



Norwegian University
of Life Sciences

Master's Thesis 2016 60 ECTS
Department of Animal and Aquaculture Science

Climate change resilience through enhanced reproduction and lactation performance in Malawian Zebu cattle

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Master in Animal Breeding and Genetics

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Preface

The Norwegian Government and the Government of the Republic of Malawi are supporting a programme titled “*Capacity Building for Managing Climate Change in Malawi*” (CABMACC). CABMACC is a collaboration programme between Lilongwe University of Agriculture and Natural Resources (LUANAR), Malawi and the Norwegian University of Life Sciences (NMBU), Norway. CABMACC is funded by the Ministry of Foreign Affairs through the Norwegian Agency for Development Cooperation (NORAD). The overall goal of the programme is to improve livelihoods and food security through innovative responses and enhanced capacity for adaptation to climate change in Malawi. The CABMACC programme has seven projects in total, and the second project is on “*Livestock value chain, food security, and environmental quality: Transforming rural livelihoods through community based resilience in indigenous livestock management practice*”. This project is led by Professor Leonard Kamwanja of the *Trustee of Agriculture Promotion Programme* (TAPP), and NMBU Professor Olav Reksen, Department of Production Animal and Clinical Science (ProdMed).

The main motivation for me as a student to participate in the project is my interest in cattle reproduction and food production. Being a veterinarian, I wanted to acquire further knowledge about these topics combined with an opportunity to learn how veterinary science can be a key component in international development cooperation. Through participating in the project “*Livestock value chain, food security, and environmental quality: Transforming rural livelihoods through community based resilience in indigenous livestock management practice*”, I hope to obtain valuable knowledge concerning both reproductive physiology and challenges related to food production in developing countries. Working in the field conditions is a challenging task, but I have tried to learn how to put things on track under such conditions.

I am much thankful to Olav Reksen and my Supervisor Lars Olav Eik for giving me this opportunity to take part in a project that aims to search for solutions of some of the problems that Malawians are currently facing. Problems regarding agricultural management and lack of food are of high importance as climate changes will continue to affect the world in the years to come.

I would like to thank Professor Leonard Kamwanja, Winfred Chanza and Mphatso Chipandula of *Trustee of Agriculture Promotion Programme*, for their guidance and hospitality during my stays in Malawi.

My sincere gratitude goes to my supervisor Lars Olav Eik, who helped me in becoming the part of this project. I am also much thankful to Olav Reksen and without Olav's guidance; it was not going to happen. I am extremely thankful to both that they were available 24/7 for guidance.

I am thankful to Maria Stokstad and Siv Klevar for their support in analysis of blood samples and guidance in the field of infectious diseases. It has been a privilege to learn from such skilled professionals.

I am also thankful to Thea B. Blystad Klem and Harald Holm, both of them helped me in understanding the pregnancy diagnosis under field conditions and their hard work and contribution in the project.

I would like to thank Dr Gilson Njunga at the Central Veterinary Laboratory for handling of blood and faecal samples and all his support by providing the necessary apparatus to carry out the blood sampling in the field conditions.

I am thankful to Dr Liveness J. Banda (LUANAR) for her collaboration and interest in our research activities and for helping in the feed and milk analysis.

I am also very much thankful to farmers and field personnel for enjoyable days with the Zebu cows in Rumphi, Malawi.

I am much thankful to all my ASLP (Australia-Pakistan Agriculture Sector Linkage Programme) colleagues, especially Peter Wynn (Charles Stuart University, Australia), Peter Thomson & Russell Bush (The Sydney University, Australia), David McGill (The University of Melbourne, Australia) and Hassan Mahmood Warriach (University of Veterinary and Animal Sciences, Lahore-Pakistan) for their evergreen support for me, especially in this thesis review.

I owe thanks to a very special person, my wife, Mehwish for her continued and unfailing love, support and understanding during my pursuit of Master degree and I appreciate my sons (Abdullah & Saim) for abiding my ignorance and the patience they showed during my thesis writing.

Last but not least: I thank all my friends for supporting me spiritually.

Summary

Title:

Climate change resilience through enhanced reproduction and lactation performance in
Malawian Zebu cattle

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Supervisor: Lars Olav Eik (IHA)

Project leader: Olav Reksen (ProdMed)

Malawi is one of the most vulnerable countries to climate change in the world, and the site of our investigation, Bolero EPA (Extension Planning Area), Rumphi District, is particularly exposed. Climate change lead to a drier and longer dry seasons and erratic rainfall. Constrains on availability of food and water in the dry period has negative impacts on reproductive performance, milk production and calf growth. However, these problems affect farmers differently, and improved management is likely to give higher calving rates and therefore increased production of milk and meat. Through capacity building on successful Zebu cattle management in the Rumphi district in Malawi, the overall aim of the current project is to increase reproductive performance and lactation yields and thereby climate change resilience through the following sub goals:

- (1) To describe the smallholder cattle farming system and ongoing general practices regarding management of Zebu cattle in the Bolero EPA, Rumphi District.
- (2) To determine reproductive and lactation performance and important influencing factors in Zebu cattle calving in fall.
- (3) To determine the prevalence of possible infectious causes for reproduction and production inefficiency in Zebu cattle

The Norwegian Government and the Government of the Republic of Malawi are supporting the program “*Capacity Building for Managing Climate Change in Malawi*” (CABMACC), This thesis is a part of a sub-project of CABMACC entitled “*Livestock value chain, food*

security, and environmental quality: Transforming rural livelihoods through community based resilience indigenous livestock management practice”.

This study encompasses a cross sectional survey of management practices and reproductive performance in 39 Zebu cattle herds with 509 cattle, and a cohort study from the same farms following 101 fall-calving Malawian Zebu cows from calving and one year onwards. All the farmers in the study area are practicing an agro-pastoral production system.

Information on available water sources, distance to water sources, farmers perception of reproductive performance, number of bulls per farm and milk marketing was registered in the cross sectional survey by a semi-structured questionnaire in August 2015.

The cohort was divided into groups according to access to water and additional feeding. The animals were divided into near (≤ 1.5 km) and far (> 1.5 km) groups in relation to distance to water. We purchased dried leguminous leaves and maize bran for supplemental feeding of 2 kg leaves and 2 kg maize bran per cow and day. The supplement was provided during the driest months (October & November) of 2015. For the feeding trial, 31 cows were subject to treatment, and 31 cows of the same parity and with the same distance to the water sources served as the control group. The whole cohort (n=101) was included in the analyses to assess associations among several explanatory variables, namely, agricultural section, bull management, water source and alterations of body girth circumference, with pregnancy outcome (1/0) during January 2016, Zebu cow open days and lactation length. Bi-weekly milk sampling was conducted for progesterone analysis to determine the median number of days from calving to onset of ovarian (luteal) activity using the P4-Rapid test from Ridgeway Science, St Briavels, England.

Blood samples for the diagnoses of infectious diseases were collected from each study animal at two occasions; October 2015 and April 2016. Each time, blood serum from cows were analysed for antibodies to foot and mouth disease virus (FMDV), *Babesia* spp., *Theileria* spp., *Neospora caninum*, bovine virus diarrhoea virus (BVDV) and *Brucella* spp. Blood samples from calves were analysed only in April 2016 since the level of maternal antibodies was expected to influence the results of the October sampling. Thin blood smears were used for the detection of *Babesia*, *Theileria* and *Trypanosoma*.

The results of our study show that Malawian Zebu cows experience fewer days from calving to onset of luteal activity (OLA) than previously reported from Tanzanian Zebu cows. Median number of days from calving to OLA (n=96) was 61 days. A relatively short period from calving to OLA, and considerably variability in days open and pregnancy rate at 6 month between cows, herds and agricultural sections, point towards a potential to increase reproductive performance in the Zebu cattle of this region. In January 2016, there were 53 pregnant and 48 non-pregnant cows. Out of 53 pregnant cows, 49 calved between June and September of 2016, three cows experienced late abortions and one cow received a stillborn calf. Out of the remaining 49 calves, nine were confirmed dead, twelve sold, one moved and one was borrowed before the end of October 2016. Herds keeping their own bull, experienced significantly less days open than herds where the mating was restricted to periods of common grazing. The best results were achieved in herds with one bull as compared to the herds with more than one bull. Water source was significantly associated with days open and lactation length. Tap and bore water was less favourable in both cases. This may be due to the work force needed to supply enough water to the animals. Distance to water was not associated with either days open or lactation length. Supplemental feeding was significantly and positively associated with the length of the lactation period, but not associated with days open. Loss of body girth circumference was negatively associated with likelihood of pregnancy, but did not alter length of the lactation period. This study also showed that only 10% of farmers were marketing the milk from their Zebu cows. Farmers involved in milk marketing had significantly higher average milk production as compared to the farmers that did not sell milk.

Antibodies against FMDV were found in the serum from 12 cows and six calves in October 2015 and six calves in the April 2016 blood sampling. These results indicated that the disease is endemic for the region, but not widespread. Further PCR-testing showed that none of the animals had an ongoing infection of FMD. Blood sampling for *Coxiella burnetii* showed that 44 cows were positive in October 2015 and 42 cows in April 2016. Only one cow was found to be positive for BVDV and *Neospora caninum* in both samplings. *Brucella abortus* was not detected in any of the samplings, which were in contrast to our expectations. Three cows were positive for *Trypanosoma*, but no one were diagnosed with *Theileria* or *Babesia*.

Acronyms

ADF: Acid Detergent Fibre

BCS: Body Condition Score

Bolero-EPA: Bolero Extension Planning area

BVDV: Bovine Viral Diarrhoea Virus

CP: Crude Protein

DM: Dry Matter

DIM: Days in Milk

ELISA: Enzyme-Linked Immunosorbent Assay

FAO: Food and Agriculture Organization of the United Nations

FMD: Food and Mouth Disease

FMDV: Foot and Mouth Disease Virus

FSH: Follicle Stimulating Hormone

GDP: Gross Domestic Product

GE: Gross Energy

GnRH: Gonadotropin-Releasing Hormone

LH: Luteinizing Hormone

NEBAL: Negative Energy Balance

OD: Optical Density

OLA: Onset of luteal activity

PD: Pregnancy Diagnosis

P4: Progesterone

USD: United States Dollar

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1. Introduction

The domestic agriculture sector in South Asia and sub-Saharan Africa plays a major role as the basis for food supply (Del Ninno et al. 2007; Ellis et al. 2003). The need for significant productivity improvements in the agriculture sector is common to sub-Saharan African countries to meet the combined challenges of population growth and climate changes. The current project focuses on smallholder cattle farmers mitigating adverse effects of climate change through improved management of production and reproduction in Zebu cattle.

1.1 Smallholder Agriculture

The definition of smallholders varies between different ecological zones as for example, the smallholders from densely populated areas often cultivate less than 1 ha of land and those from semi-arid areas may cultivate up to 10 ha or more (Dixon et al. 2004; Jayne et al. 2003; Morton 2007). These small-scale farmers are less market-oriented and use parts of the produce for family consumption (Singh et al. 2002). More than 1.5 billion people live on smallholder farms, of which half a billion are partly or completely dependent on livestock. More than two thirds of the African population depends on smallholders agriculture in order to sustain their livelihood. In Asia and sub-Saharan Africa, more than three quarters of the food supply is provided by these smallholders (Altieri & Koohafkan 2008; Otte & Chilonda 2002).

Smallholder farmers in Africa largely depend on grazing areas as the main source of fodder for their herds. During the dry season, both the quality and quantity of pasture diminishes and the livestock struggle to maintain body condition, hence reproduction is compromised. Consequently, milk production decreases and new-born calves are not fed adequately (Klinedinst et al. 1993). Erratic rainfalls and prolonged dry season further decreases fodder availability (Morton 2007), which severely affects production and reproduction of the livestock (Thornton et al. 2009).

1.2 Malawian smallholder cattle farmers

Malawi is a landlocked country with a total area of 118 484 km² of which 20% is made up by Lake Malawi, the third largest freshwater lake in Africa and the eleventh largest in the world. (Frenken 2005; O'Reilly et al. 2003). Malawi shares borders with Tanzania, Zambia, Zimbabwe and Mozambique (Figure 1). Malawi has a population of more than 15 million people where

more than 80% of the population lives in rural areas, and their existence is largely dependent on agriculture (FAO 2006a). Malawi has a tropical climate with two distinct seasons, a dry season and a wet season (Banda et al. 2012). In 2014, GDP growth was 5.7% and figures from 2015 stated that 71% of the population lives on less than \$1.04/day (USAID Malawi 2015). The UN NEWS Centre report from 2013 indicates that more than 50% of the country remains mired in poverty, with one quarter of Malawians earning less than the estimated costs of a diet providing minimum calorie intake, and about half of the children are suffering from acute or severe malnutrition (FAO 2006a; The UN NEWS Centre 2013). In Malawi, the total expenditures on maize constitute 48-53% of the average monthly budget, irrespective of whether the household is located in rural or urban areas. Meats rank second, followed by milk and eggs (Banda 2008). Livestock is not the direct source of income for the smallholders but are mostly used as a means of mitigating the impact of drought, erratic rainfall, delayed harvest, localized flooding and waterlogging in fields (Menon 2007).

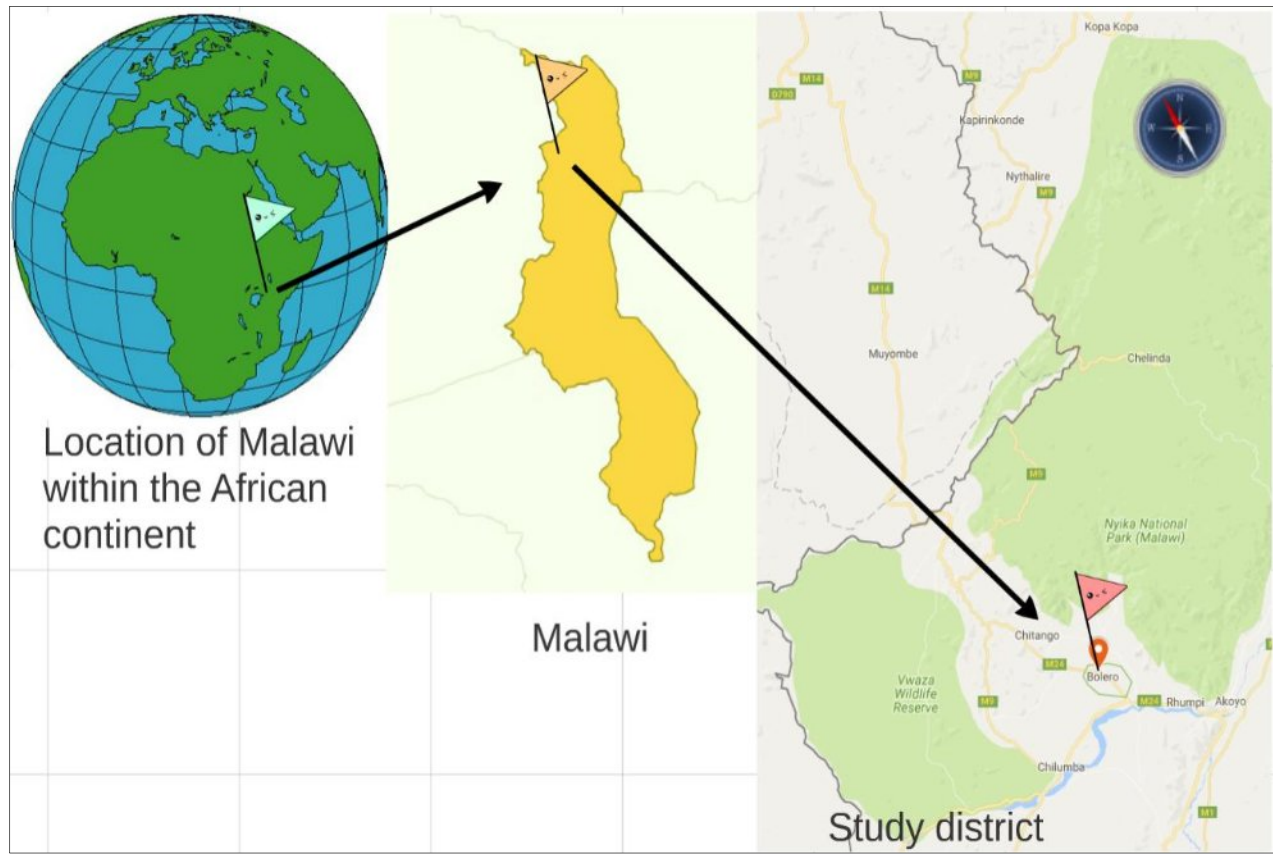


Figure 1. Location of the study district

1.3 Malawian Zebu cattle and susceptibility to climate change

The Malawian Zebu cattle provide milk mostly in the informal (non-commercial) sector and milk is an important contribution to the diet. In Malawi the average milk consumption is estimated to be 4.7 kg per capita per year (Banda 2008; FAO 2006b). The milk in the commercial sector derives mostly from crossbred and purebred Holstein Friesian dairy cattle in both smallholder and estate farms (Banda 2008) together with the importation of milk products from South Africa and EU, specially Ireland (Chalmers & Chitika 2013).

The dominant cattle breed in Malawi is the East African Zebu. In 2002, the national population was estimated to be 0.75 million head of cattle (Otte & Chilonda 2002). The Zebu is small compared to other cattle breeds. Malawian Zebu cattle are bred for meat and milk, both being important in the rural community (FAO 2000). Traditionally, Zebu cattle are kept in groups ranging from less than ten animals to more than 100. However, the majority of Malawian cattle farmers being smallholder farmers under an agro-pastoral system manage up to 10 head of livestock (Dixon et al. 2004). The rainy season continues only for a period of 4-5 months (December until end of April). In the rainy season, in the central and some parts in the south of the Malawi, grazing is restricted to the dambos (shallow wetlands), hillsides and roadsides because most of the arable land is used for crops. In the less dense populated northern Malawi, the cattle graze in natural pastures during the rainy season. At the onset of the dry season, cattle are moved to dambos, and natural pasture grazing is supplemented with crop residues and in some cases maize bran. Nearly three million hectare are available for natural grazing in Malawi (FAO 2000).

In most of the African continent, irrigation does not play any significant role in agriculture, making food production entirely dependent on rainfall and thereby susceptible to the detrimental effects of drought and erratic rainfall (You et al. 2011). Despite larger water reserves Malawi is regularly experiencing severe drought (Calow et al. 1997) and flooding has been a major problem in parts of the country due to erratic rainfall patterns (Menon 2007). There are more than 0.7 million small dams that have been reported to be in a state of disrepair in the country (Frenken 2005), indicating a poor ability to utilize the water resources. In addition, there are nine major dams in the country; seven of them were constructed for the municipal water supply and two of them were constructed for hydroelectric power generation.

1.4 Short overview of reproductive physiology in cattle

Cattle are non-seasonal polyoestrus animals. The length of the oestrus cycle usually range from 17-24 days, and is defined as the number of days between two standing oestruses (Marcel Amstalden 2015). Ovarian folliculogenesis is a complex biological process controlled by both intrafollicular factors, including locally produced growth factors, and extra-ovarian signals, such as gonadotrophins (Emori & Sugiura 2014; Marcel Amstalden 2015; Scaramuzzi et al. 2011). Every cow is born with a certain number of oocytes. The primordial follicle contains a primary oocyte surrounded by granulosa cells. A primordial follicle takes several months to reach the ovulatory stage (Scaramuzzi et al. 2011). Two-thirds of the way through this process, the follicles have transitioned to tertiary or antral follicles. Antral follicle development occurs under stimulation from follicle stimulating hormone (FSH). Further stimulation from luteinizing hormone (LH) enhances production of androgens by the theca cells. Androgen is in turn aromatized to estradiol by follicular granulosa cells. Increased blood levels of oestradiol triggers gonadotropin-releasing hormone (GnRH) release from the hypothalamus through a positive feedback mechanism that further stimulates follicular development onwards to ovulation or atresia (Marcel Amstalden 2015; Scaramuzzi et al. 2011).

Nutritional and metabolic inputs affect reproductive performance through complex interactions between the gonadotropic and somatotropic axes. Numerous hormonal and metabolic signals from the liver, pancreas, muscle and adipose tissue, including glucose, fatty acids, insulin-like growth factor-I, insulin, growth hormone, ghrelin and leptin influence brain centers that control metabolism, energy balance and feed intake (Chagas et al. 2007).

GnRH secretion is suppressed in animals experiencing severe negative energy balance, and the LH pulse frequency, which is the determining factor for the fate of the dominant follicles, may not peak. In such circumstances, the follicle undergoes atresia or in some cases, a cyst is formed (Butler 2000). When the dominant follicle produces sufficient oestradiol in a normal oestrus cycle, the positive feedback loop causes a surge of LH, and the follicle ovulates (Ball & Peters 2004).

Oestradiol is also responsible for the behavioural and physiological changes seen in cows entering oestrus. Common signs of oestrus include restlessness, flehmen, bellowing, standing to be mounted by other cows, and mucous vaginal discharge. The ruptured follicle will transform into a progesterone-producing structure known as the corpus luteum (CL) after ovulation. In a normal oestrus cycle, the CL starts producing progesterone 3-4 days after ovulation, production reaching a peak around day 8 and concentrations remain high in blood or milk until day 17. Progesterone concentrations during the different phases of the sexual cycle are shown in Figure 2.

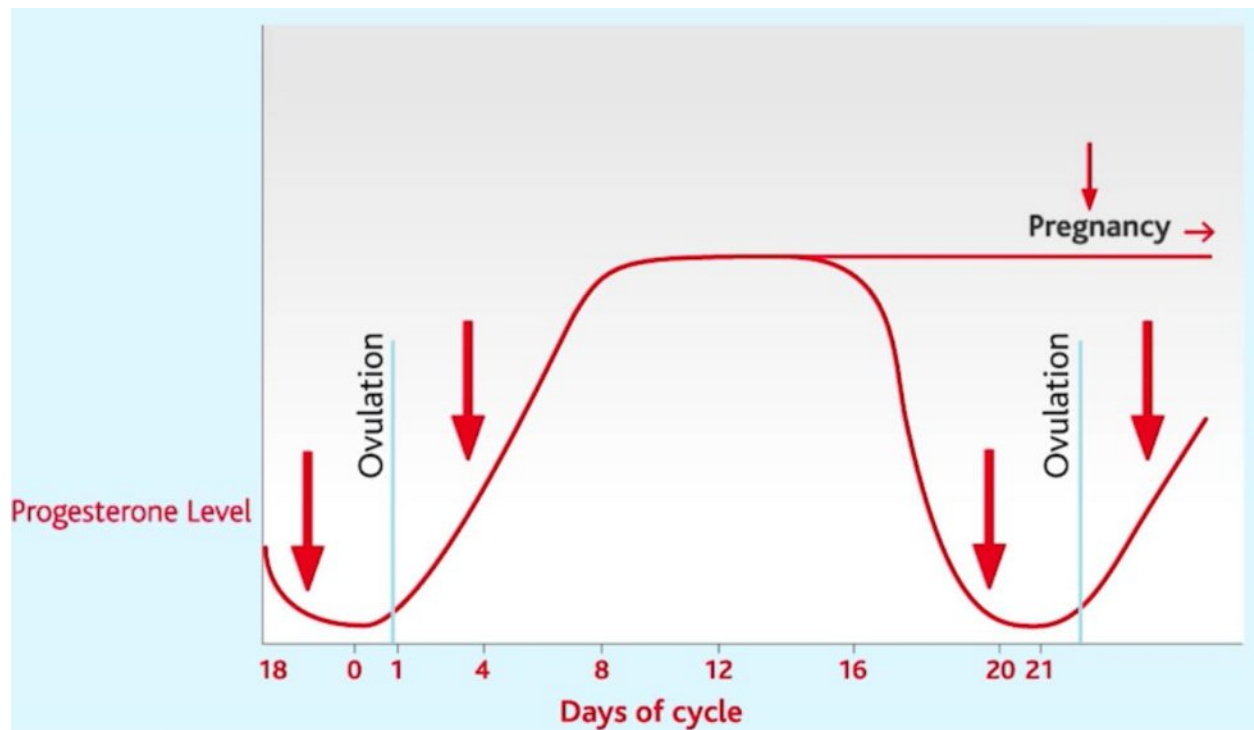


Figure 2. Diagrammatic presentation of progesterone level in bovine estrous cycle

Increased progesterone levels inhibit hypothalamic GnRH release, hence preventing LH-surge and ovulation in mid-cycle. The CL secretes oxytocin around day 17 of oestrus, which acts on the endometrium and leads to release of endometrial prostaglandin. Prostaglandin causes the CL to undergo luteolysis, ceasing production of progesterone and regressing the structure. If the oocyte is fertilized, embryonic trophoblast cells will produce interferon-tau, which prevents the increase of endometrial oxytocin receptors. Thus, oxytocin from the ovaries cannot stimulate prostaglandin synthesis and pregnancy is maintained (Ball & Peters 2004).

- **Energy:**

Cows in severe negative energy balance tend to prioritize body maintenance needs over reproduction (Chilliard et al. 1998), which leads to ovarian quiescence and an extended number of days open due to delayed (onset of luteal activity (OLA)). The median number of days from calving to OLA was 167 and 200 days for Tanzanian Zebu cows calving in the dry and the rainy seasons respectively (Matiko et al. 2008). Tanzanian Zebu cows with high body condition score (BCS) loss post-partum experienced an extended period from calving to OLA. The authors discussed that energy- and water-deficits during the dry period were most likely responsible for delayed commencement of ovarian cyclicity in Tanzanian Zebu cows, and in 11.5% of cows cessation of ovarian activity was observed (Matiko et al. 2008). Reproductive performance of Malawian Zebu cattle may be similar to the Tanzanian Zebu. In a study from Tanzania, Matiko (2008) reported an average calving interval of more than 470 days while Agyemang & Nkhonjera (1990) reported an average calving interval of more than 485 days in a study of pure- and cross bred cattle in Southern Malawi.

- **Management**

Local differences in management practices between villages in Tanzania have shown to affect the time interval from calving to OLA. Differences in herd composition, feeding practices, and seasonal breeding strategies may be part of the explanation, but other factors such as motivation, skills of the herdsman, and educational level probably also contributes to the “Village effect”. Parity was reported to affect reproductive performance in Tanzanian Zebu cows, where cows in their third or subsequent parities showed a significant reduction in the number of days to OLA (Matiko et al. 2008). Shorter interval from calving to OLA was observed by Matiko et al. (2008) in cows that lost their calf in early lactation.

A majority of smallholder farmers send their cattle for grazing and cows are exposed to available bulls while grazing. A recent study from the Mzimba District, Northern Malawi, showed that 64.6% of the farmers had no bull in their herd (Nandolo et al. 2015). Also the decision about which bull to keep in the herd was made by the owner although such a decision affected the whole breeding area because the cattle mixed freely. A relatively high inbreeding rate was estimated. However, the authors concluded that there was potential to establish a community breeding program (Nandolo et al. 2015).

- **Improved energy and protein feeding:**

Leguminous plants have been used to improve protein content in cattle feeding in several African countries. *Gliricidia sepium* leaves comprise of 21% crude protein, 23.8% crude fiber, 21-34% dry matter (Ministry of Agriculture and Fishries 2013). The high content of protein makes *Gliricidia* a great protein supplement to grass and other basal feeds. Due to high crude protein content in *Gliricidia sepium*, numerous of reports concludes with increased weight gain and milk production in both large and small ruminants after the introduction of this tree legume as a supplement (Aye & Adegun 2013).

1.5 Infectious causes of impaired production and reproduction

Health problems compromise body condition and decrease immune function. Poor health condition has been shown to exert a negative effect on milk yield (Fleischer et al. 2001) and reproductive performance (Opsomer et al. 2000). Reproductive problems can be caused by infectious agents, which exerts a direct or indirect effect on the reproductive system. The activation of immune and inflammatory responses can block oocyte function, interfere with oocyte maturation and embryonic development. The embryos might be lost due to hyperthermia, the toxic effect on the corpus luteum, reduction in the endometrial cell proliferation and interference with oocyte maturation and embryonic development (Hansen et al. 2004). In this study, we have concentrated on common agents that may decrease lactation yield and reproductive performance by influencing the cows' general health or exerting a direct effect on the reproductive system.

- **Theileria:**

Theileria spp. are protozoa which infect leukocytes and red blood cells through their life cycle (Mans et al. 2015). Nymphs and adult ticks inject the protozoa through their saliva. East coast fever, caused by *Theileria parva*, results in lymphopenia, enlarged lymph nodes, anaemia, high fever and dyspnoea. Mortality, particularly in indigenous cattle, is rare, but infection can sometimes result in progressive chronic anaemia (McKeever 2009). Milk production is decreased and the economic loss to the agricultural industry is significant (Mans et al. 2015).

- **Babesia:**

Babesia bovis and *Babesia bigemina* are hemoparasites that attack red blood cells (Kocan 1995). Babesiosis causes several symptoms such as; weakness, increased respiratory rate, fever, haemoglobinuria, jaundice, muscle wasting and tremors. The disease can be fatal. Juvenile cattle under 9 months rarely show symptoms if infected (Saad-Roy et al. 2015). The parasite is transmitted to other cattle through ticks, especially *Rhipicephalus* spp. *Babesia* can be transmitted by both larvae, nymphs and adult ticks.

- **Trypanosoma:**

Trypanosomiasis is a disease of domestic animals, wild animals and humans. The disease is caused by vector-borne haemoparasites, a protozoan belonging to the genus *Trypanosoma* (Hamilton et al. 2004). Biting flies (tsetse), fleas and leeches transmit the disease from host to host (Gibson 2007). There are several different species in this genus, in domestic livestock the most pathogenic species being *Trypanosoma vivax*, *Trypanosoma congolense* and *Trypanosoma brucei* (Dagnachew et al. 2014). Experimental infection with *T. vivax* in young Zebu cattle from tsetse and non-tsetse infested areas of northwest Ethiopia revealed an acute form of trypanosomiasis. The major clinical findings were reduced feed intake, fever, enlarged lymph nodes, edema, anemia, extreme weight loss and nervous signs (Dagnachew et al. 2014) which is likely to have a negative impact also on reproductive efficiency.

- **Neospora:**

Neospora caninum is recognized as a protozoan causing reproductive and economic loss worldwide (Anderson et al. 2000; Dubey et al. 1988; Trees et al. 1999). The parasite causes

abortions through either maternal placental inflammation, maternal and foetal placental necrosis, or foetal damage (Dubey et al. 2006). The reason why only some infected animals abort and not others is still unknown (Canton et al. 2014). Birth of weak calves and calves with neurological deficits have been seen after foetal infection, but most congenital infections are subclinical (McAllister 2014). Bovine neosporosis manifests in both epidemic and endemic transmission patterns, but a high infection prevalence of the protozoa is possible without a noticeable abortion problem. Canids, which act as definite hosts for the parasite, shed oocysts in faeces. The oocysts are capable of surviving for prolonged periods in soil and water. Cattle act as an intermediate host and are infected by ingesting oocysts. Infection of the foetus occurs through trans-placental transmission of the parasite (McAllister 2014).

- **Q fever:**

Q fever is a zoonotic disease caused by the gram-negative coccobacillus *Coxiella burnetii* (Arricau-Bouvery & Rodolakis 2005). The greatest risk of transmission occurs at parturition by inhalation, ingestion, or direct contact with birth fluids or placenta. The organism is also shed through milk, urine and feces. Ticks may transmit the disease among domestic ruminants: ixodid (hard ticks) and argasid ticks (soft ticks) can act as reservoirs of the organism. Infection in ruminants is usually subclinical but can cause anorexia and abortion (Parker et al. 2006).

- **Brucella:**

Brucella is a genus of gram-negative bacteria that causes brucellosis (Shevtsov et al. 2015). The bacteria is zoonotic and affect human and animal health worldwide (Dorneles et al. 2015). In cattle, *Brucella abortus* is the primary cause of disease (Kabi et al. 2015). Bovine brucellosis causes abortions, weight loss, stillbirth and decreased milk yield, which result in economic loss and reduced production (Dorneles et al. 2015). Aborted foetuses and unpasteurized products are sources of infection to humans and susceptible animals (Kabi et al. 2015). The seroprevalence of brucellosis amongst cattle was reported to be 15.6% in one investigation in Gairo division, Morogoro Region, Tanzania (Kanuya et al. 2006), and the disease is endemic in most African countries (Holt et al. 2011; Muma et al. 2007; Schelling et al. 2003).

- **Bovine viral diarrhoea virus (BVDV):**

BVDV is a pesti-virus belonging to the family Flaviviridae. The virus is known to cause a variety of clinical manifestations of disease in cattle (Baker 1995), and is considered to be endemic in most cattle-producing countries (Gruenberg 2014). Reproductive and immunosuppressive effects of the virus cause considerable economic impact (Houe 1999). BVDV infection can lead to reduced conception rate, congenital abnormalities, early embryonic death and abortions (McGowan et al. 1993). In bulls, reduction of sperm density and motility, as well as increased sperm abnormalities have been reported (Paton et al. 1989). Severe clinical disease, presented as acute BVD or mucosal disease, is usually seen in cattle between 6-months and 2 years of age. Persistently infected cattle represent the main reservoir for transmission of BVDV (Gruenberg 2014)

- **Foot and-mouth disease (FMD):**

The disease is caused by an aptho-virus of the family of Picornviridae and there are seven different serotypes. The virus is highly contagious, and is transmitted through direct or indirect contact. The primary site of infection is in the mucosa of the pharynx, and the virus spreads along the lymphatic system. Symptoms are fever and blisters on the feet and in the mouth. Vesicles rupture within 48 hours and leave wounds causing anorexia and lameness. Morbidity can be as high as 100% in a susceptible population. Chronic post-FMD syndromes may appear four weeks after acute disease (Kitching 2002) and are also referred to as “Heat-Intolerance Syndrome” (HIS) (Artz 2011). Symptoms are heat intolerance (Barasa 2008), hirsutism (Ghanem & Abdel-Hamid 2010) and hypertrichosis from failure of seasonal shedding of hair. Pronounced panting in hot weather, increased pulse rate and elevated body temperature are also reported (Mullick 1949). FMD can disturb the reproductive cycle and can result in anoestrus, nymphomania, abortion and birth of weak or dead calves (Artz 2011).

2. Objectives

Through capacity building of successful Zebu cattle management in the Bolero-EPA, Rumphi district, Malawi, the overall aim of the current project is to increase climate change resilience by enhancing milk production and reproductive performance. We will investigate factors which may impact on reproduction and milk production in Malawian Zebu cattle from smallholder farms. It includes recommending protocols for bull management and optimal feeding of Zebu cattle for reproductive efficiency during the driest months of the year. The impact of water source and water availability on reproduction and lactation and the prevalence of infectious agents with a possible impact on reproductive failure will also be evaluated. The following sub goals have been identified:

- (1) To describe the smallholder cattle farming system and ongoing general practices regarding management of Zebu cattle in the Bolero EPA, Rumphi District through a cross sectional study.
- (2) To determine reproductive and lactation performance and important influencing factors in Zebu cattle calving in fall.
- (3) To determine the prevalence of possible infectious causes for reproduction and production inefficiency in Zebu cattle.

3. Material and methods

3.1 Timeline of activities

Field activities pertinent to the current master thesis started in August 2015 and continued until June 2016 (Figure 3.)

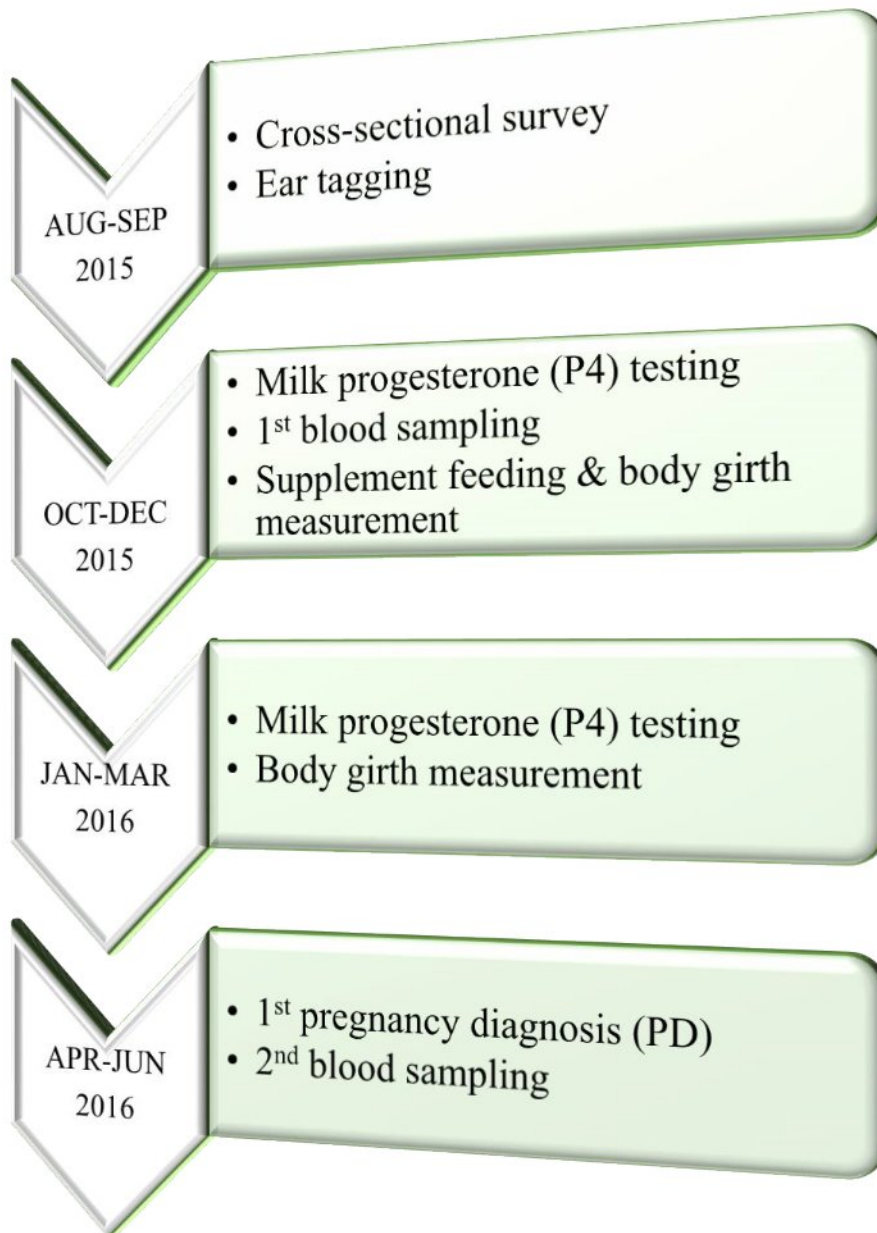


Figure 3. Timeline of field activities of the master thesis

3.2 Material and methods Objective 1

Objective 1: To describe the smallholder cattle farming system and ongoing general practices regarding management of Zebu cattle in the Bolero EPA, Rumphi District.

Due to non-availability of the concise information about the cattle herd size, herd composition and smallholder farming system in Bolero-EPA, a cross sectional survey was conducted to obtain basic information about the smallholder Zebu cattle farming system. Questions were asked focused on availability of water, fodder, breeding bulls and herd size in the Bolero-EPA, Rumphi district, Northern Malawi.

- **Site selection:**

The study was conducted in Bolero-EPA, 63 km from the Northern capital - Mzuzu. The focus area of study involved 31 villages belonging to seven sections in the Bolero - EPA: Bolero/Bolero-A, Bata, Chozoli, Chikwawa, Chirambo, Mjuma and Jalira. This area was selected due to following reasons: Rumphi is one of the five districts in Malawi where smallholder farming is predominantly practiced (Mzuzu Agriculture Development Division 2009), highly affected by the effect of climate change and soil suitable for the cultivation of leguminous plant – *Gliricidia sepium*.

- **Survey conduction:**

Before the cross sectional survey was conducted, all the farmers, belonging to 19 Community Development Committees (CDC) of Bolero-EPA, were invited for a meeting about the project activities. Approximately two hundred farmers gathered for a meeting at one of three locations: Church of Central African Presbyterian (CCAP), Chikwawa Rural Training Centre (RTC), and Bolero and Kwaza school - Kwaza, on September 1st, 3rd and 8th 2015 respectively. The aim of these meetings was to identify farmers that were willing to participate in the project activities, to increase the general awareness about the project research activities (including project theme and objectives), and to identify cows that had calved or were expected to calve between August 15th and September 19th 2015.

After the initial meetings, 39 smallholder farmers of the Bolero-EPA were selected for the cross-sectional survey. One student from the Norwegian University of Life Sciences and one student from Lilongwe University of Agriculture and Natural Resources together with the field assistant

from TAPP conducted the survey. This team worked together both in the conduct of the survey and ear tagging during farm visits that took place during September 2015.

The questionnaire was presented to the facilitators (field assistant and students from NMBU and LUANAR) before the interviews took place in order to familiarize them with the material and avoid miscommunication with farmers. During the face-to-face interview with farmers, survey questions were translated into the local language by the Malawian student and field assistant. The cross-sectional survey comprised the following sub-sections: herd size and structure, milking practices, milk yield, milk marketing, husbandry practices, source of water, distance from the water source, pregnancy status of cows, availability of breeding bulls, reproductive history and current reproductive status, feeding practices, seasonal feed and fodder management, vaccination status and land use for irrigation. Female calves older than 8-months were defined as heifers until calving. The survey questionnaire is presented in Appendix 1.

3.3 Material and methods Objective 2

Objective 2: To determine reproductive and lactation performance and important influencing factors in Zebu cattle calving in fall.

- **Cow selection:**

Initially 109 Malawian fall-calving Zebu cows owned by 39 farmers from 31 villages within seven sections of the Bolero - EPA were enrolled in this cohort study. Cows were followed from October 6th 2015 until October 22nd 2016. Cows were chosen on the following selection criteria: Calving within 5 weeks between August 15th and September 19th 2015, moderate to good body condition, free from clinical signs of diseases or other indications for compromised health. Two farmers with a total of eight cows withdrew during the first three weeks of study, such that the cohort encompassed 101 cows from 37 different households.

- **Outcome variables in statistical tests:**

The following outcomes were assessed. Pregnancy status (1/0) at January 10th 2016, days from calving to pregnancy (days open) until January 10th 2016, and length (days) of the lactation period. Pregnancy status at January 10th was assessed by rectal examination between April 11th and 16th 2016, and by calving during the summer / fall of 2016. Conception dates were approximated by subtracting 280 days from the calving date. However, date of conception was set to January 10th 2016 in two cows that were pregnant in April, but did not calve because one cow aborted in May and the other was sold. In one farm, the ear tagging of the cows were mixed up between January and April 2016 such that progesterone data were used to establish figures for conception date in four cows. Days open were calculated as the difference between approximated conception date and calving date. Calving dates were obtained from the farmers.

The date of cessation of lactation was approximated by using the date when the farmers no longer obtained milk for human consumption (n=75), or the date for the last milk sample obtained for analytical purposes (n=26) in cows that still were lactating. The last milk sampling was conducted February on 9th 2016.

- **Factors of study (explanatory variables):**

The following variables were assessed to explain variation in the outcome variables: Availability of breeding bulls, distance to water source for the livestock during October and November 2015 (near / far), type of water source (tap, bore, well, dam, pond, river), effect of additional feeding during October and November, parity (first, second and > second), changes in body girth circumference (cm) from October 2015 to February 2016.

Information on availability of bulls, distance to water, and water source for livestock were obtained at farm level from the cross sectional survey described in Section 3.1. Measures of body girth circumference (cm) were obtained monthly. However, this exercise required that the animals were restrained, which was not always achievable under the field conditions. Therefore, the difference in body girth circumference between October and February were assessable in 74 out of 101 cows.

Additional feed during the months October and November was supplied to 29 cows beginning October 9th. Another two cows were added to the “experimental group” on October 26th, 2015 as supply for additional feed had improved to some extent. These 31 cows comprised the “experimental group” and received 2 kg of dried *Gliricidia sepium* leaves and 2 kg maize bran daily in addition to pasture feeding. Additional feeding ceased on November 27th, 2015. Parity of the cows was obtained at earmarking during the cross sectional survey before the cohort study was initiated.

For the effect of additional feeding, the 31 cows in the “experimental group” were compared with a “control group” of cows that were matched in terms of parity and distance to water. After calving, cows within the same category of parity and distance to water were alternately assigned to the experimental or control groups. Farmers having access to drinking water for livestock at or within a distance of 1.5 Km were included in the “near” category while farmers with water access at a distance of more than 1.5 km were included in “far” category.

- **Onset of luteal activity (OLA) and ovarian function:**

All cows were subject to milk sampling for the assessment of milk progesterone by a cow side P4-rapid test (Ridgeway Science, UK). Sampling for progesterone measurements started between October 6th and 15th 2015. The sampling period continued until the last two weeks in December

2015. Typically, the last progesterone sample was obtained between December 15th and 30th 2015. However, among other challenges, this depended on accessibility of the farms during the rainy period and on whether cows were still lactating three to four months after calving. Cows were preferably sampled twice weekly for the period, but sampling frequency typically ranged between three and seven days. Altogether 96 cows were included in this part of the cohort study as five cows were omitted due to irregular sampling intervals.

Progesterone analyses were used to determine time from calving to OLA as defined as three consecutive values within 21 days with a positive reaction for elevated milk progesterone. The P4 Rapid test (Ridgeway Science, St Briavels, England) was used according to the manufacturer's recommendations. The following procedure was used to conduct the P4 Rapid test as shown in Figure 4.

- To ensure dispersion of the progesterone, which is accumulated in the milk fat, the milk was mixed thoroughly in a sample vial.
- The milk was transferred from the sample vial to a test tube with a clean pipette.
- The P4 rapid test stick was removed from the packet and marked with the tag number of the cow and the date.
- The stick was placed in the milk sample, red-end down. Care was taken to ensure that the red part of the stick stayed dry. The stick was left standing in the test tube for at least 5 minutes.
- The strips were interpreted by the author.

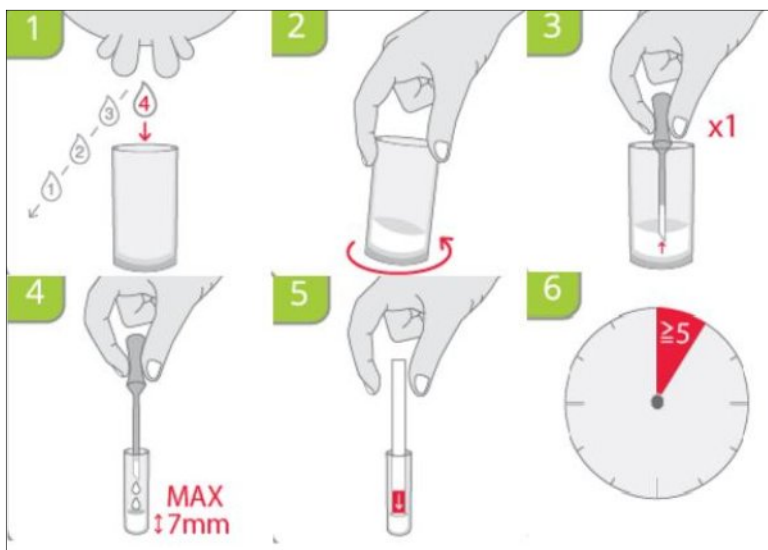


Figure 4. P4-rapid test procedure

- **Analyses of feed supplement:**

Feed samples of *Gliricidia sepium* leaves and maize bran were collected and sent to the Animal Science Department (LUANAR, Malawi) for nutritional analysis. Feed samples were analyzed for dry matter (DM), ash (oven drying) and crude protein (CP) by Kjeldhal using the AOAC 1995 procedures (Intl 1995). Gross energy (GE) was calculated using 6100 Oxygen Bomb Calorimeter (Yan & Kim 2011). Acid detergent fiber (ADF) was analysed by using an Ankom fiber analyzer and fat percentage by Ankom (xt10) extractor as described by Cao et al. (2009).

3.4 Material and methods Objective 3

Objective 3: To determine the prevalence of possible infectious causes for reproduction and production inefficiency in Zebu cattle.

Blood sampling was conducted between October 19th and 23rd 2015 and between April 11th and 16th 2016 to get two consecutive samples from cattle and their calves. In October 2015 –105 cows and their 105 calves were sampled, and in April 2016, samples from 95 cows and 88 calves were collected. The samples were analysed for antibodies against foot and mouth disease virus (FMDV), bovine viral diarrhoea virus (BVDV), *Coxiella burnetii*, *Brucella* spp. and *Neospora caninum* (Figure 4) at the Norwegian Veterinary Institute in Oslo, Norway. In April 2016, blood smears were also made on site for evaluation of the blood parasites; *Theileria* spp., *Babesia* spp. and *Trypanosoma* spp.

- **Blood sampling:**

Sera was obtained by collecting blood into sterile vacutainers without additives and cooled in the field cooler until centrifugation at 1200xg. Serum was frozen on the same day as blood sampling. EDTA (Ethylene Diamine Tetra Acetic acid) mixed blood was obtained for the evaluation of blood parasites. The blood smears were examined on site by microscopy at the same day by an experienced technician from the Central Veterinary Laboratory in Lilongwe, Malawi. The blood smears were examined in April 2016 since we expected the prevalence of blood parasites to be at a peak during the end of the rainy season.

- **Serology:**

Cows blood sampled in October 2015 as well as all cows and their calves blood sampled in April 2016 were tested for the presence of antibodies against FMDV, BVDV, *Coxiella burnetii*, *Brucella* spp. and *N. caninum* using enzyme – linked immunosorbent assays (ELISAs). There are different ELISA assay principles such as indirect ELISAs, sandwich ELISAs and competitive or inhibition ELISAs. In this study, well-established commercial ELISAs were used. ELISAs were chosen because they are among the serological methods recommended by OIE for the agents investigated (Wright et al. 1993), are commercially available, and suitable for large sample size.

The ability of a test to distinguish between infected and non-infected individuals is described by its diagnostic sensitivity and specificity. The sensitivity is defined as the proportion of infected individuals correctly identified by the test, and the specificity is the proportion of non-infected individuals correctly identified. The sensitivity and specificity of a test depend on the selected cut-off level, and alteration of the cut-off value will always affect both the sensitivity and specificity (Altman & Bland 1994).

Results of these ELISAs are given as absorbance values after correlation to a positive control; Corrected Optical Density ($OD_{(corr)}$) values and Percent Inhibition (PI) or Percent Positive values. Results of indirect ELISAs are often expressed as PP and competitive ELISAs are expressed as PI values. Some examples for calculations used for $OD_{(corr)}$, PP and PI values are given below:

$$OD_{Corr} = OD_{sample} - OD_{control}$$

$$PP = \frac{OD_{[(corr)Sample \text{ or } Negative \text{ control}]}}{OD_{[(corr)Positive \text{ control}]}} \times 100$$

$$PI = 100 - \frac{OD_{450}^1 \text{ test sample}}{OD_{450} \text{ max}^2} \times 100$$

¹ OD_{450} is Optical Density of the wells measured at 450 nm light density within 15 minutes after the colour development has been stopped.

² $OD_{450} \text{ max}$ is the mean of Corrected OD_{450} of wells

- **Antibody analysis:**

- All sera collected was screened for the presence of antibodies to FMD virus (FMDV) in the Biosafety Level 3 Laboratory at NVI, using PrioCHECK[®] FMDV NS (Prionics AG Zurich, Switzerland). This analysis identifies FMDV seropositive animals independent of serotype and vaccination status.

The optical density (OD) was measured in a Lab system multiskan EX spectrophotometer. All OD-values were correlated to a positive control serum following the manufacturer's instructions.

PI-values for the positive, weak positive and negative values are shown in the Table 1.

Table 1: Percentage Inhibition (PI) values for ELISA (PrioCHECK[®] FMDV NS) for foot and mouth disease

Category	PI-Value
Positive	PI > 70.0%
Weak positive	PI 70.0% - > 50.0%
Negative	PI 50.0% - <50.0%

- For the detection of antibodies against BVDV, an indirect ELISA (SVANOVIR[®] ELISA) was used. All OD-values were correlated to a positive control serum following the manufacturer's instructions For the BVDV ELISA, the following criterion was validated.
 - Optical Density (OD) value for positive controls should be >0.5
 - Percentage Positive (PP) values for negative should be <5

Table 2: Standard Optical Density (OD) & Percentage positive (PP) values for BVDV ELISA (SVANOVIR[®] ELISA)

Test criteria	Requirement	Category	PP value
OD positive control	OD > 0.5	Positive	≥10%
PP negative control	PP < 5	Negative	<10%

- For detection of antibodies against *Coxiella burnetii*, Q-fever IDEXX antibody test kit (IDEXX, USA) was used. All OD-values were correlated to a positive control serum following the manufacturer's instructions. Test results are presented in percentage of Sample to Positive ratio (S/P %). S/P % is calculated by using the formula below:

$$\frac{S}{P} \% = \frac{\text{Sample mean} - \text{Negative control mean}}{\text{Positive control mean} - \text{Negative control mean}} \times 100$$

Table 3: Percentage of sample to positive ratio values for *Coxiella burnetii* ELISA (IDEXX)

Category	S/P%
Positive	≥ 40%
Doubtful	30% - < 40%
Negative	< 30%

- Antibodies to *Brucella* spp. were detected using SVANOVIR[®] Brucella-Ab-I-ELISA (Boehringer Ingelheim Svanova Uppsala, Sweden) antibody test. Sera samples were considered positive having PP-value PP ≥ 40% while samples with PP < 40% were considered as negative
- Antibodies to *N.caninum* were detected using SVANOVIR[®] *N. caninum* iscom ELISA (Boehringer Ingelheim Svanova Uppsala, Sweden) antibody test following the manufactures instruction. Sera samples were considered positive when PP values where ≥ 20% while samples with PP < 20% were considered as negative.

4. Statistical analyses

Statistical analyses were performed by the statistical software package JMP Pro 12 (SAS Institute Inc., Cary, NC), RStudio (Version 0.99.896), R (Version 3.2.2) Inc. Boston MA, USA and googleVis-0.6.1 package was used for the creation of pie charts and histograms. Statistical significance was considered with a *P*-value less than 0.05. Statistical tests are referred to as borderline non-significant with a *P*-value equal to or less than 0.10.

Estimates of pregnancy percentage from the cross sectional study was compared between farms with and without their own breeding bull(s) using univariate analyses of variance (ANOVA). ANOVA was also applied for the evaluation of an association between level of milk production and income from milk marketing as obtained in the cross sectional survey.

Univariate survival models by means of Kaplan-Meier (K-M) estimators and the log rank statistics were used to assess relationships between days open and the presence of breeding bull(s) in the herd, water management, parity and provision of supplemental feed. The relationship between pregnancy (1/0) and Agricultural Sections within Bolero-EPA was assessed using the chi-squared test for categorical explanatory variables. Similarly, the association between pregnancy (1/0) and changes in body girth circumference between October and February were assessed in a univariate logistic regression analysis.

ANOVA was used when lactation length was compared between Agricultural Sections, parities and the provision of supplemental feeding. Linear regression was used to assess the relationship between lactation length and changes in body girth circumference between October and February. Loess smoothing was used to evaluate the linearity of this model.

Only univariate tests without adjustment for clustering at farm level were conducted. Significance levels should be interpreted with caution as violation of the assumption of independence may have underestimated the width of the standard errors in some models.

5. Results

5.1 Results Objective 1

Objective 1: To describe the smallholder cattle farming system and ongoing general practices regarding management of Zebu cattle in the Bolero EPA, Rumphi District.

- **Smallholder cattle population density and herd structure:**

The cattle population at the 39 farms included in the survey are summarized by section of Bolero EPA in Table 4. The highest density of cattle was found in Bata section. Average number of bulls available per registered farmer (n=39) was 0.94.

Table 4. Distribution by section of the cattle population (n=509) in 39 surveyed farms in the Bolero EPA, Rumphi, Malawi.

Sections	Adult cows			Heifers	Calves	Bulls	Total
	Milking cows	Dry cows	Conceived cows				
	No. (%)	No. (%)	No. (%)				
Chozoli	21 (43)	4 (8)	8 (16)	16 (33)	23 (28)	11 (13)	83 (16)
Chikwawa	25 (46)	4 (7)	9 (17)	16 (30)	27 (31)	6 (7)	87 (17)
Bolero	7 (47)	0 (0)	2 (13)	6 (40)	11 (41)	1 (4)	27 (5)
Bata	52 (44)	13 (11)	15 (13)	38 (32)	68 (35)	10 (5)	196 (39)
Chirmabo	24 (45)	1 (2)	8 (15)	20 (38)	23 (28)	7 (8)	83 (16)
Mjuma	5 (50)	0 (0)	2 (20)	3 (30)	6 (35)	1 (6)	17 (3)
Jalira	4 (36)	0 (0)	3 (27)	4 (36)	4 (25)	1 (6)	16 (3)
Sum	138 (27)	22 (4)	47 (9)	103 (20)	162 (32)	37 (8)	509 (100)

- **Distribution of cattle and sections with respect to water source:**

Six different water sources were used by the smallholder farmers included in the cross sectional survey from Bolero-EPA (Table 5). These included river, dam, tap water, small pond, bore water and open well. Cattle from the Chirambo section had access to all water sources except small ponds. Cattle from Chozoli, Chikwawa, Mjuma and Jalira Sections had access only to river as

the source of water while the remaining two Sections (Bolero and Bata) had access to at least three water sources (Table 5), namely river, dam and tap water/small pond.

Table 5. Overview of water sources availability with respect to location

Sections	River	Dam	Tap water	Little pond	Bore water	Open well
Chozoli	✓	-	-	-	-	-
Chikwawa	✓	-	-	-	-	-
Bolero	✓	✓	✓	-	-	-
Bata	✓	✓	-	✓	✓	-
Chirambo	✓	✓	✓	-	✓	✓
Mjuma	✓	-	-	-	-	-
Jalira	✓	-	-	-	-	-

The distribution of cows with respect to the main water sources are displayed in Figure 5.

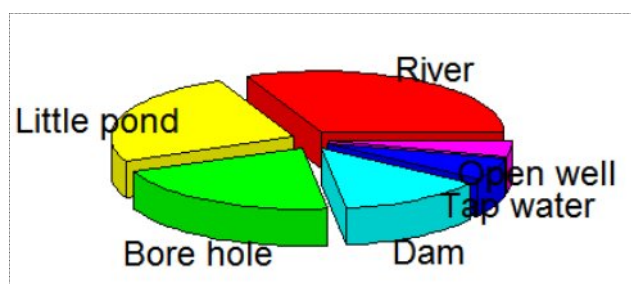


Figure 5. Cow density (%) with respect to water source.

- **Herd pregnancy percentage and bull ratio:**

Herd pregnancy percentage of the cross sectional survey was calculated from the data provided through interview with the farmers. Pregnancy percentage is determined by dividing the number of pregnant cows by the total number of adult cows in the herd. Distribution of pregnancy percentage with respect to the number of farms is shown in Figure 6, and the distribution of pregnancy percentage by Agricultural Section is displayed in Figure 7.

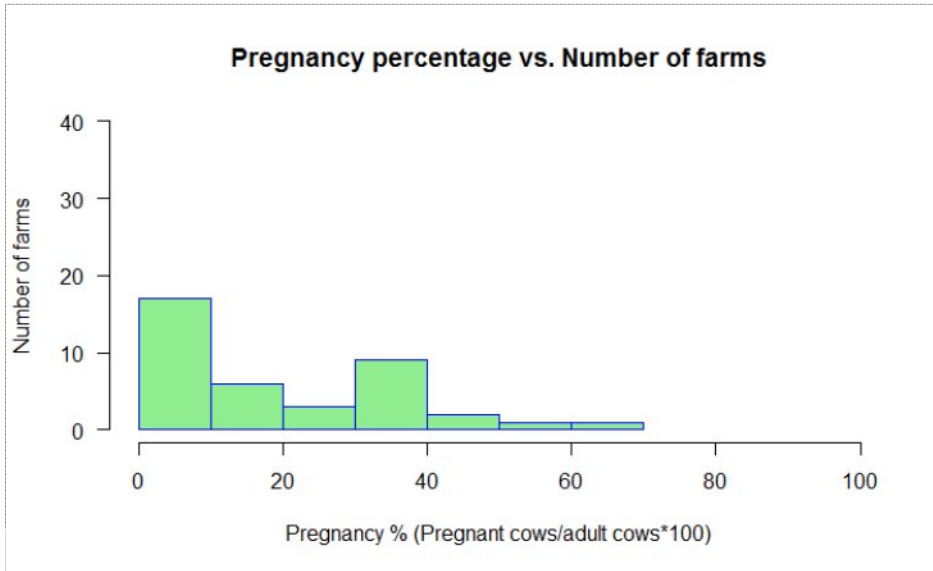


Figure 6. Pregnancy percentage distributed by number of farms

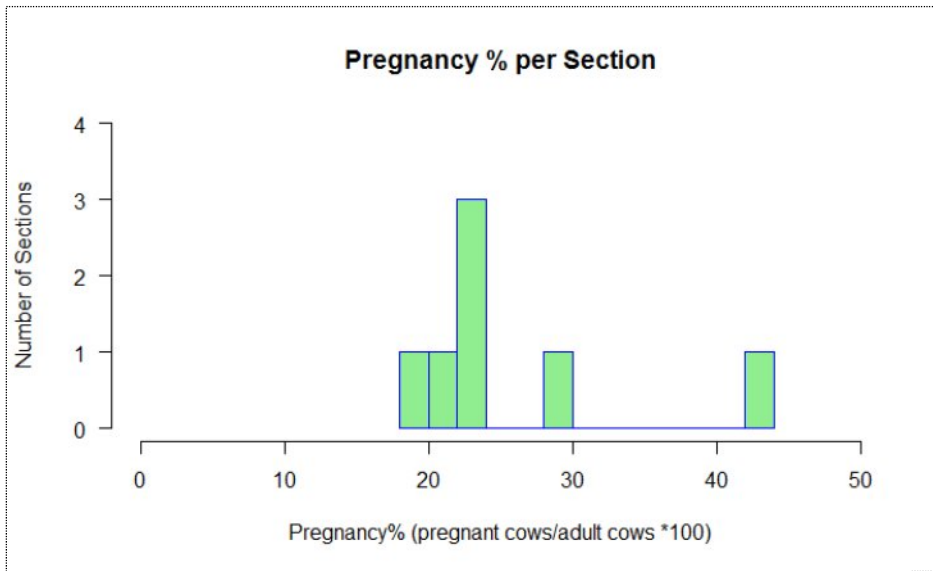


Figure 7. Pregnancy percentage distributed by number of Agricultural Sections

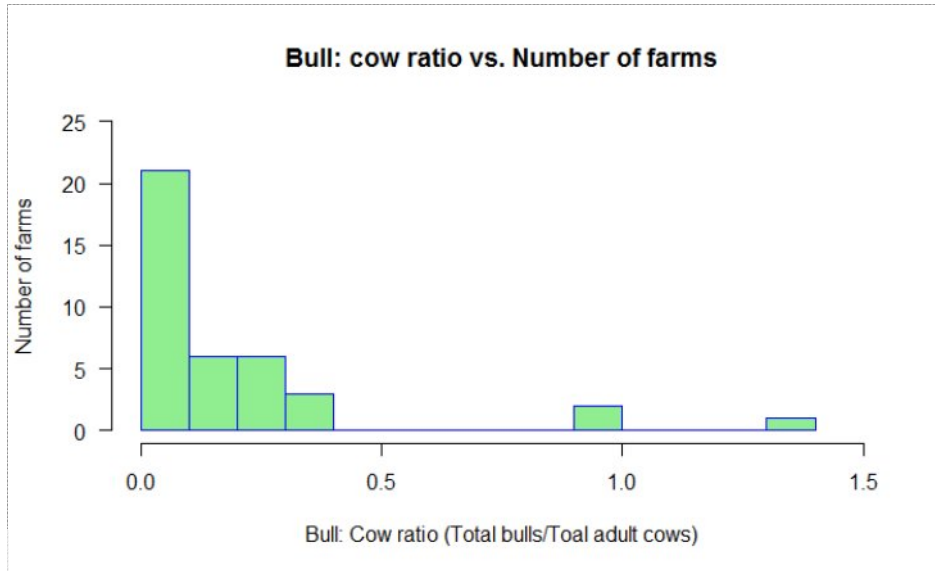


Figure 8. Bull:cow ratio distributed by number of farms

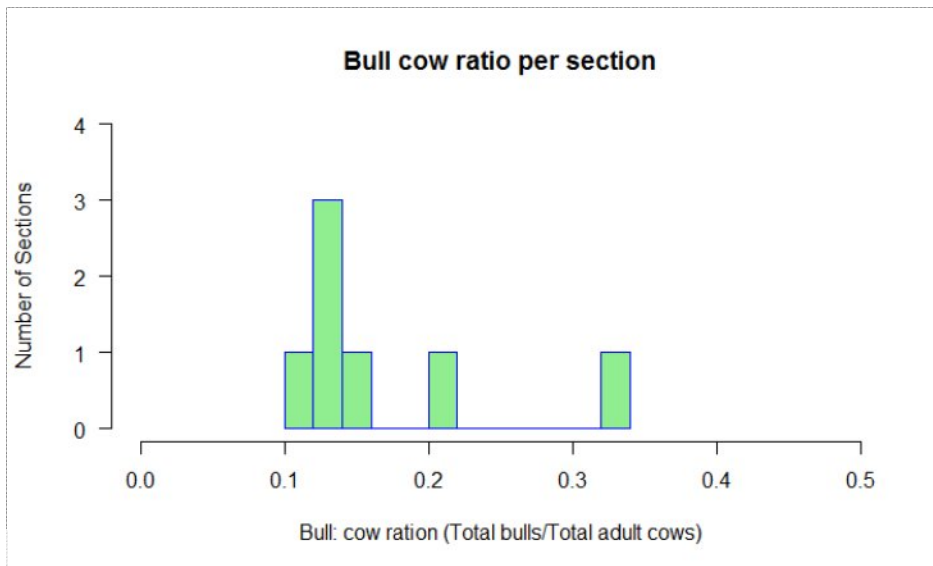


Figure 9. Bull cow ratio distributed by number of Agricultural Sections

The bull:cow ratio of the herd is determined by dividing the number of available bulls at shed by all the adult cows in the current herd. Heifers were not included in the adult cow group. The distribution of the bull:cow ratio with respect to farms and Agricultural Sections is given in Figures 8 and 9 respectively.

The difference in pregnancy percentage between herds with and without a breeding bull was assessed. The estimated difference was borderline non-significant ($P = 0.07$). Presence of a bull 24 hours a day explained 8.6% of the variance in pregnancy percentage between the adult cows included in the cross sectional survey ($n=207$). The mean pregnancy % in this population of Malawian Zebu cattle was 18.5% in September 2015. The mean of the responses in the ANOVA model relative to presence of a breeding bull (1/0) may be obtained from Table 6.

Table 6. ANOVA of pregnancy percentage with respect to presence / absence of a bull in the herd.

Bull present	Mean pregnancy %	Std error	F-ratio	<i>P</i> -Value
Yes	23.1	4.0	3.5	0.07
No	12.0	4.5		

- **Average milk production relative to marketing of milk:**

Average milk production per cow per day was calculated from the cross sectional survey data provided by the farmers. Milk production was calculated in terms of the number of bottles (300 ml) produced per day per cow. Average milk production per cow per day at the farm level is given in Figure 10. Thirty-five farmers (90%) milked their cows once per day, only one farmer milked his cows three times per week and three farmers did not milk their cows at all. The majority of the farmers ($n=35$) did not sell milk while four farmers (10%) sold milk as displayed in the ANOVA in Table 7.

Table 7. ANOVA of average milk yield per cow per day in farms (n=39) that were marketing milk versus farms not selling surplus of the milk production.

Marketing of milk	Average milk yield kg/day	Std error	F-ratio	<i>P</i> -Value
No	1.27	0.11	10.39	0.002
Yes	2.40	0.33		

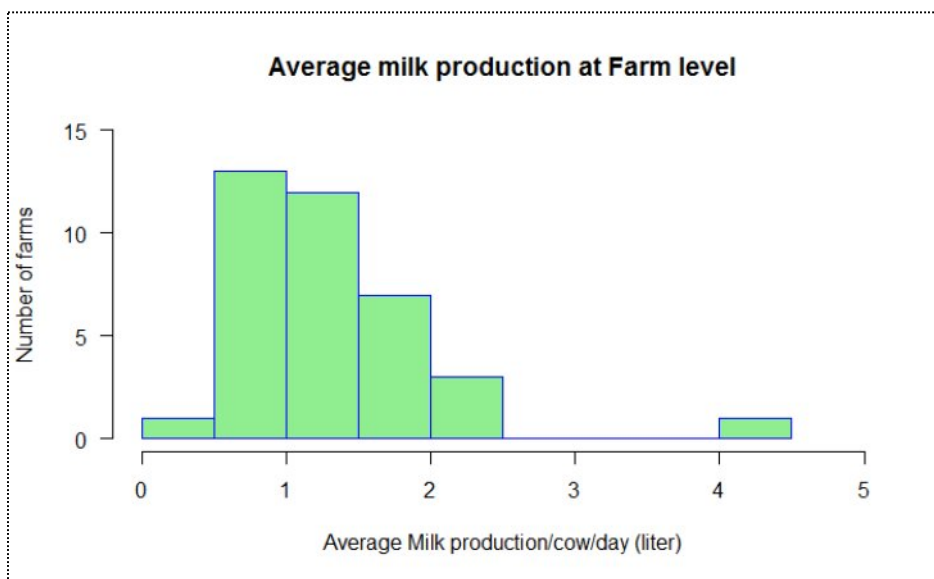


Figure 10. Average milk production per cow per day at the farm level

5.2 Results Objective 2

Objective 2: To determine reproductive and lactation performance and important influencing factors in Zebu cattle calving in fall.

- **Determining Onset of luteal activity (OLA) by progesterone testing:**

OLA in Aug-Sep calving Zebu cows was defined as three consecutive samples of elevated progesterone within 21 days. Using this definition, 43 cows already showed OLA at the beginning of the milk sampling period. The median value of OLA after calving was 61 days and this value was obtained as an average number of days to OLA in cow number 48 (60 days) and 49 (62 days). Twenty five out of 101 cows did not experience OLA before sampling for progesterone ended in the last week of December.

- **Reproductive status at January 10th 2016:**

Fifty-three out of 101 cows were found to be pregnant on January 10th 2016, and 48 cows were not pregnant. Calving was recorded in 49 of the pregnant cows and day of conception was calculated based on the date of calving. Day of conception was assessed by progesterone profiles in four cows. The mean number of days from calving to pregnancy in these 53 cows was 65 days (SD 32 days). This showed a potential for acquiring calving intervals of less than one year in the Zebu population, if best practices were utilized. Late abortion occurred in three and stillbirth in one of the 53 cows that were assessed to have been pregnant on January 10th 2016 (Figure 11 & 12).

Among 48 non-pregnant cows in April 2016, pregnancy was verified in 20 cows at the second pregnancy diagnosis (PD) between October 23rd and 27th 2016, while the absence of pregnancy (< 5 weeks pregnant) was confirmed in 18 cows in October 2016. A diagnosis was not conducted in the remaining 10 cows because the cows either had died involuntarily (n=5), were sold (n=4), and in one case the PD was not performed because of animal welfare reasons.

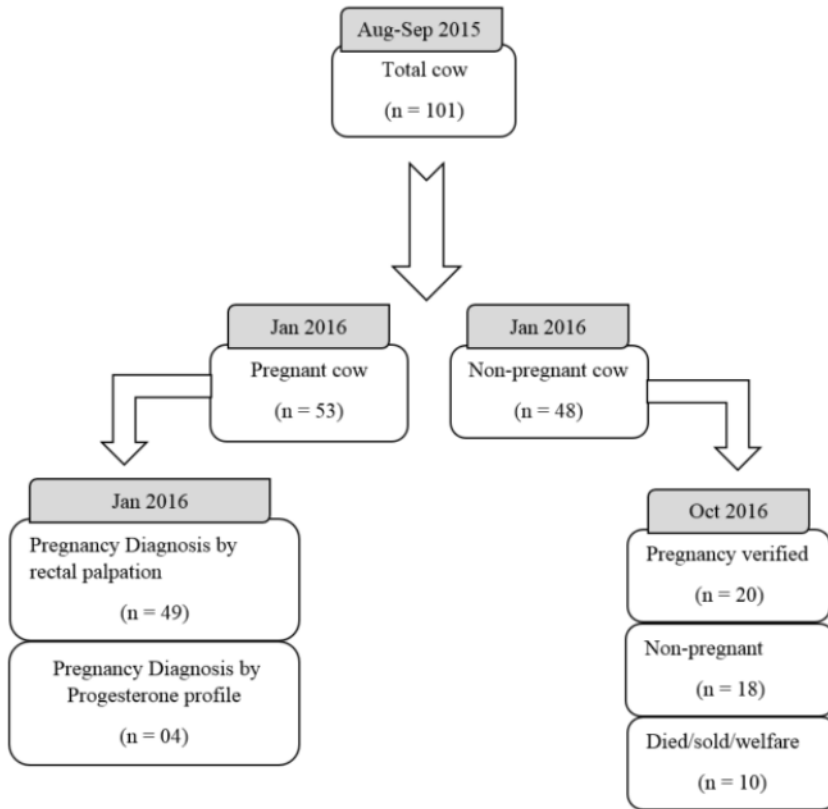


Figure 11. Reproductive status of fall (2015) calving Zebu cows

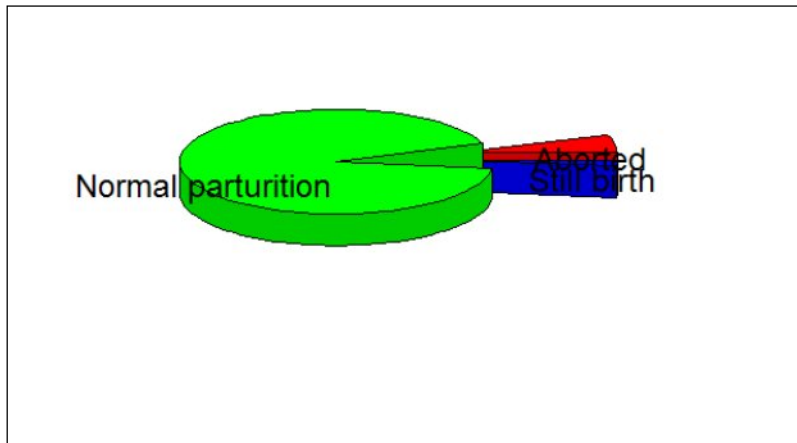


Figure 12. Late abortion and stillbirth in Malawian Zebu cows (n=53) that were pregnant in January 10th 2016.

- **Pregnancy percentage distributed by section**

The distribution of percentage of cows that were diagnosed as being pregnant by January 10th 2016 in the different Sections of the Bolero-EPA is displayed in Figure 13 and Table 8. The Pearson chi- square statistic (Table 8) showed that pregnant cows were not equally distributed by Section ($P = 0.015$).

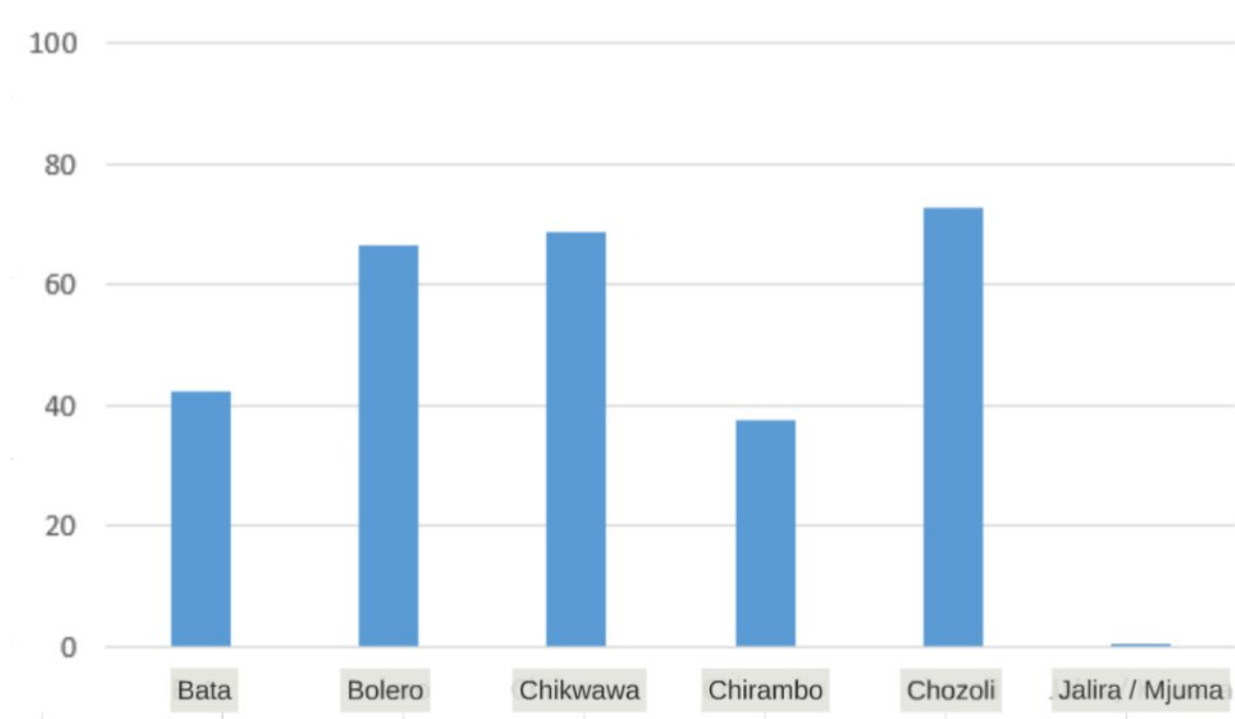


Figure 13. Distribution of pregnant Malawian Zebu cows (%) by January 10th 2016 (n=53) distributed by sections of the Bolero-EPA

Table 8. Distribution of Malawian Zebu cows (n=101) by pregnancy status and Section of the Bolero-EPA. (Pearson chi-square statistic $P = 0.015$). Distribution of expected number of cows under the null hypothesis of no association is presented in parentheses

Observed no. of cows (Expected no. if no association)	Pregnant	Not pregnant	Total
Bata	26 (23.6)	19 (21.4)	45
Bolero	2 (3.1)	4 (2.9)	6
Chikwawa	5 (8.4)	11 (7.6)	16
Chirambo	10 (8.4)	6 (7.6)	16
Chozoli	3 (5.8)	8 (5.2)	11
Mjuma / Jalira	7 (3.7)	0 (3.3)	7
Total	53	48	101

- **Likelihood of pregnancy (1/0) relative to changes in body girth circumference:**

Non-pregnant cows at January 10th had lost an average of 2.3 cm of the body girth circumference between October 2015 and February 2016. In contrast, pregnant cows had gained an average of 0.8 cm during the same period. The logistic regression analysis showed a borderline non-significant ($P = 0.08$) association between loss in body girth circumference and odds ratio of non-pregnant (Table 9). A decrease in body girth circumference of 1 cm is related to an odds ratio of 0.94 for pregnancy as compared to a cow with the same body circumference in February as in October (Odds ratio=1). Conversely, increasing body circumference by 1 cm increased the odds of pregnancy by 6%.

Table 9. Logistic regression model of likelihood of pregnancy in Malawian Zebu cows (n=74) related to loss in body circumference (cm) between October 2015 and February 2016.

Explanatory variable	Estimate	SE	ChiSquare	Prob>ChiSq	Odds ratio
Intercept	0.324	0.245	1.82	0.18	
Body girth change (cm)	-0.066	0.038	2.98	0.08	0.94

- **Days open related to bull management:**

The Kaplan-Meier survival analysis showed a significant difference in days open ($P = 0.01$) relative to number of breeding bulls in the herd (Table 10, Figure 14). Interestingly, the shorter interval to pregnancy is found in herds with one breeding bull as compared to herds with no or more than one bull.

Table 10. Kaplan-Meier statistics of days open in 101 Malawian Zebu cows exposed to none, one or more than one breeding bull per herd.

No. of bulls	Days open (median)	SE	Censored cows	Pregnant cows	Prob > ChiSq (Wilcoxon)
0	111	6.7	18	12	0.01
1	83	5.3	20	36	
>1	108	8.0	10	5	

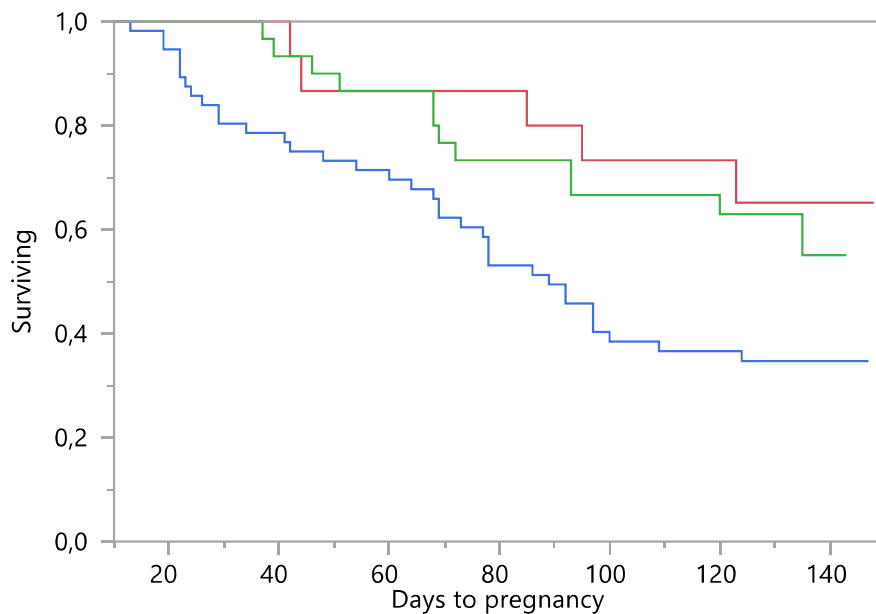


Figure 14. Kaplan-Meier plot of days open by number of breeding bulls in the herd (--- >1, --- 0, --- 1) in 101 Malawian Zebu cows

- **Days open related to water management:**

The Kaplan-Meier survival analysis showed significant differences ($P = 0.02$) in days open relative to water source for the livestock (Table 11, Figure 15). Interestingly, the optimum days to pregnancy is achieved in herds with access to open wells or small ponds. Dams and rivers are the less efficient strategies for provision of water relative to the number of days open. Less days open are also not favoured by tap and bore water as the main water sources for the livestock. The most favourable strategy, small ponds and open wells, were located further away from the households than rivers and open wells (Figure 16).

Table 11. Kaplan-Meier statistics of days open in 101 Zebu cattle relative to water source for the livestock

Water source	Days open (median)	SE	Censored no.	Pregnant no.	Prob>ChiSq (Wilcoxon)
Bore / tap water	89	5.6	10	11	
Dam / river	107	6.3	30	19	0.02
Open well / pond	73	5.2	8	23	

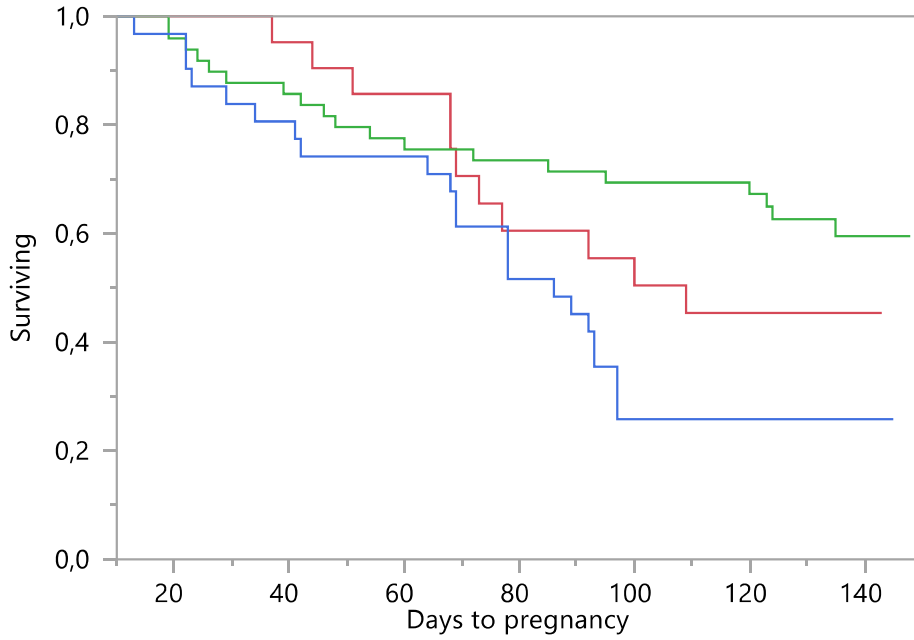


Figure 15. Kaplan-Meier plot of days open by water source for livestock in the herd (----- Bore / tap water, ----- Dam / river, ----- Open well / small pond) in 101 Malawian Zebu cows.

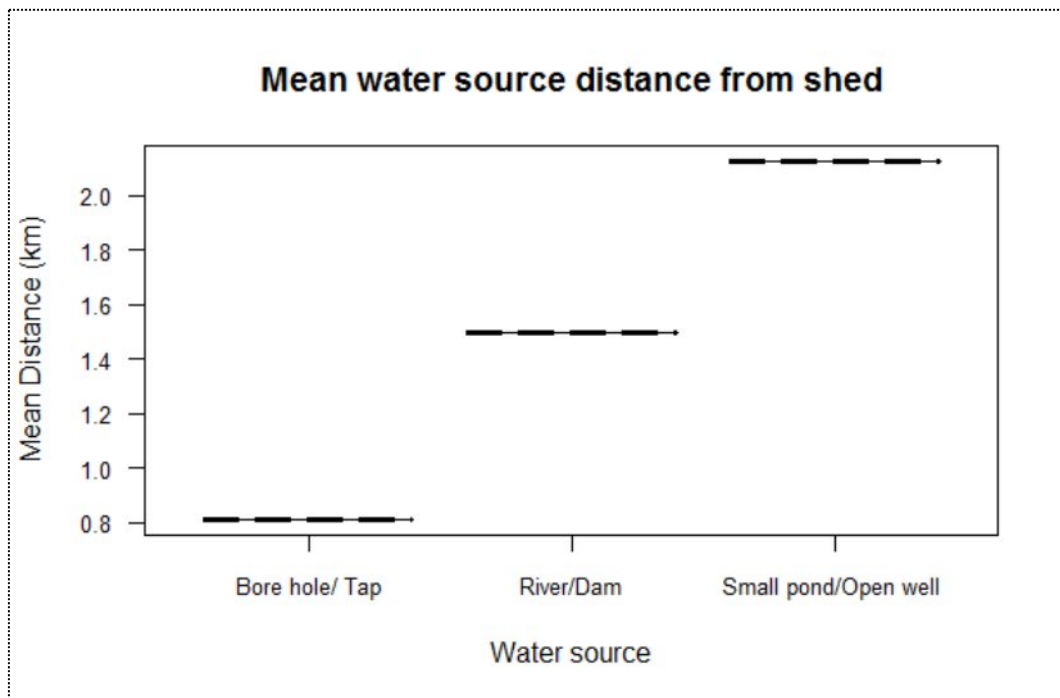


Figure 16. Mean distance (km) from the household to the water source

Distance to water (Near / far) was not significantly associated with days from calving to pregnancy when tested in a Kaplan-Mayer analysis (Wilcoxon chi-square, $P = 0.81$)

- **Days open related to parity**

The Kaplan-Meyer survival analysis showed a numerical, but not significant difference ($P = 0.18$) in days open relative to parity (Table 12, Figure 17).

Table 12. Kaplan-Mayer statistics of days open in 101 Zebu cattle exposed in their first, second or later parity

Parity	Days open (median)	SE	Censored no.	Pregnant no.	Prob > ChiSq (Wilcoxon)
First	90	8.5	13	6	0.18
Second	96	7.1	16	13	
> Second	92	5.5	19	34	

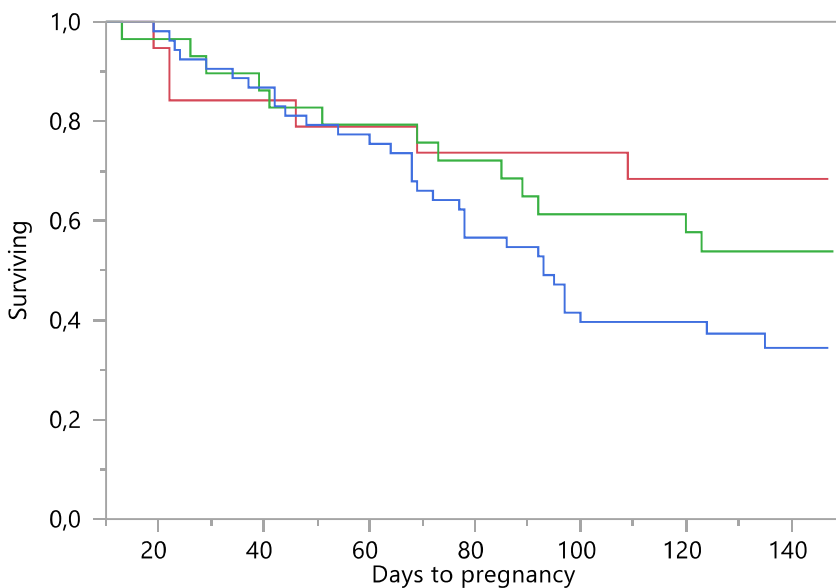


Figure 17. Kaplan-Meier plot of days open by parity (---- First, ---- Second, ---- >Second) in 101 Malawian Zebu cows.

- **Days open related to supplemental feeding:**

No significant associations were found between days open and supplemental feeding during the dry period, neither when analysed in data balanced for parity and distance to water (n=62) ($P = 0.30$), nor when analysed in the entire data set (n=101) ($P = 0.78$).

- **Feed analyses:**

The results of the feed analysis showed that the mixture of the supplement feed (*Gliricidia sepium* leaves and maize bran) contained crude protein (CP) of 20.9% and the gross energy (GE) content was 7062.2 Cal/g. The composition of supplemental feed is displayed in Table 13.

Table 13. Nutritional analysis (percentage) of supplemental feed

Sample ID	DM (%)	Ash (%)	CP (%)	Fat (%)	ADF (%)	GE (Cal/g)
<i>Gliricidia</i> leaves	85.36	08.59	17.91	11.23	45.68	6933.89
Mixed <i>Gliricidia</i> leaves and maize bran	86.94	07.28	20.91	10.76	40.07	7062.18
Maize bran	90.05	13.43	13.43	17.03	23.71	7258.13

- **Length of the lactation period by Agricultural section:**

The ANOVA showed significant differences between Agricultural Sections relative to the length of the lactation period ($P < 0.001$). Four (censored) observations with no information on lactation length were omitted from the ANOVA as shown in Table 14.

Table 14: ANOVA of lactation period length in days (d) for 97 Malawian Zebu cows distributed by Agricultural Sections.

Section	Mean (d)	SE	n	F-ratio	Prob F
Chikwawa	163	6.9	16		
Chozoli	140	8.3	11		
Bolero	135	11.3	6	9.4	<0.001
Chirambo	133	7.1	15		
Bata	124	4.3	42		
Jalira / Mjuma	82	10.5	7		

- **Length of the lactation period relative to water management strategy:**

The ANOVA showed significant differences ($P = 0.016$) in the length of the lactation period relative to water source for the livestock (Table 15). The longest lactation periods were achieved in herds with access to water from dam and rivers, which was the less favourable water source for reproductive efficiency. The least efficient water sources in terms of prolonged lactation periods were water from bore and tap water.

Table 15: ANOVA of lactation period length in days (d) for 97 Malawian Zebu cows distributed relative to water source for the livestock

Water source	Mean (d)	SE	n	F-ratio	Prob F
Bore / tap water	112	7.4	19		
Dam / river	136	4.7	47	4.4	0.016
Open well / pond	135	5.8	31		

Distance to water (near/far) was not significantly associated with length of the lactation period when tested in a corresponding ANOVA ($P=0.89$).

- **Length of the lactation period relative to parity:**

Lactation length in days was not related to parity ($n=97$) when assessed using ANOVA ($P = 0.34$).

- **Length of the lactation period relative to additional feeding:**

The ANOVA showed significant differences in lactation length relative to supplemental feeding when analysed in the data balanced for parity and distance to water ($n=62$) ($P = 0.04$) as shown in Table 16. This association was borderline non-significant ($P = 0.08$) in the dataset including all observations of lactation period length ($n=97$).

Table 16: ANOVA of lactation length in days (d) for 62 Malawian Zebu cows relative to supplemental feeding during October and November 2015

Supplemental feeding	Mean (d)	SE	n	F-ratio	Prob F
No	125	5.2	31	4.4	0.04
Yes	140	5.2	31		

- **Length of the lactation period related to changes in body girth circumference:**

The ordinary linear regression analysis showed no significant association ($P = 0.87$) between the length of the lactation period and changes in body girth between October 2015 and February 2016.

5.3 Results Objective 3

Objective 3: To determine the prevalence of possible infectious causes for reproduction and production inefficiency in Zebu cattle.

In October 2015, 105 cows and 105 calves were blood sampled. Out of 105, five (5%) cows and four/105 (4%) calves were weak positive for antibodies against FMDV. Furthermore, 35/105 (33%) cows were positive for antibodies against *Coxiella burnetii* and only one cow/105 (1%) was positive for antibodies against BVDV and *N. caninum*. There were no cows with antibodies against *Brucella* spp. (Table 17). Samples from calves collected in October 2015 were not tested for antibodies against *Coxiella burnetii*, BVDV, *N. caninum* and *Brucella* spp.

From the blood samplings in April 2016, 95 cows and 88 calves were analysed. Out of 95, two cows (2%) and five/88 (6%) calves were weak positive for antibodies against FMDV. *Coxiella burnetii* antibodies were detected in 40/95 (42%) of the cows while no calves were positive. One cow/95 (1%) was positive for antibodies against BVDV and one for *N. caninum* (Table 17). No calves had antibodies to BVDV or *N. caninum*. All animals tested in April 2016 were negative for antibodies against *Brucella* spp.

In order to confirm the status of weak positive samples, real time PCR was performed according to the OIE (2008) recommendations. All the samples were negative for FMDV such that we can conclude that there is no active presence of FMDV in the Bolero-EPA at the present.

Table 17. Prevalence of infectious agents in Malawian Zebu cows

Infectious agents	Positive animal (percentage)	
	October 2015	April 2016
FMDV	5 (5)	2 (2)
<i>Coxiella burnetii</i>	35 (33)	40 (42)
<i>Brucella</i> spp.	0 (0)	0 (0)
BVDV	1 (1)	1 (1)
<i>N. caninum</i>	1 (1)	1 (1)

6. Discussion

6.1 Discussion Objective 1

Objective 1: To describe the smallholder cattle farming system and ongoing general practices regarding management of Zebu cattle in the Bolero EPA, Rumphi District.

This cross-sectional survey provided information on the smallholder cattle herd size, composition and management practices that can describe the herd production level, reproductive status and ongoing husbandry practices related to Zebu cattle in northern Malawi. Thirty-nine smallholder farmers were considered to be a representative sample of the 200 smallholders that were included in the initial meetings. The cross-sectional survey showed that the highest density of milking cows were present in the Bata section. The survey data showed that many of the farms did not have a breeding bull in the herd. One recent study from Malawi (Nandolo et al. 2015) also reported that 64.6% farms were without any bull in the herd. Nandolo (2015) reported that more than two thirds of the farms were dependent on the neighbouring village bulls for cow mating. This situation is alarming for the area due to poor reproductive performance, and the fact that fewer available bulls can be a risk for spreading diseases from one cow to another cow during mating. The cross sectional survey showed a borderline non-significant difference between the mean pregnancy percent and presence/absence of bull in the herd. Only 8.6% of the variance in pregnancy percentage could be explained by the presence of a bull in the herd. Although this is a very rough estimate, it points towards large unexplained variances that would need to be accounted for in a future breeding program.

Water availability influenced the cows' production and hence reproduction status. Survey results showed that 40% of the cows obtained water within 2 km distance from the kraal while 60% of cows needed to walk from 2-5 km to access water. There are 0.7 million small dams in Malawi that are about to be destroyed (Frenken 2005). Building or renovation of water dams is likely to improve the cow's health and production status.

High pregnancy percentages in the herd are necessary for increasing herd size and hence production. Results from this survey showed that more than 40% of surveyed farms had no pregnant cows in the herd at the time of the survey. Low pregnancy percentage leads to fewer calf births and a decline in milk production.

Higher profitability is expected to increase sustainability in smallholder cattle farming. Higher production, improved product quality and access to market play an important role in improving the livelihood of smallholder farmers. Farmers are facing adverse effects of climate change that also results in a decline in herd milk production and limited milk sale as shown in the cross sectional survey. This study showed that only 10% of the farmers were involved in milk sale. However, farmers that sold milk owned considerably higher-producing cows than farmers that did not market their surplus milk, which shows a potential for enhanced production and improved climate change resilience.

6.2 Discussion Objective 2

Objective 2: To determine reproductive and lactation performance and important influencing factors in Zebu cattle calving in fall.

This study reported 61 days as the median value of onset of luteal activity after calving in Malawian Zebu cow. This median value is considerably lower than the 167 (dry season) and 200 (rainy season) days reported for Tanzanian Zebu cattle (Matiko et al. 2008). It showed a potential for good reproductive efficiency in Malawian Zebu cows if the farmers adopted best practices.

It is reasonable to assume that environmental effects are more pronounced in Malawi compared with western European countries. Hence a breeding plan for Zebu cattle will be markedly influenced by the feeding and environmental factors in Africa (Mulder & Bijma 2005; Ojango & Pollott 2002; Zwald et al. 2003). Applying the best management of Zebu cattle farming practices will minimize the environmental variance, and thus increase the heritability of traits such as increased milk production. However, the focus should not be only on the increased genetic gain of milk yield, but also on reproductive efficiency (fewer days open). This is because selection for high milk yield traits may influence reproduction negatively (Heringstad et al. 2003), and because short calving intervals have a positive impact on total milk yield (Chagunda 1996; Chagunda 2002). In conclusion, more effective breeding plans can be obtained by minimizing adverse environmental effects making selection for yield and reproduction more effective in Malawi. However, with large genotype x environment interactions, breeding animals in similar environmental conditions as they will be used is important. Knowledge of the impact of factors

such as parity, feeding regimens and water management are therefore needed as a first step towards recommendations on selection for given traits in the Malawian Zebu cow.

In the cohort study, there was a significant difference in number of days from calving to pregnancy in cows with 0, 1, and >1 bull in the herd. Normally it is observed that chances of conception in cows will increase with an increase in number of bulls in the herd because then one cow has access to more than one bull for mating. Surprisingly in this study, optimum days to pregnancy were achieved with only one bull in the herd. It may be coincidental but it is tempting to hypothesize that an effect of fighting between bulls may be a reason. Anyway, the results of the cohort study showed a clear negative effect of not keeping a bull together with the cows on days open also when they were not at pasture.

Water is vital for the milk production from Malawian Zebu cattle, and restoring dams will be of importance to adapt to prolonged dry periods caused by climate changes. Water and feed are in excess amount in the rainy season. Due to this the period from calving to OLA is shorter in the rainy season as compared to the dry season (Matiko et al. 2008). This study found non-significant results when days open were related to the distance travelled to the water source, when dichotomized as “far” and “near”. Our dichotomization of the distance may not have been ideal to pick up possible negative effects of distance to the main water source. Besides, it is possible that distance to water interacted with the source of water in this material. Presently, the data material is too small to provide reliable predictions of such interactions, which will improve in accuracy as the study proceeds further. Although the optimal water source for an improvement in days open and prolonged lactation period, is not obvious in this investigation, our results clearly showed that cows getting water from taps and bore water were less efficient in both production and reproduction. Similar results have been found for calf growth (Chang'a et al. 2012) in Tanzania. The authors from the Tanzanian study concluded that animals that were dependent on the work force for their water supply, did not get enough water. The same association is likely to be the case in the current study.

One of the aims of this study was to investigate whether supplemental feeding could decrease days open in Malawian Zebu cows. For this purpose, 200 smallholder farmers were screened for the presence of identifying cows that had calved in August to September 2015. One hundred and nine cows that calved in this period and their owners (n=39) were included in the study. There

was no significant association in days open between groups receiving supplemental feed and the control group, neither when data were balanced in terms of parity and distance to water ($n=62$) ($P = 0.30$) nor when the entire data set was analysed for the purpose ($n=101$) ($P = 0.78$). This indicated that factors other than supplemental feeding, such as provision of water and access to breeding bulls, are of greater importance than supplemental feeding in healthy Zebu cows of this region. However, the numerical relationship between odds of pregnancy and loss of body girth circumference showed that our research animals underwent a certain degree of metabolic stress affecting reproductive performance. Our data are presently too limited to warrant a study on interactions between feed supply, bull management and water supply, but the feeding trial continues so that we will gather the necessary power to investigate such interacting effects.

Parity was reported to affect reproductive performance in Tanzanian crossbred Zebu cows, where cows in their third or later parities showed a significant reduction in number of days to OLA (Matiko et al. 2008). Parity has a significant influence on ovarian activity after calving (Tanaka et al. 2008). The present study reported a numerical but non-significant ($P = 0.18$) negative relationship between days from calving to pregnancy in older cows, indicating that herd composition (keeping older cows) is a management strategy that may help in increasing the calf numbers and milk yield on a yearly basis.

Cows receiving supplemental feeding, and cows having dams and rivers as their main water source had the longest lactation periods in the present cohort study. Milk yield as measured in kg or litres, is the phenotype that is used in most breeding programs. We also considered having the farmers weigh milk on a monthly basis, but this was omitted as we did not think we would get comparable measures. Lactation length will inevitably be a more indirect measure of lactation performance. However, in our trial the farmers collected milk samples that were picked up by the project field assistant two times a week, such that we were well informed about which cows that had stopped milking between October 2015 and February 2016. We therefore chose to use this as a parameter for lactation yield in the current cohort study.

People in rural areas rely on the milk derived from local cows; hence, a prolonged lactation period is of great importance in indigenous zebu cattle. The average consumption of milk per capita a year in Malawi is reported to be 4-5 kg (FAO; 2006). Milk consumption is generally

seen as low if it is less than 30 kg per year (FAO 2016). Milk provides 3% of the dietary energy supply in Asia and Africa, compared with 8% to 9% in Europe and Oceania (FAO 2016).

The supplemented feed comprised a total of 4 kg a day (2 kg *Gliricidia* leaves and 2 kg maize bran) with a CP of 20.91%. The composition of feed shows that CP value of mixed supplement feed (*Gliricidia* and maize bran) is up to the recommended level (CP = 17-19%) for dairy cattle (Colmenero & Broderick 2006). Higher milk production is likely to increase milk marketing and improve farmers' livelihoods. The present study indicated that more of the high milk supply demands can be met by increasing milk production from local breeds.

6.3 Discussion Objective 3

Objective 3: To determine the prevalence of possible infectious causes for reproduction and production inefficiency in Zebu cattle.

- **Serological tests:**

PCR analysis concluded that there were no active infections for FMDV in our samples, but the serological tests detected 16 weak positive that later confirmed without active infection. With these results, we can suspect that some of the cattle have been exposed to the virus. On 2nd January 2016, there was an FMD outbreak in Nsanje Southern Malawi (OIE Malawi 2016). The last outbreak happened in the same area in September 2015. The infected area is close to the Mwabvi Game Reserve where foot and mouth disease is endemic in the wild buffalo population. This could have been the source of infection if cattle have been grazing in the same area (EuFMD January 2016). It is a long distance (>800 km) from the outbreak in the south to the Bolero EPA, Rumphi District, but caution should be taken to prevent spreading the virus further. In addition, the Bolero EPA is bordering Vwaza Marsh Wildlife Reserve and an investigation of FMDV in the wild animal population may be warranted. All transportation and trading of cattle should be stopped as quickly as possible when FMD is diagnosed. If the disease is spread over a wider geographical area, it can be more difficult and costly to control (Iqbal 2014). However, most of assays used in the present study have been validated in non-endemic regions and therefore the sensitivity and specificity of the test might differ when analysing African samples.

Hence, the interpretation of the weak positive animals in the present study could also be considered as false positive, and further investigation will be undertaken.

From the blood sampling in October 2015, and April 2016, 33% and 42% of the cattle tested positive for Q-fever respectively. This should be noted as the disease causes abortion both in cattle and more commonly in goats (Blood et al. 1989). There is a large goat population in the area, and it is likely that goats are an important reservoir for the disease. Local people who handle the cattle should be aware since the disease can be transmitted to humans through milk, urine, feces, birth fluids and placenta from infected livestock.

N. caninum is one of the major causes of reproductive problems and abortions in dairy and beef cattle worldwide (Moreno-Torres et al. 2016). Studies of the domestic life cycle of the parasite have shown that dogs are both intermediate and definitive hosts, whereas cattle are natural intermediate hosts (Ghalmi et al. 2014). One cow had antibodies to *N. caninum* in two consecutive samplings, and the sero-status of her calves are not known. However, it is unlikely that the four cases of late abortions / stillbirth in this study was caused by *N. caninum*.

Brucellosis was less prevalent than we have experienced from Tanzania (Kanuya et al. 2006). None of the animals tested positive for *Brucella* spp., which is surprising as the disease is endemic in most of the Sub Saharan countries (Holt et al. 2011; Muma et al. 2007; Schelling et al. 2003). The disease is one of the most common zoonoses worldwide, and is a major public health concern (Ducrottoy et al.). To reduce the occurrence in humans, milk pasteurization and food hygiene are important measures to be taken. Amongst smallholder dairy farmers in northern Malawi general knowledge about possible transmission of diseases between humans and animals is high, although most farmers practice risk behaviours that could potentially expose the public to milk-borne zoonotic diseases such as brucellosis (Tebug et al. 2014). The present investigation showed a low prevalence of some important infectious diseases common to the region. However, there are also other pathogens such as *Campylobacter foetus*, Bovine Herpesvirus-1 (causative agent of Infectious Bovine Rhinotracheitis) that cause reproductive disorders that could be further investigated

At the time of the sampling, the animals had been through the wet season. The risk of infection is increased during the rainy season because the number of ticks is 2-3 times higher in this period (Mooring et al. 1994). Therefore we had expected more *Babesia* spp. and *Theileria* spp. Than we

found in this population of Zebu cattle. Interestingly, *Trypanosoma* spp. were more prevalent, which also corresponds with local knowledge of prevailing tsetse flies. Thus, our investigation verified that *Trypanosoma* were endemic in Malawian Zebu cattle (Van den Bossche et al. 2000).

7. Validity and systematic errors

7.1 Clustering at farm level

Only univariate tests without adjustment for clustering at farm level have been conducted. Thus, violation of the assumption of independence may have underestimated the width of the standard errors to some extent. However, the current thesis is imperative for descriptive purposes and for hypotheses generation serving our ongoing fieldwork in Malawi. The effects observed at herd and section level may be directly related to resources such as availability of bulls, feed and water, but also mirror motivation, skills of the herdsman, and their educational levels (Matiko et al. 2008). As our work proceeds through the 2016-2017 sampling season, we expect to be able to adjust for clustering and estimate variance components at farmer and section (advisory) level.

7.2 Classification errors

Field studies in developing countries are some of the most meaningful, but also challenging tasks for a researcher. Baseline surveys have often been the first approach to become acquainted with common practices, perceptions, education level and conditions in the field. In our case, we balanced the experimental and control group of the feed trial according to known factors such as parity and distance to the main water source. Although we were guided by our cross sectional survey, we did not account for access to water in an optimal way in the blocking of the feed trial since this factor did not show significant in any of the subsequent analyses.

Furthermore, 2300 Rapid P4 strips were interpreted for the determination of milk progesterone in this thesis. Bendiktsen et al. (2015) validated this test for the determination of oestrus, and found a sensitivity of 0.91 and specificity of 0.92 for detecting absence of luteal activity at a threshold of progesterone (P4) > 4 ng/ml. A thorough evaluation of sensitivity and specificity for the detection of luteal activity has not yet been conducted such that a number of test results may be false. In order to address this problem, we have used a conservative approach for the definition of OLA in that we have required at least three consecutive tests within 21 days to indicate elevated progesterone.

7.3 Confounding/Interactions

The unique contribution of the current thesis is that it has allowed us to investigate a number of important factors while the study is still ongoing in Malawi, and the results presented should be viewed as “work in progress”. We are well aware of the fact that a number of factors presently

investigated in univariate models may act as confounding factors when included in a more thorough model. This may be illustrated by the fact that water sources are not equally distributed by Agricultural Sections and by the fact that distance to water and water sources are not independent. Likewise, the interaction between availability of breeding bulls and effect of feeding / water management on reproductive outcomes needs to be evaluated such that cows experiencing the same likelihood of service are compared. This will be taken care of as our work proceeds.

7.4 Representativeness

Although we regard the 39 herds and associated stock to be representative of the 200 farmers that were invited for the initial survey, we cannot exclude that a certain selection has been included. Our aim has been to investigate the potential of Zebu cattle production and reproduction such that we have selected healthy cows for the study and we regard the results to be representative for a healthy population of fall-calving Zebu cows, as we have experienced in northern Malawi.

8. Conclusion

This study is still in progress and will be continued with one more year of research to conclude on the most appropriate advices for the Zebu cattle management among smallholder farmers in northern Malawi. However, the current thesis have documented a number of possible points for improvement.

Milk yield per cow was directly associated with the milk marketing strategy at farm level. Loss in body girth circumference had a borderline non-significant association with the success of pregnancy, increasing body circumference by one cm increased the likelihood of pregnancy by 6%.

Herds with only one bull displayed a lower number of open days as compared to the herds with more than one bull. The optimum number of open days was achieved in herds having open wells and small ponds as the source of drinking water.

Water source and Agricultural Sections showed a significant difference relative to the length of lactation period. Bore and tap water proved least efficient water sources in terms of prolonged lactation period, probably because man power is needed in order to get water out from these sources. Lactation length was longer in cows receiving feed supplements

This study also showed that there was no active infection of foot and mouth disease virus in the study population of cows while 34.4% and 41.5% of the study population was positive for the *Coxiella burnetii* in October 2015 and April 2016 respectively. Bovine viral diarrhoea (two positive), *Neospora caninum* (two positive) and *Brucella abortus* (all negative) were not prevalent in the study population.

9. Appendix

INITIAL SURVEY PERFORMA:

Baseline questionnaire

SECTION 1: FARMER BIODATA

Farmer name

Gender

Village

Section

SECTION 2: CATTLE INFORMATION AT SHED

Tag numbers allotted

Cattle breed

Total milking cows.....

Total heifers.....

Total dry cows.....

Total conceived cows.....

Total calves.....

Total bulls.....

SECTION 3: HUSBANDRY PRACTICES

Do you milk cows?

If yes, how often do you milk cow?

Average milk production/cow/day (L)

Do you sale milk?

If yes, number of bottles sold/day (01 bottle = 270 ml)

Source of water

Distance of water source

Water source (Near/Far)

SECTION 4: INFORMATION OF INDIVIDUAL COW

Tag number

Body condition score (BCS)

Milk production/day (L)

Parity of cow

Time (month) since last calving

Number of offspring delivered until now

Age of the calf present today

SECTION 5: FEED, FODDER AND LAND

Do you provide additional feed to cattle?

If yes, what kind of additional do you provide?

How frequently do you provide additional feed?

During which time of the year you provide additional feed.

Mention the most available fodder to the cattle.....

What is the source of fodder?

What did you offer to the cows during the feed shortage?

Did you plant any leguminous tree?

Did you provide minerals to the cattle?

How much land (acres) did you manage?

How much land used to maize cultivation?

What is the fate of maize cobs?

What is the average maize production/acre?

SECTION 6: DISEASE AND PREVENTION

What are the most prevailing health problem in cows?

What are the most prevailing health problem in calves?

What treatment is given to the cows?

Did you vaccinate your cattle?

10. References

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