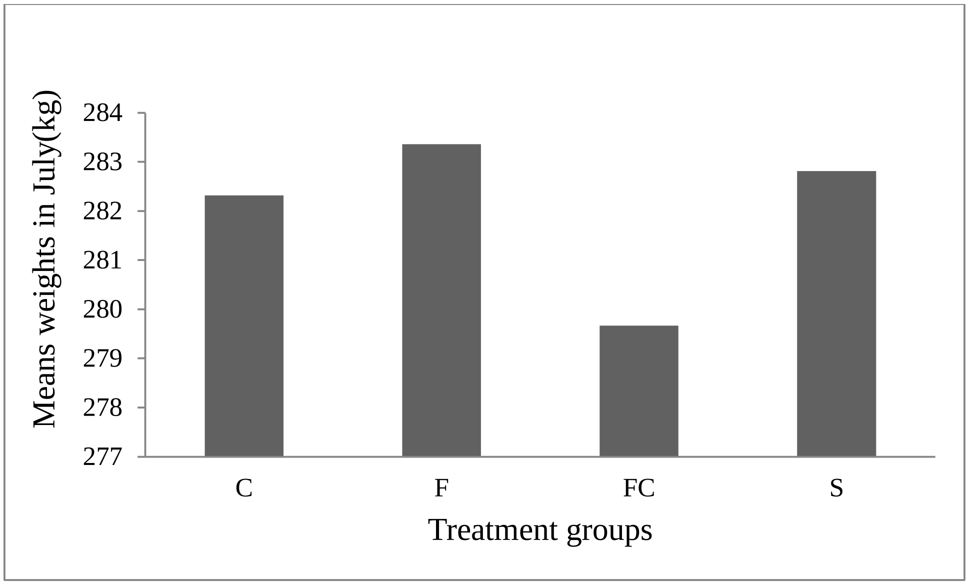
Heifers from group C showed a slight weight decrease (-0.45kg) and those from treatment group S showed also a slight weight decrease of -0.14kg compare to April, but an increase in weight was observed in heifers from the treatment groups F (+2.44kg) and FC (+3.39kg). Heifers from these groups have approximately the same average weights.



Graph 4.18 The live weights of heifers in June

C= Control; F= Fluxacur; FC= Flukazole; S= Sovereign

The average weights were negatively affected. A remarkable decrease of weights occurred in all four treatment groups. The weight losses of heifers from the four treatment groups were as follow: 16kg for heifers from control group, 17.24kg for heifers from treatment group F, -23.52kg for heifers from treatment group FC and -12.56kg for heifers from treatment group S. Weights of heifers from groups C and F were expected to improve progressively. However, a weight loss recovery is a time demanding process.

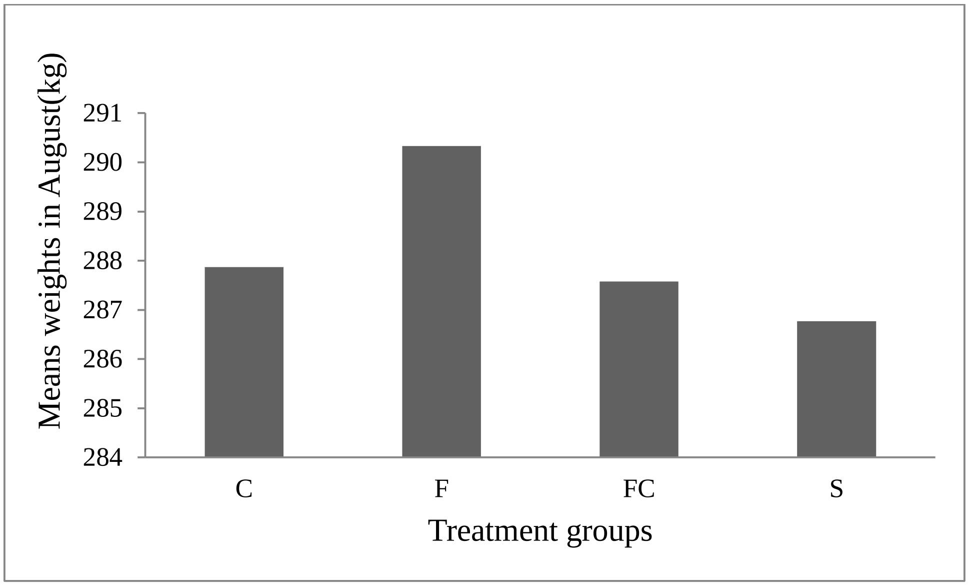


Graph 4.19 The live weights of heifers in July

C= Control; F= Fluxacur; FC= Flukazole; S= Sovereign

Compared to June, there is no sign of growth in July due to continual weights decrease. The observed weight losses were respectively -3.71 kg for heifers from groups C, -6.10 kg for heifers from treatment group F, -3.26kg for heifers from treatment group FC and -8.74 for heifers from treatment group S. Heifers from the treatment group S lost more weight during this particular experimental month of July. Heifers were not expected to gain weight (for growth) immediately one month post- treatment.

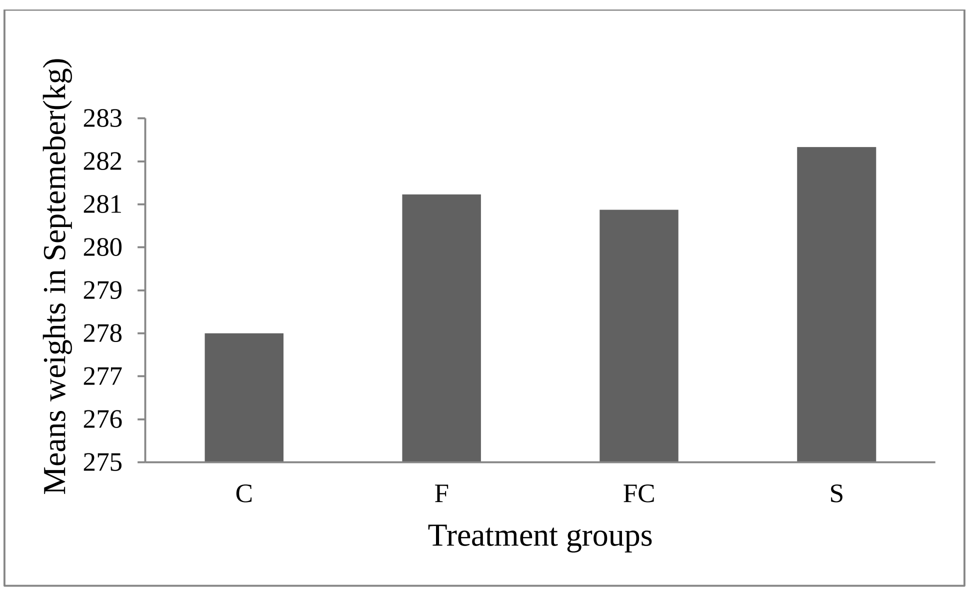
4.4. The effects of flukicidal treatments on the live weight changes from August to October



Graph 4.20 Effects of flukicidal treatments on the live weights of heifers in August

C= Control; F= Fluxacur; FC= Flukazole; S= Sovereign

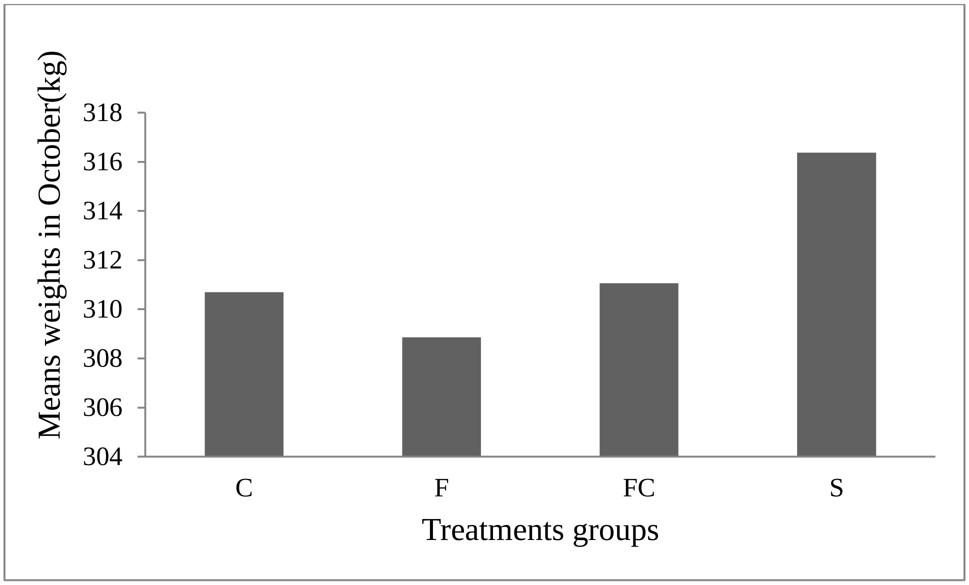
From July to August there was weight increase in all four treatment groups; heifers from the group C gained 4.15kg, those from treatment group F showed an average increase of 6.97kg; the gain of heifers from the treatment group FC was 7.91kg. Heifers from treatment group Sovereign pour-on showed a slight increase of 3.96kg. These weight increases are an indication of growth.



Graph 4.21 Effects of flukicidal treatments on the live weights of heifers in September

C= Control; F= Fluxacur; FC= Flukazole; S= Sovereign

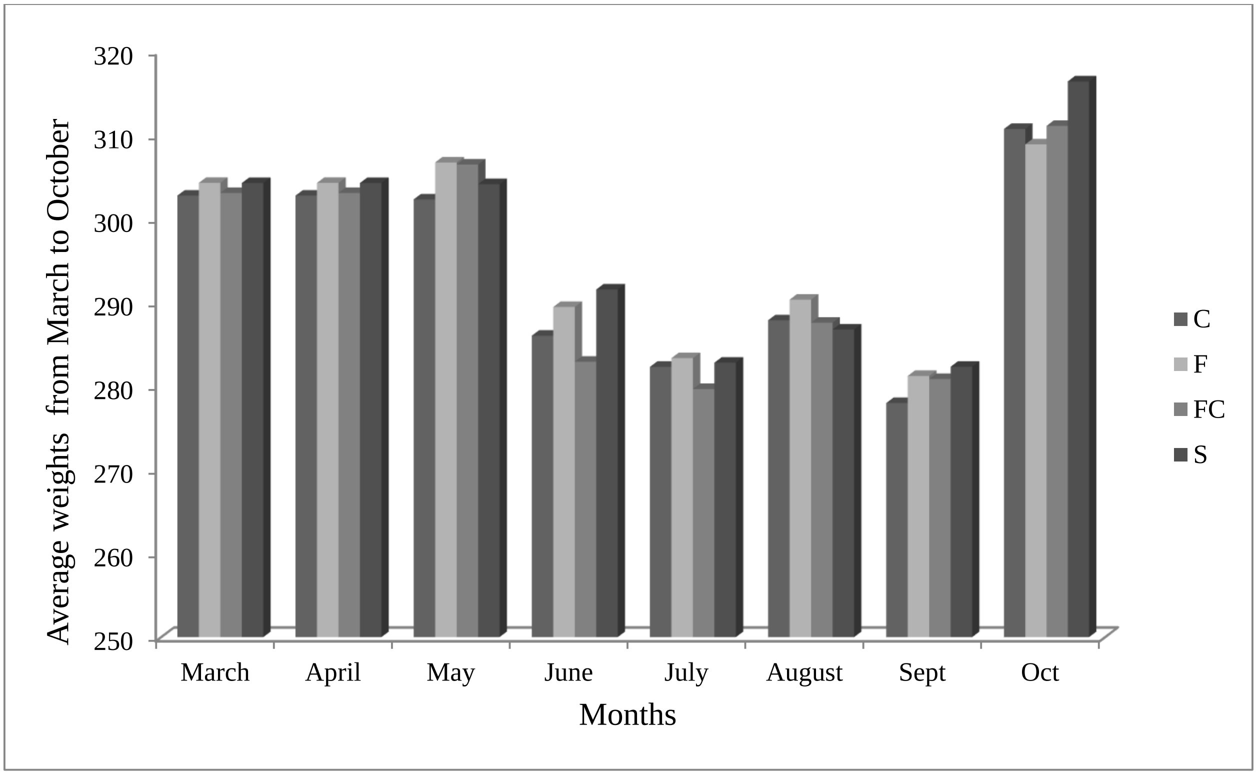
A decrease in average weights was noted in all four treatment groups. The weight losses were expressed respectively as follow: heifers from group C lost (-8kg), those from treatment group F lost (-9.10kg), heifers from treatment group FC lost (-6.71kg) and those from treatment group S lost (-4.44kg). The highest weight loss occurred in heifers from treatment group F. However, heifers from treatment group S showed a slight weight loss.



Graph 4.22 Effects of flukicidal treatments on the live weights of heifers in October

C= Control; F= Fluxacur; FC= Flukazole; S= Sovereign

During this particular month heifers from all treatment groups had a weight increase. Unmedicated heifers gained 22kg, heifers treated with Fluxacur gained 26.77kg, those treated with Flukazole C increased by 30.19kg and those from treatment group Sovereign pour- on showed the highest weight gain +34.44kg and had the most beneficial effect of the treatment.

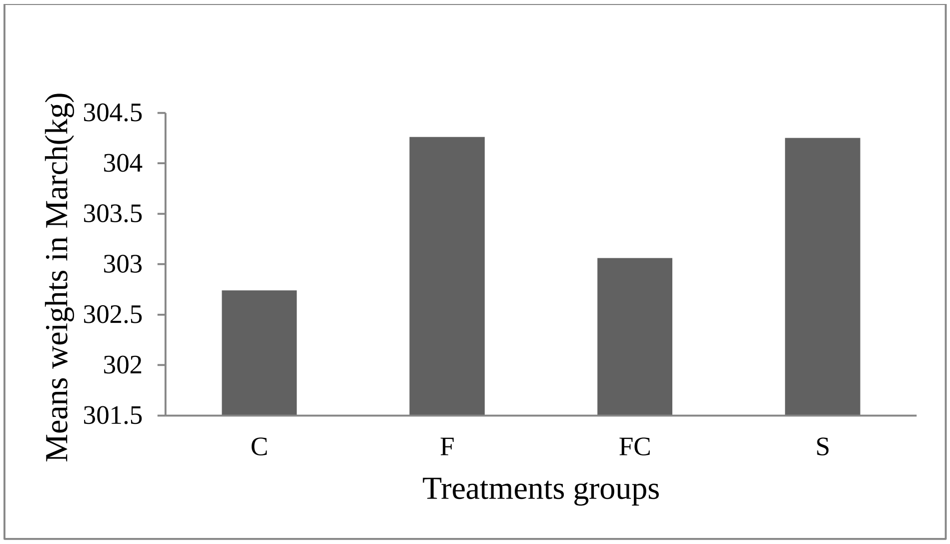


Graph 4.23 The combined data of live weights of heifers from March to Jully and the effects of Flukicidal treatment on the live weights of heifers from August to October.

C= Control; F= Fluxacur; FC= Flukazole; S= Sovereign

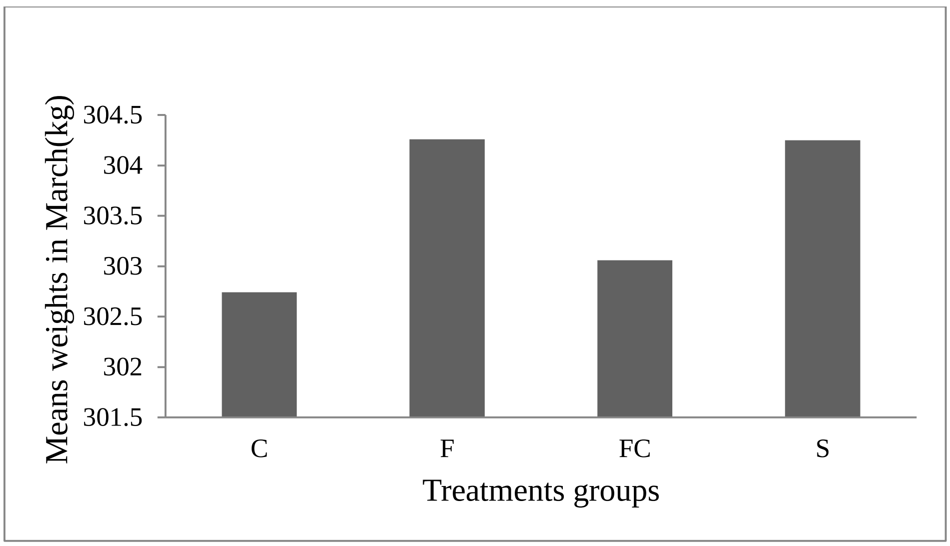
4.5 Data on the growth of heifers from March to July

The data on the growth of heifers over time following the treatment with three commercial flukicidal products are presented in the graphs 4.24 to 4.28, for each treatment group from March to July and the effects of Flukicidal treatment on the growth of heifers from August to October are presented in Graphs 4.29, 4.30 and 4.31. The combined data on the growth and effects of treatments on the growth of heifers are indicated in Graph 4.32



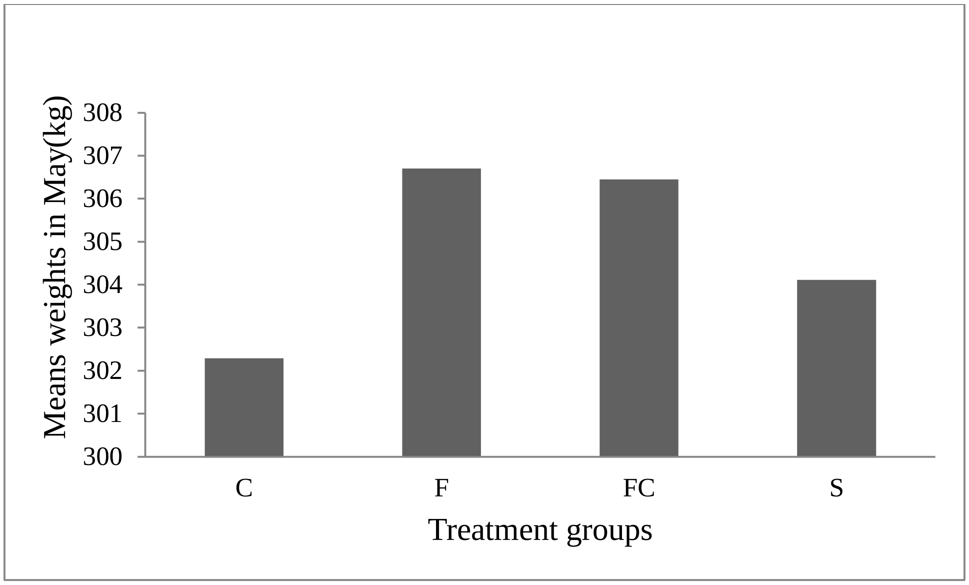
Graph 4.24 The growth of heifers in March

C= Control; F= Fluxacur; FC= Flukazole; S= Sovereign



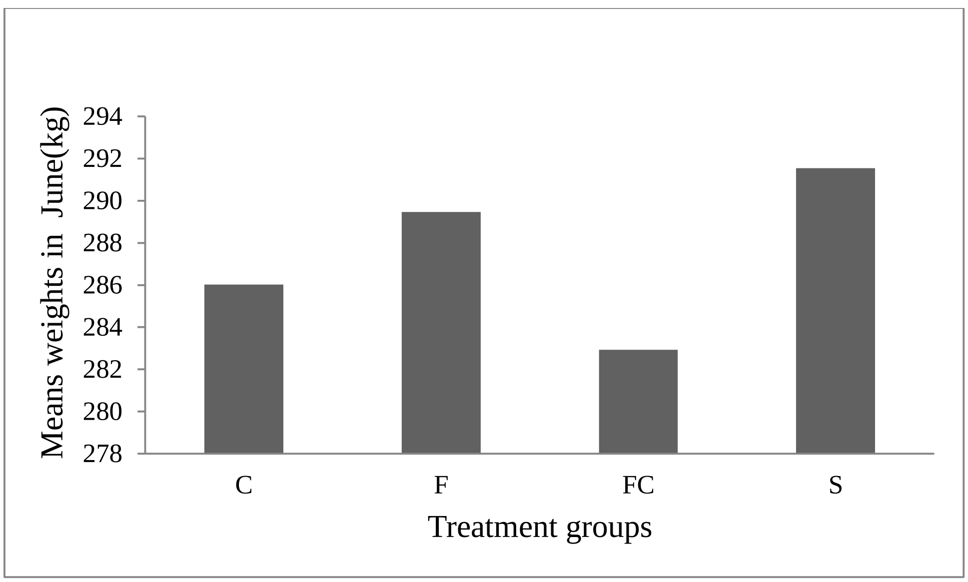
Graph 4.25 The growth of heifers in April

C= Control; F= Fluxacur; FC= Flukazole; S= Sovereign



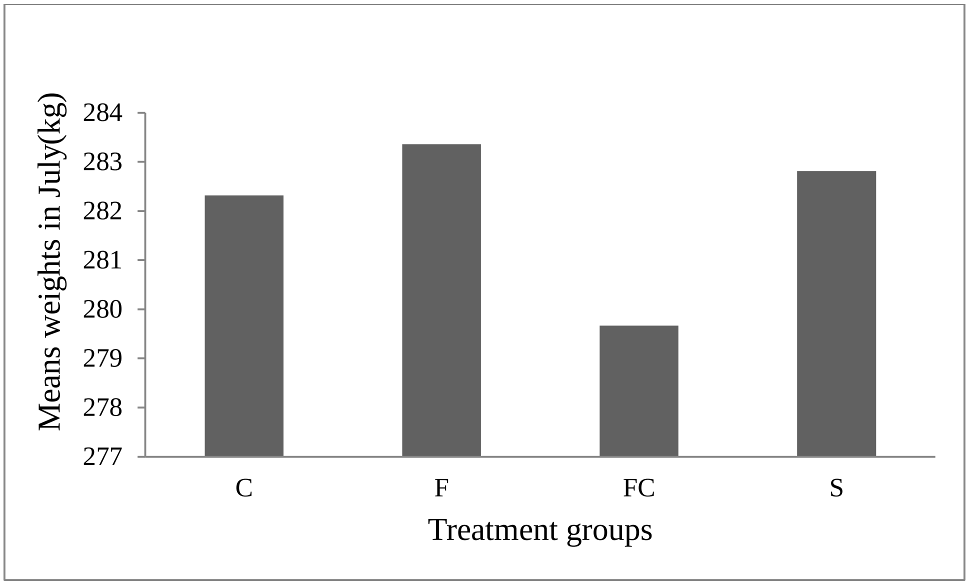
Graph 4.26 The growth of heifers in May

C= Control; F= Fluxacur; FC= Flukazole; S= Sovereign



Graph 4.27The growth of heifers in June

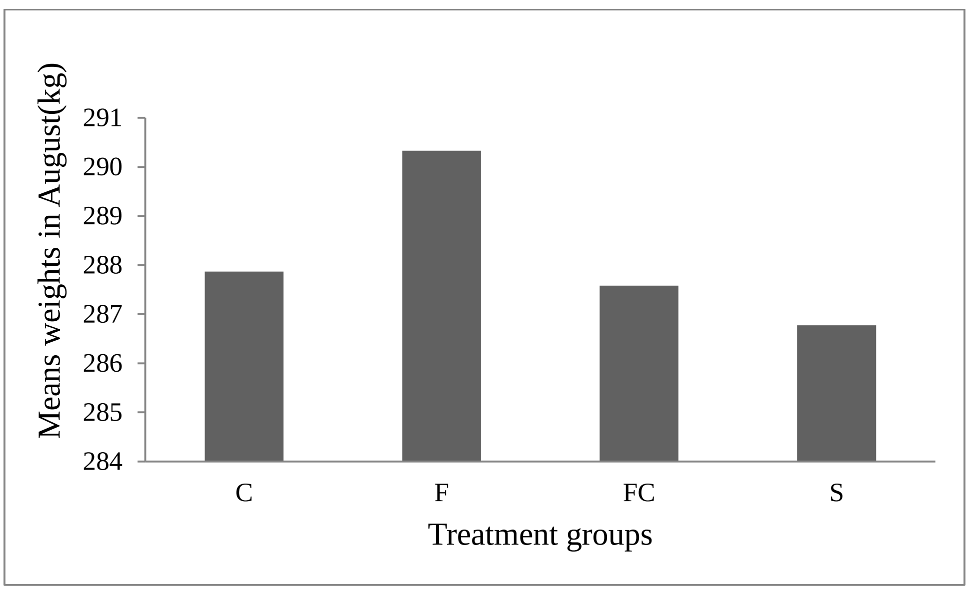
C= Control; F= Fluxacur; FC= Flukazole; S= Sovereign



Graph 4.28 The growth of heifers in July

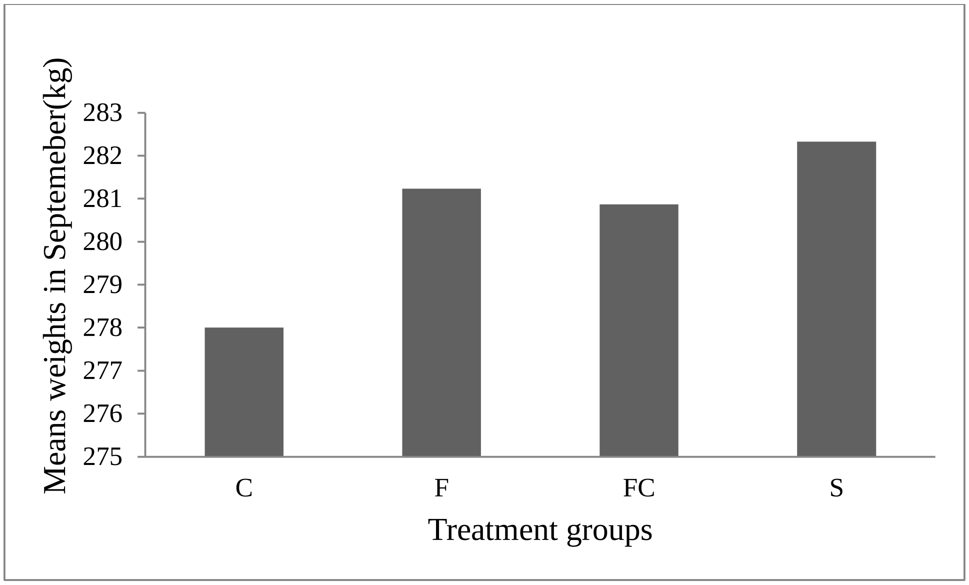
C= Control; F= Fluxacur; FC= Flukazole; S= Sovereign

4.6 The effects of Flukicidal treatment on the growth of heifers from August to October.



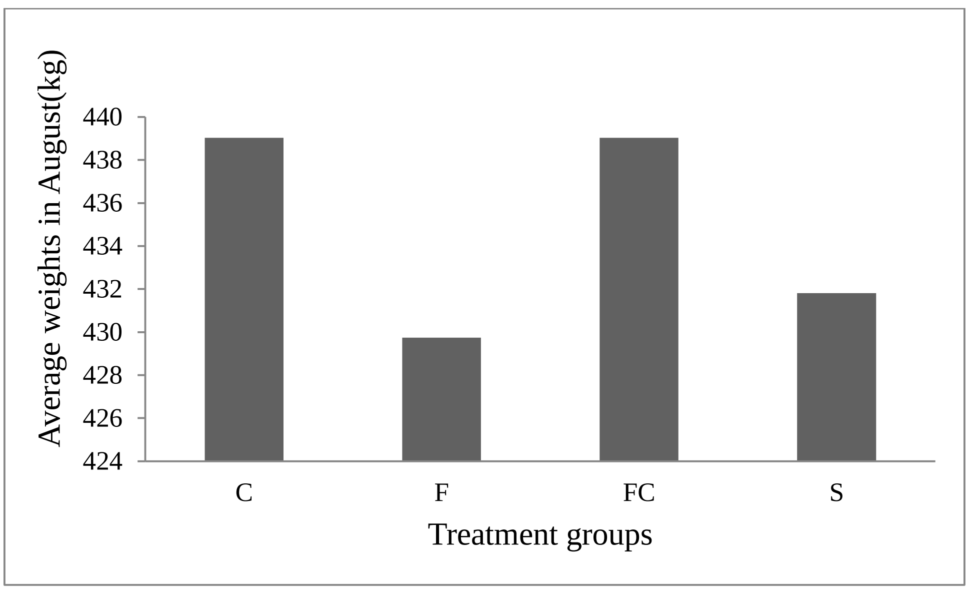
Graph 4.29 Effects of flukicidal treatments on the growth of heifers in August

C= Control; F= Fluxacur; FC= Flukazole; S= Sovereign



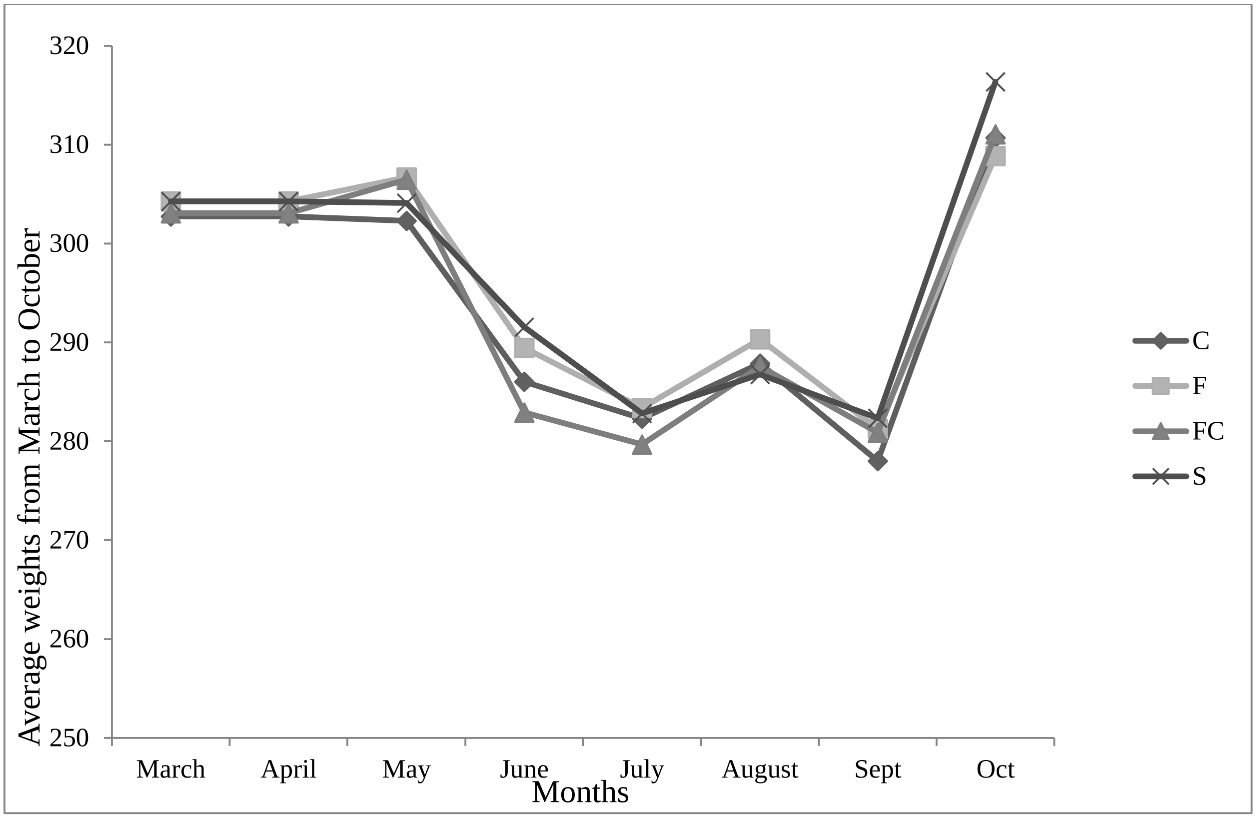
Graph 4.30 Effects of flukicidal treatments on the growth of heifers in September

C= Control; F= Fluxacur; FC= Flukazole; S= Sovereign



Graph 4.31 Effects of flukicidal treatments on the growth of heifers in October

C= Control; F= Fluxacur; FC= Flukazole; S= Sovereign



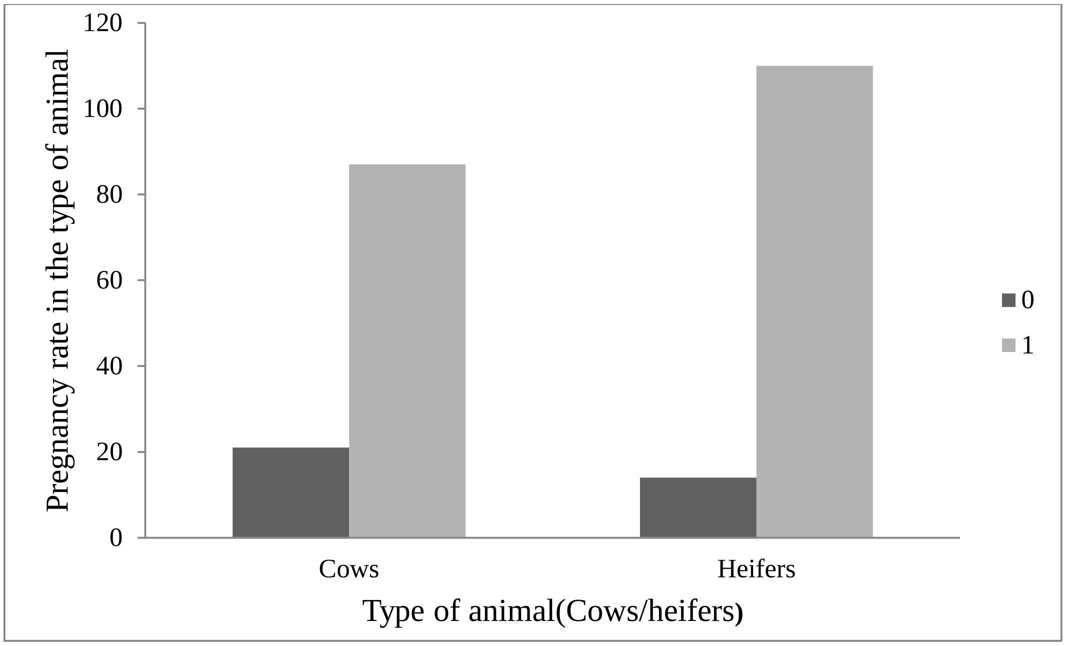
Graph 4.32 The combined data of the growth of heifers from March to July and the effects of Flukicidal treatment on the growth of heifers from August to October.

C= Control; F= Fluxacur; FC= Flukazole; S= Sovereign

4.7 Effects of type (cow/heifer) of animals on pregnancy rates

The pregnancy rate for heifers was 88.7% (i.e. 110 out of 124 heifers) and in cows it was 80.5% (i.e. 80 out of 108 cows). The results of this study indicate that pregnancy rates differed significantly between cows and heifers.

Graph 4.4 shows the results of the effect of the type (cows and heifers) of animals on pregnancy rates.



Graph 4.33 Effects of type (cow/heifer) of animals on pregnancy rates

Pregnancy: 0=Non pregnant; Pregnant.

4.8 Correlation(r) between parameters in Cows and heifers

Table 4.3 Correlation(r) between dependent parameters in cows

Variables	Weight	Weight diff. July-August	Weight diff. March-August
Weight	1	0.95	0.13
Weight diff. July-August	0.95	1	0.63
Weight diff. March -August	0.13	0.63	1

r= coefficient of correlation

Table 4.3 shows different values of correlation between variables considered in cows.

Table 4.4 Correlation(r) between dependent variables in heifers

Variables	Weight	Weight diff. July-August	Weight diff. March-October
Weight	1	-0.23	0.14
Weight diff. July-August	-0.23	1	0.28
Weight diff March -October	0.14	0.28	1

r = coefficient of correlation

Different values of correlation between variables are shown in the Table 4.4 in heifers.

CHAPTER 5

DISCUSSION AND CONCLUSIONS

This study used a large sample of beef cattle both cows and heifers treated once over a six month period to investigate the impact of treatment on their growth and live weight changes. The results show no effects of treatments on the growth and on the weight gain.

5.1 Effects of flukicidal treatments on cows

5.1.1 Effects of flukicidal treatments on live weights of cows

Many studies have reported an increase in weight and weight gain following treatment with a chemical anthelmintic when compared to untreated animals (Zinsstag, 1997). According to Bianca, (2005) upon a review of over 60 studies where anthelmintic effect on growth or weight gain were tested, the majority of them found increases in weight and weight gain in treated animals. Ryan et al. (1997) reported a 34% increase ($P < 0.02$) in weight gain of treated calves (n=29) compared to untreated calves (n=29), which was 33.9kg over 168 days after turnout. Dimander *et.al* (2003) study proved that chemically treated cattle performed better than untreated ones that were raised in identical set- stocked conditions.

However, the results from this study (section 4.1 and Table 4.1) suggest that there is no significant difference (p=1,000) between treatment groups, F, FC S and C. This result is in agreement with the reports of Bianca (2005) who found that there was no effect of treatment using a chemical dewormer on weight or weight gain of cattle. This result is also supported by Barger (1981) who demonstrated that there was a failure of regular anthelmintic treatment to increase weight in calves and by Zinsstag (1997).

Results obtained in this study (Table 4.1) show fluctuations of weight characterised by weight losses especially from April to July. During the trial month of May the farmer ran short of molasses meal stock to supplement the cows. There was a strike action at Voermol Feeds Company. This meant that the cows went without the necessary winter lick. Subsequently the group had weight loss occurrences during the first part of the trial set up. Besides, one will expect the weights of all cows to change negatively from March to end of August due to changes in season. The cows enter winter, quality of grazing deteriorates and weights are expected to decrease somewhat. This result is supported by Bohman (1995) who found that weight loss occurrences are a result of winter feeding which is more restrictive and reduces weight gains of the animals because of the seasonal variation in the quantity, quality and availability of forage. Furthermore, most the cows were with suckling calves at hand, thus weight gain and growth were compromised.

All cows were exposed to similar environmental conditions, but cows in some treatment groups have responded better to these deteriorating grazing and environmental conditions, such as weight gain noted from July after treatment until August in groups C, similar observations were made by Coirdia *et al*. (1982, 1984, 1987), Bumgarner *et al*. (1986) and Stromberg *et al*. (1987) who found that treated cows gained less weight than the unmedicated cows. Cows from FC treatment group also increased weight in August; this observation could be a result of the effect of seasonal variation in availability, quantity and quality of available forage, which can bring a greater change on cows as supported also by Roberts (2011) on his research on feed efficiency on a cow herd. From these results and analyses it can be concluded that live weight (growth is quantified by weight gain) is not affected by the treatment, using these three commercial flukicidal products.

Knowing the fact that, the investigation on live weight changes and growth of beef cattle, following treatment with a topical flukicidal product and flukicidal drenches has not yet been established, from the results of this study, it can therefore be concluded that there is no significant difference in weight gain (growth) between cows from treatment groups, F, FC S and C and there is no effect of treatment on the live weight (growth) of the cows.

5.1.2 Correlation between parameters in cows

From the results of correlation (Table 4.3), weight was strongly and positively correlated with weight difference from July to August. The positive correlation between these two variables is directly proportional; it indicates that if weight increases, then weight difference from July to August increases also. The weight was poorly correlated to weight difference from March to August. This poor correlation is due to the fact that any change in the weight of August will cause a proportional change in weight difference from March to August which will not be correlated to the weight (Starting weight of March).

The coefficient of correlation shows that the weight difference from July to August was strongly and positively correlated to weight difference from March to August. This correlation indicates that the weight differences from March to August and from July to August are in a direct proportional relation (when one increases the other increases as well).

5.2 Effects of flukicidal treatments on heifers

5.2.1 Effects of flukicidal treatments on live weight (growth) of heifers

There have been more efficient reports of deworming effects on weight gain (growth) in cattle. Brown (2001) investigated on the effect of combined treatment with an avermectin endectocide and flukicide on productivity of replacement heifers , he claimed that strategic treatment of replacement heifers with a flukicide and an avermectin resulted in significantly (P<0.05) better weight gains and improved body condition scores at palpation compared to cattle treated with an avermectin only. Loyacano *et a*l (2001) findings on the effects of parenteral administration of Doramectin or a combination of Ivermectin and Clorsulon on control of gastrointestinal nematode and liver fluke infections and on growth performance in cattle indicated that cattle with nematode infections and low overall *F. hepatica* burdens that were treated in the fall with Doramectin had significantly greater body weight gains during the 140-day study period. In their study on the effect of gastrointestinal nematode and liver fluke infections on weight gain and reproductive performance of beef heifers, Loyacano *et al* (2002) confirmed weight gains (P < 0.01).

A similar study done by Bullent *et.al* (2006) reported that a therapeutic suppression of liver fluke resulted in significant body weight and body weight gain in replacement of the animals.

The results obtained in this investigation (Table 4.2) reveal that there is no significant difference (P>0.05) between heifers from the four treatment groups in general. This result is supported by Bianca (2005), Barger (1981) and Zinsstag (1997).The late author claimed that treatment with dewormers failed to increase weights in treated cattle. Besides, these weight losses also occurred in the first set of the trial due to a delay in supplement supply experienced by the farmer in May following a strike action at the Voermol feed company.

Moreover, heifers endured the hardship of winter feeding which has been said to restrict and reduce animal weight gains following the availability, quantity and quality of available forage, this observation is supported by Bohman (1995).

In addition, a couple of factors may lead to the failure of anthelmintic treatment to increase weight in cattle. These factors could be extrinsecal to cattle (wrong dose or wrong anthelmintic applied) or intrinsecal to cattle (the parasites load, resistance etc.)The results obtained from this study reveal that most of animals stayed below 500epg (Table 5appendix). This observation is supported by Bianca (2005)

All cattle were exposed to similar environmental conditions, but cows in some treatment groups had responded better to these deteriorating grazing and environmental conditions. Some levels of significance were observed in March and April for heifers from treatment group FC (P= 0.031), in June for heifers from group C (P=0.022) and for heifers from treatment group F (P=0.047), in August for untreated heifers (P=0.035), for heifers treated with Fluxacur (P=0.009) and for heifers from the treatment group FC (P=0.003), in September for heifers from the treatment group FC (0.004) and in October for heifers from the treatment group C (P=0.030) and those from treatment group FC (P=0.009). From this investigation, the heifers from group C (unmedicated) gained more weight in June and in October at the end of trial than any other treatment group. These observations are in agreement with those of Ciordia *et a*l. (1982, 1984, and 1987); Bumgarner *et a*l. (1986) and Stromberg *et a*l. (1987) who found that treated cattle gained less weight than the untreated ones. The weight gain occurrences in these different treatment groups during different months of the trial, may be the effect of feeding (winter lick and supplement supply) on the weight. This observation agrees with the report of Roberts (2011) which established that the seasonal change cause an effect on the availability, the quantity and the quality of available forage which can bring a weight gain in animals which was observed in this study from August to October.

From the results of this investigation and from literature, it can be concluded that there is no significant difference in weight gain between heifers from the four treatment groups(C, F, FC, and S) and there is no effect of treatment on the body weight (growth) of heifers.

5.2.2 Correlation between parameters in heifers

According to values obtained from the Table 4.4, the weight was not correlated with the weight difference from July to August. The weight and weight difference from March to October were poorly correlated. The lack of a correlation between weight and the weight differences from July to August or from March to October is due to the fact that any change (increase or decrease) in the weight of the last month (July or October) will cause a change in the weight difference but will not be in relation with the starting weight (Weight of March). No correlation was observed between weight difference from July to August and weight difference from March to October, which is due to the fact that the period between July and August is shorter than the period between March and October during which a change in weight is more likely to occur.

5.3 Conclusion

Liver flukes have been shown to affect the performance of all classes of cattle, but young growing animals, including replacement beef heifers, seem to be the most susceptible. In areas where this parasite is known to be a threat, parasite control should be tailored to control it, using locally developed strategies for optimal timing of treatments. Although previous research (Bianca, 2005) suggests that anthelmintic treatment on faecal egg counts does not affect the growth of pastured dairy steers, no information was available on the effects of such anthelmintics treatments on the performance of extensive beef cows and heifers. The present study indicates that the treatments with liver fluke combination control products such as Fluxacur NF (Triclabendazole & Abamectin), Flukazole C (Triclabendazole & Oxfendazole) and Sovereign-Pour on (Triclabendazole & Ivermectin) did not affect the body mass of cattle in this specific trial, contrary to concerns of cattle owners due to rumors spread by certain veterinary pharmaceutical companies. The lack of differences in live weights or weight gains between treated and untreated cattle suggest that flukicidal treatments do not affect the live weights or weight gain in extensive beef cows or heifers. This single trial under the prevailing eastern Highveld conditions does not necessarily provide a true reflection of the efficacy and potential weight gains of cattle, because of the different stages of pregnancy and suckling calves that were present at the onset of the trial, despite randomization of cattle in different treatment groups. Perhaps if a more accurate model such as an installed test environment was used it might have provided a more accuratesimilar assessment of the effects of flukicidal treatments. Nevertheless, the present study indicates that neither of the flukicidal remedies utilized outperforms the other in extensive conditions in the eastern Hiveld.

5.4 Recommendations or suggestions

In this study, all cows and heifers were managed together under similar extensive conditions (pen, pasture, feed supplement) and all the cattle were exposed to similar grazing conditions with a confirmed internal parasite presense. However, when comparing the effect of flukicidal treatments in different treatment group, more than one option is available to manage the control group. In this experiment the exposure of untreated cattle in the control group to the same parasite infested grazing was justified, but cattle in this groups contributed to pasture recomtamination. In follow-up studies, control animals could be kept away and isolated from other cattle to minimize the pasture recontamination, but subjected to the same treatments and feeding.

Further studies need to be undertaken to investigate and better explain the effects and interactions of anthelmintic or flukicidal treatments in different seasons and nutritional regimes, and possibly conducted over more than one year, because the ambient conditions such as weather, season, duration of the trial and nutrition may affect the effectiveness and production responses of such treatments in cattle. The present study could only be conducted over one year due to financial constraints, but if the study is conducted over two or more years, the carry-over effects on growth of heifers, cows and weaners could be better quantified, as well as the possible effects on reproductive performce.

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APPENDIX

Table 1 Cow and heifer herd identification

H.R.ID Tag	Animal type	Animal breed	Animal gender	Animal colour	Animal first weight	Weighing date
Q01 37 271	Cattle	Bonsmara	Cow	Red	391	3/28/2012
Q01 37 272	Cattle	Bonsmara	Cow	Red	486	3/28/2012
Q01 37 273	Cattle	Bonsmara	Cow	Red	471	3/28/2012
Q01 37 274	Cattle	Bonsmara	Cow	Red	529	3/28/2012
Q01 37 275	Cattle	Bonsmara	Cow	Red	580	3/28/2012
Q01 37 276	Cattle	Bonsmara	Cow	Red	597	3/28/2012
Q01 37 277	Cattle	Bonsmara	Cow	Red	452	3/28/2012
Q01 37 278	Cattle	Bonsmara	Cow	Red	596	3/28/2012
Q01 37 279	Cattle	Bonsmara	Cow	Red	392	3/28/2012
Q01 37 280	Cattle	Bonsmara	Cow	Red	429	3/28/2012
Q01 37 281	Cattle	Bonsmara	Cow	Red	428	3/28/2012
Q01 37 282	Cattle	Bonsmara	Cow	Red	545	3/28/2012
Q01 37 283	Cattle	Bonsmara	Cow	Red	497	3/28/2012
Q01 37 284	Cattle	Bonsmara	Cow	Red	475	3/28/2012
Q01 37 285	Cattle	Bonsmara	Cow	Red	514	3/28/2012
Q01 37 286	Cattle	Bonsmara	Cow	Red	385	3/28/2012
Q01 37 287	Cattle	Bonsmara	Cow	Red	424	3/28/2012
Q01 37 288	Cattle	Bonsmara	Cow	Red	414	3/28/2012
Q01 37 289	Cattle	Bonsmara	Cow	Red	427	3/28/2012
Q01 37 290	Cattle	Bonsmara	Cow	Red	508	3/28/2012
Q01 37 291	Cattle	Bonsmara	Cow	Red	569	3/28/2012
Q01 37 292	Cattle	Bonsmara	Cow	Red	396	3/28/2012
Q01 37 293	Cattle	Bonsmara	Cow	Red	379	3/28/2012
Q01 37 294	Cattle	Bonsmara	Cow	Red	440	3/28/2012
Q01 37 295	Cattle	Bonsmara	Cow	Red	455	3/28/2012
Q01 37 296	Cattle	Bonsmara	Cow	Red	540	3/28/2012
Q01 37 297	Cattle	Bonsmara	Cow	Red	417	3/28/2012

Q01 37 298	Cattle	Bonsmara	Cow	Red	460	3/28/2012
Q01 37 299	Cattle	Bonsmara	Cow	Red	508	3/28/2012
Q01 37 300	Cattle	Bonsmara	Cow	Red	436	3/28/2012
Q01 37 301	Cattle	Bonsmara	Cow	Red	440	3/28/2012
Q01 37 302	Cattle	Bonsmara	Cow	Red	439	3/28/2012
Q01 37 303	Cattle	Bonsmara	Cow	Red	433	3/28/2012
Q01 37 304	Cattle	Bonsmara	Cow	Red	457	3/28/2012
Q01 37 305	Cattle	Bonsmara	Cow	Red	469	3/28/2012
Q01 37 306	Cattle	Bonsmara	Cow	Red	442	3/28/2012
Q01 37 307	Cattle	Bonsmara	Cow	Red	460	3/28/2012
Q01 37 308	Cattle	Bonsmara	Cow	Red	390	3/28/2012
Q01 37 309	Cattle	Bonsmara	Cow	Red	468	3/28/2012
Q01 37 310	Cattle	Bonsmara	Cow	Red	461	3/28/2012
Q01 37 311	Cattle	Bonsmara	Cow	Red	465	3/28/2012
Q01 37 312	Cattle	Bonsmara	Cow	Red	469	3/28/2012
Q01 37 313	Cattle	Bonsmara	Cow	Red	455	3/28/2012
Q01 37 314	Cattle	Bonsmara	Cow	Red	516	3/28/2012
Q01 37 315	Cattle	Bonsmara	Cow	Red	445	3/28/2012
Q01 37 316	Cattle	Bonsmara	Cow	Red	507	3/28/2012
Q01 37 317	Cattle	Bonsmara	Cow	Red	514	3/28/2012
Q01 37 318	Cattle	Bonsmara	Cow	Red	437	3/28/2012
Q01 37 319	Cattle	Bonsmara	Cow	Red	458	3/28/2012
Q01 37 320	Cattle	Bonsmara	Cow	Red	409	3/28/2012
Q01 37 321	Cattle	Bonsmara	Cow	Red	442	3/28/2012
Q01 37 322	Cattle	Bonsmara	Cow	Red	441	3/28/2012
Q01 37 323	Cattle	Bonsmara	Cow	Red	666	3/28/2012
Q01 37 324	Cattle	Bonsmara	Cow	Red	455	3/28/2012
Q01 37 325	Cattle	Bonsmara	Cow	Red	486	3/28/2012
Q01 37 326	Cattle	Bonsmara	Cow	Red	489	3/28/2012
Q01 37 327	Cattle	Bonsmara	Cow	Red	429	3/28/2012
Q01 37 328	Cattle	Bonsmara	Cow	Red	435	3/28/2012

Q01 37 329	Cattle	Bonsmara	Cow	Red	523	3/28/2012
Q01 37 330	Cattle	Bonsmara	Cow	Red	417	3/28/2012
Q01 37 331	Cattle	Bonsmara	Cow	Red	446	3/28/2012
Q01 37 332	Cattle	Bonsmara	Cow	Red	459	3/28/2012
Q01 37 333	Cattle	Bonsmara	Cow	Red	472	3/28/2012
Q01 37 334	Cattle	Bonsmara	Cow	Red	370	3/28/2012
Q01 37 335	Cattle	Bonsmara	Cow	Red	455	3/28/2012
Q01 37 336	Cattle	Bonsmara	Cow	Red	517	3/28/2012
Q01 37 337	Cattle	Bonsmara	Cow	Red	483	3/28/2012
Q01 37 338	Cattle	Bonsmara	Cow	Red	514	3/28/2012
Q01 37 339	Cattle	Bonsmara	Cow	Red	462	3/28/2012
Q01 37 340	Cattle	Bonsmara	Cow	Red	437	3/28/2012
Q01 37 341	Cattle	Bonsmara	Cow	Red	453	3/28/2012
Q01 37 342	Cattle	Bonsmara	Cow	Red	417	3/28/2012
Q01 37 343	Cattle	Bonsmara	Cow	Red	536	3/28/2012
Q01 37 344	Cattle	Bonsmara	Cow	Red	512	3/28/2012
Q01 37 345	Cattle	Bonsmara	Cow	Red	541	3/28/2012
Q01 37 346	Cattle	Bonsmara	Cow	Red	463	3/28/2012
Q01 37 347	Cattle	Bonsmara	Cow	Red	482	3/28/2012
Q01 37 348	Cattle	Bonsmara	Cow	Red	463	3/28/2012
Q01 37 349	Cattle	Bonsmara	Cow	Red	469	3/28/2012
Q01 37 350	Cattle	Bonsmara	Cow	Red	387	3/28/2012
Q01 37 351	Cattle	Bonsmara	Cow	Red	516	3/28/2012
Q01 37 352	Cattle	Bonsmara	Cow	Red	559	3/28/2012
Q01 37 353	Cattle	Bonsmara	Cow	Red	441	3/28/2012
Q01 37 354	Cattle	Bonsmara	Cow	Red	371	3/28/2012
Q01 37 355	Cattle	Bonsmara	Cow	Red	438	3/28/2012
Q01 37 356	Cattle	Bonsmara	Cow	Red	370	3/28/2012
Q01 37 357	Cattle	Bonsmara	Cow	Red	540	3/28/2012
Q01 37 358	Cattle	Bonsmara	Cow	Red	406	3/28/2012
Q01 37 359	Cattle	Bonsmara	Cow	Red	417	3/28/2012

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Q01 37 363	Cattle	Bonsmara	Cow	Red	501	3/28/2012
Q01 37 364	Cattle	Bonsmara	Cow	Red	508	3/28/2012
Q01 37 365	Cattle	Bonsmara	Cow	Red	455	3/28/2012
Q01 37 366	Cattle	Bonsmara	Cow	Red	489	3/28/2012
Q01 37 367	Cattle	Bonsmara	Cow	Red	438	3/28/2012
Q01 37 368	Cattle	Bonsmara	Cow	Red	561	3/28/2012
Q01 37 369	Cattle	Bonsmara	Cow	Red	495	3/28/2012
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Q01 37 371	Cattle	Bonsmara	Cow	Red	548	3/28/2012
Q01 37 372	Cattle	Bonsmara	Cow	Red	504	3/28/2012
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Q01 37 378	Cattle	Bonsmara	Cow	Red	438	3/28/2012
Q01 37 379	Cattle	Bonsmara	Cow	Red	447	3/28/2012
Q01 37 380	Cattle	Bonsmara	Cow	Red	370	3/28/2012
Q01 37 381	Cattle	Bonsmara	Cow	Red	511	3/28/2012
Q01 37 382	Cattle	Bonsmara	Cow	Red	536	3/28/2012
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Q01 37 387	Cattle	Beef master	Heifer	Red and white	289	3/28/2012
Q01 37 388	Cattle	Beef master	Heifer	Red and white	271	3/28/2012

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Q01 37 392	Cattle	Beef master	Heifer	Red and white	304	3/28/2012
Q01 37 393	Cattle	Beef master	Heifer	Red and white	290	3/28/2012
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Q01 37 408	Cattle	Beef master	Heifer	Red and white	307	3/28/2012
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Q01 37 417	Cattle	Beef master	Heifer	Red and white	265	3/28/2012
Q01 37 418	Cattle	Beef master	Heifer	Red and white	316	3/28/2012
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Q01 37 426	Cattle	Beef master	Heifer	Red and white	288	3/28/2012
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Q01 37 430	Cattle	Beef master	Heifer	Red and white	299	3/28/2012
Q01 37 431	Cattle	Beef master	Heifer	Red and white	336	3/28/2012
Q01 37 432	Cattle	Beef master	Heifer	Red and white	314	3/28/2012
Q01 37 433	Cattle	Beef master	Heifer	Red and white	305	3/28/2012
Q01 37 434	Cattle	Beef master	Heifer	Red and white	293	3/28/2012
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Q01 37 436	Cattle	Beef master	Heifer	Red and white	292	3/28/2012

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Q01 37 440	Cattle	Beef master	Heifer	Red and white	364	3/28/2012
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Q01 37 462	Cattle	Beef master	Heifer	Red and white	341	3/28/2012
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Q01 37 465	Cattle	Beef master	Heifer	Red and white	286	3/28/2012
Q01 37 466	Cattle	Beef master	Heifer	Red and white	279	3/28/2012
Q01 37 467	Cattle	Beef master	Heifer	Red and white	303	3/28/2012
Q01 37 468	Cattle	Beef master	Heifer	Red and white	350	3/28/2012

Q01 37 469	Cattle	Beef master	Heifer	Red and white	308	3/28/2012
Q01 37 470	Cattle	Beef master	Heifer	Red and white	315	3/28/2012
Q01 37 471	Cattle	Beef master	Heifer	Red and white	302	3/28/2012
Q01 37 472	Cattle	Beef master	Heifer	Red and white	263	3/28/2012
Q01 37 473	Cattle	Beef master	Heifer	Red and white	288	3/28/2012
Q01 37 474	Cattle	Beef master	Heifer	Red and white	267	3/28/2012
Q01 37 475	Cattle	Beef master	Heifer	Red and white	304	3/28/2012
Q01 37 476	Cattle	Beef master	Heifer	Red and white	266	3/28/2012
Q01 37 477	Cattle	Beef master	Heifer	Red and white	311	3/28/2012
Q01 37 478	Cattle	Beef master	Heifer	Red and white	303	3/28/2012
Q01 37 479	Cattle	Beef master	Heifer	Red and white	340	3/28/2012
Q01 37 480	Cattle	Beef master	Heifer	Red and white	276	3/28/2012
Q01 37 481	Cattle	Beef master	Heifer	Red and white	307	3/28/2012
Q01 37 482	Cattle	Beef master	Heifer	Red and white	294	3/28/2012
Q01 37 483	Cattle	Beef master	Heifer	Red and white	321	3/28/2012
Q01 37 484	Cattle	Beef master	Heifer	Red and white	308	3/28/2012

Q01 37 485	Cattle	Beef master	Heifer	Red and white	323	3/28/2012
Q01 37 486	Cattle	Beef master	Heifer	Red and white	292	3/28/2012
Q01 37 487	Cattle	Beef master	Heifer	Red and white	265	3/28/2012
Q01 37 488	Cattle	Beef master	Heifer	Red and white	290	3/28/2012
Q01 37 489	Cattle	Beef master	Heifer	Red and white	301	3/28/2012
Q01 37 490	Cattle	Beef master	Heifer	Red and white	298	3/28/2012
Q01 37 491	Cattle	Beef master	Heifer	Red and white	309	3/28/2012
Q01 37 492	Cattle	Beef master	Heifer	Red and white	291	3/28/2012
Q01 37 493	Cattle	Beef master	Heifer	Red and white	303	3/28/2012
Q01 37 494	Cattle	Beef master	Heifer	Red and white	322	3/28/2012
Q01 37 495	Cattle	Beef master	Heifer	Red and white	285	3/28/2012
Q01 37 496	Cattle	Beef master	Heifer	Red and white	284	3/28/2012
Q01 37 497	Cattle	Beef master	Heifer	Red and white	322	3/28/2012
Q01 37 498	Cattle	Beef master	Heifer	Red and white	302	3/28/2012
Q01 37 499	Cattle	Beef master	Heifer	Red and white	317	3/28/2012
Q01 37 500	Cattle	Beef master	Heifer	Red and white	268	3/28/2012

Q01 37 501	Cattle	Beef master	Heifer	Red and white	284	3/28/2012
Q01 37 502	Cattle	Beef master	Heifer	Red and white	310	3/28/2012
Q01 37 503	Cattle	Beef master	Heifer	Red and white	308	3/28/2012
Q01 37 504	Cattle	Beef master	Heifer	Red and white	288	3/28/2012
Q01 37 505	Cattle	Beef master	Heifer	Red and white	312	3/28/2012
Q01 37 506	Cattle	Beef master	Heifer	Red and white	274	3/28/2012
Q01 37 507	Cattle	Beef master	Heifer	Red and white	360	3/28/2012
Q01 37 508	Cattle	Beef master	Heifer	Red and white	272	3/28/2012
Q01 37 509	Cattle	Beef master	Heifer	Red and white	298	3/28/2012
Q01 37 510	Cattle	Beef master	Heifer	Red and white	281	3/28/2012
Q01 37 511	Cattle	Beef master	Heifer	Red and white	332	3/28/2012
Q01 37 512	Cattle	Beef master	Heifer	Red and white	338	3/28/2012
Q01 37 513	Cattle	Beef master	Heifer	Red and white	313	3/28/2012
Q01 37 514	Cattle	Beef master	Heifer	Red and white	292	3/28/2012
Q01 37 515	Cattle	Beef master	Heifer	Red and white	296	3/28/2012
Q01 37 516	Cattle	Beef master	Heifer	Red and white	287	3/28/2012

Q01 37 517	Cattle	Beef master	Heifer	Red and white	275	3/28/2012
Q01 37 518	Cattle	Beef master	Heifer	Red and white	286	3/28/2012
Q01 37 519	Cattle	Beef master	Heifer	Red and white	261	3/28/2012
Q01 37 520	Cattle	Beef master	Heifer	Red and white	282	3/28/2012

Table 3 Cows and heifers' treatment group ranking

H.R.ID Tag	Ranking Group	Animal type	Animal breed	Animal gender	Animal colour	Animal first weight	Weighing date
Q01 37 271	**2**	Cattle	Bonsmara	Cow	Red	391	3/28/2012
Q01 37 272	**3**	Cattle	Bonsmara	Cow	Red	486	3/28/2012
Q01 37 273	**2**	Cattle	Bonsmara	Cow	Red	471	3/28/2012
Q01 37 274	**2**	Cattle	Bonsmara	Cow	Red	529	3/28/2012
Q01 37 275	**3**	Cattle	Bonsmara	Cow	Red	580	3/28/2012
Q01 37 276	**1**	Cattle	Bonsmara	Cow	Red	597	3/28/2012
Q01 37 277	**3**	Cattle	Bonsmara	Cow	Red	452	3/28/2012
Q01 37 278	**2**	Cattle	Bonsmara	Cow	Red	596	3/28/2012

Q01 37 279	**1**	Cattle	Bonsmara	Cow	Red	392	3/28/2012
Q01 37 280	**3**	Cattle	Bonsmara	Cow	Red	429	3/28/2012
Q01 37 281	**1**	Cattle	Bonsmara	Cow	Red	428	3/28/2012
Q01 37 282	**2**	Cattle	Bonsmara	Cow	Red	545	3/28/2012
Q01 37 283	**4**	Cattle	Bonsmara	Cow	Red	497	3/28/2012
Q01 37 286	**1**	Cattle	Bonsmara	Cow	Red	385	3/28/2012
Q01 37 287	**2**	Cattle	Bonsmara	Cow	Red	424	3/28/2012
Q01 37 288	**3**	Cattle	Bonsmara	Cow	Red	414	3/28/2012
Q01 37 289	**3**	Cattle	Bonsmara	Cow	Red	427	3/28/2012
Q01 37 290	**3**	Cattle	Bonsmara	Cow	Red	508	3/28/2012
Q01 37 291	**1**	Cattle	Bonsmara	Cow	Red	569	3/28/2012
Q01 37 292	**1**	Cattle	Bonsmara	Cow	Red	396	3/28/2012
Q01 37 293	**2**	Cattle	Bonsmara	Cow	Red	379	3/28/2012
Q01 37 294	**4**	Cattle	Bonsmara	Cow	Red	440	3/28/2012
Q01 37 295	**4**	Cattle	Bonsmara	Cow	Red	455	3/28/2012
Q01 37 296	**3**	Cattle	Bonsmara	Cow	Red	540	3/28/2012

Q01 37 297	**4**	Cattle	Bonsm ara	Cow	Red	417	3/28/2012
Q01 37 298	**2**	Cattle	Bonsm ara	Cow	Red	460	3/28/2012
Q01 37 299	**1**	Cattle	Bonsm ara	Cow	Red	508	3/28/2012
Q01 37 300	**1**	Cattle	Bonsm ara	Cow	Red	436	3/28/2012
Q01 37 301	**3**	Cattle	Bonsm ara	Cow	Red	440	3/28/2012
Q01 37 302	**3**	Cattle	Bonsm ara	Cow	Red	439	3/28/2012
Q01 37 303	**1**	Cattle	Bonsm ara	Cow	Red	433	3/28/2012
Q01 37 304	**4**	Cattle	Bonsm ara	Cow	Red	457	3/28/2012
Q01 37 306	**2**	Cattle	Bonsm ara	Cow	Red	442	3/28/2012
Q01 37 307	**1**	Cattle	Bonsm ara	Cow	Red	460	3/28/2012
Q01 37 308	**4**	Cattle	Bonsm ara	Cow	Red	390	3/28/2012
Q01 37 309	**4**	Cattle	Bonsm ara	Cow	Red	468	3/28/2012
Q01 37 310	**2**	Cattle	Bonsm ara	Cow	Red	461	3/28/2012
Q01 37 311	**1**	Cattle	Bonsm ara	Cow	Red	465	3/28/2012
Q01 37 312	**1**	Cattle	Bonsm ara	Cow	Red	469	3/28/2012
Q01 37 313	**2**	Cattle	Bonsm ara	Cow	Red	455	3/28/2012

Q01 37 314	**3**	Cattle	Bonsmara	Cow	Red	516	3/28/2012
Q01 37 315	**3**	Cattle	Bonsmara	Cow	Red	445	3/28/2012
Q01 37 316	**1**	Cattle	Bonsmara	Cow	Red	507	3/28/2012
Q01 37 317	**3**	Cattle	Bonsmara	Cow	Red	514	3/28/2012
Q01 37 318	**2**	Cattle	Bonsmara	Cow	Red	437	3/28/2012
Q01 37 319	**4**	Cattle	Bonsmara	Cow	Red	458	3/28/2012
Q01 37 320	**2**	Cattle	Bonsmara	Cow	Red	409	3/28/2012
Q01 37 321	**2**	Cattle	Bonsmara	Cow	Red	442	3/28/2012
Q01 37 322	**4**	Cattle	Bonsmara	Cow	Red	441	3/28/2012
Q01 37 323	**4**	Cattle	Bonsmara	Cow	Red	666	3/28/2012
Q01 37 324	**1**	Cattle	Bonsmara	Cow	Red	455	3/28/2012
Q01 37 325	**4**	Cattle	Bonsmara	Cow	Red	486	3/28/2012
Q01 37 326	**3**	Cattle	Bonsmara	Cow	Red	489	3/28/2012
Q01 37 327	**2**	Cattle	Bonsmara	Cow	Red	429	3/28/2012
Q01 37 328	**3**	Cattle	Bonsmara	Cow	Red	435	3/28/2012
Q01 37 329	**3**	Cattle	Bonsmara	Cow	Red	523	3/28/2012

Q01 37 330	**2**	Cattle	Bonsm ara	Cow	Red	417	3/28/2012
Q01 37 331	**2**	Cattle	Bonsm ara	Cow	Red	446	3/28/2012
Q01 37 332	**4**	Cattle	Bonsm ara	Cow	Red	459	3/28/2012
Q01 37 333	**4**	Cattle	Bonsm ara	Cow	Red	472	3/28/2012
Q01 37 334	**2**	Cattle	Bonsm ara	Cow	Red	370	3/28/2012
Q01 37 335	**1**	Cattle	Bonsm ara	Cow	Red	455	3/28/2012
Q01 37 336	**4**	Cattle	Bonsm ara	Cow	Red	517	3/28/2012
Q01 37 337	**3**	Cattle	Bonsm ara	Cow	Red	483	3/28/2012
Q01 37 338	**2**	Cattle	Bonsm ara	Cow	Red	514	3/28/2012
Q01 37 339	**2**	Cattle	Bonsm ara	Cow	Red	462	3/28/2012
Q01 37 341	**3**	Cattle	Bonsm ara	Cow	Red	453	3/28/2012
Q01 37 342	**4**	Cattle	Bonsm ara	Cow	Red	417	3/28/2012
Q01 37 343	**1**	Cattle	Bonsm ara	Cow	Red	536	3/28/2012
Q01 37 344	**3**	Cattle	Bonsm ara	Cow	Red	512	3/28/2012
Q01 37 345	**3**	Cattle	Bonsm ara	Cow	Red	541	3/28/2012
Q01 37 346	**3**	Cattle	Bonsm ara	Cow	Red	463	3/28/2012

Q01 37 347	**2**	Cattle	Bonsm ara	Cow	Red	482	3/28/2012
Q01 37 348	**1**	Cattle	Bonsm ara	Cow	Red	463	3/28/2012
Q01 37 349	**3**	Cattle	Bonsm ara	Cow	Red	469	3/28/2012
Q01 37 350	**3**	Cattle	Bonsm ara	Cow	Red	387	3/28/2012
Q01 37 351	**4**	Cattle	Bonsm ara	Cow	Red	516	3/28/2012
Q01 37 352	**4**	Cattle	Bonsm ara	Cow	Red	559	3/28/2012
Q01 37 353	**4**	Cattle	Bonsm ara	Cow	Red	441	3/28/2012
Q01 37 354	**4**	Cattle	Bonsm ara	Cow	Red	371	3/28/2012
Q01 37 355	**4**	Cattle	Bonsm ara	Cow	Red	438	3/28/2012
Q01 37 356	**4**	Cattle	Bonsm ara	Cow	Red	370	3/28/2012
Q01 37 357	**2**	Cattle	Bonsm ara	Cow	Red	540	3/28/2012
Q01 37 358	**2**	Cattle	Bonsm ara	Cow	Red	406	3/28/2012
Q01 37 359	**4**	Cattle	Bonsm ara	Cow	Red	417	3/28/2012
Q01 37 360	**1**	Cattle	Bonsm ara	Cow	Red	502	3/28/2012
Q01 37 361	**1**	Cattle	Bonsm ara	Cow	Red	521	3/28/2012
Q01 37 362	**2**	Cattle	Bonsm ara	Cow	Red	423	3/28/2012

Q01 37 363	**1**	Cattle	Bonsm ara	Cow	Red	501	3/28/2012
Q01 37 364	**4**	Cattle	Bonsm ara	Cow	Red	508	3/28/2012
Q01 37 365	**2**	Cattle	Bonsm ara	Cow	Red	455	3/28/2012
Q01 37 366	**3**	Cattle	Bonsm ara	Cow	Red	489	3/28/2012
Q01 37 367	**1**	Cattle	Bonsm ara	Cow	Red	438	3/28/2012
Q01 37 368	**1**	Cattle	Bonsm ara	Cow	Red	561	3/28/2012
Q01 37 369	**4**	Cattle	Bonsm ara	Cow	Red	495	3/28/2012
Q01 37 370	**1**	Cattle	Bonsm ara	Cow	Red	390	3/28/2012
Q01 37 371	**4**	Cattle	Bonsm ara	Cow	Red	548	3/28/2012
Q01 37 372	**1**	Cattle	Bonsm ara	Cow	Red	504	3/28/2012
Q01 37 373	**3**	Cattle	Bonsm ara	Cow	Red	467	3/28/2012
Q01 37 375	**1**	Cattle	Bonsm ara	Cow	Red	422	3/28/2012
Q01 37 376	**2**	Cattle	Bonsm ara	Cow	Red	486	3/28/2012
Q01 37 377	**1**	Cattle	Bonsm ara	Cow	Red	476	3/28/2012
Q01 37 378	**3**	Cattle	Bonsm ara	Cow	Red	438	3/28/2012
Q01 37 379	**1**	Cattle	Bonsm ara	Cow	Red	447	3/28/2012

Q01 37 380	**3**	Cattle	Bonsmara	Cow	Red	370	3/28/2012
Q01 37 381	**2**	Cattle	Bonsmara	Cow	Red	511	3/28/2012
Q01 37 382	**4**	Cattle	Bonsmara	Cow	Red	536	3/28/2012
Q01 37 383	**4**	Cattle	Bonsmara	Cow	Red	481	3/28/2012
Q01 37 387	**4**	Cattle	Beef master	Heifer	Red and white	289	3/28/2012
Q01 37 388	**1**	Cattle	Beef Master	Heifer	Red and white	271	3/28/2012
Q01 37 389	**1**	Cattle	Beef master	Heifer	Red and white	329	3/28/2012
Q01 37 390	**4**	Cattle	Beef master	Heifer	Red and white	368	3/28/2012
Q01 37 391	**4**	Cattle	Beef master	Heifer	Red and white	269	3/28/2012
Q01 37 392	**3**	Cattle	Beef master	Heifer	Red and white	304	3/28/2012
Q01 37 393	**1**	Cattle	Beef master	Heifer	Red and white	290	3/28/2012
Q01 37 394	**4**	Cattle	Beef master	Heifer	Red and white	315	3/28/2012
Q01 37 395	**1**	Cattle	Beef master	Heifer	Red and white	308	3/28/2012
Q01 37 396	**3**	Cattle	Beef master	Heifer	Red and white	317	3/28/2012
Q01 37 397	**1**	Cattle	Beef master	Heifer	Red and white	290	3/28/2012
Q01 37 398	**1**	Cattle	Beef master	Heifer	Red and white	313	3/28/2012

Q01 37 399	**3**	Cattle	Beef master	Heifer	Red and white	326	3/28/2012
Q01 37 400	**2**	Cattle	Beef master	Heifer	Red and white	394	3/28/2012
Q01 37 401	**2**	Cattle	Beef master	Heifer	Red and white	275	3/28/2012
Q01 37 402	**4**	Cattle	Beef master	Heifer	Red and white	264	3/28/2012
Q01 37 403	**1**	Cattle	Beef master	Heifer	Red and white	354	3/28/2012
Q01 37 404	**2**	Cattle	Beef Master	Heifer	Red and white	322	3/28/2012
Q01 37 405	**3**	Cattle	Beef master	Heifer	Red and white	282	3/28/2012
Q01 37 406	**4**	Cattle	Beef master	Heifer	Red and white	272	3/28/2012
Q01 37 407	**4**	Cattle	Beef master	Heifer	Red and white	343	3/28/2012
Q01 37 408	**1**	Cattle	Beef master	Heifer	Red and white	307	3/28/2012
Q01 37 409	**4**	Cattle	Beef master	Heifer	Red and white	310	3/28/2012
Q01 37 410	**1**	Cattle	Beef master	Heifer	Red and white	352	3/28/2012
Q01 37 411	**1**	Cattle	Beef master	Heifer	Red and white	305	3/28/2012
Q01 37 412	**1**	Cattle	Beef master	Heifer	Red and white	297	3/28/2012
Q01 37 413	**4**	Cattle	Beef master	Heifer	Red and white	319	3/28/2012
Q01 37 414	**4**	Cattle	Beef master	Heifer	Red and white	306	3/28/2012

Q01 37 415	**1**	Cattle	Beef master	Heifer	Red and white	268	3/28/2012
Q01 37 416	**2**	Cattle	Beef master	Heifer	Red and white	325	3/28/2012
Q01 37 417	**3**	Cattle	Beef master	Heifer	Red and white	265	3/28/2012
Q01 37 418	**2**	Cattle	Beef master	Heifer	Red and white	316	3/28/2012
Q01 37 419	**2**	Cattle	Beef master	Heifer	Red and white	302	3/28/2012
Q01 37 420	**1**	Cattle	Beef Master	Heifer	Red and white	286	3/28/2012
Q01 37 421	**2**	Cattle	Beef master	Heifer	Red and white	334	3/28/2012
Q01 37 422	**2**	Cattle	Beef master	Heifer	Red and white	309	3/28/2012
Q01 37 423	**2**	Cattle	Beef master	Heifer	Red and white	272	3/28/2012
Q01 37 424	**3**	Cattle	Beef master	Heifer	Red and white	293	3/28/2012
Q01 37 425	**1**	Cattle	Beef master	Heifer	Red and white	286	3/28/2012
Q01 37 426	**1**	Cattle	Beef master	Heifer	Red and white	288	3/28/2012
Q01 37 427	**1**	Cattle	Beef master	Heifer	Red and white	298	3/28/2012
Q01 37 428	**1**	Cattle	Beef master	Heifer	Red and white	278	3/28/2012
Q01 37 429	**2**	Cattle	Beef master	Heifer	Red and white	299	3/28/2012
Q01 37 430	**2**	Cattle	Beef master	Heifer	Red and white	299	3/28/2012

Q01 37 431	**1**	Cattle	Beef master	Heifer	Red and white	336	3/28/2012
Q01 37 432	**2**	Cattle	Beef master	Heifer	Red and white	314	3/28/2012
Q01 37 433	**2**	Cattle	Beef master	Heifer	Red and white	305	3/28/2012
Q01 37 434	**4**	Cattle	Beef master	Heifer	Red and white	293	3/28/2012
Q01 37 435	**3**	Cattle	Beef master	Heifer	Red and white	303	3/28/2012
Q01 37 436	**4**	Cattle	Beef Master	Heifer	Red and white	292	3/28/2012
Q01 37 438	**4**	Cattle	Beefmaster	Heifer	Red and white	275	3/28/2012
Q01 37 439	**2**	Cattle	Beef master	Heifer	Red and white	385	3/28/2012
Q01 37 441	**1**	Cattle	Beef master	Heifer	Red and white	332	3/28/2012
Q01 37 442	**1**	Cattle	Beef master	Heifer	Red and white	288	3/28/2012
Q01 37 443	**4**	Cattle	Beef master	Heifer	Red and white	356	3/28/2012
Q01 37 444	**2**	Cattle	Beef master	Heifer	Red and white	282	3/28/2012
Q01 37 445	**1**	Cattle	Beef master	Heifer	Red and white	287	3/28/2012
Q01 37 446	**3**	Cattle	Beef master	Heifer	Red and white	313	3/28/2012
Q01 37 447	**1**	Cattle	Beef master	Heifer	Red and white	319	3/28/2012
Q01 37 448	**4**	Cattle	Beef master	Heifer	Red and white	334	3/28/2012

Q01 37 449	**3**	Cattle	Beef master	Heifer	Red and white	326	3/28/2012
Q01 37 450	**3**	Cattle	Beef master	Heifer	Red and white	294	3/28/2012
Q01 37 451	**3**	Cattle	Beef master	Heifer	Red and white	283	3/28/2012
Q01 37 452	**3**	Cattle	Beef master	Heifer	Red and white	328	3/28/2012
Q01 37 453	**4**	Cattle	Beef master	Heifer	Red and white	304	3/28/2012
Q01 37 454	**4**	Cattle	Beef Master	Heifer	Red and white	280	3/28/2012
Q01 37 455	**1**	Cattle	Beef master	Heifer	Red and white	279	3/28/2012
Q01 37 456	**4**	Cattle	Beef master	Heifer	Red and white	296	3/28/2012
Q01 37 457	**1**	Cattle	Beef master	Heifer	Red and white	355	3/28/2012
Q01 37 458	**2**	Cattle	Beef master	Heifer	Red and white	269	3/28/2012
Q01 37 459	**4**	Cattle	Beef master	Heifer	Red and white	278	3/28/2012
Q01 37 460	**4**	Cattle	Beef master	Heifer	Red and white	323	3/28/2012
Q01 37 461	**3**	Cattle	Beef master	Heifer	Red and white	339	3/28/2012
Q01 37 462	**3**	Cattle	Beef master	Heifer	Red and white	341	3/28/2012
Q01 37 463	**1**	Cattle	Beef master	Heifer	Red and white	281	3/28/2012
Q01 37 464	**2**	Cattle	Beef master	Heifer	Red and white	319	3/28/2012

Q01 37 465	**1**	Cattle	Beef master	Heifer	Red and white	286	3/28/2012
Q01 37 466	**1**	Cattle	Beef master	Heifer	Red and white	279	3/28/2012
Q01 37 467	**4**	Cattle	Beef master	Heifer	Red and white	303	3/28/2012
Q01 37 468	**2**	Cattle	Beef master	Heifer	Red and white	350	3/28/2012
Q01 37 469	**4**	Cattle	Beef master	Heifer	Red and white	308	3/28/2012
Q01 37 470	**1**	Cattle	Beef Master	Heifer	Red and white	315	3/28/2012
Q01 37 471	**4**	Cattle	Beef master	Heifer	Red and white	302	3/28/2012
Q01 37 472	**2**	Cattle	Beef master	Heifer	Red and white	263	3/28/2012
Q01 37 473	**3**	Cattle	Beef master	Heifer	Red and white	288	3/28/2012
Q01 37 474	**1**	Cattle	Beef master	Heifer	Red and white	267	3/28/2012
Q01 37 475	**2**	Cattle	Beef master	Heifer	Red and white	304	3/28/2012
Q01 37 476	**2**	Cattle	Beef master	Heifer	Red and white	266	3/28/2012
Q01 37 477	**2**	Cattle	Beef master	Heifer	Red and white	311	3/28/2012
Q01 37 478	**4**	Cattle	Beef master	Heifer	Red and white	303	3/28/2012
Q01 37 479	**1**	Cattle	Beef master	Heifer	Red and white	340	3/28/2012
Q01 37 480	**4**	Cattle	Beef master	Heifer	Red and white	276	3/28/2012

Q01 37 481	**4**	Cattle	Beef master	Heifer	Red and white	307	3/28/2012
Q01 37 482	**2**	Cattle	Beef master	Heifer	Red and white	294	3/28/2012
Q01 37 483	**1**	Cattle	Beef master	Heifer	Red and white	321	3/28/2012
Q01 37 484	**2**	Cattle	Beef master	Heifer	Red and white	308	3/28/2012
Q01 37 485	**2**	Cattle	Beef master	Heifer	Red and white	323	3/28/2012
Q01 37 486	**3**	Cattle	Beef Master	Heifer	Red and white	292	3/28/2012
Q01 37 487	**3**	Cattle	Beef master	Heifer	Red and white	265	3/28/2012
Q01 37 488	**3**	Cattle	Beef master	Heifer	Red and white	290	3/28/2012
Q01 37 489	**3**	Cattle	Beef master	Heifer	Red and white	301	3/28/2012
Q01 37 490	**3**	Cattle	Beef master	Heifer	Red and white	298	3/28/2012
Q01 37 491	**3**	Cattle	Beef master	Heifer	Red and white	309	3/28/2012
Q01 37 492	**1**	Cattle	Beef master	Heifer	Red and white	291	3/28/2012
Q01 37 493	**3**	Cattle	Beef master	Heifer	Red and white	303	3/28/2012
Q01 37 494	**3**	Cattle	Beef master	Heifer	Red and white	322	3/28/2012
Q01 37 495	**4**	Cattle	Beef master	Heifer	Red and white	285	3/28/2012
Q01 37 496	**3**	Cattle	Beef master	Heifer	Red and white	284	3/28/2012

Q01 37 497	**2**	Cattle	Beef master	Heifer	Red and white	322	3/28/2012
Q01 37 498	**4**	Cattle	Beef master	Heifer	Red and white	302	3/28/2012
Q01 37 499	**1**	Cattle	Beef master	Heifer	Red and white	317	3/28/2012
Q01 37 500	**3**	Cattle	Beef master	Heifer	Red and white	268	3/28/2012
Q01 37 501	**4**	Cattle	Beef master	Heifer	Red and white	284	3/28/2012
Q01 37 502	**4**	Cattle	Beef Master	Heifer	Red and white	310	3/28/2012
Q01 37 503	**4**	Cattle	Beef master	Heifer	Red and white	308	3/28/2012
Q01 37 504	**2**	Cattle	Beef master	Heifer	Red and white	288	3/28/2012
Q01 37 505	**2**	Cattle	Beef master	Heifer	Red and white	312	3/28/2012
Q01 37 506	**1**	Cattle	Beef master	Heifer	Red and white	274	3/28/2012
Q01 37 507	**2**	Cattle	Beef master	Heifer	Red and white	360	3/28/2012
Q01 37 508	**3**	Cattle	Beef master	Heifer	Red and white	272	3/28/2012
Q01 37 509	**3**	Cattle	Beef master	Heifer	Red and white	298	3/28/2012
Q01 37 510	**2**	Cattle	Beef master	Heifer	Red and white	281	3/28/2012
Q01 37 511	**1**	Cattle	Beef master	Heifer	Red and white	332	3/28/2012
Q01 37 512	**2**	Cattle	Beef master	Heifer	Red and white	338	3/28/2012

Q01 37 513	**2**	Cattle	Beef master	Heifer	Red and white	313	3/28/2012
Q01 37 514	**3**	Cattle	Beef master	Heifer	Red and white	292	3/28/2012
Q01 37 515	**4**	Cattle	Beef master	Heifer	Red and white	296	3/28/2012
Q01 37 516	**3**	Cattle	Beef master	Heifer	Red and white	287	3/28/2012
Q01 37 517	**2**	Cattle	Beef master	Heifer	Red and white	275	3/28/2012
Q01 37 518	**3**	Cattle	Beef Master	Heifer	Red and white	286	3/28/2012
Q01 37 519	**2**	Cattle	Beef master	Heifer	Red and white	261	3/28/2012
Q01 37 520	**4**	Cattle	Beef master	Heifer	Red and white	282	3/28/2012

Table 4 Allocation of experimental animals in different treatment groups

H.R.ID Tag	Ranking Group	Treatment Group	Animal type	Animal breed	Animal gender	Animal colour	Animal first weight
Q01 37 271	**2**	**FC**	Cattle	Bonsmara	Cow	Red	391
Q01 37 272	**3**	**F**	Cattle	Bonsmara	Cow	Red	486
Q01 37 273	**2**	**FC**	Cattle	Bonsmara	Cow	Red	471
Q01 37 274	**2**	**FC**	Cattle	Bonsmara	Cow	Red	529
Q01 37 275	**3**	**F**	Cattle	Bonsmara	Cow	Red	580
Q01 37 276	**1**	**S**	Cattle	Bonsmara	Cow	Red	597
Q01 37 277	**3**	**F**	Cattle	Bonsmara	Cow	Red	452
Q01 37 278	**2**	**FC**	Cattle	Bonsmara	Cow	Red	596
Q01 37 279	**1**	**S**	Cattle	Bonsmara	Cow	Red	392
Q01 37 280	**3**	**F**	Cattle	Bonsmara	Cow	Red	429
Q01 37 281	**1**	**S**	Cattle	Bonsmara	Cow	Red	428
Q01 37 282	**2**	**FC**	Cattle	Bonsmara	Cow	Red	545
Q01 37 283	**4**	**C**	Cattle	Bonsmara	Cow	Red	497
Q01 37 286	**1**	**S**	Cattle	Bonsmara	Cow	Red	385
Q01 37 287	**2**	**FC**	Cattle	Bonsmara	Cow	Red	424
Q01 37 288	**3**	**F**	Cattle	Bonsmara	Cow	Red	414
Q01 37 289	**3**	**F**	Cattle	Bonsmara	Cow	Red	427
Q01 37 290	**3**	**F**	Cattle	Bonsmara	Cow	Red	508
Q01 37 291	**1**	**S**	Cattle	Bonsmara	Cow	Red	569
Q01 37 292	**1**	**S**	Cattle	Bonsmara	Cow	Red	396
Q01 37 293	**2**	**FC**	Cattle	Bonsmara	Cow	Red	379
Q01 37 294	**4**	**C**	Cattle	Bonsmara	Cow	Red	440
Q01 37 295	**4**	**C**	Cattle	Bonsmara	Cow	Red	455
Q01 37 296	**3**	**F**	Cattle	Bonsmara	Cow	Red	540
Q01 37 297	**4**	**C**	Cattle	Bonsmara	Cow	Red	417
Q01 37 298	**2**	**FC**	Cattle	Bonsmara	Cow	Red	460
Q01 37 299	**1**	**S**	Cattle	Bonsmara	Cow	Red	508

QO1 37 300	**1**	**S**	Cattle	Bonsmara	Cow	Red	436
Q01 37 301	**3**	**F**	Cattle	Bonsmara	Cow	Red	440
Q01 37 302	**3**	**F**	Cattle	Bonsmara	Cow	Red	439
Q01 37 303	**1**	**S**	Cattle	Bonsmara	Cow	Red	433
Q01 37 304	**4**	**C**	Cattle	Bonsmara	Cow	Red	457
Q01 37 306	**2**	**FC**	Cattle	Bonsmara	Cow	Red	442
Q01 37 307	**1**	**S**	Cattle	Bonsmara	Cow	Red	460
Q01 37 308	**4**	**C**	Cattle	Bonsmara	Cow	Red	390
Q01 37 309	**4**	**C**	Cattle	Bonsmara	Cow	Red	468
Q01 37 310	**2**	**FC**	Cattle	Bonsmara	Cow	Red	461
Q01 37 311	**1**	**S**	Cattle	Bonsmara	Cow	Red	465
Q01 37 312	**1**	**S**	Cattle	Bonsmara	Cow	Red	469
Q01 37 313	**2**	**FC**	Cattle	Bonsmara	Cow	Red	455
Q01 37 314	**3**	**F**	Cattle	Bonsmara	Cow	Red	516
Q01 37 315	**3**	**F**	Cattle	Bonsmara	Cow	Red	445
Q01 37 316	**1**	**S**	Cattle	Bonsmara	Cow	Red	507
Q01 37 317	**3**	**F**	Cattle	Bonsmara	Cow	Red	514
Q01 37 318	**2**	**FC**	Cattle	Bonsmara	Cow	Red	437
Q01 37 319	**4**	**C**	Cattle	Bonsmara	Cow	Red	458
Q01 37 320	**2**	**FC**	Cattle	Bonsmara	Cow	Red	409
Q01 37 321	**2**	**FC**	Cattle	Bonsmara	Cow	Red	442
Q01 37 322	**4**	**C**	Cattle	Bonsmara	Cow	Red	441
Q01 37 323	**4**	**C**	Cattle	Bonsmara	Cow	Red	666
Q01 37 324	**1**	**S**	Cattle	Bonsmara	Cow	Red	455
Q01 37 325	**4**	**C**	Cattle	Bonsmara	Cow	Red	486
Q01 37 326	**3**	**F**	Cattle	Bonsmara	Cow	Red	489
Q01 37 327	**2**	**FC**	Cattle	Bonsmara	Cow	Red	429
Q01 37 328	**3**	**F**	Cattle	Bonsmara	Cow	Red	435
Q01 37 329	**3**	**F**	Cattle	Bonsmara	Cow	Red	523
Q01 37 330	**2**	**FC**	Cattle	Bonsmara	Cow	Red	417
Q01 37 331	**2**	**FC**	Cattle	Bonsmara	Cow	Red	446

Q01 37 332	**4**	**C**	Cattle	Bonsmara	Cow	Red	459
Q01 37 333	**4**	**C**	Cattle	Bonsmara	Cow	Red	472
Q01 37 334	**2**	**FC**	Cattle	Bonsmara	Cow	Red	370
Q01 37 335	**1**	**S**	Cattle	Bonsmara	Cow	Red	455
Q01 37 336	**4**	**C**	Cattle	Bonsmara	Cow	Red	517
Q01 37 337	**3**	**F**	Cattle	Bonsmara	Cow	Red	483
Q01 37 338	**2**	**FC**	Cattle	Bonsmara	Cow	Red	514
Q01 37 339	**2**	**FC**	Cattle	Bonsmara	Cow	Red	462
Q01 37 341	**3**	**F**	Cattle	Bonsmara	Cow	Red	453
Q01 37 342	**4**	**C**	Cattle	Bonsmara	Cow	Red	417
Q01 37 343	**1**	**S**	Cattle	Bonsmara	Cow	Red	536
Q01 37 344	**3**	**F**	Cattle	Bonsmara	Cow	Red	512
Q01 37 345	**3**	**F**	Cattle	Bonsmara	Cow	Red	541
Q01 37 346	**3**	**F**	Cattle	Bonsmara	Cow	Red	463
Q01 37 347	**2**	**FC**	Cattle	Bonsmara	Cow	Red	482
Q01 37 348	**1**	**S**	Cattle	Bonsmara	Cow	Red	463
Q01 37 349	**3**	**F**	Cattle	Bonsmara	Cow	Red	469
Q01 37 350	**3**	**F**	Cattle	Bonsmara	Cow	Red	387
Q01 37 351	**4**	**C**	Cattle	Bonsmara	Cow	Red	516
Q01 37 352	**4**	**C**	Cattle	Bonsmara	Cow	Red	559
Q01 37 353	**4**	**C**	Cattle	Bonsmara	Cow	Red	441
Q01 37 354	**4**	**C**	Cattle	Bonsmara	Cow	Red	371
Q01 37 355	**4**	**C**	Cattle	Bonsmara	Cow	Red	438
Q01 37 356	**4**	**C**	Cattle	Bonsmara	Cow	Red	370
Q01 37 357	**2**	**FC**	Cattle	Bonsmara	Cow	Red	540
Q01 37 358	**2**	**FC**	Cattle	Bonsmara	Cow	Red	406
Q01 37 359	**4**	**C**	Cattle	Bonsmara	Cow	Red	417
Q01 37 360	**1**	**S**	Cattle	Bonsmara	Cow	Red	502
Q01 37 361	**1**	**S**	Cattle	Bonsmara	Cow	Red	521
Q01 37 362	**2**	**FC**	Cattle	Bonsmara	Cow	Red	423
Q01 37 363	**1**	**S**	Cattle	Bonsmara	Cow	Red	501

Q01 37 364	**4**	**C**	Cattle	Bonsmara	Cow	Red	508
Q01 37 365	**2**	**FC**	Cattle	Bonsmara	Cow	Red	455
Q01 37 366	**3**	**F**	Cattle	Bonsmara	Cow	Red	489
Q01 37 367	**1**	**S**	Cattle	Bonsmara	Cow	Red	438
Q01 37 368	**1**	**S**	Cattle	Bonsmara	Cow	Red	561
Q01 37 369	**4**	**C**	Cattle	Bonsmara	Cow	Red	495
Q01 37 370	**1**	**S**	Cattle	Bonsmara	Cow	Red	390
Q01 37 371	**4**	**C**	Cattle	Bonsmara	Cow	Red	548
Q01 37 372	**1**	**S**	Cattle	Bonsmara	Cow	Red	504
Q01 37 373	**3**	**F**	Cattle	Bonsmara	Cow	Red	467
Q01 37 375	**1**	**S**	Cattle	Bonsmara	Cow	Red	422
Q01 37 376	**2**	**FC**	Cattle	Bonsmara	Cow	Red	486
Q01 37 377	**1**	**S**	Cattle	Bonsmara	Cow	Red	476
Q01 37 378	**3**	**F**	Cattle	Bonsmara	Cow	Red	438
Q01 37 379	**1**	**S**	Cattle	Bonsmara	Cow	Red	447
Q01 37 380	**3**	**F**	Cattle	Bonsmara	Cow	Red	370
Q01 37 381	**2**	**FC**	Cattle	Bonsmara	Cow	Red	511
Q01 37 382	**4**	**C**	Cattle	Bonsmara	Cow	Red	536
Q01 37 383	**4**	**C**	Cattle	Bonsmara	Cow	Red	481
Q01 37 387	**4**	**C**	Cattle	Beef master	Heifer	Red and white	289
Q01 37 388	**1**	**S**	Cattle	Beef master	Heifer	Red and white	271
Q01 37 389	**1**	**S**	Cattle	Beef master	Heifer	Red and white	329
Q01 37 390	**4**	**C**	Cattle	Beef master	Heifer	Red and white	368
Q01 37 391	**4**	**C**	Cattle	Beef master	Heifer	Red and white	269
Q01 37 392	**3**	**F**	Cattle	Beef master	Heifer	Red and white	304

Q01 37 393	**1**	**S**	Cattle	Beef master	Heifer	Red and white	290
Q01 37 394	**4**	**C**	Cattle	Beef master	Heifer	Red and white	315
Q01 37 395	**1**	**S**	Cattle	Beef master	Heifer	Red and white	308
Q01 37 396	**3**	**F**	Cattle	Beef master	Heifer	Red and white	317
Q01 37 397	**1**	**S**	Cattle	Beef master	Heifer	Red and white	290
Q01 37 398	**1**	**S**	Cattle	Beef master	Heifer	Red and white	313
Q01 37 399	**3**	**F**	Cattle	Beef master	Heifer	Red and white	326
Q01 37 400	**2**	**FC**	Cattle	Beef master	Heifer	Red and white	394
Q01 37 401	**2**	**FC**	Cattle	Beef master	Heifer	Red and white	275
Q01 37 402	**4**	**C**	Cattle	Beef master	Heifer	Red and white	264
Q01 37 403	**1**	**S**	Cattle	Beef master	Heifer	Red and white	354
Q01 37 404	**2**	**FC**	Cattle	Beef master	Heifer	Red and white	322
Q01 37 405	**3**	**F**	Cattle	Beef master	Heifer	Red and white	282
Q01 37 406	**4**	**C**	Cattle	Beef master	Heifer	Red and white	272
Q01 37 407	**4**	**C**	Cattle	Beef master	Heifer	Red and white	343
Q01 37 408	**1**	**S**	Cattle	Beef master	Heifer	Red and white	307

Q01 37 409	**4**	**C**	Cattle	Beef master	Heifer	Red and white	310
Q01 37 410	**1**	**S**	Cattle	Beef master	Heifer	Red and white	352
Q01 37 411	**1**	**S**	Cattle	Beef master	Heifer	Red and white	305
Q01 37 412	**1**	**S**	Cattle	Beefmaste r	Heifer	Red and white	297
Q01 37 413	**4**	**C**	Cattle	Beef master	Heifer	Red and white	319
Q01 37 414	**4**	**C**	Cattle	Beef master	Heifer	Red and white	306
Q01 37 415	**1**	**S**	Cattle	Beef master	Heifer	Red and white	268
Q01 37 416	**2**	**FC**	Cattle	Beef master	Heifer	Red and white	325
Q01 37 417	**3**	**F**	Cattle	Beef master	Heifer	Red and white	265
Q01 37 418	**2**	**FC**	Cattle	Beef master	Heifer	Red and white	316
Q01 37 419	**2**	**FC**	Cattle	Beef master	Heifer	Red and white	302
Q01 37 420	**1**	**S**	Cattle	Beef master	Heifer	Red and white	286
Q01 37 421	**2**	**FC**	Cattle	Beef master	Heifer	Red and white	334
Q01 37 422	**2**	**FC**	Cattle	Beef master	Heifer	Red and white	309
Q01 37 423	**2**	**FC**	Cattle	Beef master	Heifer	Red and white	272
Q01 37 424	**3**	**F**	Cattle	Beef master	Heifer	Red and white	293

Q01 37 425	**1**	**S**	Cattle	Beef master	Heifer	Red and white	286
Q01 37 426	**1**	**S**	Cattle	Beef master	Heifer	Red and white	288
Q01 37 427	**1**	**S**	Cattle	Beef master	Heifer	Red and white	298
Q01 37 428	**1**	**S**	Cattle	Beef master	Heifer	Red and white	278
Q01 37 429	**2**	**FC**	Cattle	Beef master	Heifer	Red and white	299
Q01 37 430	**2**	**FC**	Cattle	Beef master	Heifer	Red and white	299
Q01 37 431	**1**	**S**	Cattle	Beef master	Heifer	Red and white	336
Q01 37 432	**2**	**FC**	Cattle	Beef master	Heifer	Red and white	314
Q01 37 433	**2**	**FC**	Cattle	Beef master	Heifer	Red and white	305
Q01 37 434	**4**	**C**	Cattle	Beef master	Heifer	Red and white	293
Q01 37 435	**3**	**F**	Cattle	Beef master	Heifer	Red and white	303
Q01 37 436	**4**	**C**	Cattle	Beef master	Heifer	Red and white	292
Q01 37 438	**4**	**C**	Cattle	Beef master	Heifer	Red and white	275
Q01 37 439	**2**	**FC**	Cattle	Beef master	Heifer	Red and white	385
Q01 37 441	**1**	**S**	Cattle	Beef master	Heifer	Red and white	332
Q01 37 442	**1**	**S**	Cattle	Beef master	Heifer	Red and white	288

Q01 37 443	**4**	**C**	Cattle	Beef master	Heifer	Red and white	356
Q01 37 444	**2**	**FC**	Cattle	Beef master	Heifer	Red and white	282
Q01 37 445	**1**	**S**	Cattle	Beef master	Heifer	Red and white	287
Q01 37 446	**3**	**F**	Cattle	Beef master	Heifer	Red and white	313
Q01 37 447	**1**	**S**	Cattle	Beef master	Heifer	Red and white	319
Q01 37 448	**4**	**C**	Cattle	Beef master	Heifer	Red and white	334
Q01 37 449	**3**	**F**	Cattle	Beef master	Heifer	Red and white	326
Q01 37 450	**3**	**F**	Cattle	Beef master	Heifer	Red and white	294
Q01 37 451	**3**	**F**	Cattle	Beef master	Heifer	Red and white	283
Q01 37 452	**3**	**F**	Cattle	Beef master	Heifer	Red and white	328
Q01 37 453	**4**	**C**	Cattle	Beef master	Heifer	Red and white	304
Q01 37 454	**4**	**C**	Cattle	Beef master	Heifer	Red and white	280
Q01 37 455	**1**	**S**	Cattle	Beef master	Heifer	Red and white	279
Q01 37 456	**4**	**C**	Cattle	Beef master	Heifer	Red and white	296
Q01 37 457	**1**	**S**	Cattle	Beef master	Heifer	Red and white	355
Q01 37 458	**2**	**FC**	Cattle	Beef master	Heifer	Red and white	269

Q01 37 459	**4**	**C**	Cattle	Beef master	Heifer	Red and white	278
Q01 37 460	**4**	**C**	Cattle	Beef master	Heifer	Red and white	323
Q01 37 461	**3**	**F**	Cattle	Beef master	Heifer	Red and white	339
Q01 37 462	**3**	**F**	Cattle	Beef master	Heifer	Red and white	341
Q01 37 463	**1**	**S**	Cattle	Beef master	Heifer	Red and white	281
Q01 37 464	**2**	**FC**	Cattle	Beef master	Heifer	Red and white	319
Q01 37 465	**1**	**S**	Cattle	Beef master	Heifer	Red and white	286
Q01 37 466	**1**	**S**	Cattle	Beef master	Heifer	Red and white	279
Q01 37 467	**4**	**C**	Cattle	Beef master	Heifer	Red and white	303
Q01 37 468	**2**	**FC**	Cattle	Beef master	Heifer	Red and white	350
Q01 37 469	**4**	**C**	Cattle	Beef master	Heifer	Red and white	308
Q01 37 470	**1**	**S**	Cattle	Beef master	Heifer	Red and white	315
Q01 37 471	**4**	**C**	Cattle	Beef master	Heifer	Red and white	302
Q01 37 472	**2**	**FC**	Cattle	Beef master	Heifer	Red and white	263
Q01 37 473	**3**	**F**	Cattle	Beef master	Heifer	Red and white	288
Q01 37 474	**1**	**S**	Cattle	Beef master	Heifer	Red and white	267

Q01 37 475	**2**	**FC**	Cattle	Beef master	Heifer	Red and white	304
Q01 37 476	**2**	**FC**	Cattle	Beef master	Heifer	Red and white	266
Q01 37 477	**2**	**FC**	Cattle	Beef master	Heifer	Red and white	311
Q01 37 478	**4**	**C**	Cattle	Beef master	Heifer	Red and white	303
Q01 37 479	**1**	**S**	Cattle	Beef master	Heifer	Red and white	340
Q01 37 480	**4**	**C**	Cattle	Beef master	Heifer	Red and white	276
Q01 37 481	**4**	**C**	Cattle	Beef master	Heifer	Red and white	307
Q01 37 482	**2**	**FC**	Cattle	Beef master	Heifer	Red and white	294
Q01 37 483	**1**	**S**	Cattle	Beef master	Heifer	Red and white	321
Q01 37 484	**2**	**FC**	Cattle	Beef master	Heifer	Red and white	308
Q01 37 485	**2**	**FC**	Cattle	Beef master	Heifer	Red and white	323
Q01 37 486	**3**	**F**	Cattle	Beef master	Heifer	Red and white	292
Q01 37 487	**3**	**F**	Cattle	Beef master	Heifer	Red and white	265
Q01 37 488	**3**	**F**	Cattle	Beef master	Heifer	Red and white	290
Q01 37 489	**3**	**F**	Cattle	Beef master	Heifer	Red and white	301
Q01 37 490	**3**	**F**	Cattle	Beef master	Heifer	Red and white	298

Q01 37 491	**3**	**F**	Cattle	Beef master	Heifer	Red and white	309
Q01 37 492	**1**	**S**	Cattle	Beef master	Heifer	Red and white	291
Q01 37 493	**3**	**F**	Cattle	Beef master	Heifer	Red and white	303
Q01 37 494	**3**	**F**	Cattle	Beef master	Heifer	Red and white	322
Q01 37 495	**4**	**C**	Cattle	Beef master	Heifer	Red and white	285
Q01 37 496	**3**	**F**	Cattle	Beef master	Heifer	Red and white	284
Q01 37 497	**2**	**FC**	Cattle	Beef master	Heifer	Red and white	322
Q01 37 498	**4**	**C**	Cattle	Beef master	Heifer	Red and white	302
Q01 37 499	**1**	**S**	Cattle	Beef master	Heifer	Red and white	317
Q01 37 500	**3**	**F**	Cattle	Beef master	Heifer	Red and white	268
Q01 37 501	**4**	**C**	Cattle	Beef master	Heifer	Red and white	284
Q01 37 502	**4**	**C**	Cattle	Beef master	Heifer	Red and white	310
Q01 37 503	**4**	**C**	Cattle	Beef master	Heifer	Red and white	308
Q01 37 504	**2**	**FC**	Cattle	Beef master	Heifer	Red and white	288
Q01 37 505	**2**	**FC**	Cattle	Beef master	Heifer	Red and white	312
Q01 37 506	**1**	**S**	Cattle	Beef master	Heifer	Red and white	274

Q01 37 507	**2**	**FC**	Cattle	Beef master	Heifer	Red and white	360
Q01 37 508	**3**	**F**	Cattle	Beef master	Heifer	Red and white	272
Q01 37 509	**3**	**F**	Cattle	Beef master	Heifer	Red and white	298
Q01 37 510	**2**	**FC**	Cattle	Beef master	Heifer	Red and white	281
Q01 37 511	**1**	**S**	Cattle	Beef master	Heifer	Red and white	332
Q01 37 512	**2**	**FC**	Cattle	Beef master	Heifer	Red and white	338
Q01 37 513	**2**	**FC**	Cattle	Beef master	Heifer	Red and white	313
Q01 37 514	**3**	**F**	Cattle	Beef master	Heifer	Red and white	292
Q01 37 515	**4**	**C**	Cattle	Beef master	Heifer	Red and white	296
Q01 37 516	**3**	**F**	Cattle	Beef master	Heifer	Red and white	287
Q01 37 517	**2**	**FC**	Cattle	Beefmaster	Heifer	Red and white	275
Q01 37 518	**3**	**F**	Cattle	Beefmaster	Heifer	Red and white	286
Q01 37 519	**2**	**FC**	Cattle	Beefmaster	Heifer	Red and white	261
Q01 37 520	**4**	**C**	Cattle	Beef master	Heifer	Red and white	282

Table 5 Faecal sample analysis for the liver fluke eggs presence in cows in May

S Bester May 2012, Cows				No Nematodes were tested for in the first collection batch – miscommunication				
						Trematodes		
Key			Test tube & dung sample no		Qty	Dung analysis		HR ID Tag
No Faecal sample			1	X	1	Negative		Q01,37348
ID Number smutched			2	X	1	Negative		Q01,37333
			3	X	1	Negative		Q01,37357
			4	X	1	Positive	1	Q01,37321
			5	X	1	Negative		Q01,37345
			6	X	1	Positive	1	Q01,37351
			7	X	1	Positive	1	Q01,37317
			8	X	1	Positive	1	Q01,37371
			9	X	1	Negative		Q01,37307
			10	X	1	Positive	1	Q01,37361
			11	X	1	Negative		Q01,37382
			12	X	1	Positive	1	Q01,37363
			13	X	1	Negative		Q01,37386
			14	X	1			Q01,37342
			15	X	1	Negative		Q01,37314
			16	X	1	Negative		Q01,37381
			17	X	1	Negative		Q01,37315
			18	X	1	Positive	1	Q01,37354
			19	X	1	Negative		Q01,37274
			20	X	1	Negative		Q01,37282
			21	X	1	Negative		Q01,37375
			22	X	1	Positive	1	Q01,37316
			23	X	1	Positive	1	Q01,37334

			24	X	1	Positive	1	Q01,37288
			25	X	1	Positive	1	Q01,37338
			26	X	1	Negative		Q01,37294
			27	X	1	Positive		Q01,37359
			28	X	1	Negative		Q01,37281
			29	X	1	Positive	1	Q01,37364
			30	X	1	Negative		Q01,37308
			31	X	1	Positive	1	Q01,37291
			32	X	1			Q01,37276
			33	X	1	Positive	1	Q01,37300
			34	X	1			Q01,37277
			35	X	1	Negative		Q01,37344
			36	X	1	Positive	1	Q01,37296
			37	X	1	Positive	1	Q01,37293
			38	X	1	Positive	1	Q01,37278
			39	X	1	Negative		Q01,37377
			40	X	1	Negative		Q01,37346
			41	X	1	Negative		Q01,37373
			42	X	1	Negative		Q01,37353
			43	X	1	Negative		Q01,37370
			44	X	1	Negative		Q01,37286
			45	X	1	Negative		Q01,37295
			46	X	1	Negative		Q01,37275
			47	X	1	Negative		Q01,37323
			48	X	1	Negative		Q01,37365
			49	X	1	Negative		Q01,37360
			50	X	1	Negative		Q01,37273
			51	X	1	Negative		Q01,37369
			52	X	1	Positive	1	Q01,37355
			54	X	1	Negative		Q01,37280
			55	X	1	Negative		Q01,37325

			55	X	1	Negative		Q01,37383
			56	X	1	Positive	1	Q01,37283
			57	X	1	Positive	1	Q01,37380
			58	X	1	Positive	1	Q01,37318
			59	X	1	Positive	1	Q01,37327
			60	X	1	Negative		Q01,37384
			61	X	1	Negative		Q01,37320
			62	X	1	Negative		Q01,37362
			63	X	1	Positive	1	Q01,37349
			64	X	1	Positive	1	Q01,37297
			65	X	1	Negative		Q01,37303
			66	X	1	Negative		Q01,37289
			67	X	1	Negative		Q01,37385
			68	X	1	Negative		Q01,37310
			69	X	1	Negative		Q01,37313
			70	X	1			Q01,37299
			71	X	1	Negative		Q01,37379
			72	X	1	Negative		Q01,37301
			73	X	1	Negative		Q01,37306
			74	X	1	Negative		Q01,37343
			75	X	1			Q01,37319
			76	X	1	Negative		Q01,37368
			77	X	1	Negative		Q01,37328
			78	X	1	Negative		Q01,37271
			79	X	1	Positive		Q01,37322
			80	X	1	Negative		Q01,37335
			81	X	1	Negative		Q01,37279
			82	X	1			Q01,37292
			83	X	1	Negative		Q01,37272
			84	X	1	Negative		Q01,37324
			85	X	1	Negative		Q01,37337

			86	X	1	Positive	1	Q01,37366
			87	X	1	Negative		Q01,37326
			88	X	1	Positive	1	Q01,37376
			89	X	1			Q01,37309
			90	X	1	Negative		Q01,37329
			91	X	1	Positive	1	Q01,37372
			92	X	1	Negative		Q01,37358
			93	X	1	Negative		Q01,37287
			94	X	1	Positive	1	Q01,37347
			95	X	1	Negative		Q01,37332
			96	X	1	Negative		Q01,37311
			97	X	1	Negative		Q01,37290
			98	X	1	Negative		Q01,37312
			99	X	1	Negative		Q01,37341
			100	X	1	Positive	1	Q01,37378
			101	X	1	Negative		Q01,37302
			102	X	1			Q01,37331
			103	X	1	Negative		Q01,37304
			104	X	1	Negative		Q01,37339
			105	X	1	Negative		Q01,37330
			106	X	1	Negative		Q01,37356
			107	X	1	Negative		Q01,37352
			108	X	1	Negative		Not on list
			109	X	1			Q01,37298
			110	X	1	Negative		Q01,37350
			111	X	1	Negative		Q01,37336
								Q01,37284
								Q01,37285
								Q01,37305
								Q01,37340
								Q01,37367

								Q01,37374
			Total epg		**111**	Total infected	**29**	**#REF!**
			Average epg /: 0 heifer					

Table 6 Serological test results (ELISA) of Liver Fluke and Nematodes (May 2012)

Species	**Specifications**	**Test Tube**	**ELISA**	**Value**	**Value**	**Corrected**	**S/P**	Result	
sample		ID	plate Nº	1	2	O D40	Ratio		
Bovine	Heifers	Negative control		0	0.026	0.026	N/A	N/A	Mean corrected positive control value
		Positive control		0.038	0.914	0.876	N/A	N/A	
		Positive control		0.001	0.769	0.768	N/A	N/A	0.822
		103	1	0.128	1.79	1.662	202.19	Positive	
		111		0.059	1.39	1.331	161.92	Positive	
		101		1.003	0.993	-0.01	-1.22	Negative	
		105		0.439	0.655	0.216	26.28	Negative	
		109		0.232	1.771	1.539	187.23	Positive	
		104		0.32	1.807	1.487	180.90	Positive	
		107		0.203	0.56	0.357	43.43	Positive	
		102		0.674	1.579	0.905	110.10	Positive	
		108		0.154	1.783	1.629	198.18	Positive	
		110		0.406	1.49	1.084	131.87	Positive	

		106		0.232	1.783	1.551	188.69	Positive		
		100		0.347	1.363	1.016	123.60	Positive		
		90		0.073	1.197	1.124	136.74	Positive		
		80		0.39	0.866	0.476	57.91	Positive		
		70		0.483	0.918	0.435	52.92	Positive		
		60		0.188	1.863	1.675	203.77	Positive		
		59		0.781	1.963	1.182	143.80	Positive		
		99		0.186	1.47	1.284	156.20	Positive		
		69		0.406	0.941	0.535	65.09	Positive		
		89		0.127	1.497	1.37	166.67	Positive		

Species	**Specifications**	**Test Tube**	**ELISA**	**Value**	**Value**	**Corrected**	**S/P**	Result		
		79		0.003	0.592	0.589	71.65	Positive		
		58		0.108	1.548	1.44	175.18	Positive		
		68		0.5	0.464	-0.036	-4.38	Negative		
		78		0.126	1.528	1.402	170.56	Positive		
		98		0.297	2.165	1.868	227.25	Positive		
		67		1.037	2.02	0.983	119.59	Positive		
		77		0.997	1.691	0.694	84.43	Positive		
		87		0.071	0.207	0.136	16.55	Negative		
		97		0.718	1.725	1.007	122.51	Positive		
		88		0.188	1.307	1.119	136.13	Positive		
		86		0.566	1.491	0.925	112.53	Positive		
		76		1.69	1.80	0.119	14.48	Negati		

					9			ve		
		66		0.213	1.784	1.571	191.12	Positive		
		56		0.189	0.564	0.375	45.62	Positive		
		57		0.159	1.012	0.853	103.77	Positive		
		96		0.267	1.83	1.563	190.15	Positive		
		55		0.139	1.774	1.635	198.91	Positive		
		65		0.061	1.725	1.664	202.43	Positive		
		75		0	0.403	1.765	1.362	165.69	Positive Mean corrected positive control value	
		Negative control		0	0	0	N/A	N/A		
		Positive control		0.023	0.705	0.682	N/A	N/A	0.7035	
		Positive control	0.0232	0.748	0.725	N/A	N/A	0.7035		
		85		0.549	1.459	0.91	129.35	Positive		
		84		0.141	1.596	1.455	206.82	Positive		
		74		0.026	0.667	0.641	91.12	Positive		

Species	**Specifications**	**Test Tube**	**ELISA**	**Value**	**Value**	**Corrected**	**S/P**	Result		
		64		0.923	1.554	0.631	89.69	Positive		
		54		0.763	1.374	0.611	86.85	Positive		
		95		0.014	0.085	0.071	10.09	Negative		
		94		0.045	1.381	1.336	189.91	Positive		
		93		0.073	1.648	1.575	223.88	Positive		
		83		0.712	1.722	1.01	143.57	Positive		
		73		0.112	1.17	1.058	150.39	Positive		

		53		0.107	1.334	1.227	174.41	Positive		
		92		0.08	0.049	-0.031	-4.41	Negative		
		91		0.074	1.46	1.386	197.01	Positive		
		82		0.026	0.143	0.117	16.63	Negative		
		81		0.104	1.221	1.117	158.78	Positive		
		72		0.228	1.747	1.519	215.92	Positive		
		71		0.015	0.927	0.912	129.64	Positive		
		62		0.02	0.437	0.417	59.28	Positive		
		61		0	1.411	1.411	200.57	Positive		
		52		0	0.782	0.782	111.16	Positive		
		51		0.43	1.73	1.3	184.79	Positive		
		50		0.011	0.824	0.813	115.57	Positive		
		40		0	1.53	1.53	217.48	Positive		
		30		0	0.594	0.594	84.43	Positive		
		20		0	1.593	1.593	226.44	Positive		
		10		0.068	1.503	1.435	203.98	Positive		
		9		0.17	1.53	1.36	193.32	Positive		
		19		0	0.307	0.307	43.64	Positive		

Specs	**Specifications**	**Test Tube**	**ELISA**	**Value**	**Value**	**Corrected**	**S/P**	Result		
		29		0.662	1.592	0.93	132.20	Positive		
		39		0.259	0.646	0.387	55.01	Positive		
		49		1.113	1.575	0.462	65.67	Positive		
		47		0	0.044	0.044	6.25	Negative		
		47		0.044	1.631	1.587	225.59	Positive		
		46		0.252	1.75	1.498	212.94	Positive		
		38		0.099	1.496	1.397	198.58	Positive		
		37		0.056	1.547	1.491	211.94	Positive		
		28		0.4	1.632	1.232	175.12	Positive		
		27		0.198	1.434	1.236	175.69	Positive		
		18		0.061	1.421	1.36	193.32	Positive		
		17		0.061	0.102	0.041	5.83	Negative		
		8		0.013	1.773	1.76	250.18	Positive		
		36		0.045	1.759	1.714	243.64	Positive		
		26		0.008	1.769	1.761	250.32	Positive		
		16		0.008	1.431	1.423	202.27	Positive		
		6		0.728	1.776	1.048	148.97	Positive		
		Negative control		0	0	0	N/A	N/A	Mean corrected positive control value	
		Positive control		0.049	0.796	0.747	N/A	N/A		
		Positive control		0.061	0.624	0.563	N/A	N/A	0.655	
		7	3	0.091	1.551	1.46	222.90	Positive		
		5		0.028	0.16	0.132	20.15	Negative		
		15		0.069	0.147	0.078	11.91	Negative		

		25		0.195	0.48	0.285	43.51	Positive		
		35		0.055	0.486	0.431	65.80	Positive		

Species	Specifications	Test Tube	ELISA	Value	Value	Corrected	S/P	Result		
		45		0.395	1.826	1.431	218.47	Positive		
		44		0.276	0.641	0.365	55.73	Positive		
		34		0.117	0.152	0.035	5.34	Negative		
		24		0.032	1.602	1.57	239.69	Positive		
		14		0.056	0.212	0.156	23.82	Negative		
		4		1.058	1.732	0.674	102.90	Positive		
		43		0.128	1.014	0.886	135.27	Positive		
		3		0.034	0.195	0.161	24.58	Negative		
		13		0.136	0.224	0.088	13.44	Negative		
		23		0.257	1.596	1.339	204.43	Positive		
		33		0.119	1.747	1.628	248.55	Positive		
		42		0.405	1.796	1.391	212.37	Positive		
		32		0.695	0.527	-0.168	-25.65	Negative		
		22		0	0.831	0.831	126.87	Positive		
		12		1.11	1.434	0.324	49.47	Positive		
		2		0.311	1.894	1.583	241.68	Positive		
		1		0.013	1.146	1.133	172.98	Positive		
		11		0.056	0.308	0.252	38.47	Positive		
		21		0.03	0.121	0.091	13.89	Negative		
		31		0.2	1.723	1.523	232.5	Positive		

							2			
		41		0.103	0.123	0.02	3.05	Negative		
		79		0	0	0	0.00	Negative		
		80		0.344	0.096	-0.248	-37.86	Negative		
		78		0.473	0.358	-0.115	-17.56	Negative		
		99		0.013	0.39	0.377	57.56	Positive		

Species	**Specifications**	**Test Tube**	**ELISA**	**Value**	**Value**	**Corrected**	**S/P**	Result		
		88		0	0.069	0.069	10.53	Negative		
		31		0.039	0.051	0.012	1.83	Negative		
		68		0.011	0.026	0.015	2.29	Negative		
		45		0	0.028	0.028	4.27	Negative		
		117		0.063	0.336	0.273	41.68	Positive		
		128		0.101	0.208	0.107	16.34	Negative		
		42		0.217	0.274	0.057	8.70	Negative		
	Cows	63		0.151	1.912	1.761	268.85	Positive		
		29		0.257	0.178	-0.079	-12.06	Negative		
		78		0.016	0.045	0.029	4.43	Negative		
		69		0.019	0.037	0.018	2.75	Negative		
		90		0.006	0	-0.006	-0.92	Negative		
		86		0.017	0.048	0.031	4.73	Negative		
		120		0.25	0	-0.25	-38.17	Negative		
		100		0.014	0.135	0.121	18.47	Negative		
		Negative control		0	0	0	N/A	N/A	Mean	

		Positive control		0.128	0.84	0.712	N/A	N/A	corrected positive control value	
		Positive control		0.103	0.854	0.751	N/A	N/A	0.7315	
		108	4	0.16	0.078	-0.082	-11.21	Negative		
		62		0.253	0.234	-0.019	-2.60	Negative		
		118		0.148	1.632	1.484	202.87	Positive		
		119		0.244	0.172	-0.072	-9.84	Negative		
		109		0.261	0.19	-0.071	-9.71	Negative		

Species	**Specifications**	**Test Tube**	**ELISA**	**Value**	**Value**	**Corrected**	**S/P**	Result		
		97		0.119	1.241	1.122	153.38	Positive		
		129		0.17	0.198	0.028	3.83	Negative		
		91		0.068	0.095	0.027	3.69	Negative		
		110		0.082	0.094	0.012	1.64	Negative		
		52		0.112	1.467	1.355	185.24	Positive		
		59		0.075	0.041	-0.034	-4.65	Negative		
		67		0.114	0.117	0.003	0.41	Negative		
		127		0.12	0.16	0.04	5.47	Negative		
		51		0.413	0.042	-0.371	-50.72	Negative		
		89		0.183	0.248	0.065	8.89	Negative		
		39		0.303	0.149	-0.154	-21.05	Negative		
		24		0.119	0.109	-0.01	-1.37	Negative		

		8		0.241	0.306	0.065	8.89	Negative		
		98		0.176	0.215	0.039	5.33	Negative		
		60		0.572	0.637	0.065	8.89	Negative		
		87		0.43	0.38	-0.05	-6.84	Negative		
		70		0	0	0	0.00	Negative		
		7		0.081	0.018	-0.063	-8.61	Negative		
		23		0.147	0.194	0.047	6.43	Negative		
		26		0	0	0	0.00	Negative		

Species	**Specifications**	**Test Tube**	**ELISA**	**Value**	**Value**	**Corrected**	**S/P**	Result		
		36		0.066	0	-0.066	-9.02	Negative		
		48		0.112	0.17	0.058	7.93	Negative		
		1		0.136	0.296	0.16	21.87	Negative		
		22		0.176	0.366	0.19	25.97	Negative		
		9		0.177	0.28	0.103	14.08	Negative		
		47		0.011	0.229	0.218	29.80	Negative		
		38		0.07	0.045	-0.025	-3.42	Negative		
		37		0.066	0	-0.066	-9.02	Negative		
		19		0.673	0.712	0.039	5.33	Negative		
		12		0	0.015	0.015	2.05	Negative		
		21		0.116	0.091	-0.025	-3.42	Negative		
		28		0.392	0.393	0.001	0.14	Negative		
		4		0.09	1.905	1.815	248.1	Positive		

							2			
		10		0.049	0.119	0.07	9.57	Negativ e		
		27		0.049	0.136	0.087	11.89	Negativ e		
		2		0	0.197	0.197	26.93	Negativ e		
		11		0.102	0.083	-0.019	-2.60	Negativ e		
		30		0.229	0.233	0.004	0.55	Negativ e		
		96		0.395	0.234	-0.161	- 22.01	Negativ e		
		14		0.462	0.266	-0.196	- 26.79	Negativ e		
		Negative control		0	0	0	N/A	**N/A**	Mean correcte d positive control value	

Species	**Specifications**	**Test Tube**	**ELISA**	**Value**	**Value**	**Corrected**	**S/P**	Result		
		Positive control	0.068	0.8	0.732	N/A	**N/A**			
		Positive control	0.042	0.8	0.758	N/A	**N/A**	0.745		
		17	5	0.307	0.486	0.179	24.03	Negative		
		5		0.048	0.033	-0.015	-2.01	Negative		
		13		0.368	0.476	0.108	14.50	Negative		
		20		0.003	0.126	0.123	16.51	Negative		
		49		0	0	0	0.00	Negative		
		46		0.177	0	-0.177	-23.76	Negative		
		43		0.187	0.002	-0.185	-24.83	Negative		
		18		0.502	0.566	0.064	8.59	Negative		
		3		0.207	0	-0.207	-27.79	Negative		
		15		0.205	0.127	-0.078	-10.47	Negative		
		50		0.288	0.077	-0.211	-28.32	Negative		
		44		0.13	0	-0.13	-17.45	Negative		
		6		0.212	0.025	-0.187	-25.10	Negative		
		25		0.004	0.059	0.055	7.38	Negative		

		40		0	0	0	0.00	Negative		
		33		0.212	0.248	0.036	4.83	Negative		
		34		0.064	0.239	0.175	23.49	Negative		
		41		0	0.068	0.068	9.13	Negative		
		32		0	0.098	0.098	13.15	Negative		
		35		0	0	0	0.00	Negative		
		16		0.284	0.4	0.116	15.57	Negative		

Species	**Specifications**	**Test Tube**	**ELISA**	**Value**	**Value**	**Corrected**	**S/P**	Result		
		58		0.096	1.724	1.628	218.52	Positive		
		76		0.71	0.767	0.057	7.65	Negative		
		66		0.193	0.199	0.006	0.81	Negative		
		57		0.058	0.043	-0.015	-2.01	Negative		
		85		1.574	1.735	0.161	21.61	Negative		
		64		0	0	0	0.00	Negative		
		55		0	0	0	0.00	Negative		
		65		0.27	0.259	-0.011	-1.48	Negative		
		56		0	0	0	0.00	Negative		
		75		0.07	0.022	-0.048	-6.44	Negative		
		95		0	0.027	0.027	3.62	Negative		
		84		0.024	0	-0.024	-3.22	Negative		
		74		0.042	0.08	0.038	5.10	Negative		
		94		0.101	0.035	-0.066	-8.86	Negative		
		73		0.108	0.177	0.069	9.26	Negative		
		63		0.253	0.263	0.01	1.34	Negative		
		82		0.183	0.173	-0.01	-1.34	Negative		
		92		0	0	0	0.00	Negative		
		93		0.06	0.211	0.151	20.27	Negative		
		83		0	0.062	0.062	8.32	Negative		
		54		0	0.017	0.017	2.28	Negative		
		53		0	0.016	0.016	2.15	Negative		
		72		0	0.235	0.235	31.54	Positive		

Species	**Specifications**	**Test Tube**	**ELISA**	**Value**	**Value**	**Corrected**	**S/P**	Result		
		61		0.279	0.3	0.021	2.82	Negative		
		Negative control	0	0.041	0.041	N/A	**N/A**	Mean corrected positive control value		
		Positive control	0.064	0.666	0.602	N/A	**N/A**			
		Positive control	0.102	0.824	0.722	N/A	**N/A**	0.662		
		71	6	0.062	0.107	0.045	6.80	Negative		
		81		0.135	0.088	-0.047	-7.10	Negative		
		130		0.028	0.076	0.048	7.25	Negative		
		116		0.515	0.557	0.042	6.34	Negative		
		125		0.067	0.039	-0.028	-4.23	Negative		
		107		0	0.052	0.052	7.85	Negative		
		133		0.07	0.163	0.093	14.05	Negative		
		122		0.019	0.068	0.049	7.40	Negative		
		124		0.359	0.426	0.067	10.12	Negative		
		106		0.127	0.331	0.204	30.82	Positive		
		132		0.106	0.141	0.035	5.29	Negative		
		123		0.214	0.351	0.137	20.69	Negative		
		134		0	0.001	0.001	0.15	Negative		
		121		0.095	0.121	0.026	3.93	Negative		
		131		0.062	0.1	0.038	5.74	Negative		
		115		0.044	0.024	-0.02	-3.02	Negative		
		101		0.084	0.112	0.028	4.23	Negative		

		126		1.25 4	1.24 4	-0.01	- 1.51	Negative		

Species	**Specifications**	**Test Tube**	**ELISA**	**Value**	**Value**	**Corrected**	**S/P**	Result		
		104		0.082	0.326	0.244	36.86	Positive		
		114		0.042	1.657	1.615	243.96	Positive		
		105		0.246	0.336	0.09	13.60	Negative		
		112		0.233	0.217	-0.016	-2.42	Negative		
		103		0.029	0	-0.029	-4.38	Negative		
		102		0.147	0.005	-0.142	-21.45	Negative		
		111		0.059	0.087	0.028	4.23	Negative		
		113		0.065	0.18	0.115	17.37	Negative		
I If the SP Ratio is higher than 30% **the animal is considered positive**										

			Pos % of heifer samples		####	

Table 7 Faecal sample analysis for the liver fluke eggs presence in cows in July 2012

HR.ID Tag	Treatment groups	e.p.g	Positive results	Trematodes	Positive results
Q 01, 37387	C				
Q 01, 37388	S	250	1	Negative	0
Q 01, 37389	S				
Q 01, 37390	C				
Q 01, 37391	C				
Q 01, 37392	F	300	1	Negative	0
Q 01, 37393	S				
Q 01, 37394	C				
Q 01, 37395	S				
Q 01, 37396	F				
Q 01, 37397	S				
Q 01, 37398	S				
Q 01, 37399	F				
Q 01, 37400	FC				
Q 01, 37401	FC				
Q 01, 37402	C				
Q 01, 37403	S	100	1	Negative	
Q 01, 37404	S				
Q 01, 37405	F				
Q 01, 37406	C				
Q 01, 37407	C	150	1	Negative	0
Q 01, 37408	S	100	1	Negative	0
Q 01, 37409	C				
Q 01, 37410	F				
Q 01, 37411	F				
Q 01, 37412	F				
Q 01, 37413	C				
Q 01, 37414	C				
Q 01, 37415	S	300	1	Negative	0
Q 01, 37416	FC				
Q 01,37417	F				
Q 01, 37418	FC				
Q 01, 37419	FC				
Q 01, 37420	S				
Q 01, 37421	FC				
Q 01, 37422	FC	100	1	Negative	0
Q 01, 37423	FC				
Q 01, 37424	FC				
Q 01, 37425	S				
Q 01, 37426	S				
Q 01, 37427	S				
Q 01, 37428	S				
Q 01, 37429	FC	100	1	Negative	0

Q 01, 37430	FC				
Q 01, 37431	S				
Q 01, 37432	FC				
Q 01, 37433	FC				
Q 01, 37434	C				
Q 01, 37435	F	50	1	Negative	0
Q 01, 37436	C				
Q 01, 37437					
Q 01, 37438	C				
Q 01, 37439	FC				
Q 01, 37440					
Q 01, 37441	S				
Q 01, 37442	S				
Q 01, 37443	C				
Q 01, 37444	FC				
Q 01, 37445	S				
Q 01, 37446	F				
Q 01, 37447	S				
Q 01, 37448	C				
Q 01, 37449	F				
Q 01, 37450	F				
Q 01, 37451	F				
Q 01, 37452	F				
Q 01, 37453	C				
Q 01, 37454	C				
Q 01, 37455	S				
Q 01, 37456	C	350	1	Negative	0
Q 01, 37457	F	50	1	Negative	0
Q 01, 37458	FC				
Q 01, 37459	C				
Q 01, 37460	C				
Q 01, 37461	F				
Q 01, 37462	F				
Q 01, 37463	S				
Q 01, 37464	FC				
Q 01, 37465	S				
Q 01, 37466	S				
Q 01, 37467	C				
Q 01, 37468	FC				
Q 01, 37469	C				
Q 01, 37470	S				
Q 01, 37471	C				
Q 01, 37472	FC				
Q 01, 37473	F				
Q 01, 37474	S				
Q 01, 37475	FC				
Q 01, 37476	FC				

Q 01, 37477	FC				
Q 01, 37478	C				
Q 01, 37479	S				
Q 01, 37480	C				
Q 01, 37481	C				
Q 01, 37482	FC				
Q 01, 37483	S	250	1	Negative	0
Q 01, 37484	FC				
Q 01, 37485	FC				
Q 01, 37486	F				
Q 01, 37487	F				
Q 01, 37488	F				
Q 01, 37489	S				
Q 01, 37490	F				
Q 01, 37491	F	50	1	Negative	0
Q 01, 37492	S				
Q 01, 37493	F	600	1	Negative	0
Q 01, 37494	F				
Q 01, 37495	C				
Q01, 37496	F				
Q01, 37497	FC				
Q01, 37498	C				
Q01, 37499	S				
Q01, 37500	F				
Q01, 37501	C				
Q01, 37502	C				
Q01, 37503	C				
Q01,37504	FC				
Q01,37505	FC				
Q01,37506	S				
Q01, 37507	FC				
Q01, 37508	FC				
Q01, 37509	F				
Q01, 37510	FC				
Q01, 37511	S				
Q01, 37512	FC				
Q01, 37513	FC				
Q01, 37514	F	400	1	Negative	0
Q01, 37515	C				
Q01, 37516	F				
Q01, 37517	FC				
Q01, 37518	F	300	1	Negative	0
Q01, 37519	FC				
Q01, 37520	C				

Table 8 Individual animal weight based dosage calculation tables for Cow herd

HR Tag Number	Animal Status	Animal Type	Animal Gender	Standing Days	Weight	Ranking	Treatment Grp.	Treat Vol. ml	Applied Dose Vol ml.	Qty
Q01-37276	Open	Cattle	Cow	468	570	**1**	**S**	**57**	**60**	1
Q01-37279	Open	Cattle	Cow	468	396	**1**	**S**	**39.6**	**40**	1
Q01-37281	Open	Cattle	Cow	468	330	**1**	**S**	**33**	**35**	1
Q01-37286	Open	Cattle	Cow	468	367	**1**	**S**	**36.7**	**40**	1
Q01-37291	Open	Cattle	Cow	468	547	**1**	**S**	**54.7**	**55**	1
Q01-37292	Open	Cattle	Cow	468	367	**1**	**S**	**36.7**	**40**	1
Q01-37299	Open	Cattle	Cow	468	496	**1**	**S**	**49.6**	**50**	1
Q01-37300	Open	Cattle	Cow	468	406	**1**	**S**	**40.6**	**45**	1
Q01-37303	Open	Cattle	Cow	468	380	**1**	**S**	**38**	**40**	1
Q01-37307	Open	Cattle	Cow	468	435	**1**	**S**	**43.5**	**45**	1
Q01-37311	Open	Cattle	Cow	468	455	**1**	**S**	**45.5**	**50**	1
Q01-37312	Open	Cattle	Cow	468	445	**1**	**S**	**44.5**	**45**	1
Q01-37316	Open	Cattle	Cow	468	487	**1**	**S**	**48.7**	**50**	1
Q01-37324	Open	Cattle	Cow	468	445	**1**	**S**	**44.5**	**50**	1
Q01-37335	Open	Cattle	Cow	468	435	**1**	**S**	**43.5**	**45**	1
Q01-37343	Open	Cattle	Cow	468	415	**1**	**S**	**41.5**	**45**	1
Q01-37348	Open	Cattle	Cow	468	452	**1**	**S**	**45.2**	**50**	1
Q01-37360	Open	Cattle	Cow	468	469	**1**	**S**	**46.9**	**50**	1
Q01-37361	Open	Cattle	Cow	468	415	**1**	**S**	**41.5**	**45**	1
Q01-37363	Open	Cattle	Cow	468	406	**1**	**S**	**40.6**	**45**	1
Q01-37367	Open	Cattle	Cow	468	422	**1**	**S**	**42.2**	**45**	1
Q01-37368	Open	Cattle	Cow	468	423	**1**	**S**	**42.3**	**45**	1
Q01-37370	Open	Cattle	Cow	468	376	**1**	**S**	**37.6**	**40**	1
Q01-37372	Open	Cattle	Cow	468	469	**1**	**S**	**46.9**	**50**	1
Q01-37375	Open	Cattle	Cow	468	430	**1**	**S**	**43**	**45**	1
Q01-37377	Open	Cattle	Cow	468	396	**1**	**S**	**39.6**	**40**	1
Q01-37379	Open	Cattle	Cow	468	429	**1**	**S**	**42.9**	**45**	1
Q01-37272	Open	Cattle	Cow	468	458	**2**	**F**	**45.8**	**50**	1
Q01-37275	Open	Cattle	Cow	468	571	**2**	**F**	**57.1**	**60**	1
Q01-37277	Open	Cattle	Cow	468	435	**2**	**F**	**43.5**	**45**	1
Q01-37280	Open	Cattle	Cow	468	399	**2**	**F**	**39.9**	**40**	1
Q01-37288	Open	Cattle	Cow	468	415	**2**	**F**	**41.5**	**45**	1
Q01-37289	Open	Cattle	Cow	468	416	**2**	**F**	**41.6**	**45**	1
Q01-37290	Open	Cattle	Cow	468	484	**2**	**F**	**48.4**	**50**	1
Q01-37296	Open	Cattle	Cow	468	529	**2**	**F**	**52.9**	**55**	1
Q01-37301	Open	Cattle	Cow	468	431	**2**	**F**	**43.1**	**45**	1
Q01-37302	Open	Cattle	Cow	468	429	**2**	**F**	**42.9**	**45**	1
Q01-37314	Open	Cattle	Cow	468	407	**2**	**F**	**40.7**	**45**	1
Q01-37315	Open	Cattle	Cow	468	355	**2**	**F**	**35.5**	**40**	1

Q01-37317	Open	Cattle	Cow	468	446	**2**	**F**	**44.6**	**45**	1
Q01-37326	Open	Cattle	Cow	468	421	**2**	**F**	**42.1**	**45**	1
Q01-37328	Open	Cattle	Cow	468	370	**2**	**F**	**37**	**40**	1
Q01-37329	Open	Cattle	Cow	468	436	**2**	**F**	**43.6**	**45**	1
Q01-37337	Open	Cattle	Cow	468	375	**2**	**F**	**37.5**	**40**	1
Q01-37341	Open	Cattle	Cow	468	442	**2**	**F**	**44.2**	**45**	1
Q01-37344	Open	Cattle	Cow	468	502	**2**	**F**	**50.2**	**55**	1
Q01-37345	Open	Cattle	Cow	468	424	**2**	**F**	**42.4**	**45**	1
Q01-37346	Open	Cattle	Cow	468	405	**2**	**F**	**40.5**	**45**	1
Q01-37349	Open	Cattle	Cow	468	465	**2**	**F**	**46.5**	**50**	1
Q01-37350	Open	Cattle	Cow	468	381	**2**	**F**	**38.1**	**40**	1
Q01-37366	Open	Cattle	Cow	468	394	**2**	**F**	**39.4**	**40**	1
Q01-37373	Open	Cattle	Cow	468	470	**2**	**F**	**47**	**50**	1
Q01-37378	Open	Cattle	Cow	468	451	**2**	**F**	**45.1**	**50**	1
Q01-37380	Open	Cattle	Cow	468	342	**2**	**F**	**34.2**	**35**	1
Q01-37271	Open	Cattle	Cow	468	372	**3**	**FC**	**37.2**	**40**	1
Q01-37273	Open	Cattle	Cow	468	425	**3**	**FC**	**42.5**	**45**	1
Q01-37274	Open	Cattle	Cow	468	480	**3**	**FC**	**48**	**50**	1
Q01-37278	Open	Cattle	Cow	468	588	**3**	**FC**	**58.8**	**60**	1
Q01-37282	Open	Cattle	Cow	468	520	**3**	**FC**	**52**	**55**	1
Q01-37287	Open	Cattle	Cow	468	421	**3**	**FC**	**42.1**	**45**	1
Q01-37293	Open	Cattle	Cow	468	375	**3**	**FC**	**37.5**	**40**	1
Q01-37298	Open	Cattle	Cow	468	451	**3**	**FC**	**45.1**	**50**	1
Q01-37306	Open	Cattle	Cow	468	431	**3**	**FC**	**43.1**	**45**	1
Q01-37310	Open	Cattle	Cow	468	442	**3**	**FC**	**44.2**	**45**	1
Q01-37313	Open	Cattle	Cow	468	458	**3**	**FC**	**45.8**	**50**	1
Q01-37318	Open	Cattle	Cow	468	402	**3**	**FC**	**40.2**	**45**	1
Q01-37320	Open	Cattle	Cow	468	394	**3**	**FC**	**39.4**	**40**	1
Q01-37321	Open	Cattle	Cow	468	350	**3**	**FC**	**35**	**40**	1
Q01-37327	Open	Cattle	Cow	468	416	**3**	**FC**	**41.6**	**45**	1
Q01-37330	Open	Cattle	Cow	468	413	**3**	**FC**	**41.3**	**45**	1
Q01-37331	Open	Cattle	Cow	468	446	**3**	**FC**	**44.6**	**45**	1
Q01-37334	Open	Cattle	Cow	468	345	**3**	**FC**	**34.5**	**35**	1
Q01-37338	Open	Cattle	Cow	468	503	**3**	**FC**	**50.3**	**55**	1
Q01-37339	Open	Cattle	Cow	468	403	**3**	**FC**	**40.3**	**45**	1
Q01-37347	Open	Cattle	Cow	468	471	**3**	**FC**	**47.1**	**50**	1
Q01-37357	Open	Cattle	Cow	468	434	**3**	**FC**	**43.4**	**45**	1
Q01-37358	Open	Cattle	Cow	468	385	**3**	**FC**	**38.5**	**40**	1
Q01-37362	Open	Cattle	Cow	468	409	**3**	**FC**	**40.9**	**45**	1
Q01-37365	Open	Cattle	Cow	468	436	**3**	**FC**	**43.6**	**45**	1
Q01-37376	Open	Cattle	Cow	468	464	**3**	**FC**	**46.4**	**50**	1
Q01-37381	Open	Cattle	Cow	468	426	**3**	**FC**	**42.6**	**45**	1
Q01-37283	Open	Cattle	Cow	468	476	**4**	**C**	**47.6**		1
Q01-37294	Open	Cattle	Cow	468	425	**4**	**C**	**42.5**		1

Q01-37295	Open	Cattle	Cow	468	390	**4**	**C**	**39**		1
Q01-37297	Open	Cattle	Cow	468	347	**4**	**C**	**34.7**		1
Q01-37304	Open	Cattle	Cow	468	432	**4**	**C**	**43.2**		1
Q01-37308	Open	Cattle	Cow	468	403	**4**	**C**	**40.3**		1
Q01-37309	Open	Cattle	Cow	468	448	**4**	**C**	**44.8**		1
Q01-37319	Open	Cattle	Cow	468	412	**4**	**C**	**41.2**		1
Q01-37322	Open	Cattle	Cow	468	425	**4**	**C**	**42.5**		1
Q01-37323	Open	Cattle	Cow	468	631	**4**	**C**	**63.1**		1
Q01-37325	Open	Cattle	Cow	468	464	**4**	**C**	**46.4**		1
Q01-37332	Open	Cattle	Cow	468	418	**4**	**C**	**41.8**		1
Q01-37333	Open	Cattle	Cow	468	373	**4**	**C**	**37.3**		1
Q01-37336	Open	Cattle	Cow	468	390	**4**	**C**	**39**		1
Q01-37342	Open	Cattle	Cow	468	410	**4**	**C**	**41**		1
Q01-37351	Open	Cattle	Cow	468	473	**4**	**C**	**47.3**		1
Q01-37352	Open	Cattle	Cow	468	450	**4**	**C**	**45**		1
Q01-37353	Open	Cattle	Cow	468	431	**4**	**C**	**43.1**		1
Q01-37354	Open	Cattle	Cow	468	348	**4**	**C**	**34.8**		1
Q01-37355	Open	Cattle	Cow	468	402	**4**	**C**	**40.2**		1
Q01-37356	Open	Cattle	Cow	468	373	**4**	**C**	**37.3**		1
Q01-37359	Open	Cattle	Cow	468	418	**4**	**C**	**41.8**		1
Q01-37364	Open	Cattle	Cow	468	515	**4**	**C**	**51.5**		1
Q01-37369	Open	Cattle	Cow	468	505	**4**	**C**	**50.5**		1
Q01-37371	Open	Cattle	Cow	468	438	**4**	**C**	**43.8**		1
Q01-37382	Open	Cattle	Cow	468	440	**4**	**C**	**44**		1
Q01-37383	Open	Cattle	Cow	468	461	**4**	**C**	**46.1**		1

Table 9 Individual animal weight based dosage calculation tables for heifer herd

HRTag Number	Animal Type	Animal Gender	Weighing Date	Weight	Ranking	Treatment Grp	Treat Vol ml	Applied dose vol/ml	Qty.
Q01-37388	Cattle	Heifer	2012/07/24 09:28	247	1	S	24.7	25	1
Q01-37389	Cattle	Heifer	2012/07/24 09:28	305	1	S	30.5	35	1
Q01-37393	Cattle	Heifer	2012/07/24 09:28	269	1	S	26.9	30	1
Q01-37395	Cattle	Heifer	2012/07/24 09:28	293	1	S	29.3	30	1
Q01-37397	Cattle	Heifer	2012/07/24 09:28	281	1	S	28.1	30	1
Q01-37398	Cattle	Heifer	2012/07/24 09:28	293	1	S	29.3	30	1
Q01-37403	Cattle	Heifer	2012/07/24 09:28	329	1	S	32.9	35	1
Q01-37404	Cattle	Heifer	2012/07/24 09:28	286	1	S	28.6	30	1
Q01-37408	Cattle	Heifer	2012/07/24 09:28	278	1	S	27.8	30	1
Q01-37415	Cattle	Heifer	2012/07/24 09:28	247	1	S	24.7	25	1
Q01-37420	Cattle	Heifer	2012/07/24 09:28	278	1	S	27.8	30	1
Q01-37425	Cattle	Heifer	2012/07/24 09:28	264	1	S	26.4	30	1
Q01-37426	Cattle	Heifer	2012/07/24 09:28	264	1	S	26.4	30	1
Q01-37427	Cattle	Heifer	2012/07/24 09:28	273	1	S	27.3	30	1
Q01-37428	Cattle	Heifer	2012/07/24 09:28	273	1	S	27.3	30	1
Q01-37431	Cattle	Heifer	2012/07/24 09:28	317	1	S	31.7	35	1
Q01-37441	Cattle	Heifer	2012/07/24 09:28	317	1	S	31.7	35	1
Q01-37442	Cattle	Heifer	2012/07/24 09:28	259	1	S	25.9	30	1
Q01-37445	Cattle	Heifer	2012/07/25 09:28	256	1	S	25.6	30	1
Q01-37447	Cattle	Heifer	2012/07/24 09:28	300	1	S	30	30	1
Q01-37455	Cattle	Heifer	2012/07/24 09:28	279	1	S	27.9	30	1
Q01-37463	Cattle	Heifer	2012/07/24 09:28	226	1	S	22.6	25	1
Q01-37465	Cattle	Heifer	2012/07/24 09:28	269	1	S	26.9	30	1
Q01-37466	Cattle	Heifer	2012/07/24 09:28	258	1	S	25.8	25	1
Q01-37470	Cattle	Heifer	2012/07/24 09:28	295	1	S	29.5	30	1
Q01-37474	Cattle	Heifer	2012/07/24 09:28	250	1	S	25	25	1
Q01-37479	Cattle	Heifer	2012/07/24 09:28	329	1	S	32.9	35	1

Q01-37483	Cattle	Heifer	2012/07/24 09:28	298	1	S	29.8	30	1
Q01-37489	Cattle	Heifer	2012/07/24 09:28	279	1	S	27.9	30	1
Q01-37492	Cattle	Heifer	2012/07/24 09:28	281	1	S	28.1	30	1
Q01-37499	Cattle	Heifer	2012/07/24 09:28	294	1	S	29.4	30	1
Q01-37506	Cattle	Heifer	2012/07/24 09:28	250	1	S	25	25	1
Q01-37511	Cattle	Heifer	2012/07/24 09:28	305	1	S	30.5	35	1
Q01-37400	Cattle	Heifer	2012/07/24 09:28	280	2	FC	28	30	1
Q01-37401	Cattle	Heifer	2012/07/24 09:28	264	2	FC	26.4	30	1
Q01-37416	Cattle	Heifer	2012/07/24 09:28	292	2	FC	29.2	30	1
Q01-37418	Cattle	Heifer	2012/07/24 09:28	293	2	FC	29.3	30	1
Q01-37419	Cattle	Heifer	2012/07/24 09:28	298	2	FC	29.8	30	1
Q01-37421	Cattle	Heifer	2012/07/24 09:28	317	2	FC	31.7	35	1
Q01-37422	Cattle	Heifer	2012/07/24 09:28	279	2	FC	27.9	30	1
Q01-37423	Cattle	Heifer	2012/07/24 09:28	250	2	FC	25	25	1
Q01-37429	Cattle	Heifer	2012/07/24 09:28	277	2	FC	27.7	30	1
Q01-37430	Cattle	Heifer	2012/07/24 09:28	268	2	FC	26.8	30	1
Q01-37432	Cattle	Heifer	2012/07/24 09:28	285	2	FC	28.5	30	1
Q01-37433	Cattle	Heifer	2012/07/24 09:28	264	2	FC	26.4	30	1
Q01-37439	Cattle	Heifer	2012/07/24 09:28	278	2	FC	27.8	30	1
Q01-37444	Cattle	Heifer	2012/07/24 09:28	260	2	FC	26	30	1
Q01-37458	Cattle	Heifer	2012/07/24 09:28	247	2	FC	24.7	25	1
Q01-37464	Cattle	Heifer	2012/07/24 09:28	300	2	FC	30	30	1
Q01-37468	Cattle	Heifer	2012/07/24 09:28	328	2	FC	32.8	35	1
Q01-37472	Cattle	Heifer	2012/07/24 09:28	246	2	FC	24.6	25	1
Q01-37475	Cattle	Heifer	2012/07/24 09:28	274	2	FC	27.4	30	1
Q01-37476	Cattle	Heifer	2012/07/24 09:28	237	2	FC	23.7	25	1
Q01-37477	Cattle	Heifer	2012/07/24 11:22	295	2	FC	29.5	30	1
Q01-37482	Cattle	Heifer	2012/07/24 09:28	272	2	FC	27.2	30	1
Q01-37484	Cattle	Heifer	2012/07/24 09:28	286	2	FC	28.6	30	1
Q01-37485	Cattle	Heifer	2012/07/24 09:28	317	2	FC	31.7	35	1
Q01-37497	Cattle	Heifer	2012/07/24 09:28	294	2	FC	29.4	30	1

Q01-37504	Cattle	Heifer	2012/07/24 09:28	282	2	FC	28.2	30	1
Q01-37505	Cattle	Heifer	2012/07/24 09:28	280	2	FC	28	30	1
Q01-37507	Cattle	Heifer	2012/07/24 09:28	329	2	FC	32.9	35	1
Q01-37510	Cattle	Heifer	2012/07/24 09:28	269	2	FC	26.9	30	1
Q01-37512	Cattle	Heifer	2012/07/24 09:28	303	2	FC	30.3	35	1
Q01-37513	Cattle	Heifer	2012/07/24 09:28	305	2	FC	30.5	35	1
Q01-37517	Cattle	Heifer	2012/07/24 09:28	257	2	FC	25.7	30	1
Q01-37519	Cattle	Heifer	2012/07/24 09:28	250	2	FC	25	25	1
Q01-37392	Cattle	Heifer	2012/07/24 09:28	277	3	F	27.7	30	1
Q01-37396	Cattle	Heifer	2012/07/24 09:28	298	3	F	29.8	30	1
Q01-37399	Cattle	Heifer	2012/07/24 09:28	307	3	F	30.7	35	1
Q01-37405	Cattle	Heifer	2012/07/24 09:28	263	3	F	26.3	30	1
Q01-37410	Cattle	Heifer	2012/07/24 09:28	332	3	F	33.2	35	1
Q01-37411	Cattle	Heifer	2012/07/24 09:28	288	3	F	28.8	30	1
Q01-37412	Cattle	Heifer	2012/07/24 09:28	279	3	F	27.9	30	1
Q01-37417	Cattle	Heifer	2012/07/24 09:28	240	3	F	24	25	1
Q01-37424	Cattle	Heifer	2012/07/24 09:28	296	3	F	29.6	30	1
Q01-37435	Cattle	Heifer	2012/07/24 09:28	280	3	F	28	30	1
Q01-37446	Cattle	Heifer	2012/07/24 09:28	287	3	F	28.7	30	1
Q01-37449	Cattle	Heifer	2012/07/24 09:28	301	3	F	30.1	35	1
Q01-37450	Cattle	Heifer	2012/07/24 09:28	293	3	F	29.3	30	1
Q01-37451	Cattle	Heifer	2012/07/24 09:28	264	3	F	26.4	30	1
Q01-37452	Cattle	Heifer	2012/07/24 09:28	302	3	F	30.2	30	1
Q01-37457	Cattle	Heifer	2012/07/24 09:28	320	3	F	32	35	1
Q01-37461	Cattle	Heifer	2012/07/24 09:28	312	3	F	31.2	35	1
Q01-37462	Cattle	Heifer	2012/07/24 09:28	328	3	F	32.8	35	1
Q01-37473	Cattle	Heifer	2012/07/24 09:28	266	3	F	26.6	30	1
Q01-37486	Cattle	Heifer	2012/07/24 09:28	278	3	F	27.8	30	1
Q01-37487	Cattle	Heifer	2012/07/24 09:28	247	3	F	24.7	25	1
Q01-37488	Cattle	Heifer	2012/07/24 09:28	280	3	F	28	30	1
Q01-37490	Cattle	Heifer	2012/07/24 09:28	271	3	F	27.1	30	1

Q01-37491	Cattle	Heifer	2012/07/24 09:28	284	3	F	28.4	30	1
Q01-37493	Cattle	Heifer	2012/07/24 09:28	290	3	F	29	30	1
Q01-37494	Cattle	Heifer	2012/07/24 09:28	294	3	F	29.4	30	1
Q01-37496	Cattle	Heifer	2012/07/24 09:28	251	3	F	25.1	30	1
Q01-37500	Cattle	Heifer	2012/07/24 09:28	243	3	F	24.3	25	1
Q01-37508	Cattle	Heifer	2012/07/24 09:28	249	3	F	24.9	25	1
Q01-37509	Cattle	Heifer	2012/07/24 09:28	283	3	F	28.3	30	1
Q01-37514	Cattle	Heifer	2012/07/24 09:28	269	3	F	26.9	30	1
Q01-37516	Cattle	Heifer	2012/07/24 09:28	275	3	F	27.5	30	1
Q01-37518	Cattle	Heifer	2012/07/24 09:28	256	3	F	25.6	30	1
Q01-37387	Cattle	Heifer	2012/07/24 09:28	263	4	Control	0		1
Q01-37390	Cattle	Heifer	2012/07/24 09:28	341	4	Control	0		1
Q01-37391	Cattle	Heifer	2012/07/24 09:28	248	4	Control	0		1
Q01-37394	Cattle	Heifer	2012/07/24 09:28	294	4	Control	0		1
Q01-37402	Cattle	Heifer	2012/07/24 09:28	248	4	Control	0		1
Q01-37406	Cattle	Heifer	2012/07/24 09:28	241	4	Control	0		1
Q01-37407	Cattle	Heifer	2012/07/24 09:28	311	4	Control	0		1
Q01-37409	Cattle	Heifer	2012/07/24 09:28	288	4	Control	0		1
Q01-37413	Cattle	Heifer	2012/07/24 09:28	307	4	Control	0		1
Q01-37414	Cattle	Heifer	2012/07/24 09:28	302	4	Control	0		1
Q01-37434	Cattle	Heifer	2012/07/24 09:28	276	4	Control	0		1
Q01-37436	Cattle	Heifer	2012/07/24 09:28	271	4	Control	0		1
Q01-37438	Cattle	Heifer	2012/07/24 09:28	252	4	Control	0		1
Q01-37443	Cattle	Heifer	2012/07/24 09:28	328	4	Control	0		1
Q01-37448	Cattle	Heifer	2012/07/24 09:28	328	4	Control	0		1
Q01-37453	Cattle	Heifer	2012/07/24 09:28	296	4	Control	0		1
Q01-37454	Cattle	Heifer	2012/07/24 09:28	271	4	Control	0		1
Q01-37456	Cattle	Heifer	2012/07/24 09:28	280	4	Control	0		1
Q01-37459	Cattle	Heifer	2012/07/24 09:28	266	4	Control	0		1
Q01-37460	Cattle	Heifer	2012/07/24 09:28	293	4	Control	0		1
Q01-37467	Cattle	Heifer	2012/07/24 09:28	284	4	Control	0		1

Q01-37469	Cattle	Heifer	2012/07/24 09:28	296	4	Control	0		1
Q01-37471	Cattle	Heifer	2012/07/24 09:28	280	4	Control	0		1
Q01-37478	Cattle	Heifer	2012/07/24 09:28	284	4	Control	0		1
Q01-37480	Cattle	Heifer	2012/07/24 09:28	243	4	Control	0		1
Q01-37481	Cattle	Heifer	2012/07/24 09:28	287	4	Control	0		1
Q01-37495	Cattle	Heifer	2012/07/24 09:28	264	4	Control	0		1
Q01-37498	Cattle	Heifer	2012/07/24 09:28	278	4	Control	0		1
Q01-37501	Cattle	Heifer	2012/07/24 09:28	262	4	Control	0		1
Q01-37502	Cattle	Heifer	2012/07/24 09:28	276	4	Control	0		1
Q01-37503	Cattle	Heifer	2012/07/24 09:28	301	4	Control	0		1
Q01-37515	Cattle	Heifer	2012/07/24 09:28	278	4	Control	0		1
Q01-37520	Cattle	Heifer	2012/07/24 09:28	254	4	Control	0		1

Table 10 Cows' weight from onset to the end of trial

Number	March	April	May	June	July	August
Q01-37271	391	391	364	377	372	365
Q01-37272	391	391	460	455	458	449
Q01-37273	471	471	461	448	425	441
Q01-37274	529	529	479	470	480	499
Q01-37275	580	580	572	577	571	563
Q01-37276	597	597	598	577	570	493
Q01-37277	452	452	452	448	435	422
Q01-37278	594	594	596	605	588	507
Q01-37279	394	394	382	395	396	408
Q01-37280	429	429	402	403	399	395
Q01-37281	428	428	397	353	330	351
Q01-37282	545	545	505	518	520	511
Q01-37283	497	497	493	490	476	481
Q01-37286	385	385	361	369	367	368
Q01-37287	424	424	425	424	421	443
Q01-37288	414	414	407	413	415	434
Q01-37289	427	427	408	414	416	415
Q01-37290	508	508	490	491	484	489
Q01-37291	569	569	556	560	547	535
Q01-37292	396	396	369	370	367	372
Q01-37293	379	379	367	374	375	385
Q01-37294	438	438	414	431	425	443
Q01-37295	455	455	428	403	390	403
Q01-37296	540	540	532	532	529	527
Q01-37297	417	417	375	350	347	350
Q01-37298	460	460	445	458	451	461
Q01-37299	510	510	505	510	496	484
Q01-37300	436	436	414	404	406	406
Q01-37301	440	440	422	433	431	418
Q01-37302	439	439	421	427	429	435

Q01-37303	431	431	399	385	380	382
Q01-37304	459	459	437	445	432	450
Q01-37306	442	442	428	447	431	429
Q01-37307	460	460	426	436	435	454
Q01-37308	390	390	385	398	403	412
Q01-37309	468	468	463	468	448	463
Q01-37310	461	461	430	440	442	452
Q01-37311	465	465	457	458	455	460
Q01-37312	471	471	439	450	445	459
Q01-37313	455	455	433	459	458	469
Q01-37314	516	516	462	425	407	410
Q01-37315	445	445	390	365	355	374
Q01-37316	507	507	486	503	487	486
Q01-37317	514	514	484	446	446	452
Q01-37318	437	437	410	397	402	415
Q01-37319	458	458	432	431	412	425
Q01-37320	409	409	388	391	394	406
Q01-37321	442	442	396	368	350	359
Q01-37322	439	439	410	422	425	440
Q01-37323	666	666	645	662	631	612
Q01-37324	455	455	444	430	445	461
Q01-37325	486	486	468	484	464	466
Q01-37326	489	489	465	436	421	402
Q01-37327	429	429	403	420	416	421
Q01-37328	435	435	421	381	370	373
Q01-37329	523	523	500	457	436	431
Q01-37330	417	417	388	410	413	427
Q01-37331	446	446	419	448	446	456
Q01-37332	459	459	435	421	418	417
Q01-37333	472	472	454	384	373	381
Q01-37334	370	370	357	337	345	339

Q01-37335	455	455	439	442	435	431
Q01-37336	517	517	464	416	390	387
Q01-37337	483	483	450	402	375	375
Q01-37338	514	514	498	529	503	499
Q01-37339	462	462	437	418	403	422
Q01-37341	453	453	433	440	442	446
Q01-37342	417	417	406	417	410	436
Q01-37343	526	526	501	437	415	403
Q01-37344	512	512	501	512	505	487
Q01-37345	541	541	472	455	424	443
Q01-37346	463	463	426	415	405	416
Q01-37347	480	480	463	478	471	476
Q01-37348	463	463	444	458	452	462
Q01-37349	469	469	455	467	465	474
Q01-37350	387	387	378	379	381	381
Q01-37351	516	516	476	480	473	469
Q01-37352	559	559	510	468	450	449
Q01-37353	441	441	431	448	431	437
Q01-37354	371	371	357	349	348	344
Q01-37355	438	438	408	397	402	411
Q01-37356	370	370	359	370	373	389
Q01-37357	540	540	498	450	434	422
Q01-37358	406	406	383	383	385	382
Q01-37359	417	417	415	424	418	419
Q01-37360	502	502	471	473	469	471
Q01-37361	521	521	492	425	415	400
Q01-37362	423	423	398	410	409	413
Q01-37363	501	501	466	430	406	410
Q01-37364	526	526	520	539	515	515
Q01-37365	455	455	428	436	439	453
Q01-37366	489	489	447	426	394	395

Q01-37367	438	438	440	439	422	448
Q01-37368	561	561	507	446	423	406
Q01-37369	495	495	491	514	505	524
Q01-37370	390	390	381	385	376	384
Q01-37371	548	548	509	472	438	441
Q01-37372	504	504	474	483	469	482
Q01-37373	469	469	473	477	470	380
Q01-37375	422	422	434	435	430	429
Q01-37376	487	487	432	469	464	467
Q01-37377	476	476	451	406	396	387
Q01-37378	438	438	431	449	451	453
Q01-37379	447	447	432	441	429	427
Q01-37380	370	370	363	362	342	364
Q01-37381	511	511	484	445	426	427
Q01-37382	536	536	499	468	440	442
Q01-37383	481	481	473	480	461	448

Table 11 Heifers' weight from onset to the end of trial

Number	March	April	May	June	July	August	September	October
Q01-37387	289	289	292	265	263	264	252	288
Q01-37388	271	271	263	248	247	255	249	287
Q01-37389	329	329	335	310	305	307	288	329
Q01-37390	368	368	354	342	341	350	345	386
Q01-37391	269	269	270	249	248	252	250	281
Q01-37392	304	304	301	283	277	281	289	298
Q01-37393	290	290	288	276	269	268	280	283
Q01-37394	315	315	314	298	294	299	290	335
Q01-37395	308	308	307	298	293	294	287	335
Q01-37396	317	317	323	306	298	297	299	310
Q01-37397	298	298	298	280	281	283	281	307
Q01-37398	313	313	307	320	293	292	288	317
Q01-37399	326	326	328	378	307	313	301	335
Q01-37400	394	394	399	267	280	391	369	406
Q01-37401	275	275	287	251	264	266	256	290
Q01-37402	264	264	267	335	248	253	259	285
Q01-37403	354	354	348	294	329	340	329	361
Q01-37404	322	322	324	259	286	286	291	327
Q01-37405	282	282	284	254	263	264	259	289
Q01-37406	272	272	267	316	241	254	247	276
Q01-37407	343	343	331	282	311	316	303	345
Q01-37409	310	310	312	346	288	287	273	303
Q01-37410	352	352	357	292	332	339	331	352
Q01-37411	305	305	312	287	288	293	284	314
Q01-37412	297	297	302	315	279	276	289	306
Q01-37413	319	319	336	288	307	323	292	337
Q01-37414	306	306	307	252	302	321	297	335
Q01-37415	268	268	271	304	247	247	258	293
Q01-37416	325	325	319	237	292	294	285	325

Q01-37417	265	265	269	301	240	422	234	272
Q01-37419	302	302	309	278	298	300	311	322
Q01-37420	286	286	296	313	278	289	278	314
Q01-37421	334	334	334	288	317	313	294	348
Q01-37422	309	309	315	255	279	289	285	310
Q01-37423	272	272	284	289	250	260	272	281
Q01-37424	295	295	304	265	296	293	283	327
Q01-37425	286	286	278	274	264	261	244	275
Q01-37426	288	288	293	279	264	268	263	287
Q01-37427	298	298	299	277	273	285	278	309
Q01-37428	276	276	291	282	273	274	266	294
Q01-37429	299	299	299	286	277	274	262	303
Q01-37430	299	299	294	324	268	285	260	293
Q01-37431	336	336	340	293	317	321	309	336
Q01-37432	314	314	314	268	285	294	284	316
Q01-37433	305	305	302	274	264	268	274	303
Q01-37434	293	293	296	284	276	267	273	293
Q01-37436	292	292	295	280	271	275	264	295
Q01-37438	275	275	277	266	252	260	254	282
Q01-37439	285	285	293	353	278	278	293	313
Q01-37441	332	332	336	267	317	343	309	343
Q01-37442	288	288	284	340	259	262	264	291
Q01-37443	356	356	350	270	328	331	306	349
Q01-37444	280	280	288	267	260	266	255	297
Q01-37445	287	287	279	294	256	262	263	288
Q01-37446	313	313	319	302	287	286	270	312
Q01-37447	319	319	323	329	300	309	317	336
Q01-37448	334	334	334	299	328	328	317	343
Q01-37449	326	326	331	284	301	311	292	310
Q01-37450	294	294	306	266	293	238	279	314
Q01-37451	283	283	283	309	264	263	260	285

Q01-37452	328	328	331	292	302	310	282	335
Q01-37453	304	304	305	277	296	296	287	319
Q01-37454	280	280	280	277	271	275	266	297
Q01-37456	296	296	299	283	280	286	272	306
Q01-37457	355	355	351	328	320	325	304	347
Q01-37458	269	269	270	248	247	252	255	272
Q01-37459	278	278	285	270	266	262	257	294
Q01-37460	323	323	324	289	293	297	300	327
Q01-37461	339	339	337	315	312	318	312	350
Q01-37462	341	341	342	331	328	331	328	340
Q01-37463	281	281	275	243	226	237	227	254
Q01-37464	319	319	313	299	300	289	290	376
Q01-37465	286	286	275	270	269	266	256	283
Q01-37467	303	303	302	291	284	294	282	299
Q01-37468	350	350	349	333	328	339	332	362
Q01-37469	308	308	310	295	296	293	287	315
Q01-37470	315	315	323	297	295	306	303	337
Q01-37471	302	302	303	286	280	276	268	303
Q01-37472	263	263	266	251	246	250	245	279
Q01-37473	288	288	288	271	266	275	273	291
Q01-37475	304	304	296	279	274	279	275	313
Q01-37476	266	266	261	249	237	257	248	288
Q01-37477	310	310	305	297	295	306	294	320
Q01-37478	303	303	345	287	284	284	281	321
Q01-37480	276	276	268	253	243	256	254	274
Q01-37481	307	307	309	298	287	293	281	308
Q01-37482	294	294	289	276	272	273	260	292
Q01-37483	323	323	319	301	298	298	289	325
Q01-37484	308	308	305	285	286	287	296	325
Q01-37485	323	323	328	314	317	320	304	330
Q01-37486	292	292	303	285	278	277	273	295

Q01-37487	265	265	272	250	247	247	248	267
Q01-37488	290	290	304	287	280	283	279	311
Q01-37489	301	301	308	279	279	279	269	313
Q01-37490	298	298	301	283	271	271	264	290
Q01-37491	309	309	303	284	284	274	269	305
Q01-37493	303	303	307	295	290	308	298	315
Q01-37494	322	322	311	295	294	306	314	328
Q01-37495	285	285	291	258	264	273	250	275
Q01-37496	284	284	282	249	251	260	239	295
Q01-37497	322	322	323	298	294	219	282	311
Q01-37498	302	302	304	279	278	291	269	301
Q01-37499	317	317	315	302	294	292	287	374
Q01-37500	268	268	276	251	243	251	249	274
Q01-37501	284	284	287	270	262	269	264	292
Q01-37502	310	310	303	287	276	283	275	311
Q01-37503	308	308	300	291	301	302	290	310
Q01-37504	288	288	296	287	282	281	277	296
Q01-37505	310	310	310	290	280	284	289	315
Q01-37506	274	274	271	264	250	246	240	273
Q01-37507	360	360	358	340	329	331	313	351
Q01-37508	272	272	269	259	249	254	256	273
Q01-37509	298	298	295	283	283	286	277	304
Q01-37510	281	281	286	273	269	277	268	299
Q01-37511	332	332	321	314	305	312	319	356
Q01-37512	338	338	342	306	303	318	307	345
Q01-37513	313	313	320	308	305	312	307	334
Q01-37514	292	292	294	276	269	276	281	305
Q01-37515	296	296	298	286	278	275	276	306
Q01-37516	287	287	297	274	275	276	284	303
Q01-37517	275	275	276	261	257	257	256	280
Q01-37518	286	286	277	253	256	256	264	300

Q01-37519	261	261	262	253	250	250	253	278
Q01-37520	282	282	281	261	254	254	256	291