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Targeted treatment and targeted selective treatment in beef calves in Brandenburg

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To my dear husband Mohamed, my parents and my lovely daughter Maya

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List of Abbreviations

ABZ	Albendazole
AR	Anthelmintic resistance
ATP	Adenosine triphosphate
bp	base pair
BCS	Body condition score
BZ	Benzimidazole
CCD	Charge coupled device
CI	Confidence interval
C. oncophora	*Cooperia oncophora*
DISCO	Diarrhoea score
df	Degree of freedom
DNA	Desoxyribonucleic acid
DWG	Daily weight gain
EC_{50}	Effective concentration 50%
EHA	Egg Hatch Assay
F	Female
FAMACHA	FAffaMAlanCHArts
F. hepatica	*Fasciola hepatica*
FEC	Faecal egg count
FECRT	Faecal egg count reduction test
FSG	First season grazing
GI	Gastro intestinal
HPD	Highest posterior distribution
H. contortus	*Haemonchus contortus*
IVM	Ivermectin
ITS-2	Second internal transcribed spacer
L1, L2, L3	First, second, third and fourth larval stage
LDA	Larval Development Assay
LMIA	Larval Migration Inhibition Assay

M	Male
MLs	Macrocyclic lactones
NaCl	Sodium chloride
NC	Negative control
O. circumcincta	*Ostertagia circumcincta*
O. ostertagi	*Ostertagia ostertagi*
O. leptospicularis	*Ostertagia leptospicularis*
OR	Odds ratio
PC	Positive control
PCR	Polymerase chain reaction
PPi	Pyrophosphate
®	Registered trademark
RFLP	Restriction fragment length polymorphism
SE	Standard error
SNPs	Single nucleotide polymorphisms
spp.	subspecies
TAE	TRIS-acetate-EDTA
TBZ	Thiabendazole
TST	Targeted selective treatment
TT	Targeted treatment
T. colubriformis	*Trichostrongylus colubriformis*
T. circumcincta	*Teladorsagia circumcincta*
WAAVP	World Association for the Advancement of Veterinary Parasitology

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1. Introduction

Gastrointestinal (GI) nematodes infections in grazing cattle are the most widely occurring parasite infections in temperate regions worldwide. They are the major causes of subclinical diseases and production losses in meat and milk in grazing livestock (Van Dijk et al., 2010). Control or prevention of these infections are commonly based on heavily usage of anthelmintics, which led to the development of anthelmintic resistance (AR) due to repeated treatment of all animals of the herds often combined with poor grazing management. The widespread deworming strategy of entire herds is considered not to be sustainable anymore by experts because of the emergence of AR. Particularly in small ruminants AR is of worldwide concern (Kaplan, 2004, Wolstenholme et al., 2004, Jackson and Coop, 2000), but it is starting to be of importance also in the cattle farming industries. Resistance against the most commonly used classes of broad spectrum anthelmintics such as the benzimidazoles (BZs), levamisole (LEV) and the macrocyclic lactones (MLs) becomes more common (Demeler et al., 2009, Stenhouse, 2007, Kaplan, 2004, Vickers et al., 2001, Familton et al., 2001, Leathwick et al., 2000, Waller, 1997, Leathwick et al., 1995). For this reason, strategic herd treatment should be either replaced or at least complemented by alternative control strategies. Farmers must learn to "live with worms"(Coles, 2002) and accordingly new treatment and control strategies have to be developed. One approach is the "Targeted Selective Treatment" (TST), where only those animals in need of treatment receive treatment, while the rest of the herd is left untreated. Another approach is the "Targeted Treatment" (TT). Here, treatment of all animals within one herd is carried out if at least one animal met the treatment criteria. Therefore, the most important factor for the use of TST and TT is to find suitable treatment parameters and establish respective ways of identifying animals in need for treatment. Such decision parameters must be sensitive, cost effective and easy to use. The aim of these alternative control strategies (TST, TT) is to prolong the effectiveness of existing anthelmintics and delay the development of resistance. The approach is based on the attempt to obtain and/or maintain a refugia to ensure the genetic diversity in the worm population. The refugia consists of the untreated parasites in the host and the free living generation in the environment, which are not affected by anthelmintic treatments and thus transfer susceptible genes to the next generation (Kenyon et al., 2009). In recent years, a considerable number of studies have been carried out concerning the applicability of TST or TT strategies. However, most of these research projects focused on small ruminants, as AR is more widespread and economically more threatening (Gaba et al., 2010, Stafford et al., 2010, Gallidis et al., 2009, Kenyon et al., 2009, Cabaret et al., 2009). In contrast, less work has been

performed regarding the feasibility of applying TST/TT concepts in cattle. Within the available studies addressing these strategies in cattle most focused on TT/TST in dairy herds and accordingly the application of TT/TST in beef based systems has not been evaluated in greater detail yet.

The aim of the present work was to establish decision parameters and evaluate those parameters in commercially beef farming systems in Germany. The application of TST and TT in comparison to either fully covered (positive) or untreated (negative control) animals was performed in first season grazing (FSG) calves. The main objective of this approach is to reduce the use of anthelmintics. However, for the farmers the most important factor is ensuring no losses in the productivity of calves. In order to assess the impact of the different anthelmintic treatments on animal productivity, the life weight gain and carcass weight were compared between treatment groups and untreated animals. Performance of the animals was assessed by recording the body condition score (BCS) and as a parasitological indicator for infection with GI nematodes faecal samples were taken and examined by MiniFlOTAC technique. Additionally sedimentation was performed aiming at the detection of *Fasciola hepatica* eggs. Quantitative methods were used to assess the number of parasite eggs per gram faeces (EPG). Herds were visited in regular intervals (4-6 weeks) and all parameters were used for statistical analysis. The results show that production in those FSG calves treated either following the TT or TST concept was not impaired if compared to fully treated animals.

In parallel, another part of the current study focused on the investigation of resistance to BZs in cattle parasitic nematodes, mainly *Cooperia oncophora* and *Ostertagia ostertagi*, present on German cattle farms. Detection of AR on cattle farms against BZs was carried out using the Faecal Egg Count Reduction Test (FECRT) and was accompanied with molecular genus specific analysis. Additionally, molecular analysis of resistance associated changes in the β-tubulin in the two above mentioned species by single nucleotide polymorphisms (SNPs) detection using pyrosequencing was envisaged.

2. Literature review

2.1 Parasitic nematodes of cattle

Pasture borne parasites of ruminants and their likely impact on cattle health and productivity have been subjected to intensive research activities in the last years (Charlier et al., 2009, Dimander et al., 2003, Dimander et al., 2000). In Europe, the two genera that are mostly involved are *Cooperia oncophora* and *Ostertagia ostertagi* (Höglund, 2010). They parasitise the small intestine and abomasum of animals and can usually be found in all grazing cattle (Borgsteede et al., 2000). The two genera *Cooperia* and *Ostertagia* belong to the family of trichostrongylidae. The most common *Cooperia* species occurring in cattle in central Europe is *C. oncophora*, but also infections with *Cooperia punctata*, *Cooperia pectinata* and *Cooperia curticei* can occur. The most common *Ostertagia* species in Europe is *O. ostertagi* but occasionally *Ostertagia leptospicularis* or *Teladorsagia circumcincta* can be found. The following parts only focus on the species *C. oncophora* and *O. ostertagi* because they are the most important pathogens of parasitic gastroenteritis in cattle in the temperate climate of central Europe.

2.1.1 *Cooperia oncophora*

C. oncophora is a nematode which parasitises the small intestine of cattle. The female worms reach a length of 6-11 mm, while the males are only 5-8 mm long. *C. oncophora* is easily identified microscopically by its coiled appearance which has been described as like a "watch spring". The cephalic vesicle is found in all *Cooperia* species, giving the head end a slight bulbous look. In addition the anterior cuticle has transverse striations in the oesophageal region. The body possess a longitudinal ridges. The male has a large bursa comparatively to the size of the body, it has a small dorsal lobe and the brown coloured spicules are short with only 240-300 µm in length. They display distinct expansions in the middle region, which often bear transverse ridged striations. There is no gubernaculum. The females have a long tube ring tail and the vulva has a vulvar flab located posterior to the middle part of the body.

2.1.1.2 Pathogenicity

The pathogenicity *of C. oncophora* is generally considered to be relatively low, as it causes only minor damage in the host's intestine (Henriksen, 1981, Coop et al., 1979). However, it usually occurs as a mixed infection with other parasites such as *O. ostertagi.* The combined occurrence of both species enhances the respective pathogenic aspects of either one and

accordingly this can lead to severe losses in the beef and dairy cattle industry (Hawkins, 1993). The parasites inhabits particularly the proximal portions of the small intestine, but in the very heavy infections it can also infect parts of the distal region. The parasites penetrate the mucosa and change the structure of small intestinal villi. The infection causes local hyperaemia, oedema, thickening of the mucosa and maybe enteritis. The clinical sings are diarrhoea, loss of appetite and decreased weight gain. Although an immunity can be acquired such immunity doesn't prevent infection with the parasite itself, but result in delayed development of worms, reduces the number of established adult worms and consequently also of the number of the excreted eggs (Gasbarre et al., 2001).

2.1.2 *Ostertagia ostertagi*

These parasites are slender reddish brown worms which inhabit the abomasum of cattle. The female worms reach a length of 8-12 mm, the males are 6-8 mm long. The body is striated only in the anterior part and possess about 30 longitudinal ridges. The male has a small bursa with one accessory lobe and the accessory membrane of the bursa is supported by two divergent rays. The spicules are equal in shape and length and divide in the posterior region, where two thin lateral branches arise from the main stem. The females have vulva which is covered by vulvar flap.

2.1.2.1 Pathogenicity

Most parasitic induced gastritis cases are caused by *O. ostertagi*. Typically, first season grazing (FSG) calves are the most affected, but older animals also can be affected (Eckert and Bürger, 1978). By the penetration of the ingested third stage larvae (L3) into the glands of the abomasum the cells are damaged and replaced by undifferentiated hyperplastic cells. Also, the neighbouring cells are influenced and on the abomasal mucosa macroscopically visible nodules arise. Depending on the severity of the disease the mucosa can show a "cobblestone-like appearance". The damage to the hydrochloric acid-producing parietal cells causes an increase of the pH in the abomasum. This favours the proliferation of bacteria and on the other hand pepsinogen is not activated to pepsin and thus the protein digestion is disturbed. Another consequence of the massive mucosal damage is the increased permeability to plasma products, which are clinically reflected in a hypoalbuminemia. This hypoalbuminemia leads to collection of body fluids in lower parts of the body which causes oedema, most commonly in the jaw (bottle jaw) or chest region or ascites. The migration of the worms from the glands eventually

produces a hyperplastic gastritis with diarrhoea. The course of the disease can be subacute to chronic and the clinical symptoms are diarrhoea, anorexia, rapid weight loss and a dull coat. In severe infections it can lead to emaciation and dehydration with reduced rumen activity and ultimately may result in the death of the animal.

Classically two types of the disease have been described; the so called summer (type I) and the winter (type II) ostertagiosis. The summer type occurs at the beginning of the grazing season, when large numbers of infective L3 are ingested. Clinical manifestations of this form start mostly from mid-July and there are usually several animals of the herd affected. The winter ostertagiosis occurs in winter or spring after the first grazing season. After ingestion, the L3 can enter a hypobiotic stage by entering the gastric gland. The development into fourth stage larvae then can be inhibited (delayed). The en masse re-emergence of larvae from the gland cause the winter ostertagiosis, which is usually associated with mild clinical symptoms or even the absence of clinical disease (Myers and Taylor, 1989). This make the *Ostertagia* is the most important GI nematodes parasite economically in temperate regions of the world (Gasbarre et al., 2001). An immunity against *O. ostertagi* develops slowly and is usually protective only after the second grazing season. It results in a delayed development of the worms, the larvae and morphological changes decreased egg production and excretion of the female worms.

2.2 Life cycle

C. oncophora and *O. ostertagi* have a simple direct life cycle and are transmitted by ingestion of L3 from contaminated pasture (Anderson, 1992). The free living stages comprise of eggs, followed by three larval stages. The L3, which is the infective stage, does not feed and can therefore survive for a long period of time until ingestion by a suitable host. Within the host, the fourth larval stage (L4) and finally the adult stage develop. Male and female copulate in the host and the eggs are then laid by adult females and consequently excreted into the environment with the host faeces. The eggs hatch in the environment and the first larva develops in optimum ambient temperatures (20-25 °C) within 1-2 weeks to the infective L3 via two moults. Due to the acidic environment of the digestive tract, the L3 loses its sheath and becomes a parasitic L3, which develops in the crypts of the gastric glands or in the small intestine through the mucous membrane, via a respective further moult into (L4) and finally to the adult stage. Overall, the development of the parasite in the host takes about 2-3 weeks (Taylor et al., 2007). The complete life cycle is shown in Figure 1.

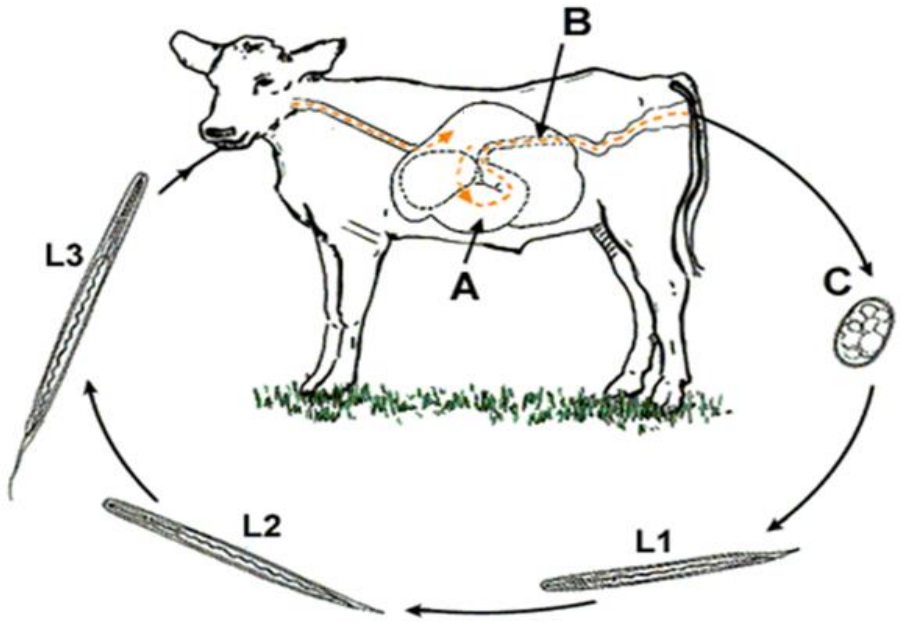


Figure 1.Trichostrongylidae life cycle. Source web site (cal.vet.upenn.edu). Depending on the species of trichostrongylidae, adults can be found in the abomasum or small intestine (A/B). Eggs are excreted with the faeces (C). The eggs hatch and the larvae develop via two larval stages (L1, L2) into the infective third larval stage (L3).

2.3 Epidemiology

Ostertagia and *Cooperia* are pasture born parasites infections. Usually all grazing cattle are infected. The infective L3s are ingested by the host with the grass. The climate conditions affect the rate L3 surviving winter as well as the hatching of the larvae from the eggs, as the larvae need a moist environment to develop rabidly. So in wet summers L3 can survive better leading to peak of infections in late summer/early autumn while in contrast most of the larvae die in very dry summers. Especially in summer and early autumn there is high infection risk due to the accumulation of L3 over the season. Clinical symptoms occur with the start of infections with *C. oncophora* in mid-July, while symptoms due to ostertagiosis usually occur between August and October. This is usually reflected in the faecal egg count (FEC) which is often relatively low after turn out and reaches the peak in summer, then followed by a decrease in late autumn and winter (Borgsteede, 1977). This decrease is caused by the development of immunity against *C. oncophora*. Consequently, the FEC can remain low during the second grazing season.

2.4 Parasite control

GI nematodes control depend on many factors including grazing pattern, host immunity and farm management system (Gauly et al., 2013). The combination of pasture management and anthelmintic treatment is considered the best way for GI nematodes control, as part of the life cycle of the parasite occur in the environment.

2.4.1 Pasture management

Good pasture management can significantly reduce the infection risk with GI nematodes. In spring, the overwintered infective L3 from the previous year are on the pastures, posing the first risk of infection. Pasture management can be achieved by:

- Reducing the number of infective larvae on pasture through cutting of the first grass growth: Although this will initially reduce the number of larvae present on pasture it has to be taken into account that the majority of larvae are in the soil (Knapp-Lawitzke et al., 2014). These larvae will not be affected.
- Grazing of older and therefore less or other non-susceptible cattle on highly contaminated pasture: This approach can be used to "clean" pasture areas before young and potentially susceptible animals are turned out. In order to maintain a low contamination this grazing time should not exceed 3 weeks so that no additional eggs will be shed onto pasture.
- Moving the animals between different pasture (Eysker et al., 1998, Brunsdon, 1980, Michel, 1976): Rotational grazing schemes have to be timed with anthelmintic treatments and adapted to the respective climate conditions. The formerly propagated "Dose and Move" system, where animals were moved to a "fresh and clean" pasture after deworming, is now considered to increase the selection pressure and therefore the development of AR. It is majorly important to control the efficacy of the used anthelmintic by using sensitive FEC methods before moving the animals. This ensures that animals, either not responding to treatment of harbouring resistant parasite population will not contaminate the new pasture.
- Annually alternating grazing with different animal species (Barger and Southcott, 1975, Southcott and Barger, 1975). Importantly, the annual change grazing of cattle and sheep has been found to not sufficiently reduced the number of L3 (Bairden et al., 1995), since most parasite species can infect both host species. Accordingly, alternating grazing should at least include non-ruminants, e.g. horses or donkeys.
- Late turnout of the calves: This aims at decreasing the risk of infection by previous grazing of the pastures with older/other animals in combination with a better immunity of the calves

due to their higher age. However, this approach is only meaningful for systems with calving during the winter/early spring and can often not be realised due to management restrictions.

- A low stocking density or increase of the ratio of food supply for animals: Contamination of the pasture is enhanced by a high stocking density. Food source is scarcer and animals may graze in areas with faecal pads which leads to a lower overall food intake but an increased uptake of infective L3. It was discussed if additional feeding of concentrates reduces the overall intake of grass and therefore also infective L3. However, Larsson et al. (2006) showed in their study that no infection reducing effects could be achieved by feeding of concentrates and forage. In the same study it was demonstrated that the previous grazing with older (second grazing season) cattle leads to reduced number of L3 on pasture (Larsson et al., 2007, Larsson et al., 2006).

Pasture management is therefore important and enables the reduction of infective L3 available for ingestion. However, it usually can't achieve parasite eradication and particularly in high producing animals it has to be combined with careful use of anthelmintic treatments.

2.4.2 Anthelmintics

Strategic use of anthelmintics to either prevent parasite infections or minimise production losses is still the main cornerstone of parasite control on most farms. In most parts of the world in cool or warm areas routine treatment approaches are used (Vercruysse and Claerebout, 2001). Some farmers may not be familiar with pasture management possibilities or the implementation of this is still considered too time and labour intense. When depending almost on the sole use of anthelmintics, timing of administration as well as correct administration of the drug is highly important.

Since most animals infected with GI nematodes acquire immunity within the first grazing season and the older animals in the second grazing season are more protected against the infections, the prophylactic medication should be administrated predominantly to the young calves during their first grazing season. Additionally the season is important for the time of treatment, as L3 levels are usually low in spring after turnout, increase until mid-July and reach the peak in late summer and early autumn. In cattle anthelmintics used against GI nematodes belong to three main groups; the BZs, imidazothiazoles and MLs. The active substance, mode of action, spectrum and administration route (Martin, 1997) of a selection of commercially available anthelmintics are listed below (Table 1).

Table 1. Commercially available anthelmintics used against GI nematode infections in cattle in Germany (Source: www.vetidata.de)

Drug group Active substance	**Administration**	**Spectrum**	**Mode of action**
Benzimidazoles Albendazole, Fenbendazole, Oxfendazole **Pro-Benzimidazole** Febantel	per os	Gastrointestinal, lung and other nematodes; trematodes	Inhibition of the tubulin polymerisation to microtubules, causing energy depletion
Imidazothiazole Levamisole	per os intramuscular pour on	Gastrointestinal, lung and other nematodes	Act as agonist on acetylcholine-esterase receptors causing spastic paralysis
MLs Doramectin, Eprinomectin, Ivermectin, Moxidectin Abamectin	subcutaneous pour on	Nematodes and ectoparasites	Selective binding to glutamate-gated chloride channels in nerve and muscle cells, causing spastic paralysis and death

2.4.2.1 Benzimidazoles (BZs)

In 1961 thiabendazole (TBZ) was the first drug from the group of the BZs which was introduced to the market (Brown et al., 1961). Then other BZs, imidazothiazoles and tetrahydropyrimidines were discovered and further developed between 1962 and 1966 and marketed in the period between 1966 and 1969 (Mehlhorn, 2008). They are effective against GI nematodes, trematodes and lungworms.

In 1980 albendazole (ABZ) synthesised by SmithKline Beecham pharmaceutical company and was used for the same purposes. Albendazole and mebendazole are closely related molecules and exert similar anthelmintic effects. These broad-spectrum anthelmintics were more efficient than the first generation drugs and have a good anthelmintic effect against most GI nematodes and a significantly improved therapeutic safety. They can also work effective against cestodes and trematodes infections, intestinal and inhibited larval stages of most strongyles and eggs. For cattle the following BZs are available on the market; ABZ, febantel, fenbendazole and oxfendazole. The structural formulas of various active ingredients from the group of BZs are shown in figure 2.

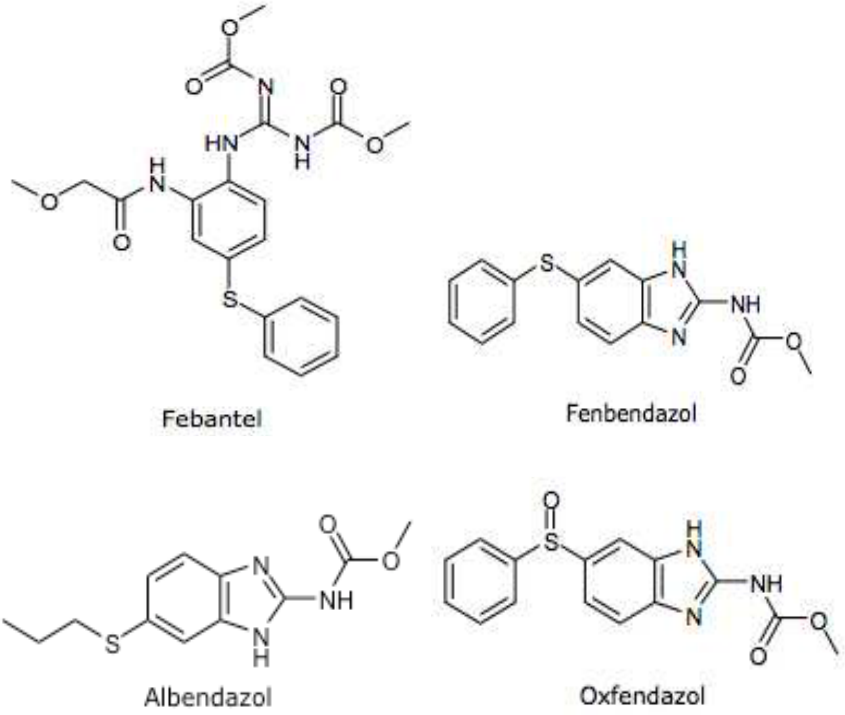


Figure 2. The structural formulas of some BZs (source website: en.wikipedia.org).

The active substance is administered orally and absorbed by less than 50% in the gastrointestinal tract. The onset of action starts within two to three days, which is why a sufficiently long contact time between anthelmintic and the animal's body is required. The effectiveness depends to a large extent on this contact time (Lanusse and Prichard, 1993). Sánchez et al. (2000) showed that feed withdrawal prior to administration of BZs can improve the bioavailability significantly. The active ingredients fenbendazole and oxfendazole in cattle reached maximum plasma concentrations after 24-36 hours (Prichard et al., 1978). The metabolites of BZs are predominantly found in plasma and tissues and they are excreted through the faeces or the urine.

2.5 Anthelmintic resistance (AR)

Prichard et al. (1980) defined resistance as: "Resistance is present when there is a greater frequency of individuals within a population able to tolerate doses of compound than in a normal population and it is heritable".

He separated two types of resistance: 1. Side-resistance: after using a drug resistance is also noted in other drugs within the same drug class (which has the same mode of action) and 2.

Multi-drug resistance: existence of resistance to different agents with different mechanism of action of two or more active groups (Prichard et al., 1980).

2.5.1 Anthelmintic resistance in ruminants

Reports of anthelmintic resistance against either long used or new substances usually occurred only a few years (~3-5) after the introduction of a new compound. Phenothiazine resistance was firstly reported in the late 1950s and early 1960s in *H. contortus* in sheep (Drudge and Wyant, 1957) . Resistance reports against thiabendazole followed in 1964, again in *H. contortus* of sheep in Kentucky, USA (Drudge et al., 1964, Conway, 1964). Those authors found no significant differences between the FECs of thiabendazole treated and untreated sheep. Reports of BZ resistance continued through the 1970s, now involving also other GI nematode species such as *O. circumcincta* and *T. colubriformis.* First resistance reports to imidazothiazoles and MLs followed in the 1980s and later (Slocombe, 1992, Waller and Prichard, 1986).

AR is now considered a serious problem in small ruminants (Kaplan and Vidyashankar, 2012, Kaplan, 2004, Wolstenholme et al., 2004, Jackson and Coop, 2000), but also in cattle a lot of studies reported AR in GI nematodes, especially to ivermectins and milbemycins (Edmonds et al., 2010, Demeler et al., 2009, Gasbarre et al., 2009, Anziani et al., 2004, Anziani et al., 2001). The most common resistant species that was reported is *Cooperia* sp., while resistance in *Ostertagia* sp. was reported much less frequently. These two species are considered the most pathogenic species and they have an economic importance (Edmonds et al., 2010, Suarez and Cristel, 2007). Most of the resistance cases reported worldwide involve MLs and in contrast, resistance reports against BZs seem to be generally less common in cattle GI nematodes. The majority of cases are reported from South America, followed by New Zealand and the USA. In Europe only few cases have been described.

In Argentina ABZ resistance was present in 32% of the investigated farms (8 farms out of 25 farms), the mean FEC reduction for ABZ was 93.3%, the most predominant resistant genera seen in 25 farms is *Ostertagia* sp. (Suarez and Cristel, 2007). Resistance to MLs especially IVM also was described by Anziani et al. (2004),(Anziani et al., 2001; Fiel et al., 2001). In Brazil, Soutello et al. (2007) found a decreased efficacy of IVM, ABZ and LEV, 19 of 25 farms had <50% reduction in FEC and the most resistant detected genera were *Cooperia* sp. and *Haemonchus* sp . In the North Island of New Zealand 62 beef cattle farms were investigated according to the results of FECRT, 7% of the farms (4 farms) showed ≥95% for all anthelmintic classes that were tested (levamisole (LEV), ABZ, IVM). Resistance to LEV was low (6%), but

IVM and ABZ resistance were reported on 92% and 76% of the farms, respectively. On 74% of the farms resistance against IVM and ABZ was found. The most often found resistant species was *Cooperia* which was identified on L3 identification obtained from faecal cultures. *Ostertagia* sp. resistant against ABZ were detected on 35% of farms in New Zealand (Waghorn et al., 2006). Mortensen et al. (2003) reported resistance to BZs in the southeast regions of the USA while Edmonds et al. (2010) detected reduced efficacy of IVM and ABZ in the western region of the USA. Coles (2005) described resistance against BZs of cattle nematodes in England.

2.5.2 Mechanism of BZ resistance

The action of BZs is preventing the tubulin polymerisation into microtubules (Lacey and Gill, 1994, Lacey, 1990). Microtubules are the major unit for cell motility and neurons synthesis (Lopata and Cleveland, 1987), they participate in cytoskeleton formation, preserving cell membrane characters and transportation inside the cell (Cleveland and Sullivan, 1985). The microtubules are also associated with most of the organelles inside the cell such as nucleus, mitochondria, and Golgi apparatus (Dustin, 1984). Action of the receptors and secretions of enzymes, neurotransmitter and hormones are also associated with microtubules (Lacey, 1988). They consist of α -tubulin and β-tubulin and 450 amino acids (Lacey and Gill, 1994). These amino acids are polymerised to form the microtubules as shown in Figure 3 (Martin, 1997). The polymerisation process occurs into two sides of the dimers, one side involve the proliferation and the other side involve the dissociation. (Ca^{2+}, GTP, Mg^{+2}, temperature changes and calmodulin affect the polymerisation process (Martin, 1997). BZs bind to the β-tubulin which inhibits the formation of microtubules at the positive pole (called capping process). The disruption of the tubulin polymerisation leads to the dysfunction of almost all vital processes associated with the microtubules such as cytoskeleton formation, intracellular nutrient transport and energy consumption. Subsequently metabolic substrates are reduced and the spindle formation in mitosis is blocked. Thus the organism subsequently dies after consumption of the remaining energy resources (Lacey, 1988).

There are two β-tubulin isotypes: 1 and 2, each one has different numbers of alleles, isotype 1 has up to six and isotype 2 has up to 12 alleles (Lubega et al., 1994, Guénette et al., 1992, Guénette et al., 1991) Resistance appears if there is a reduction in the isotype allele numbers through a mutation which leads to single nucleotide polymorphisms (SNPs) (Garg and Yadav, 2009, Rufener et al., 2009, Ghisi et al., 2007). Up to now SNPs on three different positions in the β-tubulin isotype 1 gene have been associated with BZ resistance; a mutation at positions

200 and 167 (TTC to TAC) (Kwa et al., 1994) and a mutation at position 198 (GAA to GCA) (de Lourdes Mottier and Prichard, 2008, Ghisi et al., 2007).

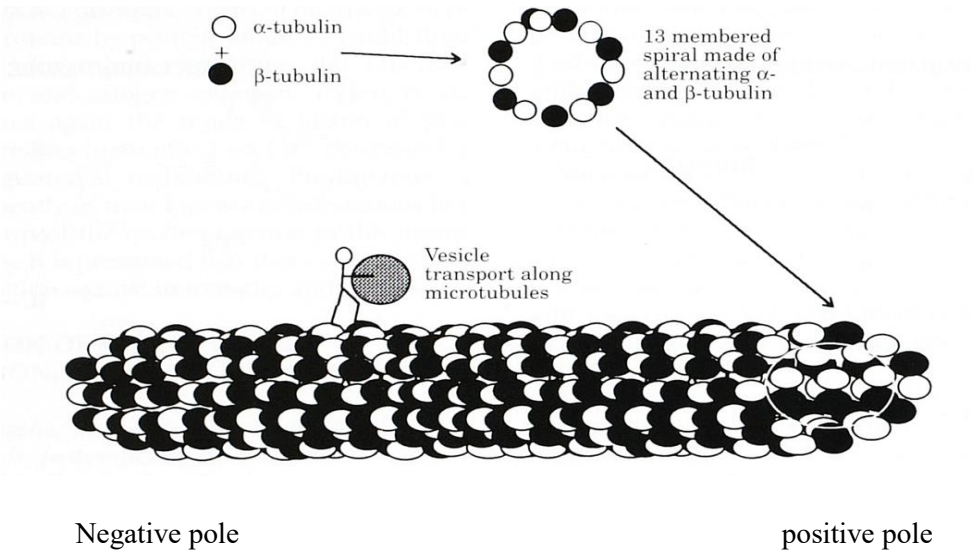


Figure 3. Microtubules formation (Martin, 1997)

2.6 Control and management of the resistance

Control of GI nematodes infection aims to minimise production losses and delay the development of AR. Even though pasture management is somehow implemented on most farms, nematode control programs were generally based on routine regularly repeated anthelmintic treatment. One fourth of cattle farms in New Zealand use the repeated treatment schemes of eight up to 12 times in the first year (Jackson et al., 2006). Several surveys in Germany and Netherlands also indicated that the control of the GI nematodes in FSG calves is rather over protective (Ploeger et al., 2000, Schnieder et al., 1999a, Borgsteede et al., 1998).

Now in many European countries, routine treatment on organic farms is forbidden (Larsson et al., 2007). Accordingly, pasture management plays again a great role in parasite control and subsequently the resistance control on sheep farms by maintaining sufficient parasites "refugia" in pasture. The refugia represent the untreated parasite stages on pasture or inside the host which dilute the upcoming resistance parasite by mating with these resistance worms (Michel, 1985). Field trails proved that using the refugia concept in worm control programs is likely to reduce the resistant worms (Waghorn et al., 2009, Besier et al., 2001, Martin et al., 1981). As the prevalence of AR is increasing (J Wolstenholme and M Kaplan, 2012, von Samson-Himmelstjerna, 2012, Edmonds et al., 2010, Condi et al., 2009, Demeler et al., 2009) current control programs have become non-beneficial

in many areas. Accordingly, more attention has been focused to introduce new treatment strategies to improve the efficacy of anthelmintic, minimise animal production losses and reduce the development of resistance. One concept relies on the management of refugia by decreasing the number of animals receiving anthelmintic treatment. This has led to the development of targeted treatments (TT), where the whole flock/herd is treated if one animal is diseased and targeted selective treatments (TST) where only single animals in the grazing group are treated (Kenyon et al., 2009, van Wyk et al., 2006).

These strategies have been first applied in small ruminant (Kenyon and Jackson, 2012, Stafford et al., 2010). The identification of diseased animals was one of the largest difficulties and hence a large of treatment decision parameters and various criteria were tested. Infections with the blood sucking parasite *H. contortus* cause anaemia. The severity of the anaemia can be classified by using the FAffaMAlanCHArt (FAMACHA) score system. Here, the colour of the conjunctiva of the eye is assessed and compared to the illustrations on a map (Van Wyk ; Bath, 2002). Other parameters are the diarrhoea score (DISCO) (Ouzir et al., 2011, Cabaret et al., 2006), milk production (Hoste et al., 2002), weight gain (Stafford et al., 2009), FEC (Cringoli et al., 2009, Leathwick et al., 2006) and body condition score (BCS) (Cornelius et al., 2014, Gallidis et al., 2009). In small ruminants successful implementation of FECs as treatment indicators have been reported from the UK with an average FEC >100 (Kenyon and Jackson, 2012), while in New Zealand treatment was performed when the mean FEC exceeded >500 in ewes (Leathwick et al., 2006) and in Greece treatment was applied when FECs exceeded 300 in sheep (Gallidis et al., 2009). Another study in Italy used the FEC as a treatment marker, they treated the ewes when their individual FEC was higher than the group mean. For the BCS contradictory results have been published with one study revealing it to be good indicator of GI nematodes infections in goats and sheep (Gallidis et al., 2009), and another study showing that non-infected ewes had a lower BCS than the infected ewes and the infected sheep (Cornelius et al., 2014).

In contrast to various trials regarding the impact of TT/TST strategies in small ruminants relatively little work has been reported for cattle. The following chapter is highlighting the different approaches so far reported for their suitability in large ruminants.

2.6.1 Targeted treatment and targeted selective treatment in calves

The studies conducted on TST in large ruminants are limited and have mainly been performed in young animals and different parameters were used for application of the TST/TT strategies.

in different studies.

Weight gain: Greer et al. (2010) and Höglund et al. (2013) demonstrated that a weight based TST program tested in dairy FSG calves was acceptable from the productivity point of view and they demonstrated that there is a reduction in anthelmintics but combined with small production losses. A lot of studies proved the relationship between GI nematodes control and weight gain in FSG calves, but in adult cows the effect of the treatment seems not to be that important from a parasitological point of view whilst more in terms of production (Charlier et al., 2009). Increased weight gain following anthelmintic treatment is the most often observed benefit. Höglund et al. (2009a) investigated the use of the DWG in FSG calves as a treatment marker. Three trails performed in Sweden between 1997 and 2004 were retrospectively analysed regarding the applicability of DWG as a TST marker. The suggested treatment threshold was 0.75 kgper day and confirmed in more recent trials (Larsson et al., 2006, Dimander et al., 2003, Dimander et al., 2000). In another study Höglund et al. (2013) showed again that the TST group had a significantly higher weight gain compared to the untreated group. These results are similar to the result of a meta-analysis published by Shaw et al. (1998b). These authors analysed data from a total of 85 control groups (untreated) and 95 treatment groups (~2250 FSG calves) over a period of 26 years. The results showed that treated animals had a significantly higher weight gain than untreated control animals. Additionally, within the treatment groups, animals with clinical symptoms of parasite related gastroenteritis had a lower weight gain compared to sub-clinically diseased animals.

FEC: Other studies used FECs as a treatment indicator because the FECs are the most widely used parameter in diagnosis of GI nematodes infection in FSG calves. It has been recommended to evaluate infection levels in young animals approximately 5 and 10 weeks after turnout (Eysker and Ploeger, 2000, Shaw et al., 1998a, Shaw et al., 1998b, Shaw et al., 1997) and subsequently to decide on further worm control measures. As reported for small ruminants the FEC treatment thresholds vary between different studies. In the same meta-analysis mentioned above a FEC ≥200 (measured 8 weeks after turnout) was found to be the most sensitive indicator for the occurrence of parasite related gastroenteritis (Shaw et al., 1998a) while other studies used cut-off values of 100 (Areskog et al., 2013), 200 (O'Shaughnessy et al., 2015) and 250 (Fahrenkrog, 2013) . In older cattle, FEC has been reported not to be a good indicator for the GI nematodes infection level (Gross et al., 1999, Michel, 1968) since at the end of the first grazing season there is a reduction in egg production by the worms as the animals begin to acquire immunity against the GI nematodes (Claerebout and Vercruysse, 2000). Even though FEC seem to be easy to obtain and to use, there are some important disadvantages associated.

First, it is relatively time and labour intense. Secondly, the correlation between the number of eggs in the faeces and the actual worm burden extremely low, particularly in *Cooperia* and *Ostertagia* species. Thirdly, the egg production is different between females of the species so the threshold setting remains difficult. These might be part of the reason, why FEC have not been successfully implemented in TT/TST studies in large ruminants yet.

BCS: The BCS can be used as an indicator of the general condition and body reserves (Van Burgel et al., 2011) in cattle. It is generally used for the assessment of general health parameters in adult cattle and when used in calves the respective scoring system has to be adapted. In a study reported by Höglund et al. (2013) the BCS was not good enough as an indicator in FSG calves. Another study performed in Germany used the BCS scoring table for Holstein dairy cows (Edmonson et al., 1989) with adaptations made to FSG calves. Here BCS was shown to be useful in combination with FECs as a TST marker (Fahrenkrog, 2013).

Serum pepsinogen test: The serum pepsinogen concentration was tested as a possible decision parameters by Berghen et al. (1993). Dorny et al. (1999) found that high serum pepsinogen concentration caused by infections with *O. ostertagi* is responsible for production losses in cattle. In a study conducted by Charlier et al. (2011) they used the serum pepsinogen level to monitor the GI nematodes infection level in FSG calves. Pooled serum samples from herds were examined and depending on the serum pepsinogen concentration divided into three categories. The first category had a serum pepsinogen concentration of <1.2 units tyrosine (UT) which refers to low parasitic infection and these animals were recommended to receive less anthelmintics. The second category had a serum pepsinogen concentration of 1.2-3.5 UT and these group was recommended to maintain the current anthelmintic treatment that were used. The third category had a serum pepsinogen concentration >3.5 UT and for this group it was recommended to increase the anthelmintic treatment.

2.6.2 Targeted treatment and targeted selective treatment in adult cows

Another decision parameter that was tested in adult cattle is the concentration of *O. ostertagi* antibodies in the milk, which is determined by using of enzyme-linked immunosorbent Assay (ELISA). Sanchez and Dohoo (2002) and Charlier et al. (2005) reported that the antibodies in milk could be used as an indicator for the infection level in the herd. Sanchez et al. (2004) detected a negative impact of GI nematode infections on milk yield of dairy cows. The average milk yield increased after anthelmintic treatment by 1 kg/cow per day.

In a trial in five European countries (Belgium, Germany, Sweden, England, Ireland) 3600 samples were examined and it was found that nematode infection is an important factor for production losses in dairy farming (Bennema et al., 2010). So the antibodies concertation in milk could be considered as a parameter for the TST treatment approach (Charlier et al., 2011, Charlier et al., 2010b, Charlier et al., 2010a).

2.7 Diagnosis of AR

The efficacy of an anthelmintic drug is an important prerequisite for effective parasite control. Decreasing efficacy need to be identified early and then, if necessary, a change of measurements has to be made to avoid further development or spread of resistance. For the detection of resistances some methods have been recently reviewed (Taylor et al., 2002). They can generally be divided to *in vitro* (involving the living parasites) and *in vivo* (involving the living host) methods, which are briefly described below.

2.7.1 Controlled test

This *in vivo* test was first introduced by Whitlock et al. (1980). Guidelines for the implementation of this test were provided by the World Association of the Advancement of Veterinary Parasitology (W.A.A.V.P) (Wood et al., 1995). The animals were experimentally infected, treated and the worm burden determined in a post mortem section and compared to untreated control animals. Is the reduction of the worms less than 90% or if over 1.000 worms survived anthelmintic treatment, resistance is present. The disadvantage of this test is that it requires large numbers of animals, the killing of those animals and is therefore expensive as well as time and labour intense.

2.7.2 Faecal Egg Count Reduction Test (FECRT)

The FECRT is the most widely used *in vivo* test for GI nematode resistance diagnosis. It relies on the concept of comparing the number of eggs before and after the treatment (Boersema, 1983). Initial recommendations for this test were provided by the W.A.A.V.P (Coles et al., 1992). According to the W.A.A.V.P. resistance is present when the egg reduction is below 95% and the lower limit of 95% confidence interval (CI) is lower than 90%. If only one of these parameters is within this range, there is a risk of resistance development. However, it needs to be highlighted that hose recommendations are only for sheep GI nematodes and until to date respective recommendations for cattle are not available. The interpretation of the FECRT in

cattle is furthermore complicated by the generally low fecundity of some species (e.g. *Ostertagia*) and the fact that there is no correlation between egg output and number of worms present in the host. Also the sensitivity of detection method (Wang, 2015) as well as the statistical analysis used are very important. The classical method for FECRT as described by Coles et al. (1992) can lead to some statistical errors: if for example all the post treatment FECs are zero the reduction will be 100% with a 95% confidence interval (CI) of 100-100% which can't be true because a zero FEC after treatment doesn't prove that there are no eggs in the faeces. It only indicates that no eggs were seen as the eggs were randomly distributed in the faecal sample even it is well mixed (Torgerson et al., 2014). So a Bayesian hierarchical model was developed by Paul et al. (2014) to overcome the disadvantages of the classical statistical method and Wang (2015) developed a model to include zero FECs.

2.7.3 *In vitro* assays

Compared to *in vivo* tests these methods have the advantages that they are often faster, less expensive and more reliable. They also require only one single sampling and can be performed for more than one drug in parallel (Lacey, 1990).

The first assay published by Le Jambre (1976) involved the incubation of eggs in a range of drug concentration and assessed respective hatching rates. It is used for the efficacy evaluation of mainly TBZ and LEV, as these drugs have an ovicidal activity. They inhibit egg hatching so the resistance can be detected by comparing the proportion of eggs and the hatched larvae. Another possibility to measure drug effects *in vitro* is the larval development assay (LDA). Here eggs are incubated in a range of drug concentrations for one week. The inhibition of larval development is believed to be caused by the inhibition of the pharynx musculature which leads to paralysis and subsequently to the death of the nematodes through starvation (Gill et al., 1995). In contrast to the Egg Hatch Assay (EHA) this assay is not limited to ovicidal drugs and accordingly can be used also for MLs. It has first been developed for sheep GI nematodes (Lacey, 1990) but recently it has been adapted also for cattle GI nematodes (Demeler et al., 2012).

A third way of investigating effects of drugs is the incubation of L3 followed by the assessment of motility. Several different assay types have been reported including the observation of motility by eye (Gill et al., 1991), the migration of L3 through sieves (Sangster et al., 1988) or movement detection by electronic devices (Folz et al., 1987). For cattle parasitic nematodes on the larval migration inhibition assay (LMIA) has been adapted and evaluated (Demeler et al.,

2012). The suitability of the EHA and the LMIA to be used in the field was reported to be reasonably good, providing similar results as the FECRT with the ability to detect decreased anthelmintic efficacy much earlier than the FECRT (Demeler et al., 2012).

2.7.4 Molecular resistance detection

The limitations of the *in vivo* tests as well as the problem of applying the *in vitro* assays to field samples has increased the research intensity in the molecular field to overcome those shortages (Lind et al., 2005, Tandon and Kaplan, 2004, Craven et al., 1999).

Molecular methods such as conventional and real time polymerase chain reaction (PCR) have opened new views for detection of AR in several populations of nematodes of small and large ruminants as well as horses (von Samson-Himmelstjerna, 2006). For the detection of AR with molecular methods the species or at least the genus needs to be determined first. Gasser et al. (1994) described a species specific PCR for parasite differentiation and restriction fragment length polymorphism (RFLP). The second internal transcribed spacer (ITS-2) of different species was amplified in the PCR, subjected to restriction enzyme digestion and then the bands were separated by gel electrophoresis. With this method a lot of species such as *C. oncophora*, *H. contortus*, *Trichostrongylus axei*, *T. circumcincta* and *Trichostrongylus colubriformis* can be differentiated. Specific sequence primers were developed for the ITS-2 regions which enable a species specific differentiation of the GI nematodes of ruminants (Schnieder et al., 1999b). A real time species specific PCR used for the detection of *C. curticei*, *C. oncophora*, *H. contortus*, *O. leptospicularis* and *T. colubriformis* published by von Samson-Himmelstjerna et al. (2002a).

The knowledge regarding the molecular mechanisms of AR is still sparse and until now respective test systems are only available for the BZs (von Samson-Himmelstjerna, 2006, Taylor et al., 2002), where changes in three codons of the β-tubulin isotype 1 gene have been associated with a resistant phenotype. First, a conventional PCR was used for detection of the resistance SNP in codon 200 of the β-tubulin isotype 1 in different studies and different parasites; allele specific PCR was used to detect BZs resistance in adult *H. contortus* (Kwa et al., 1994) and also in adult and larval stages of *T. circumcincta* (Elard et al., 1999, Humbert and Elard, 1997, Elard et al., 1996). Additionally allele specific PCR was used by Silvestre and Humbert (2002) to detect BZs in *T. circumcincta, H. contortus*, and *T. colubriformis.* Two PCRs were performed; the first PCR to amplify the first isotype 1 fragment of β-tubulin and the 2nd nested PCR to amplify the second isotype 1 fragment of the β-tubulin. Then RFLP was used for species identification and finally resistance was detected by allele specific PCR. Moreover,

a similar approach was reported by von Samson-Himmelstjerna et al. (2002b); they established an allele specific PRC for the detection of β-tubulin isotype 1 mutation at codon 200 in small strongyles in horses through using 2 primers ; one for the BZ resistant allele and the second for BZ susceptible allele. Winterrowd et al. (2003) detected a mutation in codon 200 in *C. oncophora* through amplification of partial genomic sequences from *C. oncophora* and *O. ostertagi*. In another study a full length cDNAs from *C. oncophora* containing isotype 1 and 2 of β-tubulin was cloned and sequenced resulting in finding resistant isotype 1 alleles in a small proportion of individuals but codon 167 and 198 were not analysed in this study (Njue and Prichard, 2003). A real time PCR method was also described by Alvarez-Sanchez et al. (2005) to determine frequencies of alleles in β-tubulin isotype 1 in codon 200 in trichostrongyles of sheep and von Samson-Himmelstjerna et al. (2009) used the real time PCR to diagnose the BZs resistance in *H. contortus* at codon 200.

In the past years, several pyrosequencing assays to detect BZ resistance associated changes on positions 167, 198 and 200 in the β-tubulin isotype 1 gene in a number of nematodes have been developed (Demeler et al., 2013b, Von Samson-Himmelstjerna et al., 2007).

2.7.4.1 Pyrosequencing

This sequencing by synthesis method is based on the luminometric detection of pyrophosphate (PPi) released due to the incorporation of the nucleotides, as light is emitted in an amount equal to the amount of the adenosine triphosphate (ATP) (Figure 4).

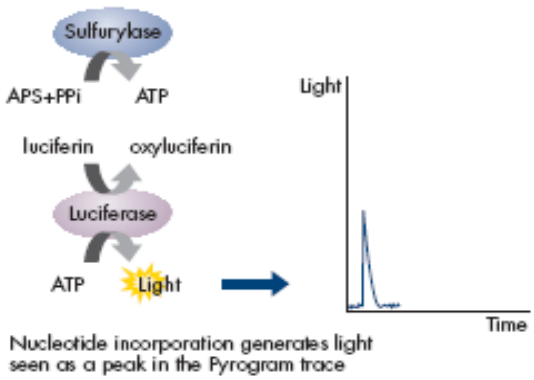


Figure 4. The pyrophosphate (PPi) with the substrate adenosine 5′ phosphosulfate (APS) is converted to adenosine triphosphate (ATP), leading to luciferin conversion to oxyluciferin which produces amounts of visible light that are relative to the ATP amount (source: PyroMark Q24 user manual 12.2012).

Pyrosequencing can be used for quantitative DNA sequences analysis and SNP detection (von Samson-Himmelstjerna and Blackhall, 2005, Troell et al., 2003), as e.g. in the case of BZ resistance. Here the mutations in the β-tubulin isotype 1 gene at codon 200 lead to a change of phenylalanine to tyrosine, which is the most commonly found SNP in BZ resistant trichostrongyles in small ruminants (Elard and Humbert, 1999, Elard et al., 1996, Kwa et al., 1994) and in small strongyles in horses (Pape et al., 2003, von Samson-Himmelstjerna et al., 2002b, von Samson-Himmelstjerna et al., 2001, Pape et al., 1999). Another mutation at codon 167 leads to the same change in amino acids (Pape et al., 2003, Silvestre and Cabaret, 2002, Prichard, 2001) and in codon 198 the glutamate is replaced by alanine.

The pyrosequencing technique offers several advantages. It is a rapid and accurate method to analyse the allele frequency in nematodes (Alderborn et al., 2000, Ronaghi et al., 1996), it can detect more than one SNP in one target (Lötsch et al., 2003, Pourmand et al., 2002), which makes it very useful in the analysis of BZ resistance, as often more than one SNP is present. Another advantage of this technology compared with other techniques such as real time PCR, MALDI-TOF mass spectrometry and mini-sequencing is, that it allows to assess the templet quantity through the light signal intensity measurements (von Samson-Himmelstjerna, 2006). In a study conducted in Germany L3 of *H. contorts* from susceptible and resistant isolates were used for comparative performance in the pyrosequencing. The comparison of peak heights at the same positions between a susceptible and resistant isolate showed a reduction in height of the primary peak combined with appearance of a secondary peak at a nucleotide site which represent a SNP in the resistance isolate. Therefore heights of the peaks can estimate the SNP frequencies as shown in Figure 5.

BZ susceptible **BZ resistant**

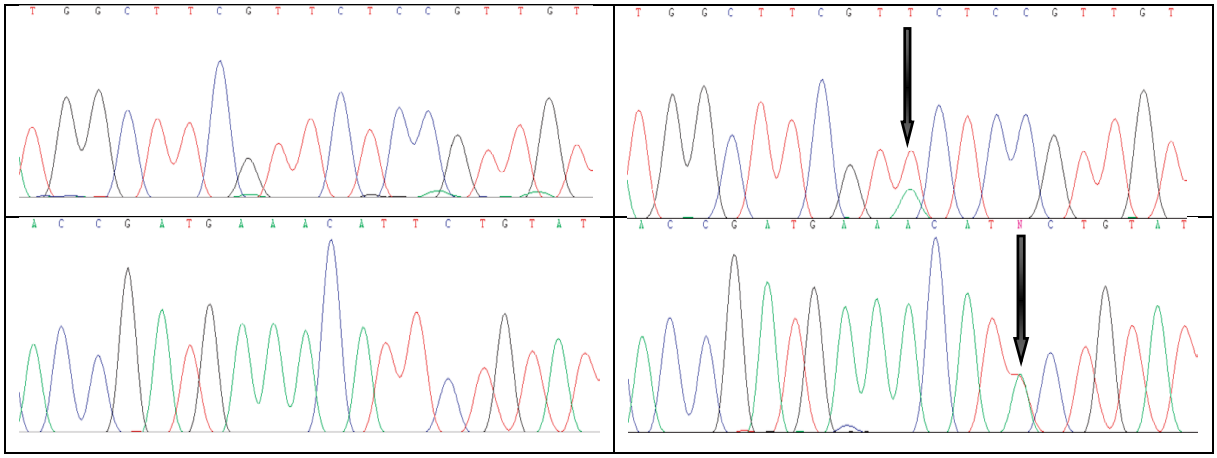


Figure 5. The upper part shows the chromatogram for codon 167, the lower for codon 198/200. The BZ susceptible population is displayed on the left, the resistant on the right side. The arrow indicate the thymine to adenine transversion in the resistant population (Von Samson-Himmelstjerna et al., 2007).

2.7.4.2 Pyrosequencing in cattle parasitic nematodes

The first pyrosequencing assay for the cattle parasites (*C. oncophora* and *O. ostertagi*) was described by Demeler et al. (2013a). Detailed pyrosequencing assays for the detection of all three SNPs in the β-tubulin isotype 1 (codon 176, 200 and 198) were described. These authors investigated the occurrence of all three SNPs in nematode populations collected in the field in Argentina, Australia, Columbia and Germany as well as in laboratory isolates. SNPs were detected in all codons but with at a different frequency for the two species. The most prevalent SNP in *Ostertagia* was in codon 167 followed by codon 200 and 198 and in *Cooperia* the most prevalent SNP was detected in codon 198 and 200 in Columbian populations. In Argentina population the most prevalent SNPs was detected in codon 176 and 200. These authors also describe the full length of β-tubulin gene isotype 1 and 2 and α-tubulin sequences in *O. ostertagi* and α-tubulin in *C. oncophora.*

Knapp-Lawitzke et al. (2015) used the pyrosequencing assay for BZs resistance detection in a study that was conducted in Germany. Two groups of calves were examined; group 1 had four experimentally infected calves with pure *O. ostertagi* showing 50% of TAC allele. Group 2 had 8 naturally infected calves by contaminated pasture divided into 4 groups of two calves in each, sub therapeutic doses of ABZ was used in increasing percentages (35–65%) for each group. Species specific PCR and pyrosequencing was performed to detect the allele frequency. *Ostertagia*, *Cooperia*, *Haemonchus* and *Trichostrongylus* was detected by the PCR in pre-treatment samples while the post-treatment samples had predominantly *Ostertagia* and some *Trichostrongylus*. The SNP (TAC) at codon 200 significantly increased after the treatment comparing to the pre-treatment samples.

3. Materials and methods

3.1 Materials

3.1.1 Chemicals

Materials	Supplier
Valbazen 10 %®	Pfizer, Berlin
DNA Loading Dye (6x)	Fermentas/Thermo Scientific, Waltham, USA
GR Green nucleic acid gel Stain	Lab gene scientific SA, Chatel-St.Denis, Switzerland
5x Phusion HF Buffer	Fermentas/Thermo Scientific, Waltham, USA
Betaine stain	Sigma –Aldrich, Munich , Germany
DNTPS	Fermentas/Thermo Scientific, Waltham, USA

3.1.2 Kits

NucleoSpin® Tissue Kit	Macherey und Nagel, Düren
Phusion® Hot Start II	Fermentas/ Thermo Scientific, Waltham, USA
Water	Roth, Karlsruhe Germany
Pyro Mark Gold Q24 Reagents	QIAGEN, Hilden, Germany

3.1.3 Primers und Markers

Cooperia SH forward (for.) (5'-ATGGCATTTGTCTACATCTGTTT-3')	(Synthesised by Invitrogen, Darmstadt)
Cooperia SH reverse (rev.) (5'AAATGATAACGAATACTACTATCTCCA-3')	
Ostertagia ostertagi 200 neull for. (5'-CGTTGTTGAGCCCTACAACGCC-3')	
Ostertagia ostertagi 200 neull rev. (5'-CGAAGATCAGCATTCAACTGC-3') with biotin	
Ostertagia ostertagi 167 for. (5'-TCGCCAAAATTCGTGAGGA-3')	
Ostertagia ostertagi 167 rev. (5'-GAGACCTTGGGCGAAGGAA-3') with biotin	
Ostertagia ostertagi 198+200 for. (5'-GTTCCTTCGCCCAAGGTCT-3')	
Ostertagia ostertagi 198+200 rev. (5'-TGTGCGGAAGCAGATATCGTA-3') with biotin	
Cooperia oncophora 167 for. (5'-TATGGGCACTTTGCTTATTTCA-3')	
Cooperia oncophora 167 rev. (5'-ACGTTTCATCGGTATTTTCTACCA-3') with biotin	

Cooperia oncophora 198+200 for. (5'-CGACACCGTTGTGGAACCTTAC-3')	
Cooperia. oncophora 198+200 rev. (5'-CCGGACATTGTGACAGACACTAG-3') with biotin	
Cooperia. oncophora SH for. (5'-ATGGCATTTGTCTACATCTGTTT-3')	
Cooperia. oncophora.198+200 sequencing primer (5'-ACTGGTAGAAAATACCGAT-3')	
Cooperia. oncophora 167 sequencing primer (5'-ACAGAATTATGGCTTCGT-3')	
Ostertagia ostertagi 167 sequencing primer (5'-CGGATAGAATCATGGCTT-3')	
Ostertagia ostertagi 198+200 sequencing primer (5'-TGGTGGAAAATACTGATG-3')	
100 bp DNA ladder	Fermentas/Thermo Scientific, Waltham, USA

3.1.4 Buffers and Solutions

Agarose gel (1. 5 %)	Boil 7.5 g agarose in 500 ml of TAE buffer
TRIS-acetate-EDTA (TAE) buffer	50 ml TAE stock solution + 450 ml of distilled water
Sodium chloride solution (saturated) (NaCl)	360 g NaCl dissolved in 1000 ml of water
PyroMark Binding Buffer, Wash Buffer, Annealing Buffer	QIAGEN, Hilden, Germany
PyroMark Denaturation Solution	QIAGEN, Hilden, Germany

3.1.5 Consumables

Falcons 15 ml, 50 ml	SARSTEDR, Nümbrecht, Germany
Filter tips 0.5-10 µl, 10-100 µl, 100-1000 µl;	Biozym Hessisch Oldendorf, Germany
Para-film	Pechiney Plastic Packaging, Chicago, USA
Pipette tips 0.5-10 ul, 10-100 ul, 100-1000 ul	SARSTEDR, Nümbrecht, Germany
Eppendorf (0.5 µl, 1.5 µl, 2 µl)	SARSTEDR, Nümbrecht, Germany
Gloves Rotiprotect-latex (nitryl)	ROTH, Karlsruhe , Germany
PCR Eppendorf (0.2 µl ,0.5 µl, 1.5 µl, 2 µl)	ROCH, Mannheim , Germany
PCR 96-multiwell plates	BIOZYM, Hessisch- Oldendorf, Germany
Wooden sticks	Roth, Karlsruhe, Germany
Glass slides	Glaswarenfabrik Karl Hecht GmbH& Co KG , Germany
Ventilated cell culture flask	SARSTEDR, Nümbrecht, Germany

3.1.6 Reusable items

McMaster Counting Chamber (2 chambers)	FIBL, Switzerland
Mini FLOTAC counting chamber	University of Naples Federico, Naples, Italy

PCR-Rack	Roth, Karlsruhe
Petri dishes	SARTEDT, Nümbrecht, Germany
Plastic measuring cylinder 100 ml	Roth, Karlsruhe
Transfer pipettes 3.5 ml	SARTEDT, Nümbrecht, Germany
Honey jars 500 ml	German Beekeepers Association e.V.
Falcon tubes Rack	Kartell , Italy
Centrifuge tube racks	Roth, Karlsruhe
Glass beakers 20 ml, 50 ml, 100 ml, 250 ml	Schott-Duran, West Germany
T- sieves	West Germany
Pipettes 10 µl, 100 µl, 1000 µl, Multi pipette® x stream	EPPENDORF, Hamburg Germany

3.1.7 Devices

C1000 TM Thermal Cycler	Bio-Rad, Munich, Germany
Epoch	Biotek, Bad Friedrichshall, Germany
Incubator	Binder , Germany
Refrigerator	C. Bomann GmbH, Kempen
Microscope	Carl Zeiss, Jena, west Germany
Stemi DV4 Microscope	Carl Zeiss, Jena, west Germany
Synergy 4	Biotek, Bad Friedrichshall
IKA®Vortex genius 3	SIGNA-ALDRICH, Munich, Germany
IKA® shaker MTS	JANKE&KUNKEL, Staufen, Germany
Mini-centrifuge RF- Heraeus	SEPA-TECH Engen, Germany
Centrifuge 54 30 R	EPPENDORF, Hamburg Germany
Incubator with shaker TMix x220	Analytik Jena , Germany
Electrophoresis gel chamber, Wide Mini sub cell GT	Bio-Rad, Munich, Germany
Take 3TM MULTI VOLUME plate	Biotek, Bad Friedrichshall, Germany
G box	SYNGENE, Cambridge , UK
Centrifuge	Multifuge® X3R; Thermo Scientific, Waltham, USA
PyroMarkQ24 work station, cartridge, instrument	QIAGEN, Hilden, Germany

3.1.8 Software

Gene Snap	Syngene, Cambridge, UK
Endnote®	Version X7; Thomson Reuters, New York, USA
Gen5®	Biotek, Bad Friedrichshall, Germany
Microsoft Excel®, Word®	Microsoft
PyroMarkQ24 soft ware	QIAGEN, Hilden, Germany
IBM SPSS Statistics 22	Armonk, NY
Web interface software	University of Zurich website http://shiny.math.uzh.ch/user/furrer/shinyas/shiny-eggCounts/

3.2 Methods

Within this thesis two studies were conducted. The first and main study comprised of the evaluation of possible effects of new anthelmintic treatment strategies in beef calves in Germany. The second study was the assessment of resistance to BZs on German cattle farms. The following section will first provide all details for the first study followed by the second study.

The first study was conducted on three farms which were all located in the federal state of Brandenburg, Germany. The selection criteria for inclusion were: total number of FSG calves ≥36, pasture access for all FSG calves, turned out onto pasture at least one month before the first sampling and no anthelmintic treatment. Two of the farms were visited in the year 2013: Linum (farm 1) and Groß Kreutz (farm 2), and a third farm was visited in the year 2014: Schlepzig (farm 3). Farm visits were undertaken from May to October 2013 and from June to November 2014.

3.2.1 Animals

Farm 1 (Linum): This farm was located approximately 60 km northwest of Berlin. It is a large commercial beef cattle farm with a permanent herd of about 400 Limousine breed adult cattle. Initially 39 calves were included. Depending on age the calves were allocated in two groups. In the first group calves were born between December and February. These calves were born inside the stable and then turned out onto pasture in May/ June. In the second group calves were born between March and April and turned out subsequently until June/July. Two calves died (which was not related to parasitic diseases) during the study so finally data of 37 calves was included.

Farm 2 (Groß Kreutz) was located around 60 km west of Berlin. This enterprise focussed on the maintenance of the Uckermärker crossbreed. Forty calves were included and again were allocated into two groups depending on their age. The calves of the first group were born between March and April in the stable and the second group were born in May on pasture. This farm is running an extensive farming management system called “Mutterkuh-Haltung”. This involves the rearing of mother cows and their offspring together, meaning that the animals are permanently out on pasture for most of the year. There are no arranged calving times and calves are with their mothers until slaughter. One animal died during the study (which was not related to parasitic disease), so finally 39 calves were included.

Farm 3 (Schlepzig) was located around 85 km southeast of Berlin. It is part of a large dairy and beef farming network near Schlepzig which consists of various organic and conventional stock farming systems. On this farm 73 calves were included. Animals were allocated into two similar sized groups with calves of the first group born between December and March and calves of the second group calves between May and August. The calves were beef calves (Uckermärker x Blonde Aquitaine mixed breed) and were grazed on two different pasture sites. Both sites were situated in the same area within 5 km of each other.

3.2.2. Study design

On the first two farms the animals were grouped into four groups. Allocation of animals to the particular groups was performed based on gender and FECs taken before the start of the study. The groups were treated as follows: 1) TT, where the whole group treated when at least one animal met the criteria, 2) TST, with only the single animals meeting the criteria were treated, 3) no treatment (negative control; NC), where none of the animals treated for the whole study duration and 4) prophylactic treatment (positive control; PC), where all animals treated prophylactically monthly (every visit) for the whole duration of the study. The anthelmintic used in this study was (Valbazen®, Pfizer) at a dose rate of 7.5 mg/kg. The total number of animals per group on each farm is presented in Table 2.

Table 2. Numbers of animals per farm in each treatment group

Year	2013		2014
Name of the farm	Groß Kreutz	Linum	Schlepzig
TST	13	14	24
TT	13	14	25
NC	7	5	24
PC	7	6	-

3.2.3. Sampling of animals

Every farm was visited in monthly intervals. On farm 2 calves were restrained manually, on farms 1 and 3 a crush gate was available. Fresh faecal samples were collected rectally by using a lubricated long plastic glove and then stored in small plastic cups covered with lids. These cups were marked with the ID number of each animal. Each sample was split into three parts: one was used for the determination of the FEC, one for sedimentation and the third part for the performance of a faecal culture. The first two parts were stored at 4°C until processing, the third part at room temperature. The body weight was determined using a scale and the BCS was

assessed by eye for each animal. BCS measuring was performed according to the modified body condition scoring table for Holstein dairy cows (Edmonson et al., 1989). This includes the observation of the vertical prominences of the lumber vertebrae, the depression appearing between the spinous and the transverse process, the transverse prominence of the lumber vertebrae, overhanging shelf formed by the transverse processes above the flank, bony prominence of the tuber coxae (hooks), depression between the hooks and pin bones, depression between the hooks and depression beneath the tail. Calves were scored on a scale ranging between 1 and 5 using 25 unit increments with 1 standing for severe under conditioning (emaciated) and 5 for over conditioning.

Finally for those animals which were slaughtered the carcass weight and the meat class was obtained. For the latter three classes were provided: U) very good developed muscles, R) good developed muscles and O) average developed muscles. Additionally three fat classes were determined: 1) very low fat cover, 2) little fat cover with the flesh visible almost everywhere and 3) fat appeared almost everywhere with a slight fat deposits in the thoracic cavity.

Treatment was applied when two out of the following three conditions were present: FEC>100, body weight gain <1.125 kgper day or a decrease in BCS by more than 0.5 units.

The second study was conducted from July 2014 to October 2014 on 11 farms in different locations in the state Brandenburg, Germany**.** There were no particular criteria for the selection of farms. Farmers were asked if they are willing to participate and if so, faecal samples were collected from FSG animals. The number of animals tested per farm ranged between 11 and 21. Animals were identified by the ear tag, faecal samples were collected rectally (pre-treatment) and then animals were treated using Valbazen® **(**Pfizer) at the recommended dose rate of 7.5 mg/kg. Farm were re-visited after 7-10 days a second sample was taken (post-treatment). FECs were determined using the Mini FLOTAC technique with a sensitivity of 5 eggs per gram as described in section 3.2.4.2 and a FECRT was performed.

Initially it was planned to additionally investigate BZ resistance using pyrosequencing. To enhance the number of farms for this molecular analysis as well as to investigate BZ resistance also in other European countries, farmers in Germany, Ireland and Belgium were asked to send a bulk sample containing faeces of at least 6-10 sampled animals. Faeces from Belgium and Ireland was cultured there and the obtained L3 were send to the FU Berlin. Faeces sent in by German farmers was cultured as described in section 3.2.4.5.

3.2.4 Parasitological techniques

3.2.4.1 Modified McMaster technique

In 2013 the McMaster method with a sensitivity of 25 eggs per gram was used for faecal examinations on farms 1 and 2. Briefly, four g of fresh faeces were weighed out in a petri dish and thoroughly mixed with a wooden stick in a few drops of saturated salt solution (NaCl). The suspension was filtrated through the sieve placed in a funnel into the measuring cylinder using a total volume of 60 ml NaCl. The faecal suspension was transferred into the glass bottle, mixed by vigorous shaking and then the counting chambers were filled (Whitlock, 1948). The number of strongyle eggs in both chambers (4 compartments) was counted under a microscope (10 magnification lens) and the FEC was calculated by multiplying the number of eggs in both chambers with 25 (dilution factor). On farm 3 the Mini FLOTAC technique was used.

3.2.4.2 Mini FLOTAC technique

5 g of fresh faeces were weighed out and thoroughly mixed in 45 ml saturated salt solution, the suspension was filtrated through a normal t-sieve, the 2 chamber of the Mini FLOTAC was filled with the suspension and left for 10 m to allow floating of the eggs then the slide was turned and examined under 10 magnification lens. The number of the eggs in both chambers multiplied by 5 (Cringoli et al., 2010).

3.2.4.3 Sedimentation

The sedimentation was carried out for diagnosis of trematode eggs in the faeces (Boch and Bauer, 2006). For this purpose 10 g faeces was mixed with tap water in a mortar and the suspension was filtrated through a tea strainer in a beaker (250 ml). The beaker was filled with tap water. Subsequently, the beaker stand 3 m to allow the (trematode) eggs to sediment. The next step was the decanting of the supernatant, then re-filling of the beaker with tap water and a further sedimentation 3 minutes. This operation was repeated 1 to 2 times. After the last decanting, the sediment was placed in a plexi-glass plate transferred, combined with three drops of a 1% methylene blue solution and on 40 to 100 magnification lens examined under the microscope.

3.2.4.4 Egg isolation from faeces

Generally the method published by Demeler et al. (2013b) was used with some modifications. Briefly, 4 g of faecal sample were homogenised in 60 ml NaCl and filtered through a 250 mm

stainless steel sieve, then 11 ml of the flow were removed for MiniFLOTAC analysis and the other amount was transferred to 50 ml falcon tube for centrifugation. The top supernatant solution was pipetted that contain the eggs and poured on 25 mm sieve, the eggs will captured on the sieves then discard the sediment. Washing with tap water, the eggs was collected by pipette in 15 ml falcon tube for centrifugation, the eggs will be sediment in the tap water, the supernatant was discard and the sediment was collected (about 1 ml) in 1.5 ml micro centrifuge tube. Distilled water was added to the sediment and the sample kept in the freezer -80° C.

3.2.4.5 Larval culture

Faecal cultures were performed for cultivation of L3. About 10-50 g of faeces were collected from each animal and faeces of all animals from a group (NC; PC; TT; TST) were combined, leading to up to four group-based faecal cultures. Fine chipped wood was added with the faeces and the mixture filled into honey jars. The lids were laid on loosely and then the jars were placed in an incubator for 7 days at 22–27 °C at 70-80% humidity. After 7 days, the jars were taken out of the incubator, filled up to the brim with tap water and covered with petri dishes. This dish was pressed on tight and the jar flipped over. The space between the glass and the petri dish was filled with tap water and left to stand for 24 h at room temperature. A Pasteur pipette was used to transfer the fluid (containing the larvae) from the petri dish into 50 ml falcon tube. Purification of the larval suspensions was obtained from using the Baerman funnel method. After 12-24 h the L3 were collected in ventilated cell culture flasks and stored in the refrigerator at 10 °C.

3.2.4.6 Larval counting

The culture flask was mixed thoroughly by shaking, 3 drops of 10 µl each from the larval solution were dropped onto a slide, the number of L3 in each drop was counted and the total number in 10µl was calculated.

3.2.5 DNA extraction from larvae

For DNA extraction the L3s were concentrated by transferring the larval suspension from the flask to 50 ml falcon tubes. L3 were allowed to sediment for 24 h in a fridge and then the supernatant was discarded. DNA isolation from L3 was performed using the NucleoSpin® Tissue Kits (Macharey and Nagel, Duren) following the manufacturer's instructions. Depending on the availability of L3 from faecal cultures, between 100 and 5000 L3 were used.

The DNA was eluted in 50µl BE buffer, quantified on a Take3® plate in a microplate reader (Epoch®, Biotech, Germany) and then stored at -20 °C until further use.

3.2.6 PCR identification

To differentiate the L3 genus specific PCRs, established in the lab of the Institute of Parasitology and Tropical Veterinary Medicine at Freie Universität Berlin, was performed. Genus specific primers (Invitrogen) and the Phusion® Hot Start II DNA polymerase (Fermentas/Thermo Scientific) were used with the following protocol:

-4.0 µl puffer HF

-0.4 µl (dNTPs)

-0.5µl forward primer

-0.5 µl reverse primer

-0.2 µl Phusion Hot Start II DNA polymerase

-13.40 µl DEPC water

-19.00 µl from this master mix + 1.0 µl DNA

-Positive control and negative control samples

The primers used in the PCR are presented in Table 3.

Table 3. Primer sequences used in the PCR

Primer name	primer sequence
Cooperia oncophora SH for.	(5'-ATGGCATTTGTCTACATCTGTTT-3')
Cooperia oncophora SH rev.	(5'-ATGGCATTTGTCTACATCTGTTT-3')
Ostertagia ostertagi SH for	(5'-TAACATTGTTAACGTTACTGAATGATACTG-3')
Ostertagia ostertagi SH rev.	(5'-ATATAAATGATACATCGAATATACAATAC-3')

The protocol contained of 40 cycles of denaturation at 98°C for 40 seconds, a primer hybridisation (annealing temperature) of 55°C *(O. o* 167) or 62°C (*C. o* 167), 60°C (*C. o* 198+200), 62°C (*Cooperia SH*) and 60°C (*O. o* 198+200) for 30 seconds, primer extension for 30 second at 72°C and finally the last cycle for 5 minutes at 72°C. Obtained PCR products were evaluated on agarose gels and visualised using a *gel electrophoresis box* (G Box, SYNGENE, Cambridge, UK).

3.2.7 Pyrosequencing

Pyrosequencing was performed following to the manufacturer's protocols (PyroMark Q24 manual user) with little modifications as the amount of the PCR product that used was 40 µl.

The PCRs were performed using the following protocol and the primers used in the PCRs are presented in Table 4:

-10.0 µl puffer HF

-10.0 µl betaine

-1.0 µl Deoxyribonucleoside (dNTPs)

-1.25 µl forward primer (10 µM, for use dilute 1:10: 5 µl stock solution +45 µl DEPC H_2O),

-1. 25 µl reverse primer

-0.5 µl Phusion Hot Start II DNA polymerase

-25.0 µl DEPC water

-49.0 µl from this master mix + 1.00 µl DNA

- Positive control and negative control samples

Table 4. Primer sequences used in the PCR used in Pyrosequencing

Ostertagia ostertagi 200 neu for	(5'-CGTTGTTGAGCCCTACAACGCC-3')
Ostertagia ostertagi 200 neu rev	(5'-CGAAGATCAGCATTCAACTGC-3') with biotin
Ostertagia ostertagi 167 for	(5'-TCGCCAAAATTCGTGAGGA-3')
Ostertagia ostertagi 167 rev.	(5'-GAGACCTTGGGCGAAGGAA-3') with biotin
Ostertagia ostertagi 198+200 for.	(5'-GTTCCTTCGCCCAAGGTCT-3')
Ostertagia ostertagi 198+200 rev.	(5'-TGTGCGGAAGCAGATATCGTA-3') with biotin
Cooperia oncophora 167 for.	(5'-TATGGGCACTTTGCTTATTTCA-3')
Cooperia oncophora 167 rev.	(5'-ACGTTTCATCGGTATTTTCTACCA-3') with biotin
Cooperia oncophora 198+200 for.	(5'-CGACACCGTTGTGGAACCTTAC-3')
Cooperia oncophora 198+200 rev.	(5'-CCGGACATTGTGACAGACACTAG-3') with biotin

Dispersion patterns for all assays are shown below in Table 5 and the sequencing primers used are presented in Table 6.

Table 5. Sequences and dispersion order in pyrosequencing assay

Assay	**Sequence to analyse**	**Dispersion order**
Cooperia oncophora Codon167	TACTCTGTTCCTTCAC	GATCTCTG
Cooperia oncophora Codon198+200	GACAACGTTACTGT	TGCACAGTACTG
Ostertagia ostertagi Codon 167	CATTACTCCGTT	TCGATACTC
Ostertagia ostertagi Codon 198+200	ACGACGTTACTGTA	GACGACAGTACTG
Ostertagia ostertagi Codon 200	AGACGTTACTGTA	GAGACAGTACTG

Table 6. Sequencing primers used in pyrosequencing assay

Primer name	**Primer sequence**
Cooperia oncophora 198+200	(5' ACTGGTAGAAAATACCGAT3')
Cooperia oncophora 167	(5' ACAGAATTATGGCTTCGT3')
Ostertagia ostertagi 167	(5' CGGATAGAATCATGGCTT3')
Ostertagia ostertagi 198+200	(5'TGGTGGAAAATACTGATG3')
Ostertagia ostertagi 2200	(5'GGTGGAAAATACTGATGAGACG 3')

3.3 Statistical analysis:

All data obtained from the animals (FEC, BCS, weight etc.) were entered in an Excel® (Microsoft® office 2013) spreadsheet and then exported into SPSS (version 22, IBM Corp., Armonk, NY) for statistical analysis and graphical illustrations. Data was checked for plausibility and normality using descriptive statistics (Q-Q plots) and mean, minimum and maximum values for BCS, FEC, DWG and carcass weight were calculated. The FEC was categorised into 2 categories; category 0: animals with no parasite (FEC 0-20) and category 1: animals with parasites (FEC >20). Correlation between the weight and BCS was calculated using partial correlation method and the frequency of the positive and negative FEC in calves was calculated. Generalised linear mixed model with repeated measures for 6 measurement times were built for weight, BCS, DWG and FEC as outcome variables. Treatment (i.e. experimental group NC, PC, TT and TST), gen der (male and female) and age (considered as a continuous predictor) were tested as fixed factors in the model, while farm and measurement time were treated as random factors. For carcass weight and average DWG as outcome variables, generalised linear mixed models were built using farm as random factor and treatment, gender, and age as fixed factors. Post-hoc comparisons among group were conducted using the least significant difference with an adjusted significance level of 0.05. Odds ratio (OR) was used to calculate the ratio of chances to have a positive FEC in the different groups.

The FECRT was calculated by using the web interface software on university of Zurich website (http://shiny.math.uzh.ch/user/furrer/shinyas/shiny-eggCounts/) using two sample paired Bayesian hierarchical model with zero inflation. The data was converted from the original excel file into a cvs file that can be used in the program. The mean FEC pre and post treatment, the FECR percentage and 95% highest posterior distribution (HPD) interval were calculated through the model.

The resistance status was detected on the positive farms for *O. ostertagi* and *C. oncophora* against the BZs by analysing the adenine and cytosine percentage at codon 176, 198 and 200 in β- tubulin gene using PyroMarkQ 24 software.

4. Results

4.1 The first study

4.1.1 Parasitological faecal examination procedures

On all the three farms strongyle eggs were detected in several animals throughout the year. The results for the FEC are presented in more detail in the following sections.

With the sedimentation method, *Paramphistomum* sp. were detected on farm 2 (4 animals) and farm 3 (8 animals), *Fasciola* sp. was detected in three animals on farm 2, *Monezia* (in 5 animals) and *Eimeria* sp. (in most of the animals) were detected on farm 3 (*Eimeria* was detected by both MiniFLOTAC and sedemintation)

4.1.2 Pasture experiment

In the grazing pasture different treatment programs were applied with the aim, to reduce the usage of anthelmintic without loss in productivity. There were 4 groups investigated: control groups (positive and negative), TST and TT group. The criteria for an individual animals or whole group treatment performed in this study were a combination of at least two of the following: a high FEC (>100), decrease in DWG (<1.125 kgperday) and a decrease in the BCS.

The TT groups: on farm 1 (Linum), only one animal met the criteria of high FEC and decrease in the DWG. On farm 2 (Groß Kreutz) again one animal showed a decrease in BCS and DWG. On farm 3 (Schlepzig) two calves met the criteria but at the same sampling time, so all TT groups were only treated once.

The TST groups: on farm 1, one animal met the criteria of high FEC, decrease in the DWG and BCS. On farm 2 one animal decreased in the BCS and DWG and on farm 3 two animals had high FEC >100 and decrease in the BCS, so only these 4 animals were treated once.

The positive groups: on farm 1 this group was treated three times, on farm 2 four times and there was no positive group on farm 3.

4.1.3 Descriptive analysis

As a first step of analysis descriptive statistic were performed. The following tables and figures present the stepwise analysis.

First, the number of valid and missing observations for each target have been assessed (Table 7) and mean, minimum, and maximum values were calculated (Table 8).

Table 7. Case processing summary for the numbers of valid and missed observations.

	Cases					
	Valid		Missing		Total	
	Number	Percent	N	Percent	N	Percent
BCS	652	77.6%	188	22.4%	840	100.0%
Weight	691	82.3%	149	17.7%	840	100.0%
DWG	625	74.4%	215	25.6%	840	100.0%
Carcass weight [kg]	390	46.4%	450	53.6%	840	100.0%
FEC	617	73.5%	223	26.5%	840	100.0%

(BCS, DWG: daily weight gain, FEC: faecal egg count)

Table 8. Mean, minimum and maximum values for, BCS, DWG, carcass weight and FEC of all visits

	Number	Minimum	Maximum	Mean	Standard deviation
BCS	652	3.00	4.50	3.7880	0.19936
DWG	625	-0.460	2.625	1.14662	0.376491
Carcass weight [kg]	390	89.0	526.5	263.935	129.9222
FEC	617	0.0	290.0	15.365	30.2450
Valid N	256				

BCS: body condition score, DWG: daily weight gain, FEC: faecal egg count)

The FEC was categorised into two categories; category 0: animals which have no parasite (with FEC of ≤20) and category1: animals which have an FEC higher than 20. The count is illustrated in Figure 6 which shows the frequency of the two FEC categories in all calves.

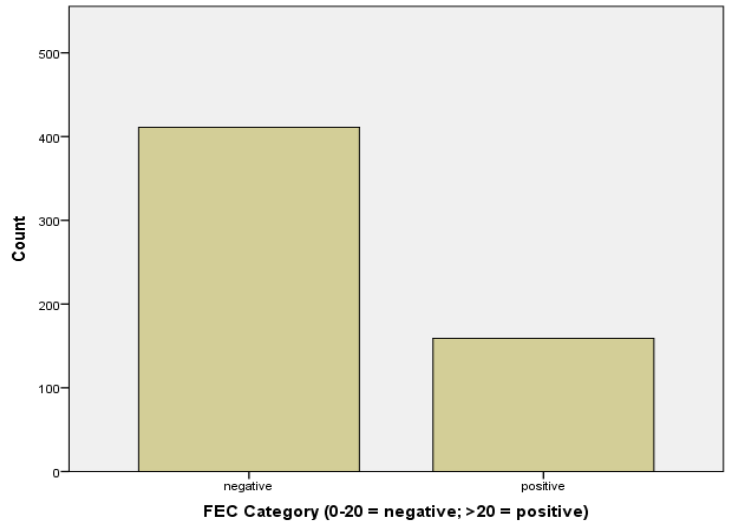
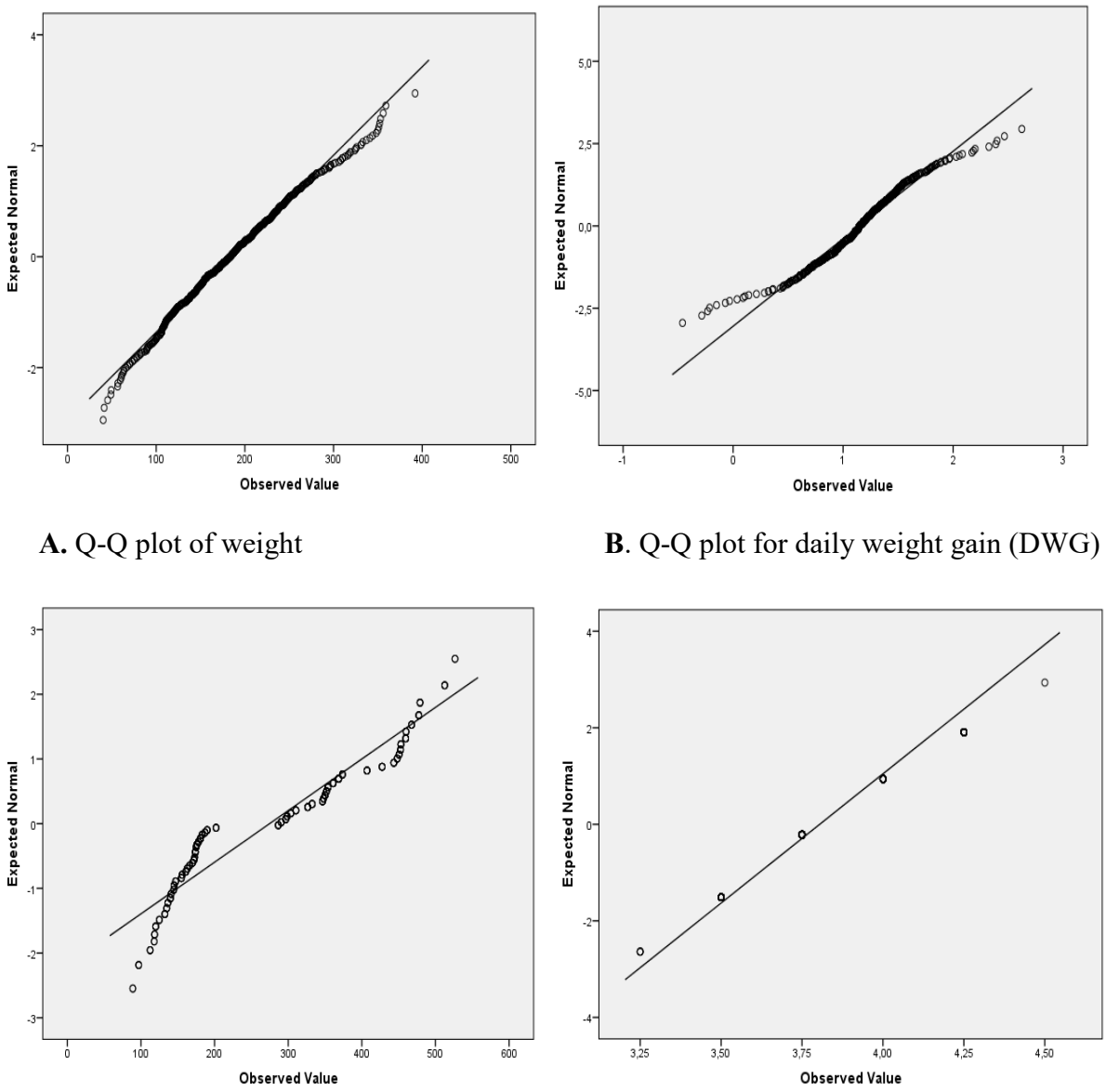


Figure 6. The frequency of the positive and negative faecal egg count (FEC) category distribution in calves.

In order to check the normality of the data QQ plots for the targets weight, DWG, carcass weight and BCS have been calculated and are presented in Figure 7.



A. Q-Q plot of weight

B. Q-Q plot for daily weight gain (DWG)

C. Q-Q plot of carcass weight

D. Q-Q plot of body condition score (BCS)

Figure 7: QQ plots of the four targets weight (A), DWG (B), carcass weight (C) and BCS (D).

4.1.4 Correlation

The partial correlation was calculated between the weight and the BCS. The result of 0.556 shows that there is a positive correlation which is significant ($p<0.001$). Detailed information is presented in Table 9 as well as in Figure 8.

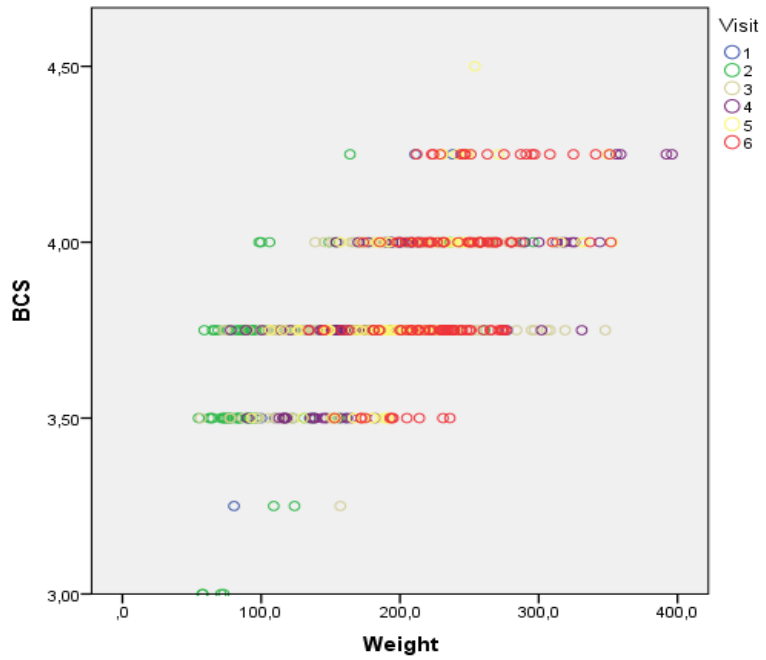


Figure 8. Positive correlation between the weight and body condition score (BCS). The colouring of the rings represents the values obtained for the 6 different visits.

Table 9. The correlation significance between the weight and BCS

Control Variables			Weight	BCS
Visit	Weight	Correlation	1.000	0.559
		Significance (2-tailed)		< 0.001
		df	0	649
	BCS	Correlation	0.559	1.000
		Significance (2-tailed)	< 0.001	
		df	649	0

(df: degree of freedom BCS: body condition score,)

4.1.5 Specific statistics

In order to assess the possible effects of gender, age and treatment group on the FEC as well as the effect of FEC on BCS, weight and carcass weight, specific statistic such as multivariable mixed models were applied.

4.1.5.1 FEC category

First the possible effect of treatment group, gender and age on the FECs in calves was tested. As shown in Table 10 and in Figure 9 the FEC categories in the different treatment groups, the anthelmintic treatment affect the FEC category (negative ≤20 and positive >20 FEC) significantly ($p<0.05$) when the treatment groups are compared to the PC group.

Additionally, the Odds Ratio (OR) (ratio of chances) was calculated; the TT group had an OR of 2.59 (meaning that the TT group has a 2.59 higher chance to have a higher FEC than the PC group), the TST group has an OR of 3.479 and the NC group of 5.0. Accordingly, the NC group has a five times higher chance of having a FEC than the PC group.

The FEC is also significantly affected by the age, when the age is increased by one day, the chance of having FEC is increasing by 0.5% (0.005). The gender also affect the FEC significantly with male calves having a 1.888 higher chance of having a FEC than female calves.

Table 10. Final multivariable mixed model predicting effect of the treatment groups, gender and age on faecal egg count in calves

Variable	Estimate	SE	P	Confidence interval		OR	Confidence interval	
				Lower	Upper		Lower	Upper
Group			<0.001					
TT	0.953	0.428	0.026	0.112	1.794	2.593	1.118	6.013
TST	1.247	0.429	0.003	0.416	2.077	3.479	1.516	7.979
NC	1.627	0.445	<0.001	0.754	2.500	5.090	2.126	12.185
PC	Reference	-	-	-	-	-	-	-
Gender								
Male	0.636	0.208	0.002	0.228	1.043	1.888	1.256	2.838
Female	Reference	-	-	-	-	-	-	-
Age	0.005	0.0019	0.015	0.001	0.009	0.005	1.001	1.009
Intercept	-3.29	0.839	<0.001	-4.935	-1.641	0.037	0.0074	0.194

(SE: standard error, P: P value, OR: odds ratio, TT: targeted treatment, TST: targeted selective treatment, NC: negative control, PC: positive control).

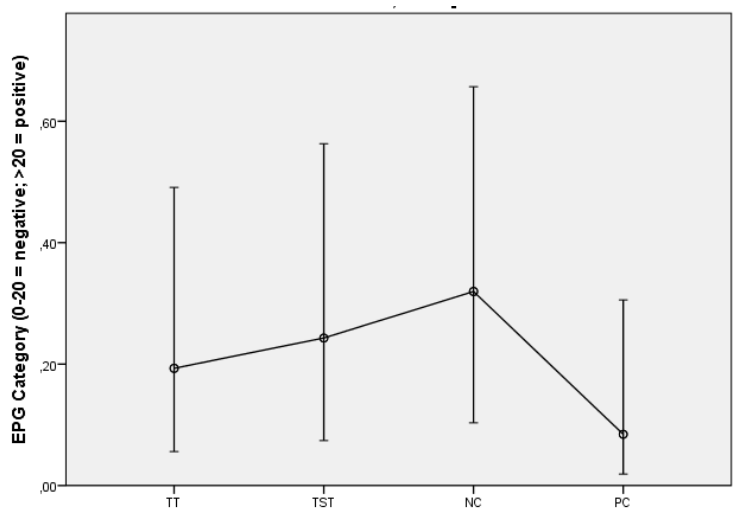


Figure 9. The standard deviation of FEC category in the different treatment groups (TT: targeted treatment, TST: targeted selective treatment, NC: negative control, PC: positive control).

Since the FEC did not affect the weight, DWG, carcass weight and the BCS significantly ($p>0.05$ for all factors, for details see Table 11) it was removed from the generalised linear mixed model as a factor affecting these targets.

Table 11. Significance of the FEC category on the weight, BCS, DWG and carcass weight

Source	Target	df1	df2	Significance
FEC category	weight	1	631	0.523
FEC category	BCS	1	633	0.840
FEC category	DWG	1	585	0.092
FEC category	Carcass weight	1	0.285	0.497

(BCS: body condition score, DWG: daily weight gain, FEC: faecal egg count, df: degree of freedom).

4.1.5.2 Calf productivity

The productivity of calves was assessed through weight gain, weight, and carcass weight. For each factor again possible effects of treatment group, gender and age were investigated using multivariable mixed models.

4.1.5.2.1 Weight

Weight was significantly affected by the variables gender and age but not by treatment group (Table 12). The gender affect the weight significantly ($p<0.001$) with males having a higher weight than females by 9.816 kg (Figure 10). The age also affect the weight significantly ($p<0.001$); the calves' weight was increasing by 1.147 kg with every day increasing in age.

Table 12. Final multivariable mixed model predicting effect of the treatment groups, gender and age on weight gain in calves.

Model Term	Coefficient	SE	P	95% Confidence interval	
				Lower	Upper
Intercept	42.899	8.5972	<0.001	26.017	59.780
TT	3.304	3.1544	0.295	-2.890	9.499
TST	-5.621	3.1457	0.074	-11.798	0.556
NC	3.210	3.3705	0.341	-3.409	9.828
PC	0b	.	.	.	.
Gender: male	9.816	1.7339	<0.001	6.411	13.221
Gender: female	0b	.	.	.	.
age	1.147	0.0177	<0.001	1.113	1.182

(SE: standard error; probability distribution: Normal, b. this coefficient is set to zero because it is redundant, TT: targeted treatment, TST: targeted selective treatment, NC negative control, PC: positive control).

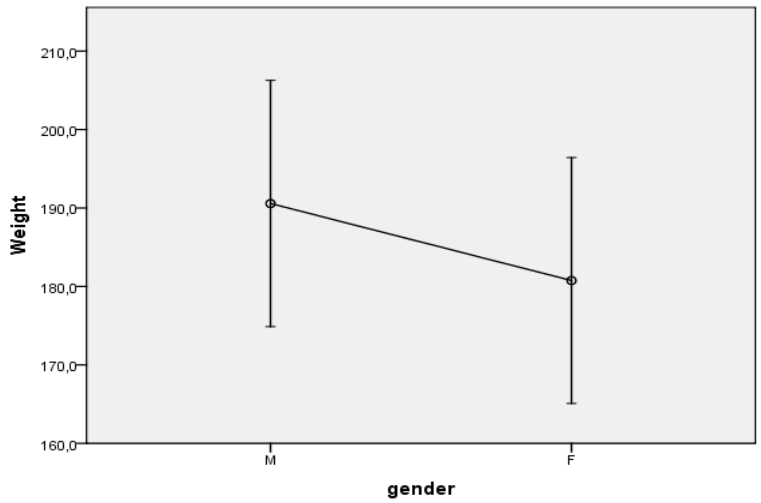


Figure 10. The mean weight for males and females calves (M: male, F: female)

The pairwise contrast between all the treatment groups were performed to compare between all the treatment groups and not only between one particular treatment group and the PC group. A significance differences appears between TST and NC and between TST and TT ($p<0.01$ for both, Table 13). The calves in the TST group had a lower weight by -8.830 kg than those in the NC group and a lower weight by -8.925 kg than the animals in the TT group.

Table 13. Pairwise contrasts between different treatment groups in weight.

Groups	Contrast Estimate	SE	df	Adjusted Significance	95% Confidence interval	
					Lower	Upper
TT - TST	8.925	2.026	647	<0.001	4.946	12.904
TT - NC	0.095	2.262	647	0.967	-4.346	4.536
TT - PC	3.304	3.154	647	0.295	-2.890	9.499
TST - NC	-8.830	2.237	647	< 0.001	-13.224	-4.437
TST - PC	-5.621	3.146	647	0.074	-11.798	0.556
NC - PC	3.210	3.371	647	0.341	-3.409	9.828

(SE: standard error, df: degree of freedom, TT: targeted treatment, TST: targeted selective treatment, NC negative control, PC: positive control) least significant difference adjusted significance level is 0.05.

To investigate how much difference in weight is between the groups, the mean weight of the different treatment groups was calculated and is presented in Table 14 and Figure 11. The results show that the differences between the groups are only small with mean weights between 179.82 and 188.74 kg.

Table 14. The mean weight value for different treatment groups

Groups	Mean	Standard Error	95%Confidence interval	
			Lower	Upper
TT	188.743[a]	8.012	173.009	204.476
TST	179.818[b]	8.006	164.096	195.539
NC	188.648[a]	8.065	172.811	204.485
PC	185.438[a,b]	8.377	168.989	201.888

(Continuous predictors are fixed at the following values: age=120, a, b= different letters show significant differences p <0.05, TT: targeted treatment, TST: targeted selective treatment, NC negative control, PC: positive control

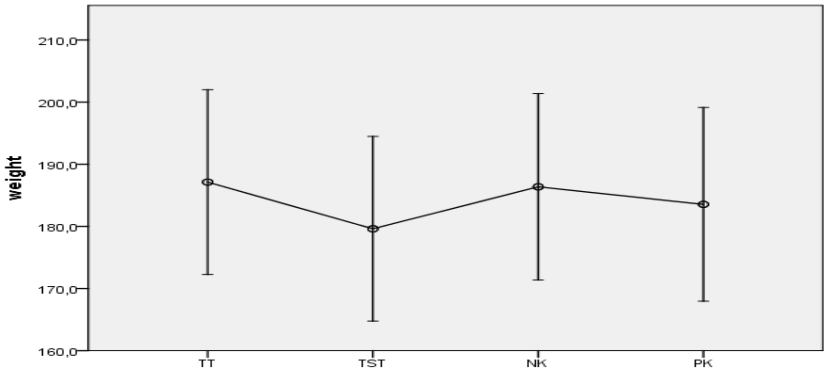


Figure 11. Estimated weight value in different treatment groups (TT: targeted treatment, TST: targeted selective treatment, NC: negative control, PC: positive control).

4.1.5.2.2 Weight at 6th visit (last visit)

To investigate the effect on the weight at the end of the pasture study phase, analysis was performed by only taking the weight of the last visit (6th weight) into account. Analysing the effect of the treatment on the last weight measurement at the end of the farm visits revealed no significant effect (p>0.05). As seen before, the 6th weight was affected by gender and age significantly (Table 15). Male calves gain 16 kg more weight than female calves and the weight was increased by 1.02 kg with the increasing in the age by one day.

Table 15. Final multivariable mixed model predicting weight at 6th visit in calves

Variable	Estimate	SE	P	Confidence interval	
				Lower	Upper
Group			0.98		
TT	4.25	8.79	0.63	-13.17	21.66
TST	-6.05	8.78	0.492	-23.43	11.33
NC	3.84	9.38	0.683	-14.74	22.42
PC	Reference	-	-	-	-
Gender					
Male	16.0	5.13	0.002	5.84	26.14
Female	Reference	-	-	-	-
age	1.02	0.084	<0.001	0.85	1.18
intercept	53.9	18.3	0.004	17.7	90.1

(SE: standard error, P: P value, TT: targeted treatment, TST: targeted selective treatment, NC: negative control, PC: positive control)

All the treatment groups were compared pairwise and no significant differences were observed between them as described in Table 16 which presents the pairwise contrasts analysis results between all different treatments groups at the last farm visit.

Table 16. Pairwise contrasts between different treatments groups in 6th weight

Groups	Contrast Estimate	SE	df	Adjusted Significance	95% Confidence interval	
					Lower	Upper
TT - TST	10.294	5.942	119	0.086	-1.472	22.060
TT - NC	0.404	6.611	119	0.951	-12.686	13.494
TT - PC	4.246	8.795	119	0.630	-13.169	21.661
TST - NC	-9.890	6.492	119	0.130	-22.744	2.964
TST - PC	-6.048	8.776	119	0.492	-23.426	11.329
NC - PC	3.842	9.381	119	0.683	-14.735	22.418

(SE: standard error df: degree of freedom, TT: targeted treatment, TST: targeted selective treatment, NC: negative control, PC: positive control) The least significant difference adjusted significance level is .05

4.1.5.2.3 Daily Weight Gain (DWG)

A generalised mixed model was performed with 5 measurements (2nd to 6th DWG) only, since the first DWG was excluded from the model (weight gain between the birth weight and the first weight measurement at the first farm visit). Table 17 provides details obtained for the effect of the treatment group, gender and age on the DWG.

No significant effect (all p values >0.05) on the DWG was found for the treatment groups (TST, TT and NC) when compared to the PC group. The DWG was significantly affected by the

gender (p= 0.011) and age (p= 0.023). The daily weight gain of the male calves is 0.098 kg higher than that of the female calves (Figure 12). With every day increase in age the DWG is decreasing by 0.002 kg, so the calves gain less weight when they are older compared to younger calves. This change is significant but very small. Over 100 days that would be a change in DWG of 0.2 kg

Table 17. Final multivariable mixed model predicting Daily weight gain (DWG) in calves

Variable	Estimate	SE	P	Confidence interval	
				Lower	Upper
Group			0.612		
TT	0.035	0.061	0.565	-0.085	0.155
TST	-0.022	0.061	0.712	-0.143	0.098
NC	0.008	0.066	0.905	-0.123	0.13
PC	Reference	-	-	-	-
Gender			0.011		
Male	0.098	0.038	0.011	0.023	0.172
Female	Reference	-	-	-	-
age	-0.002	0.0009	0.023	-0.004	-0.0003
intercept	1.419	0.173	<0.001	1.076	1.763

(SE: standard error, P: P value, TT: targeted treatment, TST: targeted selective treatment, NC: negative control, PC: positive control)

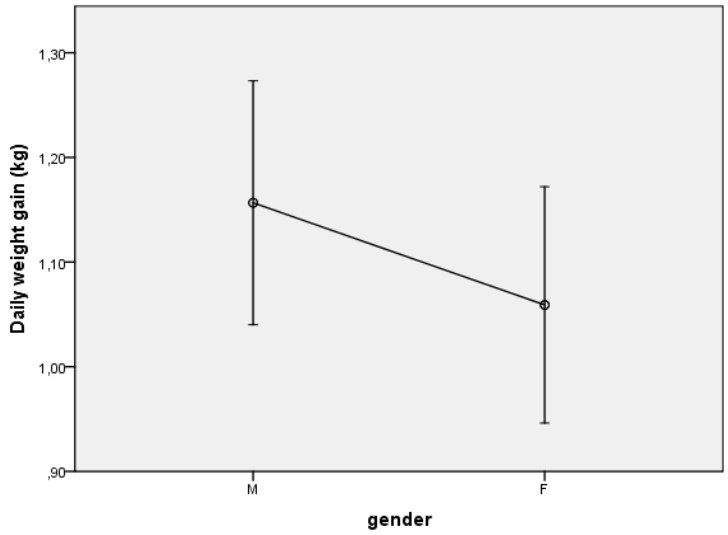


Figure 12. The mean daily weight gain for males and females calves (M: male, F: female)

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When the DWG was pairwise compared for all the treatment groups, again no significance differences between them were found (all p values > 0.05; Table 18)

Table 18. Pairwise contrasts between different treatments groups in DWG.

Groups	Contrast Estimate	SE	df	Adjusted Significance	95% Confidence interval	
					Lower	Upper
TT - TST	0.057	0.043	96	0.183	-0.028	0.142
TT - NC	0.027	0.049	96	0.585	-0.071	0.125
TT - PC	0.035	0.061	96	0.565	-0.085	0.155
TST - NC	-0.030	0.048	96	0.532	-0.126	0.066
TST - PC	-0.022	0.061	96	0.712	-0.143	0.098
NC - PC	0.008	0.066	96	0.905	-0.123	0.139

(SE: standard error df: degree of freedom, TT: targeted treatment, TST: targeted selective treatment, NC: negative control, PC: positive control). The least significant difference adjusted significance level is 0.05.

Additionally the mean DWG was calculated for all treatment groups, but no significant difference between the groups was found (Table 19; Figure 13).

Table 19. The mean daily weight gain value for different treatment groups

Groups	Mean	Standard. Error	95%Confidence Interval	
			Lower	Upper
TT	1.138	0.060	1.019	1.256
TST	1.080	0.059	0.963	1.198
NC	1.111	0.063	0.986	1.236
PC	1.103	0.074	0.956	1.250

(Continuous predictors are fixed at the following values: age=183, TT: targeted treatment, TST: targeted selective treatment, NC: negative control, PC: positive control).

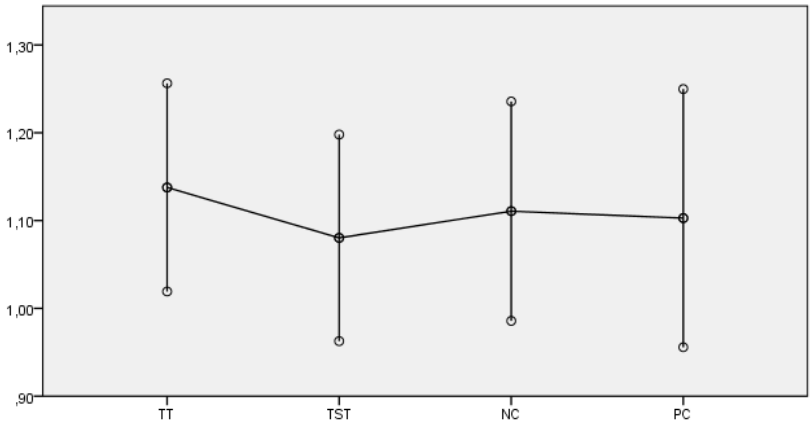

Figure 13. Estimated daily weight gain value in different treatment groups (TT: targeted treatment, TST: targeted selective treatment, NC: negative control, PC: positive control).

4.1.5.2.4 Carcass weight

The carcass weight could only be obtained from 65 calves as not all the calves went to the slaughter house and some of them were used in reproduction. Table 20 presents the effect of the treatment group, gender and age on the carcass weight of the calves obtained in a multivariable mixed model.

For the carcass weight no significant differences between the treatment groups (all p values >0.05) were obtained, therefore the carcass weight is not affected by the treatment. Table 21 shows the result of the calculation of the mean carcass weight for all treatment groups and Figure 14 shows the results in a graph.

Table 20. Final multivariable mixed model predicting effect of the treatment groups, gender and age on carcass weight in calves.

Variable	Estimate	SE	P	Confidence interval	
				Lower	Upper
Intercept	22.399	30.4019	0.464	-38.436	83.233
TT	-21.733	20.7200	0.298	-63.194	19.727
TST	-30.578	21.2910	0.156	-73.181	12.025
NC	-9.964	21.7852	0.649	-53.556	33.628
PC	Reference	-	-	-	-
Gender			0.001		
Male	46.275	13.3168	0.001	19.628	72.922
Female	Reference	-	-	-	-
Slaughter age	0.671	0.0598	<0.001	0.551	0.790

(SE: standard error, P: P value, TT: targeted treatment, TST: targeted selective treatment, NC: negative control, PC: positive control).

Table 21. The mean carcass weight value for different treatment groups.

Groups	Mean	Standard Error	95%Confidence Interval	
			Lower	Upper
TT	257.975	15.574	226.812	289.138
TST	249.131	16.529	216.057	282.205
NC	269.745	16.152	237.425	302.065
PC	279.709	22.366	234.955	324.463

(Continuous predictors are fixed at the following values: Slaughter Aged=349, TT: targeted treatment, TST: targeted selective treatment, NC negative control, PC: positive control

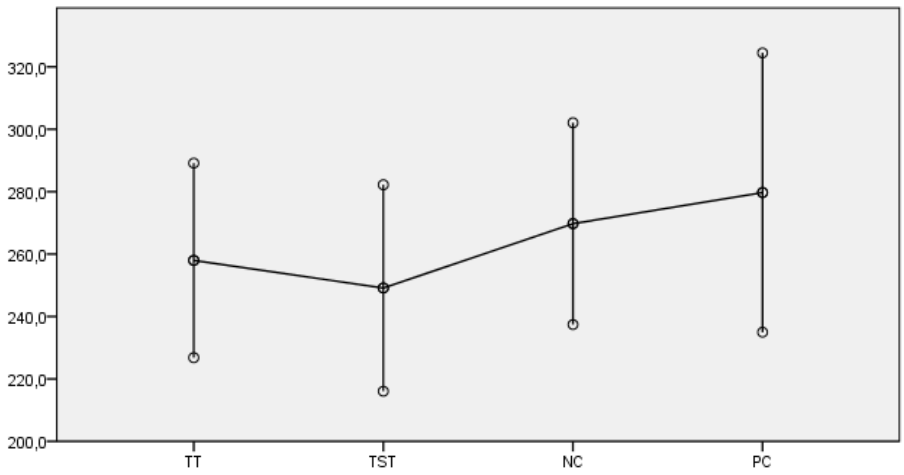

Figure 14. Estimated carcass weight value in different treatment groups (TT: targeted treatment, TST: targeted selective treatment, NC: negative control, PC: positive control).

Gender and age both affected the carcass weight significantly (0.001 and <0.001, respectively). The male calves had a 46.275 kg higher mean slaughter weight than the female calves (Table 22, Figure 15).

Table 22. The mean carcass weight for males and females calves.

Gender	Mean	Standard Error	95% Confidence Interval	
			Lower	Upper
Male	287.278	15.214	256.835	317.720
Female	241.002	15.787	209.413	272.592

(Continuous predictors are fixed at the following values: Slaughter Aged=349).

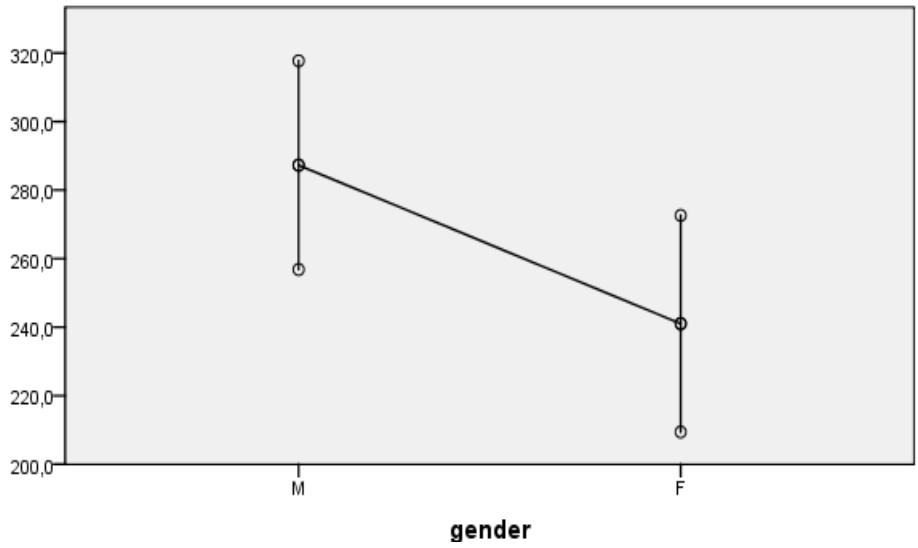

Figure 15. The mean carcass weight for males and females calves (M: male, F: female).

With every day increase in slaughter age, the slaughter weight increased by 0.671 kg (Table 19). The slaughter age varied between the calves. Some of them were slaughtered at a young age and their weight ranged from 100-200 kg. Other calves were slaughtered at an older age and their weight ranged from 300-550 kg. The scattered plot in Figure 16 illustrates the slaughter weight distribution.

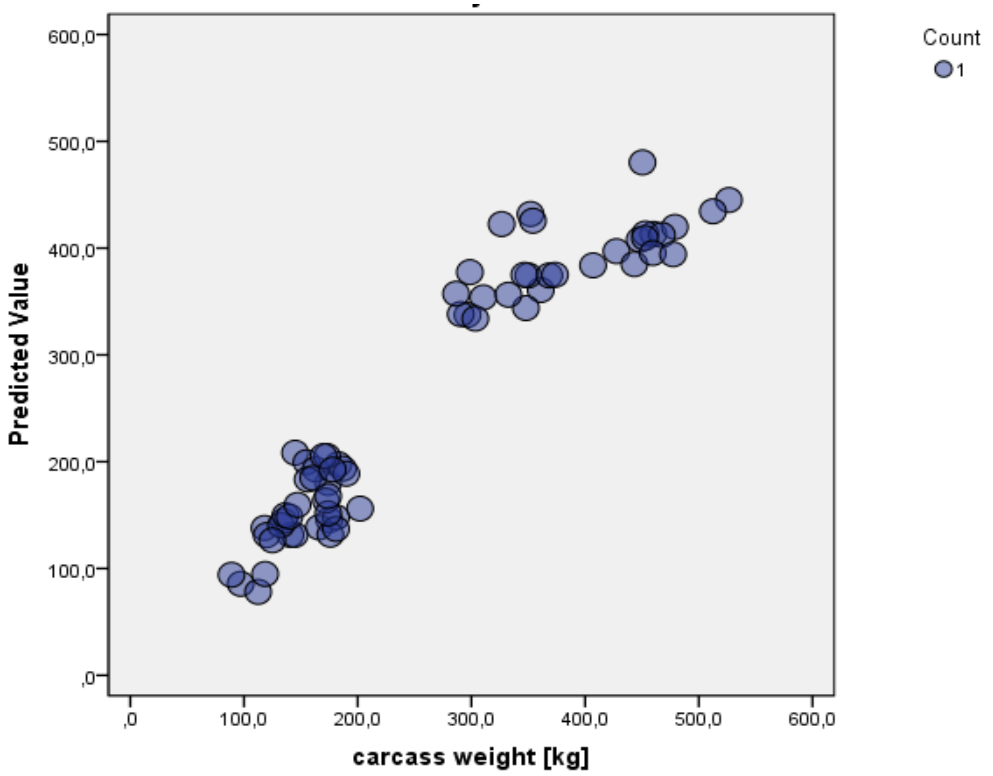

Figure 16. Scattered plot showing the distribution of the slaughtered calves in relation to their weight.

4.1.5.2.5 Fat layer

The influence of the treatment on the fat layer was also analysed. The data which was provided by the slaughter house regarding fat layer measurements was unfortunately very scarce and so analysis could only be performed on 31 calves. Even though this is not representative for all calves included in the study, as shown in Table 23 there were no significant differences between the treatment groups (all p values >0.05). Gender had also no significant effect on the fat layer so it was removed from the analysis, but the age affect the fat layer significantly p <0.05, the fat layer is increasing by 0.006 cm with every day increasing in the slaughter age.

Table 23. Final multivariable mixed model predicting effect of the treatment groups and age on fat layer.

Variable	Estimate	SE	P	Confidence interval	
				Lower	Upper
Intercept	-1.86	1.02	0.081	-3.97	0.243
Group			0.860		
TT	0.129	0.199	0.523	-0.280	0.537
TST	0.060	0.206	0.772	-0.362	0.483
NC	0.036	0.209	0.865	-0.394	0.466
PC	Reference	-	-	-	-
Slaughter Age	0.006	0.0019	0.006	0.002	0.009

(SE: standard error, P: P value, TT: targeted treatment, TST: targeted selective treatment, NC: negative control, PC: positive control).

4.1.5.3 Calf performance

As an indicator for the performance of the animals the BCS chosen and possible effects of treatment group, gender and age were investigated.

4.1.5.3.1 BCS

As shown in Table 24 the multivariable mixed model revealed a significant effect ($p < 0.001$) for the treatment groups on the BCS when comparing the treatment groups (TT, TST and NC) to the PC group. While the TST and NC groups did not differ from the PC group, the TT group displayed a significance difference to the PC group ($p = 0.002$). The calves in the TT group had a higher BCS than the PC calves by an average of 0.073. However, all calves in the T, TST and NC groups had a slightly higher BCS than calves in the PC groups.

Table 24. Final multivariable mixed model predicting effect of the treatment groups, gender and age on BCS in calves**.**

Model Term	Coefficient	SE	P	95% confidence interval	
				Lower	upper
Intercept	3.511	0.0318	< 0.001	3.448	3.573
TT	0.073	0.0236	0.002	0.026	0.119
TST	0.026	0.0235	0.264	-0.020	0.072
NC	0.053	0.0252	0.037	0.003	0.102
PC	0[b]	.	.	.	.
Gender: Male	0.033	0.0132	0.013	0.007	0.059
Gender: female	0[b]	.	.	.	.
age	0.002	0.0001	< 0.001	0.002	0.002

(SE: standard error, P: P value, b. this coefficient is set to zero because it is redundant, TT: targeted treatment, TST: targeted selective treatment, NC: negative control, PC: positive control).

To compare between the all groups and not only to the PC, pairwise contrasts between different treatments groups using the least significant difference adjusted significance level of 0.05 were performed (Table 25). Significance differences were obtained between TT – TST, TT – PC and NC – PC groups, but no significance differences were found between TST – PC, TT-NC and TST-NC.

Table 25: Pairwise contrasts for the BCS between different treatment groups.

Groups	Contrast estimate	SE	df	Adjusted Significance	95% Confidence interval	
					Lower	Upper
TT - TST	0.046	0.016	646	0.003	0.016	0.077
TT - NC	0.020	0.017	646	0.250	-0.014	0.054
TT - PC	0.073	0.024	646	0.002	0.026	0.119
TST - NC	-0.026	0.017	646	0.123	-0.060	0.007
TST - PC	0.026	0.023	646	0.264	-0.020	0.072
NC - PC	0.053	0.025	646	0.037	0.003	0.102

(SE: standard error, df: degree of freedom, TT: targeted treatment, TST: targeted selective treatment, NC: negative control, PC: positive control) The least significant difference adjusted significance level is 0.05.

Table 26 presents the estimated mean BCS values for each group; statistically very small differences between the treatment groups were found, but as the BCS was measured through a scale ranging from 1 to 5 and increasing by 0.25 points, the statistical values obtained cannot be applied meaningfully and therefore were classified as not significant.

Table 26. Estimates of the mean BCS for the different treatment groups.

Groups	Mean	Standard Error	95% Confidence interval	
			Lower	Upper
TT	4.00	0.020	3.779	3.859
TST	4.00	0.020	3.733	3.812
NC	4.00	0.021	3.757	3.841
PC	3.75	0.027	3.693	3.800

(Continuous predictors are fixed at the following values: age=120, TT: targeted treatment, TST: targeted selective treatment, NC: negative control, PC: positive control).

Again a significant effect for gender ($p= 0.013$) and age ($p <0.001$) on the BCS was found in the model with male calves having a higher BCS than female calves by an average of 0.033. Figure 17 shows the mean BCS for males and females calves. The BCS was also increasing with increasing age (by 0.2 with every 100 days).

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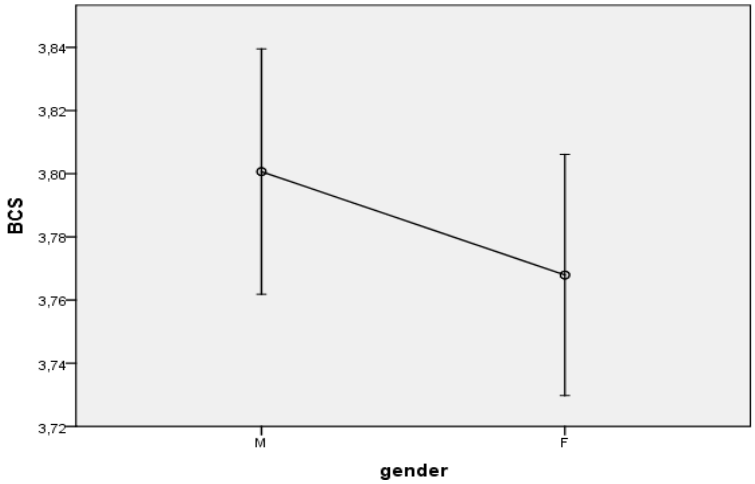

Figure 17. The mean BCS for males and females calves (M: male, F: female).

4.1.5.3.2 BCS at the 6th visit

Similar to the analysis of the weight, also possible effects of treatment group, gender and age on the BCS were analysed for the last measurement only using a multivariable mixed model (Table 27). No significant effects were found for the treatment groups (all p values >0.05) and gender (p =0.778). Only the age affected the BCS significantly as the BCS was found to increase by 0.003 points with every day increasing in age.

Table 27. Final multivariable mixed model predicting effect of the treatment groups, gender and age on 6th BCS in calves.

Model term	Coefficient	SE	P	95% confidence interval	
				Lower	upper
Group			0.273		
TT	0.083	0.052	0.115	-0.021	0.186
TST	0.025	0.052	0.636	-0.079	0.128
NC	0.053	0.056	0.341	-0.057	0.164
PC	Reference	-	-	-	-
Gender			0.778		
Male	0.009	0.030	0.778	-0.052	0.069
Female	Reference	-	-	-	-
Age	0.003	0.0005	< 0.001	0.002	0.004

(SE: standard error, P: P value; TT: targeted treatment, TST: targeted selective treatment, NC: negative control, PC: positive control).

4.1.6 Genus differentiation by qualitative polymerase chain reaction

A genus specific PCR was performed by using the DNA which was isolated from the L3 obtained from the faecal cultures. All farms were positive for the *C. oncophora* and only one farm (Schlepzig) was positive for *O. ostertagi.*

4.2 The second study

4.2.1 Diagnosis and detection of BZs resistance

4.2.1.1 Parasitological faecal examination procedures

Pre and post-treatment faecal samples were collected from 11 farms in Brandenburg, Germany. The treatment was performed by using an oral formulation of ABZ (Valbazen ® suspension, Pfizer 7.5 mg/kg). The faecal samples were examined by using MiniFLOTAC technique (sensitivity 5 FEC). Strongyle eggs were detected on all farms in a total 176 animals.

4.2.1.2 Faecal Egg Count Reduction Test (FECRT)

The mean FECs were calculated based on the number of animals per farm for all 11 farms (Table 28). Generally mean FECs were low with only one farm exceeding 100 FEC before treatment.

Table 28. Number of animals included per farm and the minimum, maximum and mean FECs before and after treatment on 11 farms in Brandenburg

Farm Number	No. of animals included	Mean FEC before treatment	Minimum FEC before treatment	Maximum FEC before treatment	Mean FEC after treatment (7-10) d.
GER. 1	11	10	0	35	0
GER. 2	21	15	0	105	0
GER. 3	21	31	0	155	0
GER.4	12	14	0	110	3
GER.5	13	9	0	35	0
GER.6	17	109	25	295	0
GER.7	18	2	0	15	0
GER.8	20	44	0	150	0
GER.9	13	5	0	35	0
GER.10	16	30	5	75	0
GER.11	14	27	0	65	0

(GER: Germany, FEC: faecal egg count).

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Even though individual FECs were not high on all farms, all farms with a maximum FEC >35 were included in the FECRT analysis (for complete FEC results for each animal in each farm before and after the treatment see Table 9 in the appendix). The FECR was calculated using the two sample paired (pre-treatment and post-treatment) Bayesian hierarchical model with zero inflation on the user web interface of the University of Zürich. The percentage of the reduction and the 95% Highest Posterior Density (HPD) interval were also calculated and results are presented in Table 29.

Table 29. FECR percentage and the 95 % HPD interval on 11 farms in Brandenburg.

Farm number	**FECR %**	95 % HPD interval
GER. 2	99.7	94.2 – 100
GER. 3	99.2	96.2 – 100
GER. 4	76.0	41.1 – 91.1
GER. 6	99.4	98.2 – 99.9
GER. 8	99.9	98.3 – 100
GER. 10	98.8	96.7 – 100
GER. 11	98.7	92.8 – 100

(GER: Germany, FECR: faecal egg count reduction, HPD: highest posterior distribution).

On six out of the seven farms included in the analysis the FECR was above 95%. On a total of six out of those seven farms, the lower HDP interval was higher than 90%. Only farm GER.4 had FECR less than 95% (76%) with an HDP interval below 90% and therefore resistance is likely to be present.

4.2.1.3 Polymerase Chain Reaction (PCR) for genus differentiation

Bulk faecal culture were performed for all 11 farms before treatment. In order to increase the number of farms in this analysis, an additional nine farmers were asked to send in bulk faecal samples from their animals. So the total number of farms included was 20. PCR results for each farm are presented in appendix 9. Examples for the gel analysis are given in Figure 18 (*Cooperia*) and Figure 19 (*Ostertagia*).

Additionally L3 from another 17 farms from Ireland and eight farms from Belgium were sent to the Institute of Parasitology and Tropical Veterinary Medicine at Freie Universität Berlin to be included in the analysis. DNA was isolated from L3 and genus specific PCR was performed for all 45 farms (20 Germany, 17 Ireland and eight Belgium).

In Germany 15 farms were positive for *Ostertagi*a, 14 farms were positive for *Cooperia* and on nine farms mixed infections were detected.

In Ireland eight farms were positive for *Ostertagia*, 10 farms were positive for *Cooperia* and on four farms mixed infections were found.

In Belgium three farms were positive for *Ostertagia* and another three farms positive for *Cooperia.* No mixed infections were detected.

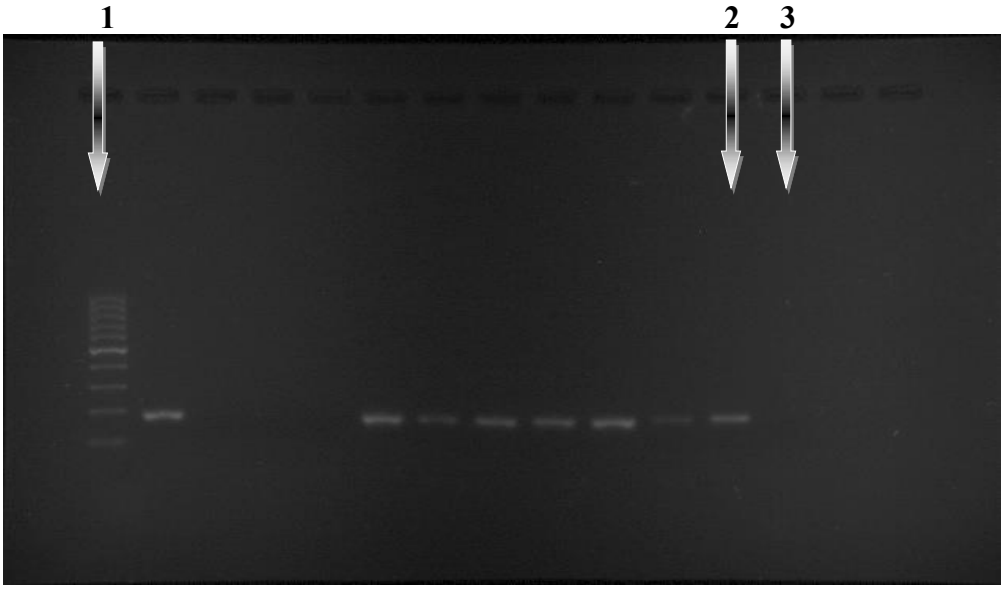

Figure 18. Qualitative PCR for *Cooperia oncophora* 1: (1:100 base pair marker), 2: positive control, 3: negative control.

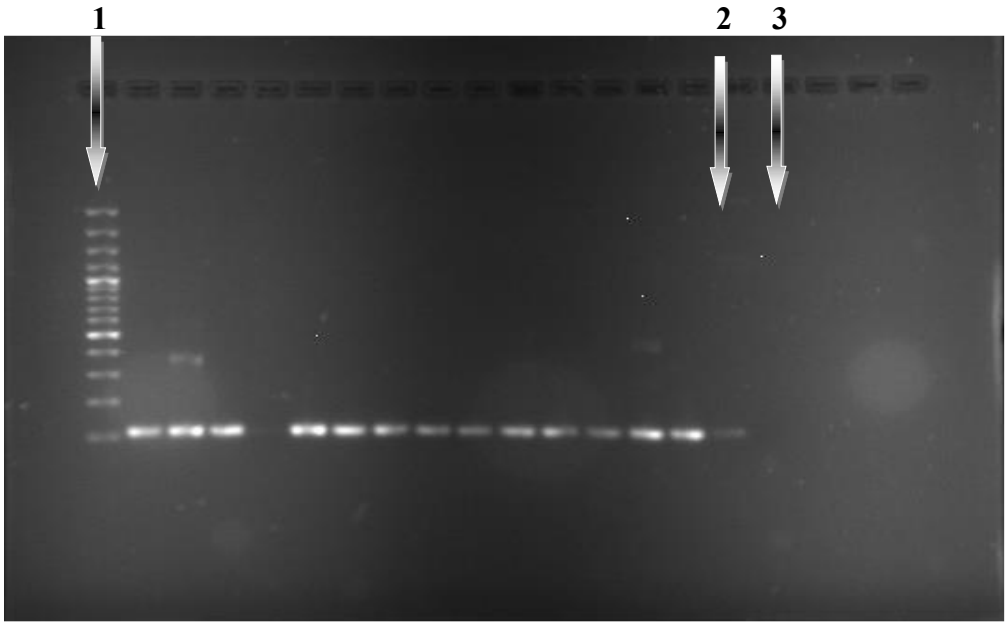

Figure 19. Qualitative PCR for *Ostertagia ostertagi* 1: (1:100 base pair marker), 2: positive control, 3: negative control.

4.2.1.4 Pyrosequencing

From all samples positive for *Cooperia* codon specific PCRs were performed (*C.o.* 176, *C.o.* 198 and *C.o*. 200) and respectively the same for *Ostertagia* positive samples. Except three farms in Germany which were negative for *C.o.* codon 176, for all other farms PCR products were obtained for *C.o.* 198 and 200. For *O.o.* on all farms PCR products were obtained for all three codons except three farms in Germany and two farms in Ireland, which were negative for codon 176 and another three farms in Germany were negative for codons 198 and 200.

The pyrosequencing technique was envisaged to be applied to all PCR products. One a single run was performed per sample and only in a limited number of samples this was successful. Repeating this run again numerous samples were not generating any results. Due to time limitations this work could not be finalised and accordingly the results presented in Table 30 below are only for a small fraction of samples. Additionally they are derived from a single run only so they have to be interpreted as very preliminary results. The data were analysed using PyroMarkQ 24 program and examples are represented in Figure 20.

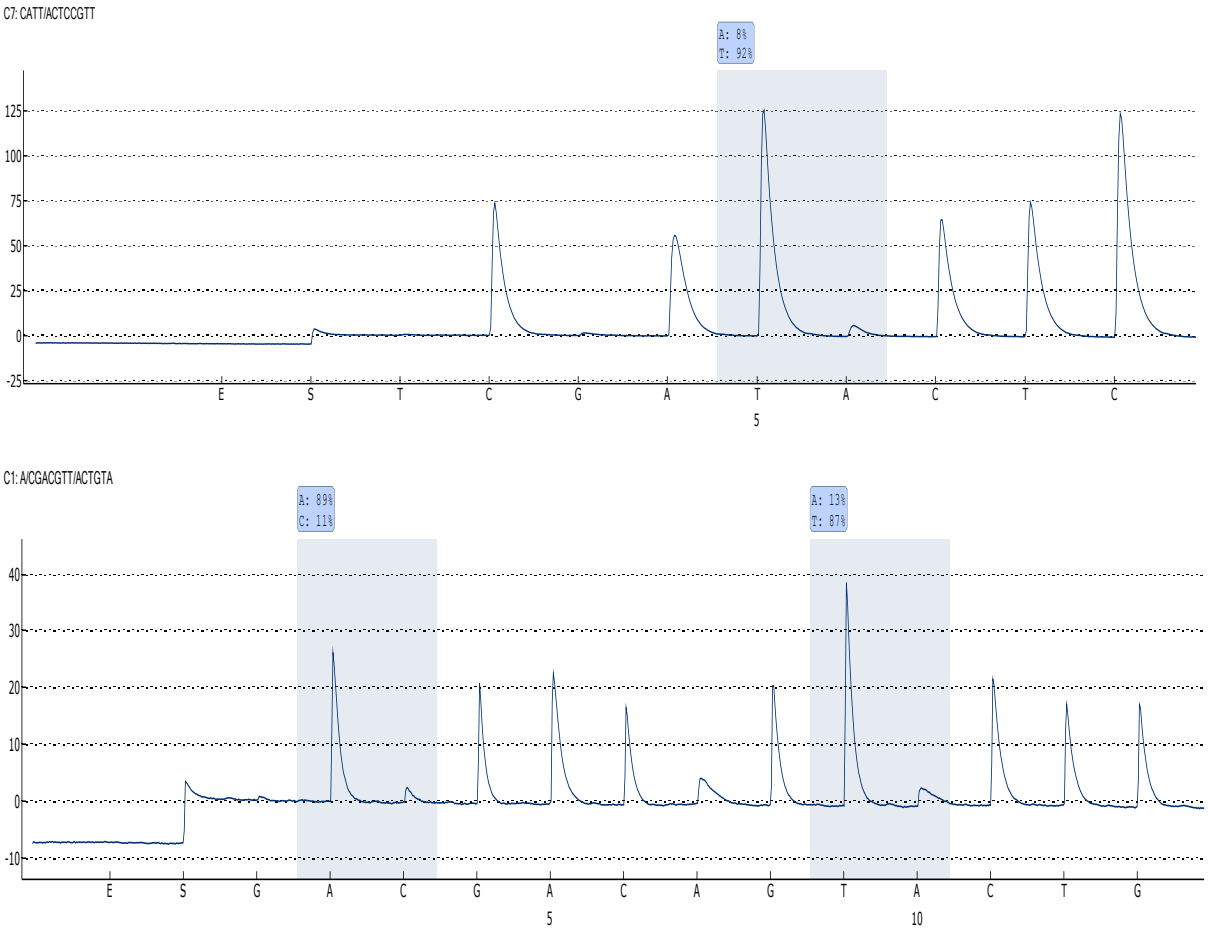

Figure 20. Pyrograms showing the percentages of the adenine (8%) versus tyrosine (92%) in this sample for *Ostertagia* codon 176 (A) and respectively for codons 198 and 200 (B).

Table 30. Frequency of the resistance associated SNP in codons 167, 198 and 200 in *Ostertagia* and/or *Cooperia* positive samples.

country	*O. ostertagi*			*C. oncophora*		
	167 (%A)	198 (%C)	200 (%A)	167 (%A)	198 (%C)	200 (%A)
IRL-03	0	12	5	2	0	6
IRL-04	1	5	4	3	0	3
IRL-06	0	-	12			
IRL-08	0	8	5			
IRL-09				11	1	4
BEL-03	0	5	2			
BEL-07				2	0	4
GER-01				2	-	8
GER-02				2	0	12
GER-06	1	-	3			
GER-07				10	-	-
GER-15	0	11	13			
GER-19	0	1	5	2	1	3

(IR: Ireland, BE: Belgium, GER: Germany, *O. o: Ostertagia ostertagi,* A: adenine, C: cytosine*).*

As shown in Table 30 only on five farms the percentage for the resistance associated SNP increased over the background value, which has been set to 10 following the recommendations of Demeler et al. (2013b). Even though these results are preliminary they are in accordance with previous reports that BZ resistance does not seem to be a real problem in the two investigated species in Europe.

5. Discussion

The aim of this study was to evaluate the effect of different anthelmintic treatment programmes on animal productivity (weight gain, DWG and carcass weight) and performance (BCS and FEC) in young beef cattle. While respective data is widely available for small ruminants, data regarding cattle are still sparse. In the face of increasing threats of anthelmintic resistant nematode population maintaining productivity in combination with low cost and animal welfare are key elements of livestock farming. Therefore it was important to also assess, which programme can achieve economic benefits to the farmers.

The nature of pasture borne parasites has been studied widely over the last century. Particularly productivity related problems due to GI parasites of large ruminants have been the focus of researchers in Europe within the last twenty years (Charlier et al., 2009, Dimander et al., 2003, Dimander et al., 2000). Control of these parasite infections in cattle depends on many factors involving host immunity, grazing system, and farm management procedures (Gauly et al., 2013). However, anthelmintic treatment is still the major tool used in GI nematode control. According to the environmental conditions in different climate zones treatment intervals are very different between countries. A quarter of cattle farmers in New Zealand repeat anthelmintic treatments between 8 and 12 times in the first year of the calves' life (Jackson et al., 2006). In Germany and Netherlands surveys were performed focussing on FSG calves. These surveys used mainly the pepsinogen level as an indicator for parasite presence and showed that control of GI nematodes is even overprotective in Northern Europe and the low levels of infections found were not associated with significant production losses (Charlier et al., 2011, Schnieder et al., 1999a, Borgsteede et al., 1998, Ploeger et al., 1990). In combination with the risk of resistance development strategic (or prophylactic) treatment schemes are not seen as sustainable anymore. Accordingly new treatment approaches have to be developed which enable the identification of those animals in need for treatment and therefore enabling to keep a refugia of untreated parasites. Even though only very limited studies have been performed in young cattle, the results published are variable. Fahrenkrog (2013) observed a good correlation between BCS, DWG and FEC and therefore used a combination of FEC and a performance related parameter for treatment of FSG dairy calves. Höglund et al. (2009b) found only a week correlation between FEC and BCS and the mid-season DWG to be a relative good indicator for treatment of beef calves.

Most studies performed in cattle in Europe revealed rather low infection levels with GI nematodes (Charlier et al., 2010a, Dimander et al., 2003). The low FECs and the low number

of calves that met the treatment criteria in the present study in 2013 could be due to the very dry weather during the pasture season. This observation is in agreement with the data of Fahrenkrog (2013), who also only recorded very low FECs in the first year of her study which was very dry. In another experiment, the infection pressure was increased artificially. Dimander et al. (2003) infected their examined animals with 5000 L3 of *O. ostertagi* and 5000 L3 of *C. oncophora* in order to guarantee a certain risk of infection. In general, low levels of infections were not found to be associated with severe production losses (Höglund et al., 2001, Ploeger et al., 2000). This is similar to the result obtained in the present study, where infections measured by FECs were very low and animal productivity was overall not affected.

However, Charlier et al. (2010a) showed in their study that despite the low infection levels with *O. ostertagi* regular anthelmintic treatments were applied on about 80% of the farms which were involved in the study. In combination with the fact that immunity against *O. ostertagi* developed very slowly, the animals often don't have the chance to acquire sufficient immunity in the first grazing season. Since usually no anthelmintic treatment is given to second season grazers, the overprotection in the FSG animals is an important factor for the economic importance of *O. ostertagi* in temperate climates.

The anthelmintic treatment parameters that were used in this study (FEC >100 in combination with a decrease in BCS or DWG) are similar to those reported by Fahrenkrog (2013). The number of animals that met the treatment criteria varied between the TT and the TST groups of the farms but were generally very low. In the three TST groups only a total number of five animals required treatment. In the TT group one calf met the criteria on farm 2 and farm 3 and two calves) but at the same sampling occasion) on farm 1, resulting in a single treatment of all the TT groups.

The BCS was included since the majority of cattle farmers in Germany do not have access to a portable weighing device when the animals are on pasture. The present study shows a positive correlation between the BCS and weight ($r = 0.556$; $p < 0.001$) as showed in Table 9and this is in agreement with the result obtained by Fahrenkrog (2013) as the correlation between the BCS and weight was between 0.75 and 0.84. However, even if there is a strong correlation between the BCS and the weight it is difficult to use the BCS as the only parameter for the GI nematodes infections treatment, since other factors than parasite infection can affect the weight. Therefore it should be combined with other factors such as the FEC, which has a direct link to the target. In the present study no differences were obtained for the BCS between groups. FECs were highest in the untreated control groups but this did not

reflect in a difference in the BCS. The FECs were probably too low (less than 200 FEC) to affect the BCS in the untreated control animals.

The FEC treatment thresholds also vary from one study to another. O'Shaughnessy et al. (2015) proposed a FEC threshold of 200 in dairy calves, which was based on a lot of studies performed in Western Europe in FSG calves (Shaw et al., 1998b, Shaw et al., 1997). Fahrenkrog (2013) suggest a FEC threshold of ≥250 in dairy calves in Northern Germany. In the present study a FEC treatment threshold of >100 was chosen which is in agreement with the one Areskog et al. (2013) used as a treatment threshold in a study of FSG beef and dairy cattle farms in Sweden. FECs were sufficiently high on farms 1 and 3 to apply the use of the three parameters in the TT and TST groups. On farm 2 calves suffered from severe weight loss which did not seem to be related to parasite problems since the FECs of all animals were either 0 or very low. Despite the overall low FECs in the present study the treatment (TT/TST/PC) affected the FEC significantly. The NC group had a 5 times higher chance of having positive FECs than the PC. However, it is also important to consider that the excretion of eggs of adult worms is not continuous in trichostrongylids in cattle and there is no clear correlation between the FEC and the worm burden (Sangster and Dobson, 2002).

The second parameter investigated was the weight or weight gain. In sheep farms in South-West of Scotland the weight gain was used successfully as a TST treatment parameter for GI nematodes control (Busin et al., 2014). In cattle the use of weight gain as TST indicator has also been tested successfully in some studies. In Sweden three studies were conducted in FSG cattle between 1997 and 2004 to evaluate the possibility of using the DWG as a TST parameter. After data analysis they proposed that a weight gain threshold of ≤0.75 kgper day in mid-season was a suitable parameter (Höglund et al., 2009b). Fahrenkrog (2013) used a threshold of <0.25 kg for dairy calves.

According to the difference in breed the DWG parameters had to be adjusted in the present study and were set at 1.125 kg. No significant differences were found between the weight of animals in the TST/TT and PC groups on the farms. This was investigated for the actual weight on each weighing day, the DWG and the weight on the last visit. This highlights the fact that strategic continuous anthelmintic treatment is not of economic benefit in a low infection environment where animals don’t benefit from the treatment. It also shows that animals receiving no treatment or only one treatment per season performed similarly to treated animals. These results are in accordance with other field studies conducted on a total of 13 farms in Flanders and Belgium. Here, the animals were grouped into 2 groups; treated group and the

untreated control group and the FEC threshold was set to >200 FEC. Only very small differences between the treated and untreated groups were obtained with the untreated group displaying a lower weight gain of 0.04 Kg per day when compared to the treated group (Shaw et al., 1997). Another study was done in Ireland to assess the effect of the TST approach on dairy calves in their first grazing season to control GI nematodes and lung worm infections (O'Shaughnessy et al., 2015). The control group received three treatments with IVM while the TST group was treated only once. The treatment parameters were a FEC ≥200 positive lung worm detection. There was no significant effect of the treatment on the live weight gain between the TST group average live weight gain of 0.47 kg per day and the one of the control group (0.50 kg per day; $p = 0.41$). Also Fahrenkrog (2013) could not find significant difference in weight gain between the PC group and animals of the TT or TST. However, due to the slightly higher FECs a significant difference was obtained between the treated groups and the untreated control group in their study. In two trials conducted in Sweden treatment also had an effect on the weight gain in FSG calves. Two groups, an untreated group and positive control (animals treated with IVM at turnout) were investigated. At housing, the positive control group gained in average 30-65 kg more weight than the negative control group (Dimander et al., 2003, Dimander et al., 2000). In another study done by Höglund et al. (2013) FSG calves were sampled and divided into three groups, the first group wasn't treated, the second group received doramectin treatment regularly and the third group was treated on the basis of TST program, the TST group had a significantly lower DWG than the regularly treated group but the TST group had a significantly better DWG than the negative group. But it is difficult to depend only on the DWG as an indicator for the beginning of the treatment because it depends on other factors such as the nutrition, management and other diseases. So DWG could be accompanied by a decrease in FEC to be used as TST indicator.

As the live weight measurements can't differentiate between the muscle, fat, gastrointestinal content and other viscera (Van Burgel et al., 2011), the effect of the treatment on carcass weight was additionally analysed. Unfortunately it was available for only 65 calves, as not all the calves that participated in the study were slaughtered. Some of them were kept for fattening or for reproduction. However, the treatment did not affect the carcass weight as indicated by non-significant differences between the treatment groups and the PC group. Even the differences between the groups were non-significant. The meat and fat thickness (cm) and classes (U, R and O) were also provided by the slaughter house, but this information was only available for 31 calves which was insufficient to perform a meaningful analysis.

Unsurprisingly the age affected the weight gain, carcass weight and BCS in the present study. With every day of increasing in age the weight is increasing by 1.147 kg per day in beef calves in the start of the grazing season but start to decrease by 0.002 kg perday when the animals become older. This change is very small, could only be seen statistically over 100 days and is biologically not relevant. The weight gain is reflected by the changes in the BCS, which also increases in the first third of the pasture season and then stays steady (increase by 0.002 unit per day is only a statistical value and not applicable on a scale working on 0.25 increments units). As the weight affected by the age also the carcass weight increased by 0.671 kg with every day increase in the slaughter age. Slightly more surprising was an effect of age on FECs. The chance of strongyle infection was increased by 0.5% per day, which possibly reflects the increasing contamination of the pasture and therefore the higher infection risk. The gender also affected weight gain, carcass weight and FECs significantly. Male calves displayed a generally higher weight (by 9.8 kg), higher carcass weight (by 46.275 kg) and had a 1.8 times higher chance to have a FEC than female calves. The higher BCS by 0.033 obtained for male calves is again only a statistical value and biologically not relevant.

The lack of significant differences in BCS, weight gain and carcass weight in the present study show that for the investigated farming type ("Mutterkuh-Haltung") the infection pressure was low in the year when the study was conducted. The chosen FEC threshold prevented the occurrence of clinical nematode related disease. The strategic treatment of the PC group did not reveal any benefit in terms of animal health, performance or productivity. However, in comparison to the untreated NC group as well as to the TST and TT group costs for anthelmintic use were significantly higher for this group. In order to save money on one hand and also delay the onset of development of anthelmintic resistance on the other hand, TT or TST based control programmes can be beneficial to farmers. Since no significant difference was found between TT and TST treated animals, the application of the TT concept might be easier to incorporate on most farms since it does not require the capture of a targeted individual animal and is therefore cheaper to apply.

The second part of the study aimed at the detection of BZ resistance on German cattle farms. BZ resistance is widely spread in small ruminants all over the world (Kaplan, 2004, Wolstenholme et al., 2004). In contrast, the situation in large ruminants seems to be different as this drug is still reported to be effective against trichostrongylidae infection (Demeler et al., 2009). The early detection of resistance is therefore a key element for the onset of resistance development or at least delaying further resistance development (von Samson-Himmelstjerna

et al., 2009). In the present study the BZs resistance detection was achieved by using the FECRT analysis and by molecular methods (SNP detection) such as pyrosequencing. Farmers were either asked to send individual faecal samples to the laboratory before treatment or the farms were visited. The parasitological examinations of the faecal samples were carried out using a modified McMaster technique on the first 2 farms with a sensitivity of 25. But because the infection level was not very high the more sensitive Mini FLOTAC method with sensitivity of 5 (Cringoli et al., 2010) was used on all following farms. On all farms strongyle eggs were detected and larval cultures performed for genus identification using PCR and subsequent pyrosequencing analysis. A second faecal sample was analysed ten days after treatment with Albendazole ® and the FECR was calculated. A number of different methods are available for the calculation of FECR. The classic method using arithmetic means has several limitations as it is only computes the mean counts and ignores the additional variations such as the variation in the counting technique which can lead to variable results (Wang, 2015). Furthermore the sensitivity of the test is affected by the detection limit used in the counting technique especially if the pre-treatment FEC was low. Additionally the parasite distribution and egg counts are over dispersed within the host population (Grenfell et al., 1995). There is a slightly high correlation between the FEC and worm burden only in *H. contortus* and *Trichostrongylus colubriformis* but in other nematodes there is no correlation between the excretions of eggs and the worm burden in the host. Thus, false negative results can be obtained. A range of different approaches aimed at overcoming these limitation of the classical method (Denwood et al., 2010, Plummer et al., 2006, Torgerson et al., 2005, Gilks et al., 1996) but none of these attempts included the variability caused by the different egg counting techniques. Recently a Bayesian hierarchical model was designed by Paul et al. (2014) for FEC analysis, which considers sampling variability as well as the individual variation between the animals in egg counts. Paul et al. (2014) proved in their study that the hierarchical model is better than the standard FECRT through a simulation study was conducted to assess the AR status through comparing the results of FECRT and the hierarchical model. The hierarchical model can be performed in the R program within the ‘‘egg Counts’’ package. An online web interface version is available on the web site of the Zurich university (http://www.math.uzh.ch/as) which. This model was enhanced to a zero-inflated Bayesian hierarchical model by Wang (2015) to consider zero FECs in the pre-treatment samples or in uninfected animals. This online web interface version is simpler to use for non-statisticians (Torgerson et al., 2014). In the present study 11 farms were investigated and the results showed that, with the exception of one farm (GER.4) as showed in Table 29,

the FECR was well above 95%. This is in accordance with the findings of (Demeler et al., 2012;Demeler et al., 2009), where FECRTs and the assessment of BZ resistance using the egg hatch assay did not reveal reduced efficacy of the drug class on German cattle farms.
To enhance the number of farms included in the molecular analysis, farmers from Belgium and Ireland were additionally asked to send in faecal samples. Larval cultures were performed and the L3s subjected to genus differentiation followed by pyrosequencing analysis. In total material from 39 farms was available. Surprisingly no mixed infection were detected on any of the 6 investigated Belgium farms. This could be due to the overall low number of L3 available for DNA extraction. In Germany and Ireland also a relatively high number of farms had mono-infection either for *Cooperia* or *Ostertagia* but also mixed infections were present which is thought to be the normal case in natural field infections (Nilsson and Sorelius, 1973). Fahrenkrog (2013) also detected mixed infection on all samples in her study except only two samples which displayed mono infection with *Cooperia*.

As a more accurate method SNP detection by pyrosequencing was applied to the samples. SNPs at the codons 176, 198 and 200 in *C. oncophora* and *O. ostertagi* were analysed in the samples positive for *O. ostertagi* (25 farms) and *C. oncophora* (27 farms). Due to time limitations the pyrosequencing analysis originally envisaged could not be finalised. As shown by the results (Table 30) in the respective section in this thesis only one run could be performed and only for a limited number of samples this was successful. In none of the samples a highly increase of the resistance associated SNP significantly above the cut-off threshold of 10% were found, the highest value obtained was 13% and it is a slight increase to be considered as a resistance and also obtained from one run (it was not confirmed by another trail). The absence of such SNPs is in general agreement with the findings by the FECRT in this and also other studies. Demeler et al. (2013a) analysed a range of different nematode populations from cattle. While high SNP frequencies were obtained for those populations from South America, Australia and New Zealand, only low or no increase was found for German field populations.

6. Summary

Targeted treatment and targeted selective treatment in beef calves in Brandenburg

Gastrointestinal (GI) nematodes are the most common nematodes in cattle; they occur worldwide and especially in temperate countries. *Cooperia oncophora* and *Ostertagia ostertagi* are the most common species which colonise the small intestines and abomasum of cattle respectively. In the last decades anthelminthic resistance started to become an increasing problem in cattle farming industry. In Europe, New Zealand and South America (Molento et al., 2011, Demeler et al., 2009, Pomroy, 2006, Chartier et al., 2001) reduced efficacy of anthelmintics in cattle herds has already been described. For this reason, strategic herd treatment should be replaced by alternative control strategies. In the present work two studies were conducted. The first study evaluated the effect of alternative treatment programs to control GI nematodes infection in first season grazing beef calves. The second study aimed at the investigation of BZ resistance by using the faecal egg cunt reduction test (FECRT) and by applying molecular techniques for SNP detection in *C. oncophora* and *O. ostertagi.*

The first study was conducted in three beef cattle farms in the federal state of Brandenburg, Germany. The calves were grouped into four groups: Targeted Treatment (TT), Targeted Selective Treatment (TST), Positive Control (strategic regular treatment) and Negative Control (no treatment). Sampling was performed during two years: two farms were visited in 2013 from May to October and one farm was investigated in 2014 between June and November. Daily weight gain (DWG) and body condition score (BCS) according to (Edmonson et al., 1989) were determined monthly and faecal samples were collected also at each visit. Faecal samples were analysed using a modified McMaster method on the first two farms and the Mini FLOTAC method on the third farm. The carcass weight, fat and muscles thickness and meat classes were obtained from the slaughter house. For the treatment Valbazen® SUSPENSION (containing albendazole) was used. The anthelmintic treatment decision criteria were either a combination of a high FEC (>100) and decrease in DWG (<1.125 kgperday) or a high FEC in combination with a decreasing BCS. A strong positive correlation between the body weight and the BCS was found. The infection level on all farms was low with mean FECs below 100 and individual FECs rarely exceeding 200. The only mild infection didn't affect the productivity of the calves in the untreated control group but the treatment had a significant effect on the FECs in all other groups. The statistical analysis of the data revealed that there was no significant difference

between the treatment groups in the DWG, BCS or carcass weight. Allocation to a particular treatment group did not affect the productivity or performance of the animals. The PC group which was treated routinely every month did not achieve a better live weight or carcass weight but generated significantly higher costs in anthelmintic usage.

The occurrence of BZ resistance was investigated using the FECRT on 11 farms in Brandenburg, Germany. Faecal samples were collected pre and post treatment with albendazole and sampling was undertaken between July and October 2014. The statistical analysis revealed that only one farm shows a reduced efficacy with a reduction percentage of 76%. However, again the overall FECs were very low making the analysis very difficult. Additionally to those farms analysed by the FECRT farmers in Germany, Ireland and Belgium were asked to send in faecal samples from their herds before treatment. Larval cultures were performed and L3s subjected to DNA extraction and subsequent PCR analysis. Genus differentiation was performed revealing relatively high number of farms with only single infections (either *Cooperia* or *Ostertagia*) and only a moderate number of farms in Ireland and Germany with mixed infections. However, since the number of larvae obtained from the cultures and available for DNA extraction varied significantly between farms (100-50,000 L3) these results can also be due to the low numbers of L3s in some samples. Pyrosequencing targeting the SNPs at codon 167, 198 and 200 of the β-tubulin isotype 1 gene could only be performed for a small subset of the samples and due to time limitations also only data from a single run was available. The preliminary results obtained show no significant increase of the BZ resistance associated SNPs in any species in any of the samples. This is in agreement with previous reports that, unlike in South America or Australia/New Zealand, BZ resistance does not seem to be a problem in Europe in cattle nematode populations yet.

Overall, the findings of the present work show that the productivity in beef calves could be maintained by applying alternative treatment strategies such as TT or TST and an overall very low use of anthelmintics. This can lead to economic benefits through saving money and delaying the onset of resistance development. Particularly on organic based farms where strategic treatment is not possible, the implementation of TT or TST can be of benefit.

7. Zusammenfassung

Targeted treatment and targeted selective treatment in Fleischkälbern in Brandenburg

Magen-Darm-Nematoden sind die häufigsten Nematoden von Rindern; Sie sind weltweit, vor allem jedoch in Ländern mit gemäßigtem Klima verbreitet. *Cooperia oncophora* und *Ostertagia ostertagi* sind die häufigsten Arten, welche den Dünndarm und Labmagen von Rindern besiedeln. Innerhalb der letzten Jahrzehnte wurde die zunehmende Resistenz dieser Parasiten gegenüber Anthelminthika ein immer größeres Problem in der Rinderhaltung. In Europa, Neuseeland und Südamerika (Molento et al., 2011, Demeler et al., 2009, Pomroy, 2006, Chartier et al., 2001) wurde bereits eine verminderte Wirksamkeit von Anthelminthika in Rinderbeständen beschrieben. Aus diesem Grund sollte die strategische Herdenbehandlung durch alternative Kontrollstrategien ersetzt werden. In der vorliegenden Studie wurden zwei Studien durchgeführt. Innerhalb der ersten Studie wurden neue Behandlungsansätze zur Kontrolle der Infektion von erstsömmrigen Fleischrindern mit Magen-Darm-Nematoden evaluiert. Ziel der zweiten Studie war der Nachweis von BZ-Resistenz durch einerseits den Eizahlreduktionstest (FECRT) und andererseits durch die Anwendung molekularer Techniken für die Detektion von SNPs in *C. oncophora* und *O. Ostertagi*.

Die erste Studie wurde in drei Rinderbetrieben im Bundesland Brandenburg in Deutschland durchgeführt. Die Kälber wurden in vier Gruppen eingeteilt: Targeted Treatment (TT; gezielte Herdenbehandlung), Targeted Selective Treatment (TST; gezielte Einzeltierbehandlung), Positivkontrolle (regelmäßige prophylaktische Behandlung) und Negativkontrolle (keine Behandlung). Der Probenzeitraum erstreckte sich über zwei Jahre: zwei Betriebe wurden von Mai bis Oktober 2013 und ein weiterer Betrieb zwischen Juni und November 2014 beprobt. Die Gewichtszunahme (DWG) und der Body Condition Score (BCS) nach (Edmonson et al., 1989) wurden monatlich bestimmt und außerdem Kotproben bei jedem Besuch gesammelt. Die Kotproben der ersten beiden Betriebe wurden mit der modifizierten McMaster-Methode und die des dritten Betriebes mit der Mini-FLOTAC-Methode analysiert. Wenn möglich wurden außerdem Informationen zum Schlachtkörpergewicht, Fett- und Muskeldicke und den Fleischklassen aus dem Schlachthaus gesammelt. Die Behandlung wurde unter Verwendung von Valbazen® SUSPENSION (Wirkstoff Albendazol, ein BZ) durchgeführt. Die Behandlungskriterien waren entweder eine Kombination aus hoher Zahl ausgeschiedener Eier (FEC >100) und einer Abnahme in des DWG (<1,125 kg/Tag) bzw. eines Absinken des BCS.

Die Daten resultierten in einer deutlich positiven Korrelation von Körpergewicht und BCS. Die Infektion mit Magen-Darm-Nematoden war auf allen Betrieben eher gering, mit mittleren FEC-Werten meistens unter 100 und individuellen FEC-Werten, selten 200 überschritten. Diese nur geringgradigen Infektionen hatten keinen Einfluß auf die Produktivität der Kälber in der unbehandelten Kontrollgruppe, jedoch hatte die Behandlung einen signifikanten negativen Effekt auf die Höhe der Eiausscheidung in den behandelten Gruppen. Die statistische Analyse der Daten ergab, daß es keinen signifikanten Unterschied zwischen den Behandlungsgruppen in der Gewichtszunahme, dem BCS oder dem Schlachtkörpergewicht gibt. Die Zuweisung zu einer der bestimmten Behandlungsgruppe hatte also keinen Einfluß auf die Produktivität der Tiere. Die Positivkontrollgruppe, welche regelmäßig monatlich behandelt wurde, erreichte nicht eine besseres Lebend- oder Schlachtgewicht, generierte allerdings deutlich höhere Produktionskosten in Form der durchgeführten anthelminthischen Behandlungen.

Das Vorkommen von BZ-Resistenz wurde zwischen Juli und Oktober 2014 mittels FECRT auf 11 Betrieben in Brandenburg, Deutschland untersucht. Kotproben wurden vor und nach Behandlung mit Albendazol auf das Vorkommen von Nematodeneiern untersucht. Die statistische Analyse der Daten ergab nur auf einem Betrieb eine reduzierte Wirksamkeit (76%). Allerdings waren auch in 2014 die FECs durchschnittlich sehr niedrig, was die statistische Analyse grundsätzlichen eher schwierig macht. Zusätzlich zu den beprobten Betrieben wurden außerdem Betriebsleiter in Deutschland, Irland und Belgien gebeten, Kotproben von Tieren ihrer Herden (vor Behandlung) einzusenden. Von allen Betrieben wurden Larvenkulturen (auf Betriebsebene) angelegt und aus den daraus erhaltenen Drittlarven DNA gewonnen und diese anschließend in PCRs eingesetzt. Die Artdifferenzierung ergab eine relativ hohe Anzahl von Betrieben, auf denen nur eine Art (entweder *Ostertagia* oder *Cooperia*) nachgewiesen werden konnte und nur eine geringe Anzahl von Betrieben in Deutschland und Irland mit gemischten Infektionen. Da jedoch die Anzahl der aus den Kulturen gewonnenen Larven extrem zwischen den Betrieben schwankte (100-500,000 L3), können diese Ergebnisse auch auf eine sehr (zu) geringe Larvenanzahl zurückzuführen sein. Mittels der Pyrosequenzierungsmethode wurden die Ziel-SNPs in Codon 167, Codon 198 und Codon 200 des β-Tubulin-Gens Isotyp 1 durchgeführt. Diese Untersuchung konnte nur für eine beschränkte Anzahl von Betrieben durchgeführt werden und aufgrund zeitlicher Einschränkungen war nur ein Pyrosequenzierungs-Lauf pro Ziel-Gen möglich. Die Ergebnisse zeigten keine Erhöhung der mit BZ-Resistenz assoziierten SNPs in allen untersuchten Proben. Obwohl die vorliegenden Ergebnisse nur als vorläufig zu sehen sind, stimmen sie mit vorherigen Berichten überein, daß

BZ-Resistenz in Europe, im Gegensatz zu Südamerika, Neuseeland oder Australien noch kein Problem darstellt.

Insgesamt zeigen die Ergebnisse der vorliegenden Arbeit, daß die Produktivität von erstsömmrigen Fleischrindern durch die Anwendung von alternativen Entwurmungskonzepten wie TT oder TST mit einem grundsätzlich geringen Einsatz von Anthelminthika erhalten werden kann. Dies führt auch zu ökonomischen Vorteilen für den Landwirt durch einerseits finanzielle Einsparungen und andererseits einer Verzögerung der Resistenzentwicklung/-ausbreitung. Insbesondere auf Bio-Betrieben, wo prophylaktische Behandlungsansätze nicht mehr erlaubt sind, kann die Anwendung von TT oder TST sehr sinnvoll sein.

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9. Appendix

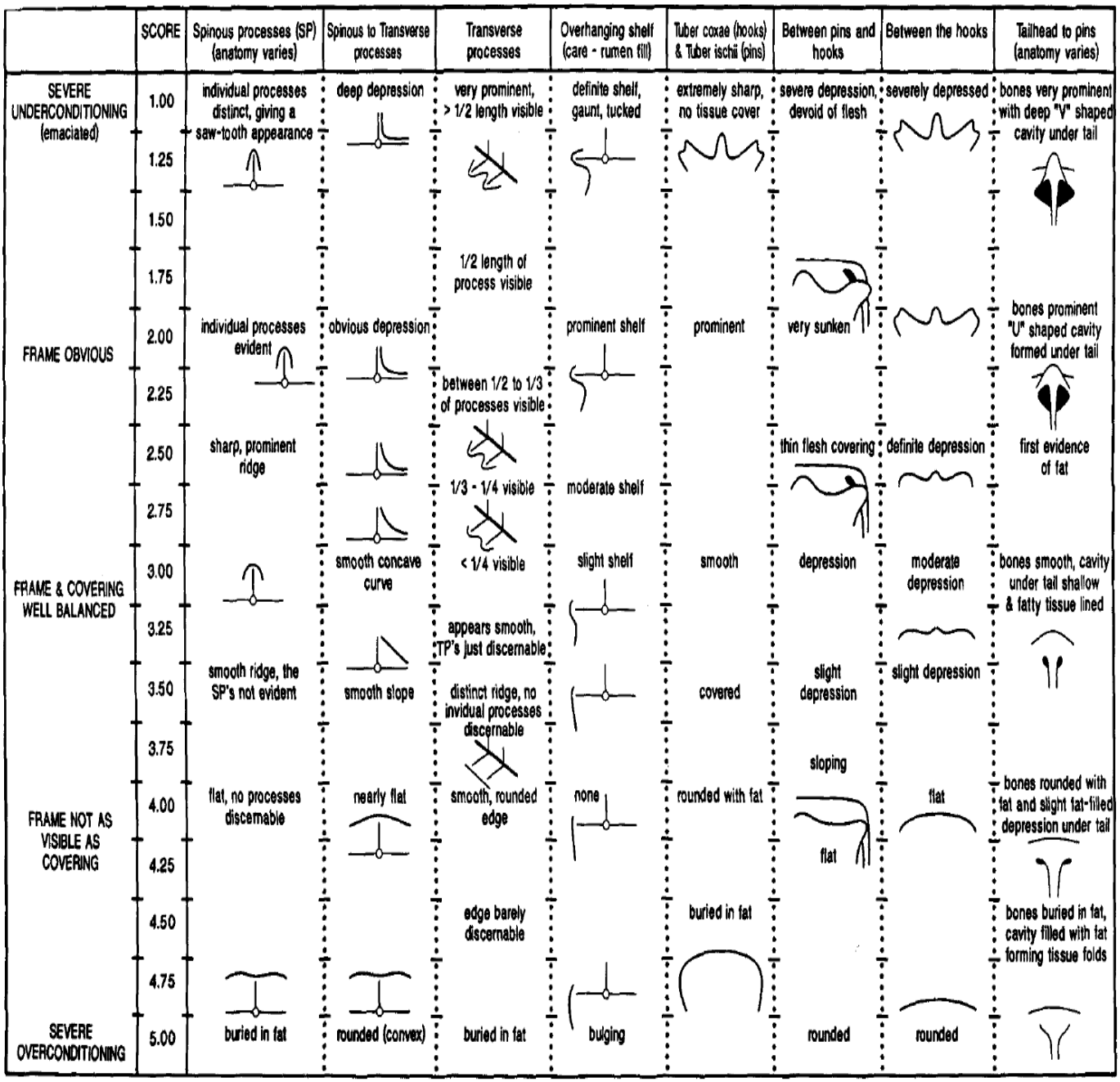

Figure S1. Diagram for BCS assessment according to (Edmonson et al., 1989)

Table S1. Individual FEC before and after the treatment on farm 1

No. of animals	FEC before treatment	FEC after treatment
1	20	0
2	5	0
3	0	0
4	5	0
5	30	0
6	15	0
7	5	0
8	5	0
9	35	0
10	5	0
11	5	0

Table S2. Individual FEC before and after the treatment on farm 2

No. of animals	FEC before treatment	FEC after treatment
1	35	0
2	0	0
3	0	0
4	50	0
5	0	0
6	0	0
7	0	0
8	0	0
9	5	0
10	105	0
11	5	0
12	0	0
13	70	0
14	5	0
15	0	0
16	5	0
17	5	0
18	15	0
19	45	0
20	5	0
21	0	0

Table S3. Individual FEC before and after the treatment on farm 3

No. of animals	FEC before treatment	FEC after treatment
1	110	0
2	5	0
3	15	0
4	70	5
5	65	0
6	10	0
7	20	0
8	35	0
9	5	0
10	155	0
11	15	0
12	20	0
13	5	0
14	5	0
15	10	0
16	15	0
17	50	0
18	30	0
19	45	0
20	10	0
21	0	0

Table S4. Individual FEC before and after the treatment on farm 4

No. of animals	FEC before treatment	FEC after treatment
1	0	0
2	5	0
3	110	40
4	40	0
5	5	0
6	10	0
7	0	0
8	20	0
9	20	0
10	5	0
11	0	0
12	0	0

Table S5. Individual FEC before and after the treatment on farm 5

No. of animals	FEC before treatment	FEC after treatment
1	15	0
2	5	0
3	20	0
4	5	0
5	5	0
6	0	0
7	5	0
8	10	0
9	25	0
10	35	0
11	0	0
12	5	0
13	5	0

Table S6. Individual FEC before and after the treatment on farm 6

No. of animals	FEC before treatment	FEC after treatment
1	25	0
2	80	0
3	95	0
4	180	0
5	295	0
6	30	0
7	45	0
8	165	0
9	230	0
10	60	0
11	120	0
12	90	0
13	140	0
14	55	0
15	80	0
16	140	10
17	135	0

Table S7. Individual FEC before and after the treatment on farm 7, which was excluded from the FECRT due to too low FECs.

No. of animals	FEC before treatment	FEC after treatment
1	0	0
2	15	0
3	5	0
4	0	0
5	5	0
6	0	0
7	0	0
8	5	0
9	0	0
10	0	0
11	0	0
12	0	0
13	0	0
14	0	0
15	5	0
16	0	0
17	5	0
18	0	0

Table S8. Individual FEC before and after the treatment on farm 8

No. of animals	FEC before treatment	FEC after treatment
1	65	0
2	90	0
3	15	0
4	5	0
5	10	0
6	0	0
7	15	0
8	10	0
9	50	0
10	125	0
11	45	0
12	60	0
13	0	0
14	30	0
15	90	0
16	115	0
17	25	0
18	30	0
19	150	0
20	15	0

Table S9. Individual FEC before and after the treatment on farm 9

No. of animals	FEC before treatment	FEC after treatment
1	5	0
2	0	0
3	5	0
4	35	0
5	10	0
6	0	0
7	20	0
8	0	0
9	0	0
10	0	0
11	15	0
12	0	0
13	0	0

Table S10. Individual FEC before and after the treatment on farm 10

No. of animals	FEC before treatment	FEC after treatment
1	35	0
2	15	0
3	20	0
4	10	0
5	5	0
6	10	0
7	40	0
8	35	0
9	25	0
10	20	0
11	40	0
12	45	0
13	55	0
14	25	0
15	75	0
16	50	0

Table S11. Individual FEC before and after the treatment on farm 11

No. of animals	FEC before treatment	FEC after treatment
1	35	0
2	50	0
3	15	0
4	50	0
5	25	5
6	0	0
7	25	0
8	0	0
9	65	0
10	30	0
11	30	0
12	10	0
13	45	0
14	40	0

Table S12. PCR results for larval cultures from farms in Ireland

Farm ID	*O. ostertagi*	*C. oncophora*
IRL-01		+
IRL-02		+
IRL-03	+	+
IRL-04	+	+
IRL-05		
IRL-06	+	
IRL-07	+	+
IRL-08	+	
IRL-09	+	+
IRL-10		+
IRL-11		+
IRL-12		
IRL-13	+	
IRL-14		+
IRL-15	+	
IRL-16		
IRL-17		+

Table S13. PCR results for larval cultures from farms in Germany

Number of farms	***O. ostertagi***	***C. oncophora***
GER-01	+	
GER-02	+	+
GER-03	+	
GER-04	+	+
GER-05	+	+
GER-06	+	
GER-07		+
GER-08	+	+
GER-09		+
GER-10		+
GER-11	+	+
GER-12	+	+
GER-13	+	+
GER-14		+
GER-15	+	
GER-16		
GER-17	+	+
GER-18	+	+
GER-19	+	
GER-20	+	+

Table S14. PCR results for larval cultures from farms in Belgium

Number of farms	***O. ostertagi***	***C. oncophora***
BEL-01		+
BEL-02		+
BEL-03	+	
BEL-04	+	
BEL-05		
BEL-06	+	
BEL-07		+
BEL-08		

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11. Selbständigkeitserklärung:

Hiermit bestätige ich, dass ich die vorliegende Arbeit selbständig angefertigt habe. Ich versichere, dass ich ausschließlich die angegebenen Quellen und Hilfen Anspruch genommen habe.

Berlin, den 24.07.2017

Walaa Mostafa Ahmed Saleh