THE EFFECT OF GARLIUM® GEM HC AS A TICK REPELLENT AGENT IN SPRINGBOK (*Antidorcas marsupialis)*

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Research proposal summary:

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DECLARATION

I, Agustina Fitte, do hereby declare that the research presented in this dissertation, was conceived and executed by myself, and apart from the necessary guidance from my supervisors, I have received no assistance.

Neither the substance, nor any part of this dissertation has been submitted in the past at this or any other University.

This dissertation is presented in partial fulfilment of the requirements for the degree MMed Vet (Fer) Wildlife.

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Signed _____ Agustina Fitte

Date _____....._ ……_.20…….____________

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BCS: body condition score

BW: body weight

cm: centimeter

DAS: diallyl sulphides

DADS: diallyl disulphide

DATS: diallyl trisulphide

DEET: N, N-diethil-m-toluamide

g/dl: grams per deciliter

Garlium: Garlium® GEM HC

GG: Garlic or treated group

CG: control group

fl: Femtoliters

g: grams

h: Hour

Hb: hemoglobin

Htc: hematocrit

HPLC: High Pressure Liquid Chromatography

IUCN: International Union for Conservation Nature

l: liter

IM: intramuscular

IV: intravenous

kg: kilogram

RBC: red blood cells

TBD: Tick borne diseases

TMR: total mix ratio

MCHb: Mean corpuscular hemoglobin concentration

mg: milligrams

Nº: number

pg: Picograms

SUMMARY

Garlic is one of the most popular edible plants in the world that has shown to have anti-microbial properties against bacteria, viruses, fungi and protozoa. Garlium® GEM HC is a product that contains garlic together with other Allium compounds. This *in vivo* study with 24 springboks (*Antidorcas marsupialis)* aimed to investigate the repellant effects of Garlium® GEM HC on immature stages of red legged ticks (*Rhipicephalus evertsi evertsi).* Engorged females of *R. e. evertsi* were collected from naturally infested domestic ruminants and were allowed to lay eggs under laboratory conditions. A representative sample of eggs have been counted and weighed. The equivalent weight of eggs represented approximately 250 larvae. They were aliquoted into separate micro-centrifuge tubes waiting for them to hatch. The hatched larvae were used to infest ears of juvenile male springbok during the study. The garlic group (GG) had an average consumption of 0.985 kg of total mix ration (TMR) containing a proportion amount of 1.36 g of Garlium® GEM HC per animal per day. Garlium® GEM HC was fed for 7 days before a total number of 250 larvae of *R. e. evertsi* were introduced per ear bag (i.e. 500 ticks in total per animal). Ear bags were then removed after a seventeen day period. Remaining attached ticks were then collected, counted and weighed. Results show that the Garlium® GEM HC treated group had a clear repellent effect on red legged ticks. The control group (CG) had a tick load of 47% more compared to the GG. Although some studies have showed that ingestion of garlic during long periods could induce methemoglobinaemia. Haematological results showed in this trial found no significant differences between the GG and the CG during the 17 day trial period. Total ear cerumen (ear wax) secretion was found to be increased in the GG, where

less ticks were found suggesting that garlic may play a role in increasing cerumen production / secretion. This finding could imply a possible physiological effect of Garlium® GEM HC to reduce tick load in springbok.

CHAPTER 1

INTRODUCTION

Garlic is one of the most popular edible plants in the world that has shown to have anti-growth properties against bacteria, viruses, fungi, protozoa, cancer (Amagase, 2006; Milner, 2006) and even helminths (Mikaili *et al.*, 2013; Ankri & Mirelman, 1999; Shyma *et al.,* 2014; Upadhyay, 2010; Watson *et al.*, 2013).

Tick borne diseases can have a major impact on commercial and financial losses in South Africa and the African continent. Wild animals have been bred extensively on game farms in the last 10 years to supply a constant increasing demand from the hunting industry and has contributed, together with climate change to the spreading of vectors throughout the industry. Consequently, *Haemonchus contortus* ("wire worm") a well-known parasite and normal inhabitant of sable and roan antelope have lately generated resistance to many of the commonly used dewormers in these species.

Garlium® GEM HC is an oil based product developed by the Swiss company Pancosma SA with garlic oil with concentrations that range between 1% and 10%. In addition, Garlium® GEM HC is formed by a series of selected carriers enriched with garlic oil and its active substances (GEM). It also possesses highly stable ingredients that makes the product an outstanding food enhancer and flavouring due to the diallyl sulphides compound.

Garlium® GEM HC is a wide used product in different animal species that has been in the market for the last 20 years. Due to the lately increase in synthetic acaricide resistance, the environment contamination that this ones produce and their increase in price to the farmer, has diverted researchers attention into natural substances mostly from plant origin. The search of “green” and natural alternatives has driven researchers to look into medicinal plants to find alternative options for a growing industry. The aims of Pancosma is to offer an economically, sustainable, ecological and efficient product that would aid in the repellency of ticks and possibly other arthropods.

This *in vivo* study using 24 springbok antelopes (*Antidorcas marsupialis*) (East, 1999) aimed to investigate the repellent effects of Garlium® GEM HC (Nchu *et al.,* 2016; Stjernberg & Berglund, 2000; Upadhyay, 2010) on immature stages of red legged ticks (*Rhipicephalus evertsi evertsi).* Engorged females of *R. e. evertsi* were collected from naturally infested wild ruminants. A group of ten selected engorged ticks were incubated using the same laboratory conditions that Rechav *et al*., (1977) used to study the life cycle of the red legged tick. Ticks were kept in a 26°C and 85% relative humidity sodium chloride warm bath to lay eggs. Once the eggs hatched, larvae were kept in the same environmental conditions until they were ready to infest the ears of juvenile male Springbok.

This experimental study included a control (no Garlium® GEM HC added) and a treatment (Garlium® GEM HC added) group. The treated group received 1 kg of feed per day, per animal total mixed ration (TMR), (CAP-CHUR© ANIMAL FEEDS, Cullinan, Gauteng, RSA) with the addition of a 1 gram of Garlium® GEM HC

compound per animal per day. Garlium® GEM HC was fed to the treated group for 7 days before a total number of 500 *R. e. evertsi* larvae (250 per ear bag) were introduced to ear bags attached to each Springbok. Ear bags were removed after a seventeen-day period and larvae and nymphs were collected, counted and weighed. Preliminary results showed that Garlium® GEM HC had a clear repellent effect on the red legged ticks. Moreover, ear wax (cerumen) secretion in the GG increased. Thus, suggesting that in addition to possible direct effects on ticks, garlic may play an indirect role through cerumen (also possibly dermal sebaceous secretion) production / secretion contributing to the repellent effects on ticks.

CHAPTER 2

LITERATURE REVIEW

2.1 The importance and impact of tick borne diseases in South Africa and the African continent

Tick borne diseases (TBD) are widely found in South Africa and in many regions of the African continent being one of the major causes of reduced animal production and financial losses (Perry *et al*., 1995). TBD affect both humans and animals, with a negative impact on animal welfare and the perpetuation of human poverty (Minjauw, & Mc Leod., 2003) in African countries.

In Africa, Asia and Latin America, TBD like Theileriosis and Babesiosis, together with rickettsial diseases such as Anaplasmosis and Heartwater are primarily health and management problems (Jongejan & Uilenberg, 2004; Peter *et al.,* 2002; Martinez Dominique *et al.,* 2012) not only for cattle and small ruminants but also for buffalo and other game species. TBD were also recently ranked as a major impact and concern in the livelihood of resource-poor farming communities in developing countries (Perry *et al.,* 2002; Minjaw & Mc Leod, 2003).

There are various reasons for the control of tick vectors around the world. Starting from the production animal's perspective: weight loss, tick abscesses that predispose to screw worm, tick worry and teat damage which consequently reduces calve numbers are some of the detrimental factors involved (Moyo & Masika, 2009). Ticks inflict direct damage on skin and hides depreciating their commercial value

and the quality of manufacturing leather (Jongejan & Uilenberg, 2004). Because of the direct damage inflicted, ticks are one of the most important ectoparasites in domestic and wild animals (Uilenbert, 1992).

Moreover, TBD are some of the most important animal diseases around Africa involving babesiosis, anaplasmosis, theileriosis, heartwater and east coast fever just to mention a few (Allsopp *et al.,*2005; Jongejan & Uilenberg, 2004; Tonetti *et al*., 2009; Ristic, 2018; Schroder & Reilly, 2013; Peter *et al.,* 2002; Worthington & Bigalke, 2001). In domestic animals as well as in some wild antelope like eland (*Taurotragus orxy*) (Moolman *et at.,*1987) ticks can also produce several other conditions; allergy, irritation, paralysis and toxicosis (Moolman *et at.,* 1987; Jongejan & Uilenberg, 2004) to mention a few.

Ticks, together with mosquitos are globally well-established vectors of pathogens of some of the most important zoonotic diseases in the world (Dantas *et al.*, 2012) such as rickettsiosis, tularemia, African tick bite fever and Borreliosis (Lyme's disease) (Coetzer *et al.,*1994; Stjernberg, 2000). These diseases receive increasing attention by human health and veterinary professions in a more dynamic world. Global warming, together with warmer winters and longer autumns and spring seasons (Dantas *et al.*, 2012) allows some tick species to thive in expansion (density) to extend outside their "normal" distribution areas (Dantas-Torres, 2015; Van den Bossche & Coetzer, 2008). Ticks spend most of their lives living off their hosts, therefore, they are highly susceptible to factors such as ambient temperature and humidity (Van den Bossche & Coetzer, 2008). Vegetation and the changing climatic conditions affect tick ecology and with this the distribution, proliferation and density of ticks (Van den Bossche & Coetzer, 2008).

In domestic cattle, European *Bos taurus* breeds have an increased susceptibility to tick borne diseases compared to zebu breeds that have a natural innate resistance (Horak, 1980; Jongejan & Uilenberg, 2004). The natural ability of zebu cattle to resist tick infestation or the capability of developing an effective immunological response to infestation is greatly determined by their genetics (Van den Bossche & Coetzer, 2008). Progressive buildup of immunity to TBD occurs in areas where ticks are present and animals are constantly exposed to protozoal pathogens that are transmitted by tick bites. This constant immune stimulation where an estimated 80% of the animal population seroconverts from the constant inoculation of protozoal diseases by ticks is known as **endemic stability** (Jongejan & Uilenberg, 2004; Regassa *et al.,* 2004; Van den Bossche & Coetzer, 2008).

Herds under **endemically stable conditions** don't show clinical disease but become carriers of tick borne pathogens (Regassa *et al*., 2004). **Endemic stable** herds with an inferior seroconversion rate below 60% of the population are considered **endemically unstable** herds. In these herds clinical disease is often seen and outbreaks occur sporadically (Regassa *et al.,* 2004).

Wild animals and livestock live in close proximity in many areas of Africa and although **endemic stability** has never been documented in wild animal populations before, it can be inferred that the same immunological principles apply to these wild populations as it does for domestic livestock (pers communication, JCA Steyl). Moreover, different wild animal species have certainly marked differences in disease susceptibility, making some of them, extremely sensitive and prone to TBD.

Furthermore, Regassa *et al* (2004) studied the progression towards **endemic stability** of *Babesia bigemina* and *Babesia bovis* in cattle when reintroduced onto a game farm after three years of keeping different wild animal species (eland, gemsbok, red hartebeest, blue wildebeest (*Connochaetes taurinus*), waterbuck, gray or common duiker (*Sylvicapra grimmia*), steenbok and klipspringer on that farm. Calves that were born on the farm and were not vaccinated against *Babesia bigemina* were bled at 7 and 8 months of age. Seventeen percent of calves showed seropositivity to *Babesia bigemina* while at eight months there was an increase to 70% seropositivity. This significant increase in antibodies for *Babesia bigemina* in the calves clearly showed a tendency of the herd moving towards **endemic stability** for *B. bigemina* (Regassa *et al.,* 2004). In this study, both livestock and antelope kudu (*Tragelaphus strepsiceros*) and impala (*Aepyceros melampus*) mainly mingled together, believing that many of the tick species attached to the cattle during this period were the offspring of adults that fed on these antelopes (Regassa *et al.,* 2004).

In South Africa most livestock are in close relation with several wildlife animal (Purnell, 1980). This situation poses a complex circulation dynamic of the tick borne pathogens between wildlife and domestic animals (Tonetti *et al.,* 2009). Wild species and specifically wild ruminants can be particularly susceptible to TBD and can play a key role as reservoirs of some of these pathogens, increasing the challenge in controlling tick borne diseases in animals (Tonetti *et al.,* 2009).

There are several wild species that carry TBD and have been specifically studied for identification of haemoparasites in South Africa. In the one study, Brothers *et al* (2011) have identified opportunistic haemoprotozoan in endangered Tsessebe

(*Damaliscus lunatus*) that were identified as *Theileria sable* and *Theileria separata* and transmitted by the *R. e. evertsi* tick, the most abundant tick species found on these animals. Preferred feeding sites for immature *R. e. evertsi* ticks are mainly the ears and perineum for adult ruminants. Moreover, subclinical infections of sheep with non-pathogenic *T. separata* transmitted by *R. e. evertsi* have been identified in South Africa (Brothers *et al*., 2011). Contrary to *T. sable* that has been reported to be the cause of fatal Theileriosis in roan and sable (*Hippotragus niger*) antelope, *T. separata* infection in antelopes has not been described before (Brothers *et al*., 2011). It is believed that due to the close phylogenetic relation between *T. separata* and Theileria sp. (Sable), they might have descended from an ancestral wild ruminant phylogenetic Theileria (sp) clade (Brothers *et al*., 2011).

Certain wild species are particularly susceptible to TBD and have particular susceptibility to Theileria spp. Mortalities had been found in several wild species like common duiker (*Syilvicapra grimmia*), greater kudu (*Tragelaphus strepsiceros*), eland (*Taurotragus oryx*), giraffe (*Giraffa cameleopardis*), roan antelope (*Hippotragus equinus*), sable (*Hippotragus niger)* antelope and in Tsessebe (*Damaliscus lunatus lunatus*) (Clift *et al.,* 2020; Horak *et al*., 2007; Steyl *et al.,* 2012). Other African species like for instance springbok (*Antidorcas marsupialis*) and impala are particularly susceptible to heartwater (Peter *et al.,* 2002). According to a study done by Horak (1982) in which the prevalence of ectoparasites in impalas was estimated, it was found that *R. e. evertsi* was the most commonly abundant tick species in these antelope (Horak, 1982). Although heartwater is mainly transmitted by *Amblyomma* spp. ticks, gene segments of *Ehrlichia ruminantium* have been found by PCR in *R. e. evertsi* ticks among others, showing that there is possibility

of heartwater transmission with *Rhipicephalus* spp (Horak, 1982; Martinez Dominique *et al.*, 2012).

R. e. evertsi is also partially responsible together with *Rhipicephalus appendiculatus* for the transmission of *Theileria* sp. in sable and roan antelope (Clift *et al*., 2020; Steyl *et al.,* 2012; Uys *et al.*, 2015). Sable and roan antelope are listed as least threatened and critically endangered, respectively by the Convention on International Trade in Endangered Species (CITES) and one of the major causes for the decrease in population is related to the unusual high rate of calf losses (Clift *et al*., 2020; Steyl *et al.,* 2012). It is believed that Theileriosis is the main cause of calf mortalities due to high susceptibility of these species (Clift *et al*., 2020; Steyl *et al.,* 2012; Nijhof, *et al.,* 2005). While a high tick load in these species can be detrimental to their health status and a sign of a susceptible immune system (disease), a reduction or a minimal tick load on the other hand could pose a beneficial effect of endemic stability against theileriosis. A reduced tick load on the animal will enhance their immune system by naturally triggering its response to the protozoal inoculation through the tick bite. In this way the stimuli of the tick bite allows the host to achieve a healthy immune response (Steyl *et al.,* 2012). Besides roan and sable antelope other species that are susceptible and have been reported with clinical Theileriosis are grey duiker, kudu and eland (Zweygarth *et al.*, 2009).

2.1.1 Acaricidal effect on ticks

Controlling tick infestation and the pathogens transmitted by them has been a priority in many countries around the world, aiming to improve human and animal health (De la Fuente *et al*., 2008). Tick control has become increasingly challenging

in recent years, and many different types of acaricide products have been used with varied effects (Li *et al*., 2007; Zaman *et al.,* 2012).

The control of ticks is mainly driven by chemotherapy with synthetic acaricides (Li, *et al.,* 2007; Zaman *et al*., 2012) but has been complicated lately by the emergence of drug resistance (Miller *et al*., 2007; Olivares-Perez *et al*., 2011; Zaman *et al*., 2012). This acaricidal resistance complicates, directly and indirectly the tick control programs in the country, resulting in significant financial losses to farmers (Corson *et al*., 2001; Shyma *et al*., 2014). With the development of tick resistance and the appearance of chemical residues in meat and milk and an increase in environment pollution, researchers are interested in finding a more ecofriendly option to control ticks (Zaman *et al*., 2012).

Several reasons exist for the increase in tick resistance to chemical acaricides, most of which could be blamed on increased application frequencies as well as over and underdosing (Shyma *et al.,* 2014). The development of more sophisticated compounds as new generation acaricides are becoming more difficult and are much more expensive to produce. These new acaricides together with an increase in price makes it more difficult to be accessed by farmers in remote and impoverished areas (Shyma *et al*., 2014).

Ticks locate hosts in two different ways, by ambushing (questing) or hunting (Bissinger & Roe, 2010). In the former, ticks will climb foliage and wait with their extended forelegs for a host to pass and will cling on to it if they come into direct contact (Bissinger & Roe, 2010). This ambushing strategy is the most commonly used by ticks (Bissinger & Roe, 2010). Moreover, ticks can respond to a stimulus

and emerge from their refuges by quickly walking towards the host. This behavior is called hunting and is triggered by different stimuli such as carbon dioxide, butyric acid, animal waste ammonia, heat, shadows and vibrations among others (Bissinger & Roe, 2010).

Tick repellency studies have shown that tick repellency to a synthetic drug is achieved through two different mechanisms, i.e., olfaction and tactile chemoreceptors (Bissinger & Roe, 2010). Olfactory repellency is achieved through the olfactory sensilla organ of the tick which is able to detect vaporized molecules. Repellency can also be achieved by means of tactile chemoreceptors occurs through tactile stimulation (Bissinger & Roe, 2010). Although difficult to differentiate between both effects, acaricides will work at both levels by inhibiting these functions in the tick. However, tick susceptibility to a tick repellent varies between different tick species such as *Ixodes ricinus (L*), *Amblyomma variegatum F*, *A. americanum* and *Boophilus microplus* (Bissinger & Roe, 2010).

Furthermore, due to the many drawbacks together with costs and the level of environmental contamination of synthetic acaricides, ethno-veterinary medicine especially biopesticides are gaining recognition in the African countries (Moyo & Masika, 2009). Natural pesticides like extracts and compounds from the neem tree, Azadirachta indica, (Schmutterer, H., 1990) show multiple benefits and are widely accepted by the farming communities due to their greater accessibility, lower environmental impact plus lower costs to the farmers (Bissinger & Roe, 2010; Moyo & Masika, 2009).

These natural occurring substances including biopeptides pose several advantages compared to the synthetic acaricides (Bissinger & Roe, 2010; Moyo & Masika, 2009; Shyma *et al*., 2014). They are cheaper products, they take less time to register, normally have a high tolerance or almost no toxicity levels, and are ecofriendly, minimizing environmental contamination (Bissinger & Roe, 2010; Moyo & Masika, 2009; Shyma *et al*., 2014).

Acaricides work in different ways, some of them act through the inhibition of the sodium, potassium ATPase of ticks like in the case of *C. procera* (Rubber tree or Rubber bush) or like in the case of Thymol, a major component of *T. ammi* that act like an allosteric modulator for the insect's GABA receptors (Zaman *et al.,* 2012) Chemical arthropod repellents like for example DEET (N, N-diethil-m-toluamide), Permethrin and Indalone are substances that cause arthropods (ticks) to move away from its source and will prevent them from attaching to the host (Bissinger & Roe, 2010).

Plants produce numerous secondary compounds that serve as repellents, such as feeding blockers or toxicants to hematophagous insects, including ticks (Bissinger & Roe, 2010; Dam *et al.,* 1998; & Singh, 2012). Furthermore, some plant base repellents contain terpenoids, plant growth regulators and anti-tick pasture plant factors which work as natural tick repellent (Bissinger & Roe, 2010). Several herbal extracts have proven to have repellent and acaricidal effects in different tick species; some of them like *Datura stramonium* (Jimson Weed)*, Azadiracha indica* (Neem tree), *Caloptris procera* (Crown flower or Giant Milkweed), *Allium sativum cloves* (Garlic), *Allium cepa* (Onion), *Carija papaya* (Pawpaw) and several others (Aboelhadid *et al*., 2013; Niebuhr *et al*., 2014; Shyma *et al*., 2014; Thorsell *et al*.,

2006) have found to kill 100% of ticks with only a 2% of an oil/alcohol concentration preparation (Aboelhadid *et al*., 2013). Others like *Achillea millefolium* (Common Yarrow), together with volatile oils of birch and/or pine tar, citronella, cloves, eucalyptus, geranium, lavender, lili of the valley and peppermint can have repellent effects similar to DEET (N, N-diethyl-m-toluamide) (Thorsell *et al.,* 2006).

2.2 Background in the development of Garlium® GEM HC

Garlium® GEM HC is a garlic compounded product produced by Pancosma, a Swiss company that is represented in South Africa by Allied Nutrition. Garlium® GEM HC is a powder product which is manufactured from both garlic powder and garlic oil which their main components are the diallyl sulphide (DAS), diallyl disulphide (DADS), diallyl trisulfide (DATS) and ajoene molecules of garlic. These compounds are added to specific carriers (salt) to enhance stability, flowability and mixability of the product. Garlium® GEM HC is a highly concentrated product suitable for inclusion in supplements, finished feed and liquid feed products. Garlium® GEM HC is suitable to add to horses (granules), cattle (powder mineral supplement), pigs (pellets), poultry (water soluble garlic) and even canine (pet food) diets.

Figure 2.1: Commercial presentation of Garlium® GEM HC powder.

The garlic oil presentation has a garlic content composition between 1% to 10% depending on the product. Moreover, garlic oil consists of a standardized, highly stable and constant levels of active ingredients allowing an easy incorporation into the feed. This stable oily solution is due to an optimized particle size and flow ability of the components. Conversely, the garlic powder in the form of dehydrated garlic granules is produced from dehydrated garlic bulb with a low garlic oil content of an average of 0.7% as the garlic process has high fluctuations in quality according to the supplier, variety, origin and season. Moreover, many actives are decomposed during manufacture due to heat treatment. Regularity in particle size is not easily achievable producing a final heterogonous mixture (Appendix B).

The following graph shows a trial to compare Garlium® GEM HC Gem 10% versus Garlic granules regarding their active components. Via a chromatographic analysis a quantitative comparison of these compounds was conducted.

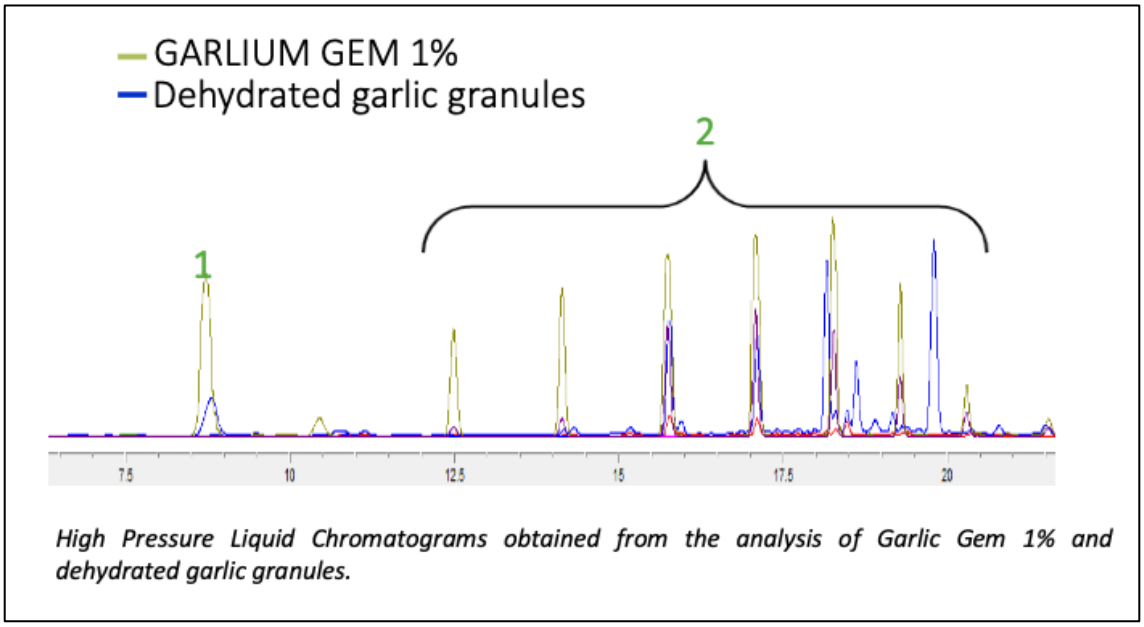

Figure 2.2: High Pressure Liquid Chromatogram comparison chart between oil and powder garlic (Abad *et al.,* 2105)

In the High Pressure Liquid Chromatography (HPLC) analysis the different peaks represent the compounds present and the area below each peak is directly correlated to the quantity of the corresponding compounds which are: **Diallyl sulphides** which in Garlium® GEM HC 10% there is approximately 10 times more of them than in the dehydrated garlic. **Triallyl sulphides** which in the Garlium® GEM HC at 10% contains similar actives and about 20% more of them than in the dried garlic (Abad *et al.,* 2105)

In order to complete the study, it would have been ideal to compare the these two components with crushed garlic and/or garlic powder available at supermarkets. This comparison would have given us a clearer idea of the potency and concentration of the Garlium® GEM HC 10% and whether pure garlic in its natural form would have been valid to be used in feed as a tick repellent. Moreover, more

research is needed to be done in order to compare all four components to be used as possible tick repellent in animals.

In the case of Garlium® GEM HC this product has been extensively used in Europe as a fly repellent for horses and it was the producer's decision to explore further markets and alternatives of use.

2.2.1 Chemical and active ingredients of garlic

When garlic clove is crushed, a protein enzyme called alliinase is released and it comes into physical contact with alliin, a compound present in the cell compartment creating the sub product allicin. Allicin is a highly volatile, unstable and short lived molecule, unless stored at -80°C. At ambient temperatures (20 h at 20°C) allicin is decomposed into diallyl sulphide (DAS), diallyl disulphide (DADS), diallyl trisulfide (DATS) and ajoene molecules which are the final and active products of garlic (Amagase *et al.,* 2001). Therefore, allicin is no longer present in processed foods but for marketing purposes in most cases allicin is sold as the key active ingredient (Amagase *et al.,* 2001).

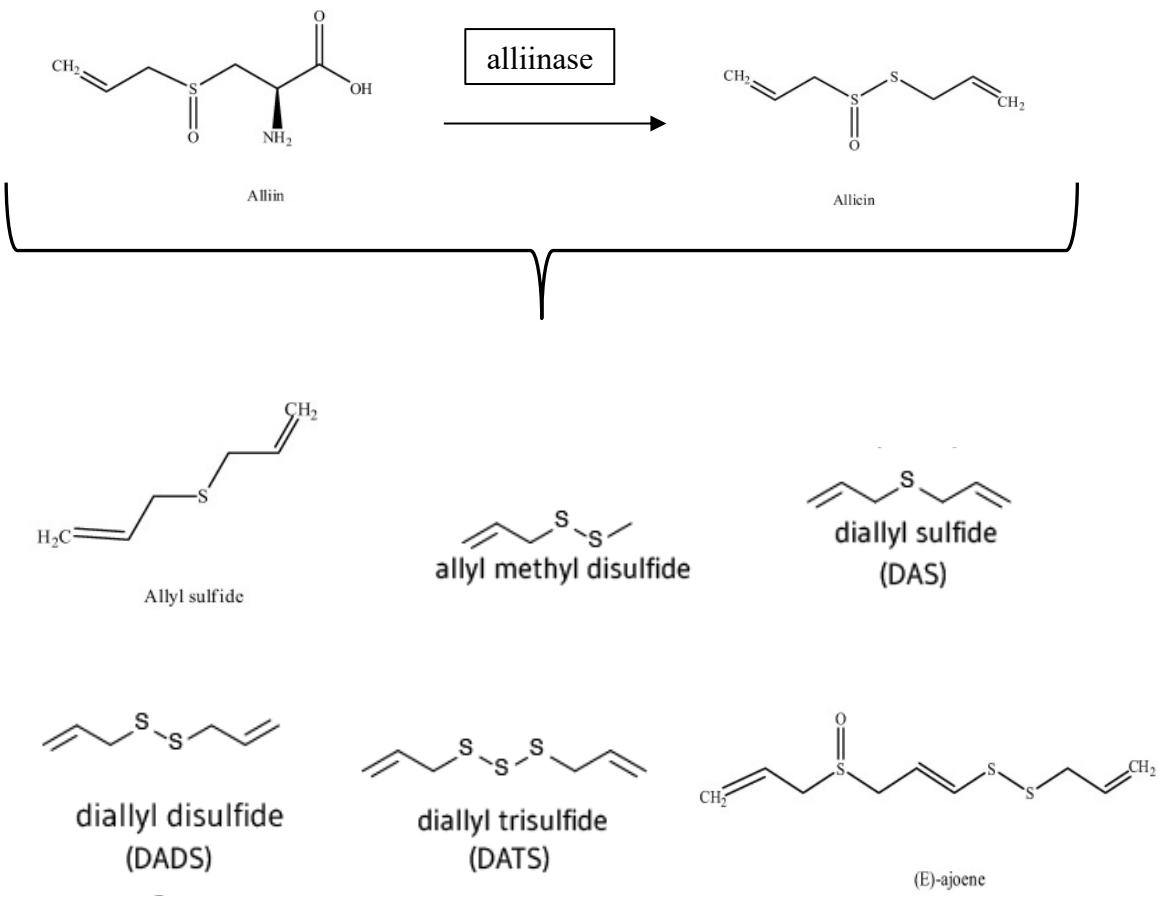

Figure 2.3: Chemical representation of garlic active components

2.2. 2 Manufacturing process of Garlic oil

Garlium® GEM HC is produced by a special production process involving extraction, distillation, concentration and filtration in order to guarantee the highest quality of garlic oil. Other extraction principals are used to isolate other essential allium compounds which are then combined with the garlic oil.

The manufacturing process of garlic oil follows certain steps like grinding, hydrolyzation, decantation, first filtration, oil extraction, final filtration and quality control. Different extraction principals are used to isolate other essential allium compounds which are then combined with the garlic oil.

The production of Garlium® GEM HC is evaluated at different points in order to comply with quality control. Aspect and colour, odour and active contents are the key points to be evaluated throughout the production process of Garlium® GEM HC (Abad *et al.,* 2015).

2.3 Why garlic ?

Garlic (*A. sativum*) belongs to the group of the *Allium* species within which we can find shallot (*A. ascalonnicum*), leek (*A. porrum*), chives (*A. schoenoprasum*) and onion (*A. cepa*) and is considered to be one of the most consumed plants in the world being widely used as spice and food additive since ancient times (Mikaiili *et al*., 2013). Originally from the Asian continent, the *Allium* species belong to the *Liliaceae* family and are also cultivated in other corners of the world like China, North Africa (Egypt), Europe and Mexico (Mikaili *et al.,* 2013). Records on the use of garlic date of 5.000 years ago where extract of bulbs were used in several folk medicine cultures (Shyma *et al*., 2014; Upadhyay, 2010).

The *Allium sativum* plants were well known to have pharmacological properties such as bactericidal, fungicidal and insecticidal effects as well as antioxidant properties among others being well documented (Huang *et al*., 2000; Kimbaris *et al*., 2009; Lanzotti, 2006; Martinez-Velázquez *et al*., 2011; Mikaili *et al*., 2013). Garlic extracts have shown to inhibit gram-positive, gram-negative and acid-fast pathogenic bacteria growth with various degrees of susceptibility (Mikaili *et al*., 2013). This effect has been shown for staphylococcus, salmonella, vibrio, mycobacteria and proteus species. (Mikaili *et al*., 2013). Ethanolic extracts of garlic showed to have

excellent inhibitory effects on bacteria such as *Escherichia coli* and *Salmonella typi* which were higher than aqueous extracts of garlic (Mikaili *et al*., 2013). Garlic extracts have shown to be effective against urinary tract infection, bacterial isolates and in a comparative study between garlic and antimicrobial drugs like cefalexin, cotrimoxazole and nalidixic acid, garlic has shown to be more effective than the above mentioned drugs (Mikaili *et al*., 2013). It is believed that the allyl methyl sulfide (AMS) compound of garlic is responsible for this effect (Mikaili *et al*., 2013). Allicin, the sub product of alliin due to the oxidative process of the enzyme alliinase, has shown to have extraordinary antifungal properties against *Candida albicans*, *Aspergillus fumigatus* and *Cryptococcus neoformans* in vitro (Khodavandi *et al.,* 2010; Mikaili *et al*., 2013).

Allicin, one of garlic's main by-product, is a powerful natural antiparasitic and acaricidal molecule with proven effects against *Schistosoma mansoni*, *Plasmodium falciparum* and *Trypanosoma brucei* (Lima *et al.*, 2011; Mikaili *et al*., 2013; Waag *et al.,* 2010). It is believed that the diallyl trisulfide molecule, which is a stable end-product of allicin is responsible for the antiparasitic and acaricidal effect of garlic. Garlic's acaricidal effects have also been shown in a number of ticks like *Rhipicephalus (Boophilus) microplus*, *Boophilus annulatus*, *Ixodes ricinus L*, *Hyalomma marginatum rufipes* and *Rhipicephalus pulchellus* just to mention a few (Aboelhadid *et al*., 2013; Nchu *et al*., 2005; Thorsell *et al*., 2006; Martinez-Velázquez *et al*., 2011). Extract preparations with garlic as one of the main components have shown to have effective anthelminthic activity against cestodes (*Hymenolepis diminuta* and *Taenia taeniaeformis*) and trematodes (*Fasciola hepatic* and *Echinostoma carponi*) (Mikaili *et al*., 2013).

It is well known that there is a high incidence of vector borne diseases in the African countries affecting both human and animal health. Mosquitos are the biggest insect group transmitting many of the most deadliest diseases to humans and animals (Gubler, 2009; Tolle, 2009; Ruter 2008). Attempts to control these insect populations using acaricides, insecticides and pesticides have become increasingly ineffective as a result of drug resistance. In 2010, Kalu *et al.,* studied the larvicidal effects of garlic in an attempt to control the *Culex quinquefasciatus* population in Nigeria, being the main cause of human filariasis (*Wuchereria bancrofti)* in the country (Kalu *et al*., 2010). Results showed that alcohol extracts of garlic were effective against the *Culex* mosquito (Kalu *et al*., 2010).

Similarly, garlic has also shown good viricidal effects against a wide range of viruses. Human viruses like *Cytomegalovirus* (HCMV), influenza B virus, *Herpes simplex* virus type 1, *Herpes simplex* virus type 2, *Parainfluenza virus* type 3, *vaccinia virus*, vesicular stomatitis virus and *Human Rhinovirus* type 2 have shown to be sensitive to garlic extracts (Mikaili *et al*., 2013; Weber *et al.,* 1992). Other studies showed that garlic's antiviral effect seen in humans is secondary to a direct toxic effect of garlic on the viruses (Abdullah *et al.,* 1988). Furthermore, garlic seems to enhance NK-cell (natural killer cell) activation to aid in the destruction of virus-infected cells (Abdullah *et al.,* 1988).

In humans, garlic has proven to produce anti-atherosclerosis effects, working well as a blood lipid and sugar modulator, being widely used in the treatment and prevention of cardiovascular diseases and as an immune system stimulator (Gao *et al.,* 2013). Moreover, in recent years the organosulfur compounds of garlic were observed to exhibit anti-carcinogenic effects on different types of tumours reducing

their incidence (Gao *et al.,* 2013). Epidemiological studies have shown that the incidence of colon, breast, lung, prostate and stomach tumors could be reduced with an increased dietary intake of garlic (Gao *et al.,* 2013). There are other positive effects of garlic on human health and these have been described as antioxidant, anti-hypertensive, anti-diabetic and hepatoprotective just to mention a few (Amagase *et al.,* 2001; Gao *et al.,* 2013).

The chemical composition of garlic is given by diallyl trisulfide (33.57%), diallyl disulfide (30.93%) and methyl allyl trisulfide (11.28%) (Amagase *et al.,* 2001; Martinez-Velázquez *et al*., 2011). It is mainly the organosulfur compounds like the diallyl di- and trisulfide that are the determinants of garlics' acaricidal properties (Martinez-Velázquez *et al*., 2011). These allyl sulfides (organosulfur) compounds, when oxygenated turned into allicin. Allicin is a poor water miscible and very volatile molecule which is responsible for the typical garlic odour (Ankri & Mirelman, 1999; Amagase *et al.*, 2001). As allicin undergoes oxidation, it forms the diallyl disulfide and trisulfide molecules. These secondary plant metabolites act as a defense mechanism against insects in herbivores (Ankri & Mirelman, 1999).

Although garlic benefits are well known and documented, it should be noted that feeding garlic to animals in large amounts and for long periods of time could cause detrimental effects (Saastamoinen *et al*., 2019). Several documents have shown that garlic could have detrimental haematological effects in the red cell line of horses, dogs and sheep (Hu *et al.,* 2002; Stevens, 1984; Saastamoinen *et al*., 2019). In horses, feeding of 32 mg/kg of body weight (BW) of garlic for 83 continuous days produced a decline in haemoglobin (Hb), haematocrit (Hct) and red blood cells (RBC) values (Saastamoinen *et al*., 2019). Moreover, this slight

anemia was associated with Heinz bodies resulting as a consequence of oxidative damage to the red blood cells RBC. These effects have also been observed and reported previously in both dogs and sheep with the prolonged treatments of garlic (Saastamoinen *et al*., 2019; Hu *et al*., 2002; Stevens, 1984).

2.4 Why *Rhipicephalus evertsi evertsi*?

Rhipicephalus e. evertsi commonly named, the "red legged tick", is one of the 896 tick species found around the world and responsible for the transmission of different diseases in wild and domestic ruminants. Ticks fall within 3 different families called the Nuttalliellidae family, the Argasidae family (or soft ticks) and the Ixodidae family (or hard ticks) in which *R. e. evertsi* is included. (Jongejan & Uilenberg, 2018). *R. e. evertsi* is easily identified by a medium size brown-dark body colour and an orange to red colouration of the legs (Jongejan & Uilenberg, 2018).

The red legged tick parasitizes a variety of hosts from domestic and wild equids like horses, mules, donkeys, and zebras to cattle, sheep, goats and wild ungulates (Jongejan & Uilenberg, 2018). There are several wild antelope species that are susceptible to the invasion of *R. e. evertsi,* among them the African buffalo (*Syncerus caffer*), kudu, wildebeest (*Connochaetes taurinus*), nyala (*Tragelaphus angasii*), reedbuck (*Redunca arundinu*), blesbok (*Damaliscus pygarus*), bushbuck (*Tragelaphus scriptus*) and impala. Eland antelope appears to be particularly vulnerable to *R. e. evertsi* infestation (Horak *et al*., 1992; Horak *et al.,* 2007; Clift *et al.,* 2020; Steyl *et al.,* 2012). *R. e. evertsi* is classified as a typical two-host tick with the larvae and nymphs usually feeding on one host animal while the adult stages feed on a different host (Jongejan & Uilenberg, 2018; Walker *et at.*, 2000). Immature

stages prefer feeding on hares (*Lepus saxatilis*) (Norval, 1981). Different stages of the red legged tick have predilection for different animal body sites (Jongejan & Uilenberg, 2018). Adult *R. e. evertsi* are normally found in the perineum and inner thigh areas of the host while larvae and nymphs prefer feeding deep in the ear canals (Jongejan & Uilenberg, 2018).

While the majority of the Ixodid tick species have a three-host life cycle, the red legged tick is one of the exceptions to the norm. During the two-host life cycle of the *R. e. evertsi,* larvae molts to nymph on the first host (Jongejan & Uilenberg, 2018). Thereafter, the nymph will engorge on a blood meal, will detach, fall off and molt again to become an adult male or female tick (Jongejan & Uilenberg, 2018). Adult ticks will wait patiently for the second host to pass and they will approach by ambushing and climbing to finally attach to the host. De Waal & Potgieter (1987) were the first to document the occurrence of transstadial transmission of *Babesia equi* (recently reclassified as *Theileria equi*) from an immature stage to the adult stage of the red legged tick. This event helped to clarify one way of infection and transmission of a hemoprotozoan to susceptible species through tick vectors (De Waal & Potgieter, 1987).

Studies done by Rechav *et al* (1977) at Rhodes University have identified the different stage periods for the red legged tick. *R. e. evertsi* has an average egg production of 10 400 eggs per cycle and its peak of production is reached on the 3rd day of oviposition (1 107 eggs/female) (Rechav *et al.,* 1977). Comparatively, similar values were also reported for *Hyalomma anatolicum anatolicum, Amblyomma americanum*, *Dermacentor variabilis*, *Rhipicephalus appendiculatus*, *Amblyomma hebreum* and *A. cajennense (*Rechav *et al.,* 1977). The larval and nymphal feeding

period averages around 17 days at 26°C, while the feeding period of larvae (usually on the inner side of the ears) is between five to eight days and the premolting stage on the host takes another four to five days (Rechav *et al.,* 1977). The larvae normally feeds slowly during the first two days of attachment, followed by a rapid increase in feeding and weight on the following days until day 6 when they molt to pharate nymphs (Rechav *et al.,* 1977). During the following days nymphs will continue to feed until they are replete and gain rapid increase in weight. There is a period of two days were both immature stages appear to be present together on the host. From day 11, larvae have moulted to nymphal stages and they are no longer present on the host (Rechav *et al.,* 1977).

The red legged tick is present in the sub-Saharan African continent and it is the most widespread of the *Rhipicephalus* spp. (Jongejan & Uilenberg, 2018). It is most commonly found in the eastern side of the African continent and it runs from Eritrea and Sudan in the north of Africa to South Africa in the south. It adapts to most climatic conditions with the only limiting factor being dryness and aridity with rainfall levels below 250-180 mm/annum (Jongejan & Uilenberg, 2018), such is the case of the western areas of South Africa.

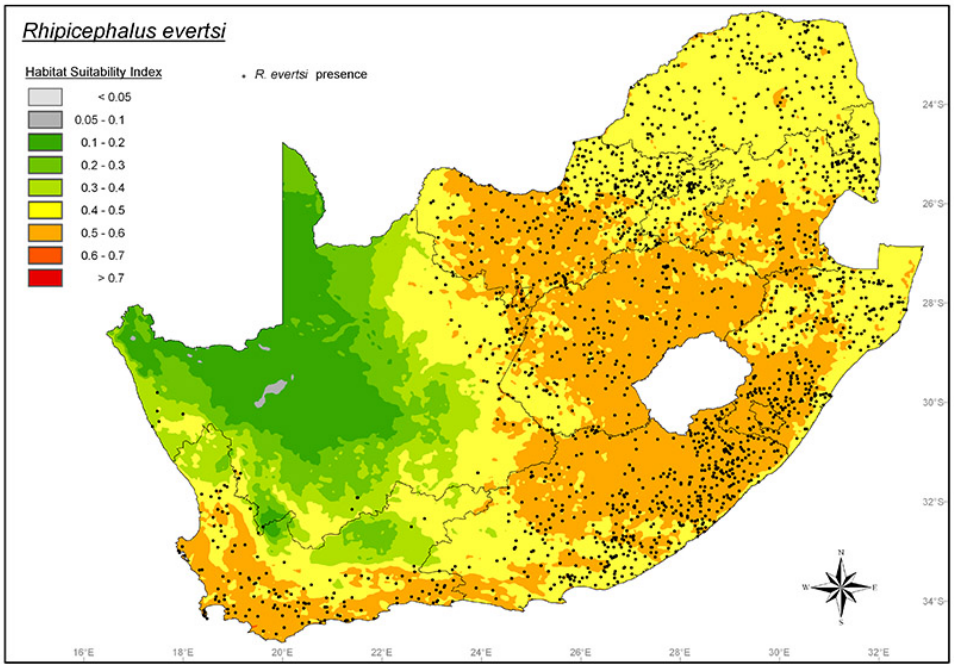

Figure: 2.4 Geographic distribution map of *R. e. evertsi* in South Africa and according to habitat suitability. Black dots represent 2.787 confirmed localities (www.Anipedia.org).

The red legged tick is the main vector of several important diseases occurring in South Africa. *Anaplasma marginale* being the cause of bovine anaplasmosis, *Babesia caballi* and *Theileria equi* the cause of equine piroplasmosis, *Theileria separata* the cause of benign ovine theileriosis, *Borrelia theileri* the cause of Borreliosis or spirochaetosis and the toxin causing spring lamb paralysis (Jongejan & Uilenberg, 2018). *R. e. evertsi* has also been responsible for transmitting Theileria sp. (sable) to sable and roan antelope (Clift *et al.,* 2020; Steyl *et al.,* 2012). According to studies done by Steyl *et al* (2012), the red legged tick could even be the main vector for the transmission of Theileria sp. (sable). This study comparing the effects of two different tick hosts (*R. appendicualtus* and *R. e. evertsi*) in roan antelope demonstrated that the first organ affected was the anorectal lymphnode that drains the perianal and tail region of the animal (Steyl *et al.,* 2012; Clift *et*

*al.,*2020). Such body area corresponds to the common location of the attachment of *R. evertsi* on the host, confirming the early transmission of the disease (Steyl *et al.,* 2012; Clift *et al.,*2020).

On the other hand, in the eastern regions of the highveld in Mpumalanga and the Free State, there is a high rise of adult stages of *R. e. evertsi* during spring time coinciding with the sheep lambing period. The adult ticks infest the lambs at a young age inoculating them with excessive salivary toxin that produces paralysis and causing the condition known as “spring lamb paralysis” in South Africa (Jongejan & Uilenberg, 2018).

Although there are no records informing of springbok being predisposed to any red legged tick associated disease, they are known to be naturally infested with the *R. e. evertsi*. Springbok are one of the most abundant antelope species in South Africa and they are listed as least concerned by the IUCN (International Union for Conservation of Nature) (Furstenburg, 2006; Hoffmann, 2014) with an increasing population trend. They are an easy species to work with and easily adaptable to captivity making them ideal for this type of research.

2.5 Problems

2.5.1 Hypothesis:

Garlium® GEM HC repels red legged ticks (*Rhipicephalus evertsi evertsi)* in sub-adult springbok. Garlium® GEM HC is acaricidal against *R. e. evertsi* in sub-adult springbok.

2.5.2 Benefits of the study

With constant increase and expansion of the wild animal industry together with the abuse and miss use of large animal parasitical dewormers, researchers and pharmaceutical companies are required to develop newer and more effective products for the constant farm animal management demand. Indiscriminate “off label” use of the domestic ruminant parasiticides in the wild ruminant population is generating parasitic resistance in these populations. Viability of alternative antiparasitic options, like in this study, requires further investigation to provide a foundation for future research and development.

Sooner or later, the farming industry will have to move into a more ecofriendly direction in order to re-establish the ecosystem balance between, animal-environment-biology, producing a healthier animal population and allowing nature to select according to fitness and resistance.

2.5.3 Research aim and objectives

Allied Nutrition in collaboration with University of Pretoria (Faculty of Veterinary Science, Onderstepoort), Cap-chur and MS Trading aimed to conduct a trial to confirm if the Garlium® GEM HC has a repellent effect on ticks in wild herbivores. This was a pilot experimental study with a low number of animals in a controlled environment (boma or enclosure).

The aim of this study was to determine whether Garlium® GEM HC has a repellent effect on red legged ticks (*R. e. evertsi*) in springbok. Secondary aims to the

repellency of the product are, body weight and haematological evaluation as determinants of springbok's wellbeing and health. Fecal egg counts were done as a mere control of the parasitic status of the antelope.

CHAPTER 3

MATERIALS AND METHODS

3.1 Model system and justification of the model

Springbok *(Antidorcas marsupialis)* was chosen as a model for this study. The reasons for this choice included the following: springbok, unlike many other antelope species, are not listed as a threatened or endangered species. Secondly, they are easily found in the vicinity of Pretoria, adapt well to captivity compared to other antelope species and are relatively easy to handle. Moreover, the commonly known red legged tick (*R. e. evertsi)* has a special attraction and predisposition for this specific antelope. Its localization in the animal is mainly in the perianal area where the skin is thinner, facilitating their attachment. All these reasons place no threat to the species itself from a conservation point of view.

The aim of this project conducted between February and April of 2017 (University of Pretoria Ethics Committee Approval number v047-14 AEC) was to determine whether the studied product had a repellent effect on the red legged tick.

This trial provided useful information for both the cattle and wild animal ruminant community benefiting the health management and possibly posing an ecofriendly solution to the game farmer. Garlium® GEM HC is easily feed presented as a powder product can be easily feed to animals, most specially game animals being kept in intensive breeding camps where they get feed supplemented.

R e. evertsi is one of the most widespread tick species distributed thorough out the Afrotropical Region. It is a two host tick and under ideal weather conditions more than one cycle can be completed in one year. Immature stages are normally located deep down the inner side of the ear canal and on the pinna of cattle, goats and small wild ruminants. Hares are also hosts of this immature stages of *R e. evertsi*. However, adults stages preferer wild and domestic equids as well as eland antelope. Contrary to immature ticks, adult ticks prefer to attach to areas like under the tail around the anus, in the groin, axillae and sternum areas of ruminants.

Larvae that are attached to the ear canal of a host normally feed for five to seven days. They molt in the host to nymphs that also feed for 5-7 days. Later on the nymphs detach from host and spend the winter in the molting stage waiting for the next host. Adults emerge in spring and they attach to a new host as they pass by them. Replete adult females attached to new host will feed for about 5-7 days and will detach and drop to the ground were they will lay approximately 7 000 eggs after a month.

Tick transstadial succession can be used to examine the effects of tick acaricides and tick repellents by looking at weight, mortality and success in maturation. In the following study ticks were classified in un-engorged ticks (nymphs -8 legs), engorged ticks (nymphs -8 legs) and larvae (6-legs). Although ticks were classified the main interest was focused on the total weight of ticks per animal in order to assess the repellent effect of the Garlium® GEM HC.

3.2 Experimental Design

Twenty-four springbok were captured from the wild in February and March of 2017 by chemical immobilization and where transported from Westlake Country Estate in Hartbeespoort Dam, North West province to Boekenhoutskloof, a farm near Cullinan, Gauteng. Although the project was held at the end of the rainy season there was no interruption due to rainy weather.

Animals were randomly allocated in two different groups of 12 animals each in two separate enclosures. Minimal amount of 10 animals per group were necessary for the following study in order to obtain results that were statistically significant. A CG and a GG were formed and given a 15 day adaptation period before commencing the trial. One week (Day -7) before the commencing of the trial, the GG started to receive Garlium® GEM HC in the feed. The reason for this was to make sure the animals adapted well to the Garlium® GEM HC and secondly to allow the Garlium® GEM HC to be in the animal's system by the time the ticks were introduced.

Once the animals were adapted, they were restraint in a custom designed, cushioned wild animal crush. This hand restrained was done on three different occasions (Day 0, 7 and 17), where they received different treatments. Springbok were scored on their body condition (BCS) and the ones that had not adapted properly to boma confinement were removed from the trial and returned to the wild. Three springbok were needed to be removed due to mal-adaption and coccidiosis infection. One animal from group B (B3) and two animals from group A (A9 and A12).

The trial commenced on Day 0 – intervention day (two weeks post adaptation). On that occasion, springboks were placed in the crush where they were hand restrained and weighed (Appendix C). Soon thereafter ear tags were place with identification numbers on them and blood samples were collected. On that same day, faecal samples for faecal egg count were collected from both groups to assess their endoparasite status as part of the health status evaluation of the groups.

Once all measurements were done and bloods drawn, each animal was hold in sternal position while ear bags were attached and ticks inoculated. To keep track of each animal's ID every animal was marked with a black water proof marker on the top of both ears bag with the same ear tag number that was given. Ear bags were firstly glued to the base of the ear with a contact adhesive (GEMKEM) to avoid ticks to crawl out of the bags and to keep the bags steady and secured. Once the ear bags were attached, 250 *R. e. evertsi* larvae were placed inside the ear bags in each of the ears (Figure 3.3). Each animal was infested with a total of 500 larvae making a total of 6 000 ticks per group. Ear bags were sealed with an elastrator band on the open end to prevent the ticks from escaping.

Intake of Garlium® GEM HC was monitored daily per group and springbok's weights together with body condition were recorded each time the animals were hand restrained (Appendix D).

On day seven, both groups were hand restrained to check on ear bags and to collect blood for blood parameter evaluation.

On day 17, all animals were hand restrained for the last time and blood was collected. Ear bags, ear tags and ticks were removed from the animals and placed in Ziploc plastic bags for tick screening at the laboratory. As ear bags were removed some of the hair came out with the glue of the ear bags. No adverse allergic reactions were notices with the ear bags throughout the 17 day study. All recovered ticks, dead and alive as well as juveniles and adults from the ear bags were preserved in 95% ethanol. Ticks were counted dead and classified according to their stadial succession (Appendix E). All animals were dipped with Bayticol dip (Bayer, Flumethrin 2%) prior to loading them for release to the veld in a game farm in Pretoria. All bomas and their surroundings were sprayed with Bayticol® (Bayer, Flumethrin 2% m/v) and F10® (Health and Hygiene (Pty) Ltd, disinfectant) a contact dip and disinfectant to avoid environmental contamination with escaped ticks.

Figure 3.1: Springbok in boma during adaptation period

Time line

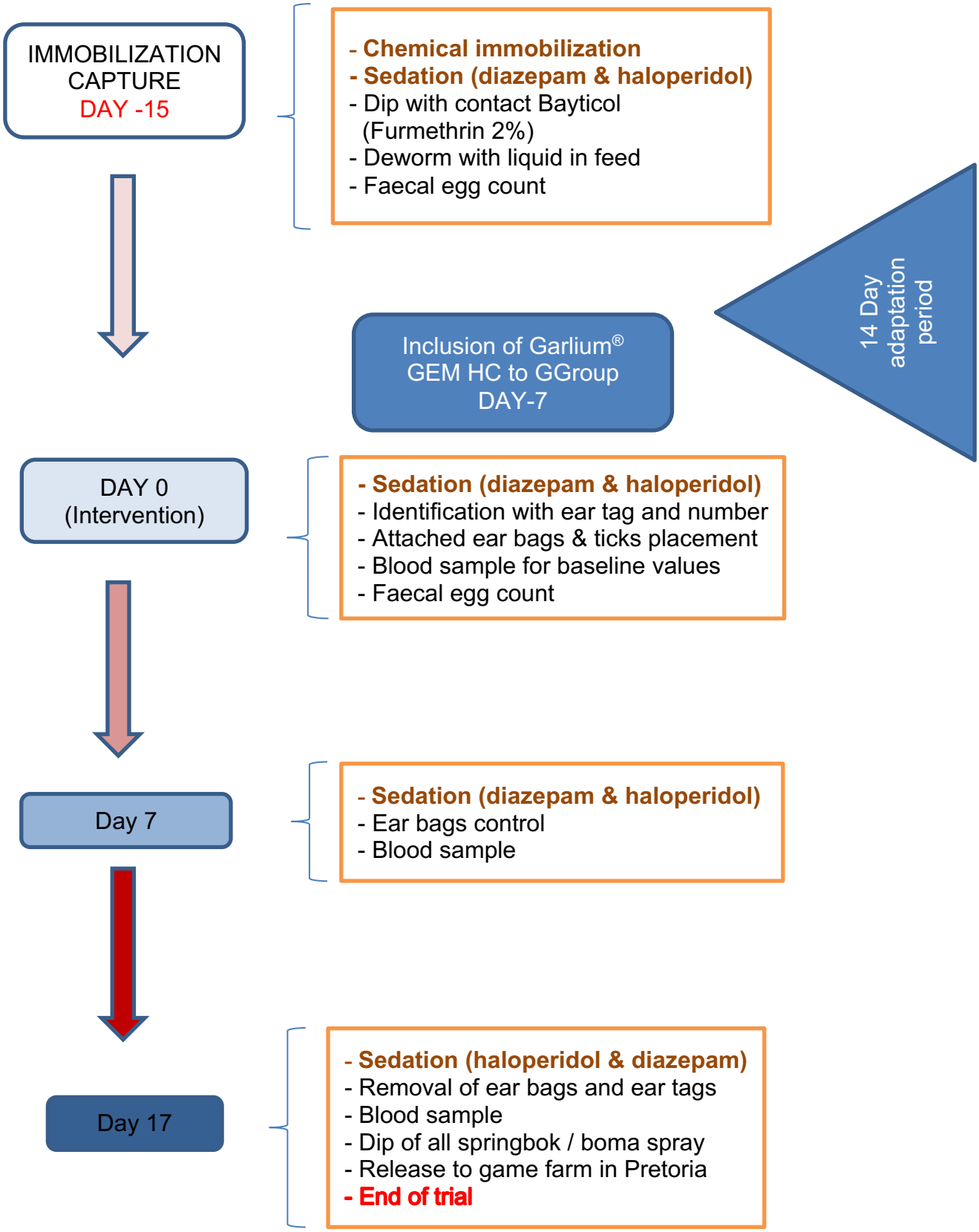

Figure 3.2: Garlium® GEM HC research project timeline.

3.3 Experimental procedures

3.3.1 Site and Subjects

The holding facilities comprised two bomas of the same rectangular shape of 15 x 5 m wide with a partition in each to facilitate handling of the animals. Both bomas were constructed of 2.4 m tall diamond mesh, covered with 100% capture shade cloth. The lower part of the boma was surrounded by conveyer belt to prevent the animals from injuring themselves. These structures were supported by 10-15cm diameter wooden poles making the entire structure sturdy to retain this antelope species.

Each boma comprised of two sections with a partition and sliding door connecting them. The smaller 5 x 5 m compartment in each boma connected to larger exercise pen through a sliding door that helped to split the animals. The smaller pen was ¾ roofed to provide the animals with some sheltering and shade. This compartment provided the animals with two feeding troughs. A single water trough that was cleaned on a daily basis was constructed in the corner of the exercise pen.

The flooring of the bomas, comprised natural soil, and was cleared from vegetation. This area was cleaned every second day to remove faeces.

3.3.2 Tick breeding and processing

Engorged red legged tick females were collected from domestic ruminants located in the Cullinan area and were relocated to the pathology laboratory of the Faculty

of Veterinary Science, UP, to be bred further under laboratory conditions. The *R. e. evertsi* were housed in a homemade acaridarium containing a sodium chloride water bath at 26°C ambient temperature with 85% relative humidity. Following oviposition, a sample batch of ova was weighed and counted. The equivalent weight of ova representing 250 eggs were separately aliquoted in Eppendorf tubes with the openings plugged with cotton wool. These tubes were left in the acaridarium for the eggs to hatch and larvae to mature prior to transfer to ear bags of springbok.

3.3.3 Animal trial

3.3.3.1 Pre-trial capture of springbok (Day -15)

Springbok were captured from the wild by a specialized game capturer (Pretoria Wildlife Services) by darting using thiafentanil oxalate (1 mg / animal / IM, A3080, Wildlife Pharmaceuticals, RSA) and azaperone (10 mg / animal / IM, Stresnil, Kyron Laboratories, Johannesburg, South Arica), followed by sedation with diazepam (5 mg / animal / IV, V-Tech, RSA) and haloperidol (5 mg / animal / IV, V-Tech, RSA) for translocation. The use of tranquilizers assisted in the boma adaptation of springbok.

During immobilization, springboks were weighed and rectal temperature together with clinical parameters (temperature, heart rate, respiration and mucus membranes) were recorded. Blood samples were taking for full blood analysis at the Onderstepoort Clinical Pathology Laboratory. All springbok were dipped with a contact dip with no residual action using Bayticol (Flumethrin 2% m/v). The Helminthology Section at the Faculty of Veterinary Science at UP did a faecal egg

count analysis in order to have baseline information about the springbok's endoparasitic and health status.

Upon arrival in the boma they were randomly allocated into the different GG, they were fed hay, lucerne and antelope cubes and were given water *ad libitum*. Both groups were fed the same diet from day -15 onwards. On day -7 before commencing the trial the Garlium® GEM HC was added the GG. This period was used as an adaption period to the change in diet from veld to a Total Mix Ration (TMR) and to allow the researches to verify that the springbok were incorporating the feed adequately. Daily feed intake from each of the groups was recorded for later analysis and comparison.

A Garlium® GEM HC powder compound from Pancosma /Allied Nutrition was added at 0.36% (2.5 kg of Garlium® GEM HC premix) in a 700 kg game feed meal composition (Table 3.1). This same feed formulation was used in the GG throughout the adaptation period from day -7 onwards and trial period. Conversely for the CG the same game feed meal composition was used but without the Garlium® GEM HC premix to feed the animals.

3.3.3.2 Animal restraint, placement of ear bags and ticks – Intervention day (Day 0)

Following the adaptation period the trial commenced on Day 0 (intervention day). On that occasion, the springboks were hand restrained in a methodical way.

Springbok were herded from their pens through a common passage to a cushioned crush. As soon as they were inside the crush, each was hand caught and restrained by farm workers to ensure the animal did not injure itself or handlers. Once restrained, blood samples were collected from the jugular, cephalic or saphenous vein. Thereafter, the springboks were sedated with diazepam (5 mg / animal / IV) using any of the accessible veins. Blood samples were drawn and stored immediately at 4°C in a portable cooler box and were submitted to the clinical pathology laboratory at the Faculty of Veterinary Science, Onderstepoort to be processed and analyzed for routine haematology and serum biochemistry parameters. The requested parameters were haemoglobin, red cell count, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, red cell distribution width, white cell count, segmented neutrophil, band neutrophil, lymphocyte, monocyte, eosinophil, basophil and platelet count.

The animal's body weights were individually recorded in order to assess their body weight gain/loss and body condition over the course of the study. As each animal was hand restrained the handler stepped on the scale and the weight was recorded in full for both. Later on the handlers weight was subtracted from the previous number obtaining the right weight for each springbok.

Once the clinical procedures were finalized, the ear bags on each springbok's ear were adapted and attached. Ear bags were composed of two fabric layers. A first and inner layer made of white cotton fabric made of a tight thread making it impossible for the immature ticks to crawl out of the bag (Figure 3.3 and 3.4). A second layer was placed on the outside and covering the inner layer of the ear bag

and was adapted from a brown plastic sheet material which would make the ear bags water proof (in case of rain) and will also assist the inner layer in avoiding the ticks to crawl out of the ear. As the white cotton fabric bag was introduced on top of the ear covering it completely, the base was glued all along the ear base to facilitate its attachment to the springbok's head (Figure 3.3). The second layer was also glued to the first layer and reinforced with Elastoplast tape (25 mm x 3 m Elastic Fabric, Elastoplast®) to keep it in place and preventing the springbok to take them off by shaking their heads. None of the springbok tried to scratch off the ear bags with their hooves and ear bags were very well tolerated by them.

Once ear bags were placed and attached the amount of 250 larvae ticks were introduced into the white cotton fabric bag. Ticks were contained in a Eppendorf tube with a small piece of cotton wool for their attachment which was introduced into the ear bag together with all the ticks in the Eppendorf tube. Once in, both inner and outer bags were closed with an elastrator rubber band to avoid ticks from crawling out (Figure 3.4).

Figure 3.3: Introduction of ticks into the ear bags

Figure 3.4: Springbok with ear bag attached.

3.3.3.3 Hand restraint for tick and ear bag control (Day 7)

On day 7 both groups (treated and control) were hand restrained for ear bag monitoring and blood sample collection. Animals were identified by a black number written on each of the ear bags on the external plastic cover (Figure 3.4). All animals were bled upon restraint in exactly the same way as done on Day 0 to obtain a complete set of venous blood samples for haematology determination. Thereafter the springboks were sedated in order to minimize stress, and the correct placement of ear bags was evaluated. Each springbok's weight was also recorded during restraint.

3.3.3.4 End of trial (Day 17)

The springbok were hand restrained for the last time in the same way as on Day 0 and 7. A final set of blood samples were taken in the exact same way as previous days and from the exact same sites. Body weight was recorded by carrying the springbok and standing on the scale (PURE PLEASURE, Model ZABSM01BK) with the handler. Both weights were recorded and then the handler's weight was subtracted to the total weight. Thereafter, springbok were sedated with diazepam with the exact same doses as used on previous days.

Ear bags and ear tags were removed from the animals. Detached ticks found in ear canal and ear pinna were collected into 50 ml plastic urine sample containers marked with the animals ID and preserved with 25 ml of 95% ethanol for further analysis. Any remaining attached ticks in the ear canal were removed from it using curved forceps and scalpel blade handle. The ticks were also deposited into the

ethanol container containing loose ticks. Once removed, the ear bags were placed inside a sealed Ziploc® bag marked with the animal ID for further processing of the contents under laboratory conditions. Consequently, all ticks were divided into the following categories engorged, un-engorged, nymphs and larvae. Total tick count and weight was recorded for each animal. No classification between dead and alive was done but all dead ticks engorged, un-engorged, nymphs and larvae were classified and counted for each antelope.

Before loading them in the truck for transport and release them back into the wild, their ear tags were removed and the animals were dipped with Bayticol® to remove any remaining tick.

Once the springbok were dispatched all bomas were sprayed and disinfected as a biosecurity measure with Bayticol® and F10® and was left to rest for 10 day before introducing new animals.

3.3.3.5 Sample collection

General clinical monitoring

Animals were scrutinized for superficial injuries that may have been sustained during physical restraint and treated if required. Special clinical attention was taken to the ears and ear canal, where the ear bags were attached. Ears and ear canals were clinically assessed in case there was any sign of ear infection or excessive and/or abnormal ear wax (cerumen) production. None of the animals presented any

skin reaction to the cement (glue) used to attach the ear bags to the antelope's ears. None of the animals suffered any injury regarding the attachment of the ear bags.

Additionally the presence of mucus secretion and/or diarrhoea was assessed individually as well.

Body condition and weight measurements

Body state was determined using the Body Condition Scoring system for Sheep (BCS) (Thompson & Meyer, 1994) where condition 1 is emaciated (BCS1), condition 2 is thin (BCS2), condition 3 is average (BCS3), condition 4 is fat (BCS4), condition 5 is obese (BCS5). Each animal was weighed each time they were hand restrained by using a manual step-on scale to weight gain/loss (PURE PLEASURE, Model ZABSM01BK). Handler and carrying springbok would step on the manual step-on scale and the total weight would be recorded. Later on the handler's weight was subtracted form that number. Feed intake was monitored daily by weighing the remaining feed from the previous day.

Venous blood samples

One 4 ml EDTA® (ethylylenediaminetetra-acetic) sample tube was filled with blood from any of the accessible veins (jugular, saphenous and / or cephalic) and was stored at 4°C for haematological analysis. Two blood smears were made from this sample and were kept for examination at the Clinical Pathology Laboratory of the Onderstepoort Veterinary Academic Hospital of the University of Pretoria.

For haematology, an ADVIA 2120 device (Haematology System, Siemens, South Africa) was used.

Two 10 ml red serum tubes were filled with blood from any of the accessible veins (jugular, cephalic or saphenous) for serum biochemistry analysis. These samples sored at 4°C and left to clot for 30 minutes before being centrifuged (Hettich zentrifugen, model D-78532 Tuttlingen) at 2000g for 8 minutes. The serum was aliquoted into 2 ml cryovials with screw tops and then frozen at the clinical pathology laboratory of the Faculty of Veterinary Science, Onderstepoort in a -20°C freezer.

Faecal egg counts were performed by the technicians at the Helminthology Section from the Faculty of Veterinary Science, Onderstepoort using the McMaster Egg Counting Technique (Vadlejch, *et al.*, 2011).

3.4 Animal Feed

The feed was formulated by a nutritionist from CAP-CHUR feeds (Table 3.1). The main components of the springbok diet was alfalfa, hominy chop, molasses meal, salt and the Garlium® GEM HC mix. Feed consumption per group was calculated by subtracting the remaining feed weight from the daily inclusion level each day. A fresh batch of feed was provided every day. Batches of mixed game feed with Garlium® GEM HC are arranged in 700kg being this one the minimum batch size in order to get a proper mixed Garlium feed composition.

Springbok were fed ad libitum at about 1kg of premix per day. For the GG this makes it a total amount of 13kg / day which in 26 day trial will add to 338 kg. The CG was

fed exactly the same feed but without the adding of the 2.5 kg of Garlium premix. Control group which only had 12 animals in it was fed a total amount of 312kg throughout the 26 day trial (12 kg / day).

The animals were fed by caretakers who were specially trained to add the right amount and to weight all the left over from the previous day. All data was recorded.

Table 3.1. The game feed meal composition with Garlium® GEM HC premix

Material	Kg inclusion (As is)	Inclusion level (%)
Alfalfa Hay	300	42.86
Hominy chop	300	42.86
Molasses meal	40	5.71
Full fat soy	50	7.14
Feed lime	2.5	0.36
P12	2.5	0.36
Salt	2.5	0.36
Garlium premix	2.5	0.36
Total	**700**	**100**

The Garlium® GEM HC premix consisted of 500 g Garlium® GEM HC and 2 kg maize meal.

Control springbok were fed the same exact feed meal composition but without the Garlium® GEM HC premix. 2 kg of maize meal was added to this groups feed in order to compensate the extra calories included in the GG by the adding of the premix.

3.5 Laboratory procedures and tick counting

Ticks attached to the ears (pinna and ear canal) and ticks that fed but dropped off into the ear bag were counted, weighed and classified. Tick classification was done based on the number of ticks inside each bag, the number of ticks collected in containers which were classified in nymphs (8 legs), engorged nymphs (8 legs) and larvae (6 legs) for each springbok (Appendix E). All ticks were dead by the time they were counted.

3.6 Data analysis.

After careful data evaluation, the two group means were tested for differences between mean effects using the two-sample t-test for normal data and heterogenous variances. Significance at the 5% level was declared when the test probability was $p < 0.05$. Data were analyzed using the statistical program GenStat® (Payne, 2015). A non-parametric Mann-Whitney test for the blood samples was performed to compare blood parameters between GG and CG before and during trial.

Boxplots were generated using SaTScan version 9.6, a shareware program.

CHAPTER 4

RESULTS

4.1 Animal feed intake and live weight results

Animals were weighed on different sampling days throughout the trial (Table 4.1). Although the animals were randomly allocated and after the intervention Day 0 when animals were hand restrained and weighted, it was noticed that the garlic or treated GG group were slightly heavier than the CG. This difference was however, not significant, (P = 0.09) but as expected, the slightly heavier animals (Garlium® GEM HC group) did consume more feed during the trial (Table 4.2). No significant negative impact of Garlium® GEM HC on feed intake could be detected. However, when evaluating the average total weight for each group, the Garlium® GEM HC group had an approximate 10% higher in body weight compared to the CG. This observation corresponds to an increase in feed consumption by the treated group as shown in Table 4.2.

Throughout the course of the trial there were three animals that died. Post mortem examination revealed coccidiosis as their cause of death in all instances due to mal-adaption.

Table 4.1 The effect of Garlium® GEM HC on the live weight of springbok. Average Total feed intake per animal per day. (Appendix D)

Parameter	Control (Kg)	Garlium (kg)	P-value
Live W (D0)	19.4	21.4	0.09
Live W (D7)	19.0	21.4	0.08
Live W (D17)	18.0	19.1	0.42
Total trial (D0-D17)	18.90	20.7	

Feed intake was also calculated as a percentage of body weight (BM). On day 7 to 14, the CG consumed 4.3% and the Garlium® GEM HC group consumed 5.2% of body weight. From day 15 to 23, the control and Garlium® GEM HC group consumed 4.4% and 4.5 %, respectively (Table 4.2).

Table 4.2 The effect of Garlium® GEM HC on feed intake of springbok

Parameter	Control (kg)	Garlium(kg)	P-value
Feed intake (Day 0 – 6)	0.84	0.99	0.04
Feed intake (Day 7 – 14)	0.82	1.11	< 0.001
Feed intake (Day 15 – 23)	0.80	0.86	0.28
Total feed intake (Day 1 – 23)	0.82	0.97	<0.01

Garlium® GEM HC fed animals had a significantly higher feed intake (26.1%) compared to the CG ($P < 0.001$). Calculations were made as follows: (100 * 0.82 / 1.11 = 73.87%), (100% - 73.87% = 26.13%). Feed intake for the whole period was also significantly higher (15.46%) for the Garlium® GEM HC group ($P < 0.01$). Calculations were made as follows: (100 * 0.82 / 0.97 = 84.53%), (100% -84.53% = 15.46%)

4.2 Tick count results

The results of the tick parameters are presented in Table 4.3. All ticks from both ears were collected and weighed at the end of the trial. In the GG the total tick weight was reduced by approximately 58% (P = 0.03; CI: 0.06 -0.15). It was observed that springbok in the GG had a significant reduction in tick load compared to the CG. The garlic group showed a decrease of approximately 56% of the number of attached ticks, which was significantly lower (P =0.02; CI: 32.9 - 57.6) compared to the CG.

At the start of the trial, ± 250 tick larvae were placed per ear bag for each animal i.e., total of 500 per animal. The amount of larvae that were both attached and in the ear bag after 17 days were reduced by approximately (43%) for the GG compared to the CG but this was not significantly different.

The stadial succession of the attached ticks were evaluated. The number of engorged nymphal stage ticks was reduced by approximately (68%) in the Garlium® GEM HC group compared to the CG (P = 0.05; CI: 5.8 - 16.7). No differences were observed in the number of un-engorged ticks between the GG and CG (P = 0.1; CI 12.7 - 34.74).

Table 4.3 The effect of Garlium® GEM HC on the different tick parameters in Garlium group and control group.

Parameter	Control - Mean	CI:95	Garlium - Mean	CI:95	P value	% change
Tick total weight (g)	0.24	0.10 - 0.4	0.10	0.06 - 0.15	0.03	58.33%
N° of ticks attached to ear (combo)	95.5	50.3 - 140.7	42.25	32.8 - 57.6	0.02	55.75%
N° of un-engorged ticks	43.7	17.8 - 69.6	23.75	12.7 - 34.7	0.11	45.65%
N° of engorged ticks	35.33	8 - 62.6	11.25	5.8 - 16.7	0.05	68.15%
N° of larvae	15.8	-10.5 - 42.1	8.91	4.71 - 13.1	0.53	43.60%

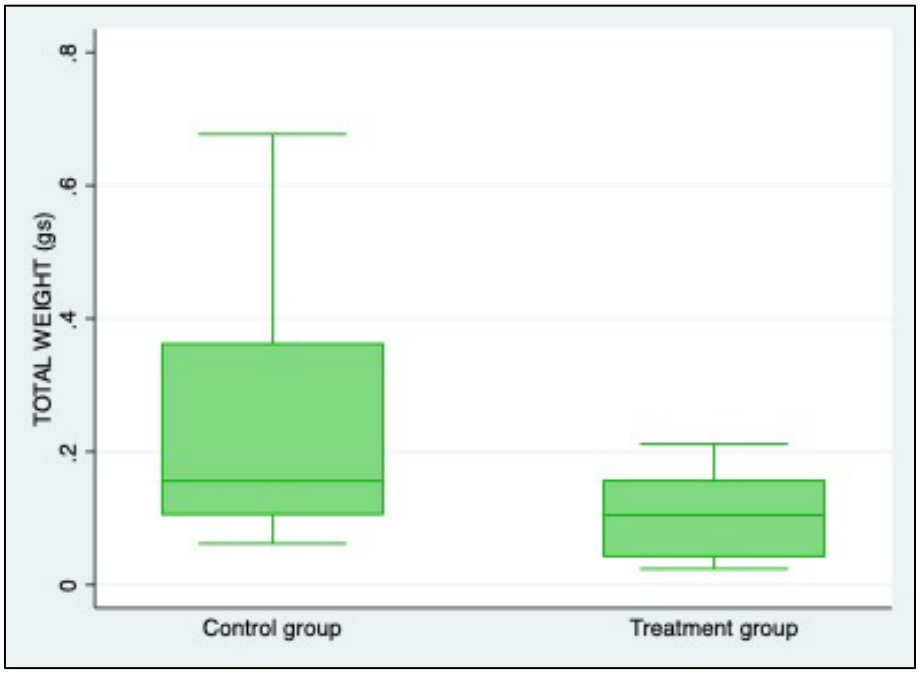

Figure 4.1: Boxplots comparing the total tick weights of 12 springbok treated with Garlium® GEM HC with 12 untreated springbok (control group).

When analyzing the total tick count in the two groups, a marked decrease was seen in the GG (P = 0.03; CI: 0.06 - 0.15) compared to the CG. This decrease in the total

tick count equated to a reduction of 56% in the GG compared to the CG (Figure 4.2).

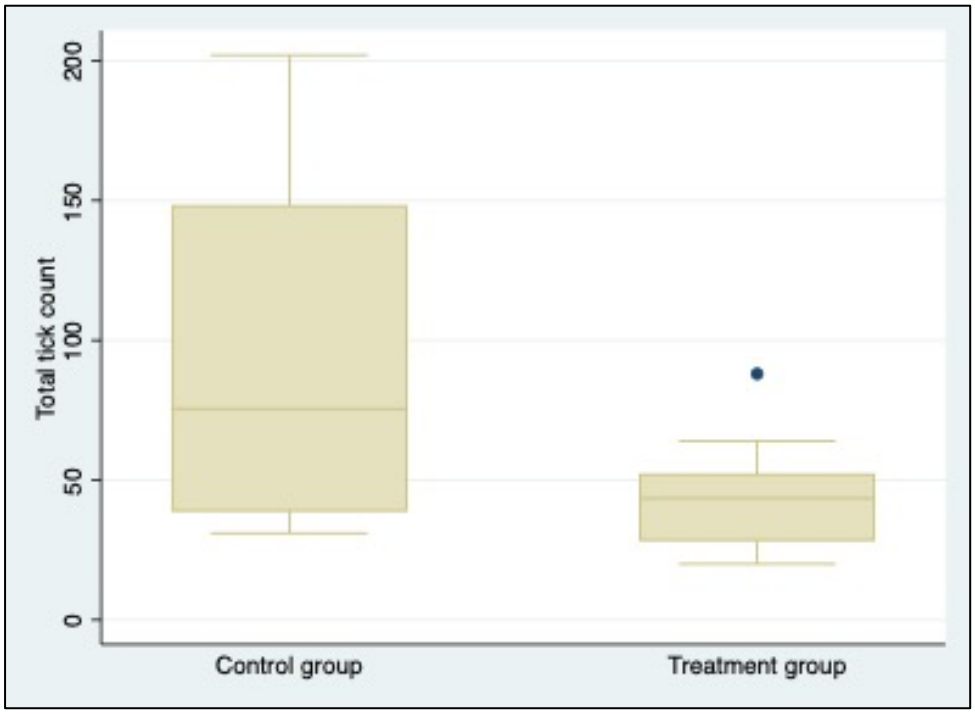

Figure 4.2: Boxplots comparing the total ticks attached to both ears of 12 springbok treated with Garlium® GEM HC with 12 untreated springbok (control group).

Ear wax from the ears was collected and weighed, an overall increase in ear wax secretion on the GG was reported (Table 4.4). However, and although not significant (P = 0.63) the inner ear wax secretion in the treated group was decreased (- 10.4%) compared to the CG while Garlium® GEM HC treated group had increased ear wax secretion (+ 40.62%) on the outer side of the ear compared to the CG. Although not significant (P = 0.24), weights of ear wax secretions were numerically higher (+ 31.46%) in the GG than in the CC.

Table 4.4 The effect of Garlium® GEM HC on the ear wax secretion in springbok

Parameter	Control	Garlium	P-value	% Change
Inner ear wax secretion (g)	0.394	0.353	0.63	-10.4
Outer ear wax secretion (g)	1.92	2.7	0.19	+40.62
Total ear wax secretion (g)	2.32	3.05	0.24	+31.46

4.3 Blood results

Blood samples were collected from all animals on Day 0 (intervention day), day 7 (day of ticks attached to ear) and day 17 (day ticks were removed from the animals) of the study. The samples were analyzed for complete blood count parameters. The results of the analyses are reported in table 4.5 and 4.6.

Only two significant differences were observed in the blood analysis. The Mean Corpuscular Haemoglobin (MCHb) concentration was slightly decreased on day 7 ($P = 0.013$) in the GG. On the other hand, no significant differences were observed on day 0 or day 17 for MCHb. Contrary, basophil levels were significantly increased ($P = 0.001$) in the GG on day 7, however no significant differences were observed in day 0 or day 17 for basophils.

Table 4.5 Whole blood analyses of springbok on day 0, 7 and 17 of the trial (part 1)

Parameter	Day	Control	Garlium	P-value
Haemoglobin (g/l)	0	184.4	176.9	0.140
	7	177.2	182.1	0.414
	17	172.3	172.0	0.969
Red cell count (x10^12/l)	0	13.70	12.97	0.100
	7	13.41	13.43	0.960
	17	13.10	12.57	0.452
Haematocrit (l/l)	0	0.525	0.515	0.190
	7	0.511	0.531	0.289
	17	0.502	0.499	0.904
Mean corpuscular Volume (fl)	0	38.6	39.8	0.250
	7	38.2	39.6	0.118
	17	38.4	39.8	0.181
Mean corpuscular haemoglobin (pg)	0	13.60	13.68	0.490
	7	13.44	13.59	0.603
	17	13.39	13.80	0.245
Mean corpuscular haemoglobin concentration (g/dl)	0	35.1	34.37	0.750
	7	35.17	34.35	0.013
	17	34.85	34.68	0.636
Red cell distribution width (%)	0	17.95	17.08	0.293
	7	17.59	17.85	0.710
	17	17.55	17.23	0.623

Table 4.6 Whole blood analyses of springbok on day 0, 7 and 17 of the trial (part 2).

Parameter	Day	Control	Garlium	P-value
White Cell count (x10^9/l)	0	5.99	5.33	0.690
	7	5.84	5.12	0.418
	17	6.63	5.92	0.422
Segmented Neutrophil (x10^9/l)	0	2.25	2.34	0.910
	7	3.32	2.64	0.423
	17	3.62	3.40	0.745
Lymphocyte (x10^9/l)	0	3.47	2.71	0.360
	7	2.13	2.00	0.592
	17	2.52	2.05	0.103
Monocytes (x10^9/l)	0	0.12	0.13	0.830
	7	0.21	0.19	0.609
	17	0.17	0.16	0.799
Eosinophil (x10^9/l)	0	0.080	0.040	0.520
	7	0.173	0.133	0.564
	17	0.211	0.154	0.559
Basophil (x10^9/l)	0	0.08	0.11	0.500
	7	0.01	0.14	0.001
	17	0.11	0.15	0.413
Platelet count (x10^9/l)	0	553.4	499.4	0.470
	7	505.7	520.4	0.781
	17	556.6	560.4	0.972

CHAPTER 5

DISCUSSION

The Garlium® GEM HC compound product from Pancosma was well tolerated in the springbok throughout the entire study and did not influence the feed intake of the animal negatively. The physical smell of the raw product is that of overwhelming garlic and on inclusion in the feed the distinct garlic smell could still be humanly detected. Garlic seemed to slightly improved feed intake, although not statistically significant. In the GG the total tick weight was reduced by more than 58%. The number of ticks that were present after 17 days (both attached and ticks in ear bag) were significantly lower for the GG (56%) than the CG. The lower tick weight could be given due to dead and disintegrated ticks or ticks that might have escaped from the ear bags. However, many *in vitro* research studies demonstrated the acaricidal properties of certain plants, of which *Allium sativum* is an example (Ankri & Mirelman, 1999; Borges *et al.,* 2011; Gao *et al.,* 2013; Martinez-Velazquez *et al.,* 2011; Mikaili *et al.,* 2013; Park *et al.,* 2006). However, there is only one study that investigated these characteristics *in vivo* in cattle (Costa-Junior & Furlong, 2011). In another study performed by Borges *et al* (2011), evaluated different types of plant extracts to determine a repellent effect on *Rhipicephalus (Boophilus) microplus* in Brazil. It was concluded that the use of plant extracts with activity against ticks have a promising future in hand of constant increasing acaricidal resistance of synthetic origin. These *in vitro* results could be supported in this study with the feeding of garlic to springbok over a period of 17 days.

As shown in the graphs (Table 4.2) the number of ticks attached to the springbok ear was also significantly decreased by 56% in the GG. This coherence in the results provides a clear indication of the repellant and/or acaricidal effects of garlic on springbok tick load.

When the number of detached ticks in the ear bags was analyzed, a large number of ticks from the garlic group were free and dead inside the bag. This finding supports a possible acaricidal effect of garlic on the ticks. Garlic lectins or agglutinins like alliinase (dimeric proteins that bind to sugars in the metabolism and cause agglutination of certain cell types) studied *in vitro* can act successfully to control certain insects (Dam *et al*., 1998). Sap-sucking planthoppers, red spider mites of tea and *Cacopsylla chinensis* that affect pear plantations and other chewing (lepidopteran) and sap-sucking (homopteran) kind of pests (Dam *et al.,* 1998; Upadhyay & Singh, 2012; Zhao *et al.,* 2013) can successfully be controlled with lectins and sulphur compounds derived from garlic. The mechanisms of action of lectins is by disrupting the midgut cells of insects through oxidative stress (Dam *et al.,* 1998). Lectins action starts in the sensory receptors, located in the mouth parts of the insect, which through oxidative stress, disrupts the membrane integrity and disturbs the ability to detect food (Upadhyay & Singh, 2012). Thereafter, lectins enter the midgut and interact with glycosylated proteins disrupting the activity of these and causing fatal disorders to the insect (Upadhyay & Singh, 2012). Similarly, the acaricidal effect of garlic on ticks may possibly be attributed not only to the effects of lectins, but also to the sulfur compounds that constitute the garlic molecule (Aboelhadid *et al.,* 2013). However, another mechanism to consider for the apparent acaricidal effect of garlic, would be the indirect effect that garlic may have on the

immune system, resulting in more effective immune-mediated killing of ticks by the host (Kiczorowska *et al.,* 2017; Yang *et al*., 2007).

As demonstrated by Costa-Junior and Furlong (2011), engorged female ticks mean weight treated with 20 g/day of Enxofre-*Allium sativum* (Gimaro Industrias Quimicas Ldta, Parana, Brazil), containing 70% mineral sulphur and 30% sulphur compounds extracted from *A. sativum,* had significantly lower mean weights than female ticks treated with other products that contain no sulphur components. Similarly, the stadial succession evaluation in the present study concluded that the percentage of un-engorged nymphal ticks were higher (46%) in the GG than in the CG (P = 0.11) but not significantly different. In addition, the number of engorged nymphal stage ticks were reduced (68%) in the GG compared to the CG (P = 0.05) although not statistically significant different. These results clearly demonstrate a direct correlation and the effectiveness of garlic (*A. sativum*) as an inhibitor of stadial development of *R. e. evertsi* ticks. Shyma *et al* (2014) were able to determine the acaricidal effect for the *Rhipicephalus* (*Boophilus*) *microplus* tick at different crude methanol extracts concentration of garlic (12.5, 25, 50 and 100 mg/ml). Natural compounds like garlic contain a wide range of active ingredients that interfere in insects biological processes thus interrupting their life cycle (Shyma *et al.,* 2014). On the other hand, essential plant oils possibly interfere with basic metabolic, biochemical, physiological and behavioral functions of ticks (Mann & Kaufman, 2012). However, little is known about the true mode of action of essential oils (Mann & Kaufman, 2012).

On the other hand, Hu *et al* (2002) studied the induction of hemolysis in dogs due to oxidative damage caused by the organosulfur compounds of *Allium sativum*. This damage to the erythrocyte resulted in the formation of eccentrocytes (red blood cells which their hemoglobin is located on one side of the cell as a result to oxidant injury to the cell membrane) and Heinz bodies which are aggregation of damaged hemoglobin located in red blood cells (Hu *et al.,* 2002). Furthermore, garlic organosulfur compounds can induce methemoglobinaemia in canine erythrocytes resulting from its oxidative properties (Hu, *et al,* 2002). Similarly, Saastamoinen *et al* (2019) supplemented horses with garlic at a dose of 32 mg /kg of BW for 83 days and they observed a decrease in hemoglobin (Hg), hematocrit (Htc) and red blood cells. However, no significant differences in hematological evaluations could be found in the current study between the treated and the control groups of springboks after 24 days. Longer term feeding trials might be indicated to see negative changes to red blood cells. Possible explanations for this finding may be related to ruminal and hepatic metabolism (species related detoxification efficiency) of the garlic compounds that is different to monogastric animals like dogs and horses, and also that the inclusion level of garlic in this trial were lower than is required to induce hematological effects.

Lastly, the specific molecular features of the active compounds in Garlium® GEM HC may be different to (less oxidative / toxic) from natural form of garlic. In this survey, the daily feed intake per animal was determined to be around 800 g, implying a Garlium® GEM HC intake of at least 1.12 g /day. Further investigation is required to determine if higher intake of Garlium® GEM HC could result in oxidative changes in the red blood cells or any other side effects affecting springbok.

Although a significant difference was observed in the mean corpuscular hemoglobin concentration (MCHC) values on day 7, the values returned to the range of the CG later in the trial. At this stage, this finding remains inexplicable, but probably not significant. Similarly, a transient increase in the basophil count of the GG was also found on day 7 of the trial recovering to normal levels on day 17 of the trial. Although no proper explanation could be found for this change, it is believed that basophils might play a key role in the antibody-mediated acquired immunity against ticks since they are released with inflammatory reaction (Wada *et al.,* 2010).

The current study was able to demonstrate in a short period of time the potential effects that Garlium® GEM HC has as tick repellent in springbok. Moreover, with the movement away from synthetic compounds to preserve human health, the livestock industry is moving to a natural alternative for growth promoters (Ogbuewu *et al.,* 2018). Garlic has shown positive and promising results to function as a growth promoter. During this trial, Garlium® GEM HC has not only proven to be an effective feed additive assisting as a natural and reliable tick repellent, but also benefited in springbok's weight gain, probably due to the enhancer properties of the product. As described by Ogbuewu *et al* (2018) garlic addition in the feed has proven to be a good stimulant of gut activity and animal health. Garlic is proved to enhance ruminal stimulation by increasing beneficial gut microbes in the gut flora that will lead to an improved microbial ecosystem (Kamra *et al.*, 2012). Furthermore, the inulin present in garlic, which is a oligomer of fructose acts as a potent probiotic (Kamra *et al.*, 2012). Inulin is not degraded in the rumen, which makes it abundantly available to the microflora of the large intestine. Its effects have been widely demonstrated, it is known to reduce levels of pathogens like *E. coli* and Clostridium spp. bacteria (Kamra *et al.*, 2012). These characteristics added to the well observed insect and

tick repellent properties makes Garlium® GEM HC a valuable supplement, that can not only aid in the control of ticks, but also support tick borne disease prophylaxis through low dose infection rates resulting from reduced tick infestations.

The live weight gain in the springbok trial has indicated the beneficial effects Garlium® GEM HC as an effective natural growth promoter and palatability enhancer. This was shown by the increase of food consumption of 5.2% of body weight on days 7 to 14 and the increase of 4.5% in the GG on days 15 to 23. Similarly but impossible to compare, studies in other animal species like broiler chickens (Kim *et al.,* 2013) and piglets (Langendijk *et al.,*2007) have clearly demonstrated positive results on the use of garlic as a food enhancer and growth promoter.

The decrease in the larval survival, both attached and in ear bags, suggests a reduction in the survival rate of tick larvae with an inhibitory effect on tick attachment and stadial development in Garlium® GEM HC fed springbok. It is clear from the statistical significant differences in tick survival that ruminal modification or degradation of the active Garlium® GEM HC ingredients did not affect its inhibitory impact on tick numbers in the GG.

Immature stages of *R. e. evertsi* have natural predilection for feeding in the ear canal and ear pinna of most domestic and wild mammals (Londt *&* Van der Bijl*,* 1977). It stands to reason that one of the host's defensive / reactive responses to this parasitic infestation will be the secretion of cerumen ("ear wax") (Quintavalla *et al*., 2004; Pata *et al*., 2003). Moreover, the attachment of ticks invariably will inflict an inflammatory reaction which could potentially stimulate cerumen production /

secretion (Londt & Van der Bijl, 1977). The total weight of the cerumen was increased in the garlic treated group by 40.6% and 31.46% respectively compared to the CG. However, these values were not statistically significant due to the small sample size of the study. As a result of the small differences in measured cerumen production between the GG and CG larger numbers of animals are required to determine statistical significance. Since dermal sebaceous secretions may have tick repellent effects (Pata *et al.,* 2003), this finding raises a possible aspect to investigate further that may help to explain a mechanism of the repellent effect of garlic derivatives. According to Horak (1982) impala and possibly other small ruminants as well, could act as wild reservoirs of immature forms of Ixodid ticks. In certain tick species like *R. evertsi,* the immature stages tend to locate mainly in the ear canal and ear pinna of the animals. As ticks molt from larvae to nymphaea there is a viability loss during this process which could be attributed to the natural cerumen secretion of the ear canal. It is through the cerumen secretion of the ear that garlic sulphur derivatives affect tick's attachment to the host (Rosen *et al.,* 2001).

It is well documented that garlic has antibacterial, antifungal and antiparasitic (like for example for tapeworms and roundworms) (Hasan *et al.*, 2015) properties in ruminants (Aboelhadid *et al*., 2013; Ankri & Mirelman,1999; Waag *et al*., 2010). Oral ingestion of Garlium® GEM HC in ruminants thus seems to be an effective route of application, however this requires further investigation. There is no clear evidence that feeding Garlium® GEM HC to wild ruminants for longer than 17 days will have negative effects in the animals. Although the exact mechanism remains unclear, this study provides a basis for further investigations into the possible mechanism/s of action of Garlium® GEM HC in wild ruminants.

Although there's very little information about coccidia species in wild ruminants, antelope can suffer from coccidiosis as a result of different stressful events (Lopez Rebollar *et al.,*1997). Springbok, as other small wild ruminants, are also prone to suffer from coccidial infections due to malnutrition, stressful situations like long periods of confinement and even transport (Junker *et al.*, 2015; Lopez Rebollar *et al*.,1997; Mapham & Vorster). In our study, 3 animals succumbed to coccidiosis during the trial, two in the control group and one in the GG. Although springboks were hosted in adequate and spacious bomas and were given sufficient time to adapt to captivity, these animals came from the wild. Their social and spatial structure was abruptly interrupted and changed, thus failing to adapt. All these cases were diagnosed at the Section of Pathology, Faculty of Veterinary Science, UP. Although it can be said that the non-treated control group was more prone to suffer disease, the trial sample of each group was too small to reach statistically significant conclusions.

Wild animals pose a natural challenge when it comes to therapy and with game farming associated intensifications on the increase in South Africa, the need for natural and practically applicable tick repellent solutions are becoming apparent. Although it would be ideal to study the effects of Garlium® GEM HC in the springbok's natural environment, this would be technically difficult and deriving in high variability with statistically significant conclusions impossible. This study provides important baseline data for extension into further investigations to the use of garlic as a tick repellent.

With the following study Pancosma has opened a spectrum of possibilities for cattle farmers, game farmers and animal keepers by offering an alternative ecological

product as a tick repellent. This study has stablished that Pancosma has a viable and safe product that does not pose a threat to wild animals while being treated for tick burdens. Garlium® GEM HC resulted to be a safe and effective product for animal consumption, however more research is advice to determine the long terms effects of it.

CHAPTER 6

CONCLUSIONS

Observations in this study showed that there was a reduced larval tick survival as well as a reduced stadial succession of ticks in the Garlium® GEM HC treated animals. There is no doubt that the feeding of garlic to springbok has impacted significantly on the number of ticks that attached to the animals. It was also found that animals with lower tick loads showed an increase in ear cerumen production / secretion, possibly indicating an *in vivo* tick repellent mechanism of garlic derivatives in ruminants.

A continual daily intake of 1.38 g Garlium® GEM HC / animal / day (1.38 g / 20.70 kg / BW / per day) was sufficient to decrease the tick load of the GG by 49%. Although red blood cell oxidation and methemoglobin (Yang *et al.,* 2003) has been observed in certain animal species, springbok did not present any negative side-effects to Garlium® GEM HC during the 24 day trial period in which they were fed garlic at the given dose. Long term and/or higher dose studies are required to determine if any detrimental hematological effects on springbok or other ruminants exists.

Endo and exo-parasitic resistance developing from misuse of synthetic acaricides is necessitating investigations into more natural and holistic alternatives to treat wild animals in intensive management farms. In this respect, Garlium® GEM HC showed promising results as a possible naturally derived alternative tick repellent. It's reduction in tick numbers rather than elimination of the total feeding tick population,

also plays an important positive role in the maintenance of endemic stability to TBD in ruminant populations. Further studies are encouraged in order to evaluate its future application/s as an alternative as a naturally derived solution to tick control for farmed wildlife populations.

Moreover, ear wax (cerumen) secretion in the GG increased. Thus, suggesting that in addition to possible direct effects on ticks, garlic may play an indirect role through cerumen (also possibly dermal sebaceous secretion) production / secretion contributing to repellent effects on ticks.

Although studies on the mechanism of action of ear cerumen have been studied previously in other species like dogs (Pata *et al.,* 2003, there is no conclusive information whether an increase of ear cerumen could prevent certain bacterial infections. However, a change in diet in dogs have been proven to produce a change in the ear cerumen constitutes which benefited the prevention of bacterial ear infections (Pata *et al*., 2003). These results need further research in order to determine the precise function of the inclusion of garlic in diets and the mechanism of action of garlic being secreted through ear cerumen as a tick repellent in springbok.

Initial pilot study on Garlium® GEM HC has shown to be a natural tick repellent in springbok and further studies are needed to evaluate the long terms effect of Garlium® GEM HC.

CHAPTER 7

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Appendix A: Animal Ethics Certificate of Approval V017-01

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	The potential effect of GARLIUM GEM HC as a tick repellent agent in male springbok
PROJECT NUMBER	V017-17
RESEARCHER/PRINCIPAL INVESTIGATOR	Dr. A Fitle

STUDENT NUMBER (where applicable)	U_14110289
DISSERTATION/THESIS SUBMITTED FOR	MMedVet

DAFF Section 20 to be obtained

ANIMAL SPECIES	Springbok	Ticks
NUMBER OF ANIMALS	24	500/animal
Approval period to use animals for research/testing purposes		February 2017-February 2018
SUPERVISOR	Dr. K Koeppel	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date 27 February 2017
CHAIRMAN: UP Animal Ethics Committee	Signature

Appendix B: Garlium® GEM HC dossier

pancosma
makes sense

GARLIUM GEM HC

Garlic type

Code	A60-9051
Description	Flavour/taste enhancing mixture for animal feed products. 5% of garlic extract on inert and vegetable carriers (salt and garlic powder).
Physical properties	Appearance: free flowing beige powder, dust free
Sensorial properties	Flavor notes: garlic, onion
Legislation	In conformity with European Union feed legislation. Free from GMO and animal products. In conformity with the directive 2002/32/CE and its modifications.
Safety	For safety information please refer to the product safety datasheet
Stability	Guaranteed 12 months when stored in original packaging and kept in a cool dry place, sheltered from light, moisture and oxygen.
Uses	In diets of horses and cattle* as an aid to repelling insects. Once ingested non metabolized garlic active ingredients are excreted via the breath and skin, surrounding the animal with a garlic aroma. In equine diets as a nutritional supplement. As a palatability enhancer: several studies reported that garlic increased the palatability of the feed offered to animals.
Application method	Mixing into feed (complete feed, herb mixture, mineral supplement, feed blocks)
Packaging	20 kg buckets

Application rates

All type of complete feeds :	0.1 – 1 kg/t
Feed blocks	5 – 15 kg/t
Adult animals (horses, ruminants*):	1 – 4 g/day
Young animals:	1 – 3 g/day

Please note that above mentioned application rates are very much dependent upon the raw materials used in the feed formulation.

In Europe higher application rates might have some legislative impact on the labelling, please contact the local Pancosma representative for further information.

**Do not feed Garlium more than 1 gram/hd/day to lactating animals where the milk is to be consumed or sold for processing*

Datasheet

30.08.2019 – MR
PANCOSMA France S.A.S.
Zone Industrielle d'Arlod - Bellegarde sur Valserine - 01200 Valserhône - France
Tel : +33 450 48 52 11 / Fax : +33 450 48 60 40
Agreement number : α FR 01 033 003 - R.C.S. Bourg-en-Bresse B 763.200.821

SAFETY DATA SHEET

(REACH regulation (EC) n° 1907/2006 - n° 2015/830)

SECTION 1 : IDENTIFICATION OF THE SUBSTANCE/MIXTURE AND OF THE COMPANY/UNDERTAKING

1.1. Product identifier

Product name : GARLIUM GEM HC, CODE A60-9051

Product code : A60-9051.

1.2. Relevant identified uses of the substance or mixture and uses advised against

This product is only for industrial use, as a supplement for animal feed.

1.3. Details of the supplier of the safety data sheet

Registered company name : PANCOSMA FRANCE S.A.S.

Address : Zone Industrielle d'Arlod - Bellegarde sur Valserine.01200.Valserhône.France.

Telephone : +33 (0)4 50 48 52 11. Fax : +33 (0)450 48 60 40.

info@pancosma.ch

www.pancosma.com

1.4. Emergency telephone number : +33 (0)1 45 42 59 59.

Association/Organisation : INRS / ORFILA http://www.centres-antipoison.net.

Emergency telephone: For hazardous materials (or dangerous goods) incident, spill, leak, fire, exposure, or accident Call Chemtrec day or night Within USA and Canada : +1-800-424-9300 Outside USA and Canada : +1-703-527-3887 (collect calls accepted)
Switzerland: poison information service 145

SECTION 2 : HAZARDS IDENTIFICATION

2.1. Classification of the substance or mixture

In compliance with EC regulation No. 1272/2008 and its amendments.

Skin irritation, Category 2 (Skin Irrit. 2, H315).

Eye irritation, Category 2 (Eye Irrit. 2, H319).

Skin sensitisation, Category 1B (Skin Sens. 1B, H317).

Specific target organ toxicity (single exposure), Category 3 (STOT SE 3, H335).

This mixture does not present a physical hazard. Refer to the recommendations regarding the other products present on the site.

This mixture does not present an environmental hazard. No known or foreseeable environmental damage under standard conditions of use.

2.2. Label elements

In compliance with EC regulation No. 1272/2008 and its amendments.

Hazard pictograms :

GHS07

Signal Word :

WARNING

Product identifiers :

	GARLIC POWDER
EC 218-548-6	ALLYL DISULFIDE

Hazard statements :

H315	Causes skin irritation.
H317	May cause an allergic skin reaction.
H319	Causes serious eye irritation.
H335	May cause respiratory irritation.

Precautionary statements - Prevention :

P264	Wash ... thoroughly after handling.
P271	Use only outdoors or in a well-ventilated area.
P280	Wear protective gloves/protective clothing/eye protection/face protection.

Precautionary statements - Response :	
P304 + P340	IF INHALED: Remove person to fresh air and keep comfortable for breathing.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P312	Call a POISON CENTER/doctor/... if you feel unwell.
P332 + P313	If skin irritation occurs: Get medical advice/attention.
P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
P337 + P313	If eye irritation persists: Get medical advice/attention.
Precautionary statements - Storage :	
P403 + P233	Store in a well-ventilated place. Keep container tightly closed.
P405	Store locked up.

2.3. Other hazards

In use, may form flammable/explosive dust-air mixture.

The mixture does not contain substances classified as 'Substances of Very High Concern' (SVHC) >= 0.1% published by the European CHemicals Agency (ECHA) under article 57 of REACH: http://echa.europa.eu/fr/candidate-list-table

The mixture fulfils neither the PBT nor the vPvB criteria for mixtures in accordance with annexe XIII of the REACH regulations EC 1907/2006.

SECTION 3 : COMPOSITION/INFORMATION ON INGREDIENTS

3.2. Mixtures

Composition :

Identification	(EC) 1272/2008	Note	%
INDEX: PAN017 GARLIC POWDER	GHS07 Wng Skin Irrit. 2, H315 Eye Irrit. 2, H319 STOT SE 3, H335		25 <= x % < 50
INDEX: I2179_57_9 CAS: 2179-57-9 EC: 218-548-6 ALLYL DISULFIDE	GHS06, GHS02, GHS07 Dgr Acute Tox. 3, H301 Eye Irrit. 2, H319 Flam. Liq. 3, H226 Skin Irrit. 2, H315 Skin Sens. 1B, H317		2.5 <= x % < 10

(Full text of H-phrases: see section 16)

SECTION 4 : FIRST AID MEASURES

As a general rule, in case of doubt or if symptoms persist, always call a doctor.

NEVER induce swallowing by an unconscious person.

4.1. Description of first aid measures

In the event of exposure by inhalation :

In the event of massive inhalation of dust, remove the person exposed to fresh air. Keep warm and at rest.

If the person is unconscious, place in recovery position. Notify a doctor in all events, to ascertain whether observation and supportive hospital care will be necessary.

If breathing is irregular or has stopped, effect mouth-to-mouth resuscitation and call a doctor.

In the event of splashes or contact with eyes :

Wash thoroughly with fresh, clean water for 15 minutes holding the eyelids open.

If there is any redness, pain or visual impairment, consult an ophthalmologist.

In the event of splashes or contact with skin :

Remove contaminated clothing and wash the skin thoroughly with soap and water or a recognised cleaner.

Watch out for any remaining product between skin and clothing, watches, shoes, etc.

In the event of an allergic reaction, seek medical attention.

If the contaminated area is widespread and/or there is damage to the skin, a doctor must be consulted or the patient transferred to hospital.

In the event of swallowing :

Do not give the patient anything orally.

In the event of swallowing, if the quantity is small (no more than one mouthful), rinse the mouth with water and consult a doctor.

Seek medical attention immediately, showing the label.

4.2. Most important symptoms and effects, both acute and delayed

No data available.

4.3. Indication of any immediate medical attention and special treatment needed

No data available.

SECTION 5 : FIREFIGHTING MEASURES

Non-flammable.

5.1. Extinguishing media

Suitable methods of extinction

In the event of a fire, use :

Water with foaming agent; Foam; Powders

Unsuitable methods of extinction

In the event of a fire, do not use :

water jet

5.2. Special hazards arising from the substance or mixture

A fire will often produce a thick black smoke. Exposure to decomposition products may be hazardous to health.

Do not breathe in smoke.

In the event of a fire, the following may be formed :

- carbon monoxide (CO)
- carbon dioxide (CO2)

5.3. Advice for firefighters

No data available.

SECTION 6 : ACCIDENTAL RELEASE MEASURES

6.1. Personal precautions, protective equipment and emergency procedures

Consult the safety measures listed under headings 7 and 8.

For non first aid worker

Avoid any contact with the skin and eyes.

Avoid inhaling dust.

If a large quantity has been spilt, evacuate all personnel and only allow intervention by trained operators equipped with safety apparatus.

For first aid worker

First aid workers will be equipped with suitable personal protective equipment (See section 8).

6.2. Environmental precautions

Prevent any material from entering drains or waterways.

6.3. Methods and material for containment and cleaning up

Retrieve the product by mechanical means (sweeping/vacuuming) : do not generate dust.

6.4. Reference to other sections

No data available.

SECTION 7 : HANDLING AND STORAGE

Requirements relating to storage premises apply to all facilities where the mixture is handled.

Individuals with a history of skin sensitisation should not, under any circumstance, handle this mixture.

7.1. Precautions for safe handling

Always wash hands after handling.

Remove and wash contaminated clothing before re-using.

Fire prevention :

Handle in well-ventilated areas.

Prevent access by unauthorised personnel.

Recommended equipment and procedures :

For personal protection, see section 8.

Observe precautions stated on label and also industrial safety regulations.

Also provide breathing apparatus for certain short tasks of an exceptional nature and for emergency interventions.

In all cases, recover emissions at source.

Avoid skin and eye contact with this mixture.

Prohibited equipment and procedures :

No smoking, eating or drinking in areas where the mixture is used.

7.2. Conditions for safe storage, including any incompatibilities

No data available.

Storage

Keep the container tightly closed in a dry, well-ventilated place.

Packaging

Always keep in packaging made of an identical material to the original.

7.3. Specific end use(s)

No data available.

SECTION 8 : EXPOSURE CONTROLS/PERSONAL PROTECTION

8.1. Control parameters

No data available.

8.2. Exposure controls

Personal protection measures, such as personal protective equipment

Use personal protective equipment that is clean and has been properly maintained.

Store personal protective equipment in a clean place, away from the work area.

Never eat, drink or smoke during use. Remove and wash contaminated clothing before re-using. Ensure that there is adequate ventilation, especially in confined areas.

- Eye / face protection

Avoid contact with eyes.

Before handling powders or dust emission, wear mask goggles in accordance with standard EN166.

Prescription glasses are not considered as protection.

Provide eyewash stations in facilities where the product is handled constantly.

- Hand protection

Wear suitable protective gloves in the event of prolonged or repeated skin contact.

Use suitable protective gloves that are resistant to chemical agents in accordance with standard EN374.

Gloves must be selected according to the application and duration of use at the workstation.

Protective gloves need to be selected according to their suitability for the workstation in question : other chemical products that may be handled, necessary physical protections (cutting, pricking, heat protection), level of dexterity required.

Recommended properties :

- Impervious gloves in accordance with standard EN374

- Body protection

Avoid skin contact.

Wear suitable protective clothing.

These clothes shall be chosen to ensure there is no inflammation or irritation of the skin at the neck and wrist by contact with the powder

Suitable type of protective clothing :

Wear protective clothing against solid chemicals and particles suspended in the air (type 5) in accordance with standard EN13982-1 to prevent skin contact.

Work clothing worn by personnel shall be laundered regularly.

After contact with the product, all parts of the body that have been soiled must be washed.

- Respiratory protection

Avoid breathing dust.

If the ventilation is insufficient, wear appropriate breathing apparatus.

When workers are confronted with concentrations that are above occupational exposure limits, they must wear a suitable, approved, respiratory protection device.

Type of FFP mask :

Wear a disposable half-mask dust filter in accordance with standard EN149.

Category :

- FFP1

Anti-gas and vapour filter(s) (Combined filters) in accordance with standard EN14387 :

- A1 (Brown)

SECTION 9 : PHYSICAL AND CHEMICAL PROPERTIES

9.1. Information on basic physical and chemical properties

General information :

Physical state :	Powder or dust.

Important health, safety and environmental information

pH :	Not relevant.
Boiling point/boiling range :	Not specified.
Flash point interval :	Not relevant.
Vapour pressure (50°C) :	Not relevant.
Density :	< 1
Water solubility :	Insoluble.
Melting point/melting range :	Not specified.
Self-ignition temperature :	Not specified.
Decomposition point/decomposition range :	Not specified.

9.2. Other information

No data available.

SECTION 10 : STABILITY AND REACTIVITY

10.1. Reactivity

No data available.

10.2. Chemical stability

This mixture is stable under the recommended handling and storage conditions in section 7.

10.3. Possibility of hazardous reactions

No data available.

10.4. Conditions to avoid

Avoid :

- formation of dusts

Dusts can form an explosive mixture with air.

10.5. Incompatible materials

No data available.

10.6. Hazardous decomposition products

The thermal decomposition may release/form :

- carbon monoxide (CO)
- carbon dioxide (CO2)

SECTION 11 : TOXICOLOGICAL INFORMATION

11.1. Information on toxicological effects

May cause irreversible damage to the skin; namely inflammation of the skin or the formation of erythema and eschar or oedema following exposure up to four hours.

May have reversible effects on the eyes, such as eye irritation which is totally reversible by the end of observation at 21 days.

Respiratory tract irritation may occur, together with symptoms such as coughing, choking and breathing difficulties.

May cause an allergic reaction by skin contact.

11.1.1. Substances

Acute toxicity :

ALLYL DISULFIDE (CAS: 2179-57-9)

Oral route :	LD50 = 260 mg/kg
Dermal route :	LD50 = 3600 mg/kg

11.1.2. Mixture

No toxicological data available for the mixture.

SECTION 12 : ECOLOGICAL INFORMATION

12.1. Toxicity

12.1.2. Mixtures

No aquatic toxicity data available for the mixture.

12.2. Persistence and degradability

No data available.

12.3. Bioaccumulative potential

No data available.

12.4. Mobility in soil

No data available.

12.5. Results of PBT and vPvB assessment

No data available.

12.6. Other adverse effects

No data available.

SECTION 13 : DISPOSAL CONSIDERATIONS

Proper waste management of the mixture and/or its container must be determined in accordance with Directive 2008/98/EC.

13.1. Waste treatment methods

Do not pour into drains or waterways.

Waste :

Waste management is carried out without endangering human health, without harming the environment and, in particular without risk to water, air, soil, plants or animals.

Recycle or dispose of waste in compliance with current legislation, preferably via a certified collector or company.

Do not contaminate the ground or water with waste, do not dispose of waste into the environment.

Soiled packaging :

Empty container completely. Keep label(s) on container.

Give to a certified disposal contractor.

SECTION 14 : TRANSPORT INFORMATION

Exempt from transport classification and labelling.

14.1. UN number

-

14.2. UN proper shipping name

-

14.3. Transport hazard class(es)

-

14.4. Packing group

-

14.5. Environmental hazards

-

14.6. Special precautions for user

-

SECTION 15 : REGULATORY INFORMATION

15.1. Safety, health and environmental regulations/legislation specific for the substance or mixture

- Classification and labelling information included in section 2:

The following regulations have been used:

- EU Regulation No. 1272/2008 amended by EU Regulation No. 487/2013.
- EU Regulation No. 1272/2008 amended by EU Regulation No. 758/2013.
- EU Regulation No. 1272/2008 amended by EU Regulation No. 944/2013.
- EU Regulation No. 1272/2008 amended by EU Regulation No. 605/2014.
- EU Regulation No. 1272/2008 amended by EU Regulation No. 1297/2014.

- Container information:

No data available.

- Particular provisions :

No data available.

15.2. Chemical safety assessment

No data available.

SECTION 16 : OTHER INFORMATION

Since the user's working conditions are not known by us, the information supplied on this safety data sheet is based on our current level of knowledge and on national and community regulations.

The mixture must not be used for other uses than those specified in section 1 without having first obtained written handling instructions.

It is at all times the responsibility of the user to take all necessary measures to comply with legal requirements and local regulations.

The information in this safety data sheet must be regarded as a description of the safety requirements relating to the mixture and not as a guarantee of the properties thereof.

Wording of the phrases mentioned in section 3 :

H226	Flammable liquid and vapour.
H301	Toxic if swallowed.
H315	Causes skin irritation.
H317	May cause an allergic skin reaction.
H319	Causes serious eye irritation.
H335	May cause respiratory irritation.

Abbreviations :

ADR : European agreement concerning the international carriage of dangerous goods by Road.

IMDG : International Maritime Dangerous Goods.

IATA : International Air Transport Association.

ICAO : International Civil Aviation Organisation

RID : Regulations concerning the International carriage of Dangerous goods by rail.

WGK : Wassergefahrdungsklasse (Water Hazard Class).

GHS07 : Exclamation mark

PBT: Persistent, bioaccumulable and toxic.

vPvB : Very persistent, very bioaccumulable.

SVHC : Substances of very high concern.

GARLIUM GEM HC, CODE A60-9051

PREMIXTURE – PRÉMÉLANGE – PREMEZCLA – VORMISCHUNG – PREMISCELA – VOORMENGSEL – PRÉ-MISTURA – PREMIKSU – FORBLANDING – ELOKEVEREKRE – ESISEOS – ΠΡΟΜΕΙΓΜΑ – PREMIKSAI – PREAMESTEC – PREMIKS – PREMIX – ПРЕМИКС – PREMIX – PREMIKSA – PREMIKSA – FORBLANDING – FÖRBLANDNING – EELSEGU.

To be used exclusively in the manufacture of feedingstuffs.

COMPOSITION: MIXTURE OF FLAVOURING COMPOUNDS (6.5%); Garlic powder, Sodium chloride.
Composition / composición / Zusammensetzung / composizione / samenstelling / composição / kompozycja / sammensætning / összetétel / koostumus / σύνθεση / sudėtis / compozije / sestava / složení / композиция / zloženie / sastav / kompozicija / sammensetning / sammansättning / koosseis

USE: All species / toutes les espèces / todos los animals / alle Tierarten, / tutte le specie / alle soorten / todas as especies de animais / wszystkie gatunki / alle dyrearter / minden faj / kaikki lajit / Όλα τα είδη / sve врсте / Visos rūšys / toate speciile / Vse vrste / Všechny druhy / Всички видове / všetky druhy / Sve vrste / Visas sugas / alle arter / alla arter / Kõik liigid: 100 - 1000 g / t (compound feed); 5 - 15 kg / t (feed block). For full specification see product MSDS and data sheet.
Utilisation / Aplicación / Anwendung / Impiego / Gebruik / Indicações / Dawkowanie / Anvendelse / Alkalmazás/ Käyttö / Χρήση / Naudokite / Utilizare / Uporaba / Použít / Употреба / Použiť / Koristi / lietošana / Bruk / Använda sig av / Kasutamine

EN	H317 May cause an allergic skin reaction. P280 Wear protective gloves/protective clothing/eye protection/face protection. P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.
FR	H317 Peut provoquer une allergie cutanée. P280 Porter des gants de protection/des vêtements de protection/un équipement de protection des yeux/du visage. P333 + P313 En cas d'irritation ou d'éruption cutanée: consulter un médecin.
ES	H317 Puede provocar una reacción alérgica en la piel. P280 Llevar guantes/prendas/gafas/máscara de protección. P333 + P313 En caso de irritación o erupción cutánea: Consultar a un médico.
DE	H317 Kann allergische Hautreaktionen verursachen. P280 Schutzhandschuhe /Schutzkleidung /Augenschutz /Gesichtsschutz tragen. P333 + P313 Bei Hautreizung oder -ausschlag: Ärztlichen Rat einholen/ärztliche Hilfe hinzuziehen.
IT	H317 Può provocare una reazione allergica cutanea. P280 Indossare guanti/indumenti protettivi/Proteggere gli occhi/il viso. P333 + P313 In caso di irritazione o eruzione della pelle: consultare un medico.
NL	H317 Kan een allergische huidreactie veroorzaken. P280 Beschermende handschoenen /beschermende kleding /oogbescherming/gelaatsbescherming dragen. P333 + P313 Bij huidirritatie of uitslag: een arts raadplegen.
PT	H317 Pode provocar uma reacção alérgica cutânea. P280 Usar luvas de protecção/vestuário de protecção/protecção ocular/protecção facial. P333 + P313 Em caso de irritação ou erupção cutânea: consulte um médico.
PL	H317 Może powodować reakcję alergiczną skóry. P280 Stosować rękawice ochronne/odzież ochronną /ochronę oczu /ochronę twarzy. P333 + P313 W przypadku wystąpienia podrażnienia skóry lub wysypki: Zasięgnąć porady/zgłosić się pod opiekę lekarza.
DK	H317 Kan forårsage allergisk hudreaktion. P280 Bær beskyttelseshandsker /beskyttelsestøj /øjenbeskyttelse/ansigtsbeskyttelse P333 + P313 Ved hudirritation eller udslet: Søg lægehjælp.
HU	H317 Allergiás bőrreakciót válthat ki. P280 Védőkesztyű/védőruha/szemvédő/arcvédő használata kötelező. P333 + P313 Bőrirritáció vagy kiütések megjelenése esetén: orvosi ellátást kell kérni.
FI	H317 Voi aiheuttaa allergisen ihoreaktion. P280 Käytä suojakäsineitä /suojavaatetusta /silmiensuojainta /kasvonsuojainta. P333 + P313 Jos ilmenee ihoärsytystä tai ihottumaa: Hakeudu lääkäriin.
EL	H317 Μπορεί να προκαλέσει αλλεργική δερματική αντίδραση. P280 Να φοράτε προστατευτικά γάντια/προστατευτικά ενδύματα/μέσα ατομικής προστασίας για τα μάτια/πρόσωπο. P333 + P313 Εάν παρατηρηθεί ερεθισμός του δέρματος ή εμφανιστεί εξάνθημα: Συμβουλευθείτε/Επισκεφθείτε γιατρό.
LT	H317 Gali sukelti alerginę odos reakciją. P280 Mūvėti apsaugines pirštines/dėvėti apsauginius drabužius/naudoti akių (veido) apsaugos priemones. P333 + P313 Jeigu sudirginama oda arba ją išberia: kreiptis į gydytoją.
RO	H317 Poate provoca o reacţie alergică a pielii. P280 Purtaţi mănuşi de protecţie/îmbrăcăminte de protecţie/echipament de protecţie a ochilor/ echipament de protecţie a feţei. P333 + P313 În caz de iritare a pielii sau de erupţie cutanată: consultaţi medicul.
SL	H317 Lahko povzroči alergijski odziv kože. P280 Nositi zaščitne rokavice/zaščitno obleko/zaščito za oči/zaščito za obraz. P333 + P313 Če nastopi draženje kože ali se pojavi izpuščaj: poiščite zdravniško pomoč/oskrbo.
CS	H317 Může vyvolat alergickou kožní reakci. P280 Používejte ochranné rukavice/ochranný oděv/ochranné brýle/obličejový štít. P333 + P313 Při podráždění kůže nebo vyrážce: Vyhledejte lékařskou pomoc/ošetření.
BG	H317 Може да причини алергична кожна реакция. P280 Използвайте предпазни ръкавици/предпазно облекло/предпазни очила/предпазна маска за лице. P333 + P313 При поява на кожно дразнене или обрив на кожата: Потърсете медицински съвет /помощ.
SK	H317 Môže vyvolať alergickú kožnú reakciu. P280 Noste ochranné rukavice/ochranný odev/ochranné okuliare/ochranu tváre. P333 + P313 Ak sa prejaví podráždenie pokožky alebo sa vytvoria vyrážky: vyhľadajte lekársku pomoc/ starostlivosť.
HR	H317 Može izazvati alergijsku reakciju na koži P280 Nositi zaštitne rukavice/zaštitno odijelo/zaštitu za oci/zaštitu za lice. P333 + P313 U slucaju nadražaja ili osipa na koži: Zatražiti savjet/pomoc lijecnika.
LV	H317 Var izraisīt alerģisku ādas reakciju. P280 Izmantot aizsargcimdus/aizsargdrēbes/acu aizsargus/sejas aizsargus. P333 + P313 Ja rodas ādas iekaisums vai izsitumi: lūdziet medķu palīdzību.
NO	H317 Kan utløse en allergisk hudreaksjon. P280 Benytt vernehansker /verneklær /vernebriller /ansiktsskjerm. P333 + P313 Ved hudirritasjon eller utslett: Søk legehjelp.
SV	H317 Kan orsaka allergisk hudreaktion. P280 Använd skyddshandskar /skyddskläder /ögonskydd /ansiktsskydd. P333 + P313 Vid hudirritation eller utslag: Sök läkarhjälp.
ET	H317 Võib põhjustada allergilist nahareaktsiooni. P280 Kanda kaitsekindaid /kaitserõivastust /kaitseprille/kaitsemaski. P333 + P313 Nahaärrituse või _obe korral: pöörduda arsti poole.

Manufacturing date: XXXXXXXXXX

Date de fabrication, fecha de fabricacion, Herstellungsdatum, data di produzione, fabricagedatum , data de fabricação, data produkcji, fremstillingsdato, gyártás dátuma, valmistuspäivämäärä, ημερομηνία κατασκευής, Pagaminimo data, data fabricaţiei, datum izdelave, Datum výroby, Дата на производство, dátum výroby, Datum proizvodnje, izgatavošanas datums, Produksjonsdato, Tillverkningsdatum, Valmistamise kuupäevi

Best use before: XXXXXXXXXX

À utiliser avant le, usar antes de, haltbar bis, da consumarsi entro, te gebruiken voor, prazo de validade, Najlepiej użyć przed, anvendes inden, minőségét megőrzi, parasta ennen, ανάλωση κατά προτίμηση πριν, geriausias iki, cel mai bun înainte de, najbolje pred, nejlépe před, най-добър преди, najlepšie pred, najbolji prije, vislabāk pirms, Best bruk før, Bästa användning innan, Parim kasutamiseks enne

Batch number: XXXXXXXXXX

Lot ,lote, Charge, lotto, batch, lote, partia, portion, hivatkozási száma, Eränumero, Τον αριθμό της παρτίδας, Partijos numeris, numārul lotului, serijska številka, číslo šarže, Партиден номер, Číslo šarže, Broj serije, Partijas numurs, Batchnummer, Partii number

Net weight: XXXXXXXXXX

Poids net, peso neto, Nettogewicht, peso netto, netto gewicht, peso liquid, masa netto, netto vægt, nettó tömeg, Nettopaino, Καθαρό βάρος, Grynas svoris, Greutate netă, Neto teža, Čistá hmotnost, Нето тегло, Čistá hmotnosť, Neto težina, neto svars, Netto vekt, Nettovikt, Netokaal

WARNING
ATTENTION
ATENCIÓN
ACHTUNG
ATTENZIONE
WAARSCHUWING
ATENÇÃO
UWAGA
ADVARSEL
FIGYELEM
VAROITUS
ΠΡΟΣΟΧΗ
ATSARGIAI
ATENŢIE
POZOR
VAROVÁNÍ
ВНИМАНИЕ
POZOR
UPOZORENJE
UZMANĪBU
OPPMERKSOMHET
VARNING
HOIATUS

Garlic powder
Allyl disulfide

Registration holder:

Allied Nutrition (Pty) Ltd
Co Reg No 2005 / 013232 / 07
89 Jean Avenue Doringkloof,
0157, RSA Tel: 012 667 4213

PANCOSMA France S.A.S.
Zone Industrielle d'Arlod
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01200 Valserhône
FRANCE
Tel: +33 450 48 52 11
Fax: +33 450 48 60 40
Agreement n°: α FR 01 033 003

COMPOSITION OF FLAVOURING AGENT

GARLIUM GEM HC
Code : A60-9051

STATE : POWDER

Flavour Fraction and carriers	**%**	**N=Natural NI=Nature identical A=Artificial**	**N°CAS**
Garlic oil	1.00	N	8000-78-0
Allyl Disulfide	5.50	NI	2179-57-9
Sodium Chloride	63.50	N	7647-14-5
Garlic powder	30.00	N	---
Total	***100.00***		

25.01.2017

Pancosma France S.A.S.

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Regulatory Affairs & Quality Manager

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certificate of analysis

Flavour: GARLIUM GEM HC

Code: A60-9051

Batch: FR022335-20/6

Evaluated Parameters:

	Specification	Result
Colour:	Beige	Conform
Odour:	Garlic, Onion	Conform
Physical Aspect:	Free flowing powder	Conform

Date of Manufacture: 24/02/2020

Best use before: 24/02/2021

Exempt from ingredients of animal origin.

Date of establishment in Bellegarde-sur-Valserine: March 09 2020

PANCOSMA France S.A.S

Sara Silva

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Quality Specialist

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Appendix C: Raw data. Live weights of springbok. Control group (A group), Treatment or Garlic group (B). Spaces in grey correspond to dead springbok.

GROUPS	DAY 0	DAY 7	DAY 17
A 1 - Control	14.40	14.1	12.8
A 2	19.30	19.3	19.7
A 3	22.00	21.9	21.2
A 4	22.80	21.8	18
A 5	20.00	20	17.5
A 6	22.20	21.7	22.5
A 7	18.00	15.6	15.4
A 8	16.40	16.6	16.3
A 9	18.90		
A 10	19.90	19.3	16.6
A 11	19.20	19.4	20
A 12	20.20	18.7	
Average	**19.4**	**18.9**	**18.0**
B 1 - Treatment	17	18.2	15.9
B 2	23.2	24.1	21.5
B 3	21	21.5	
B 4	22.4	22.9	23.1
B 5	25.8	17.4	16.8
B 6	19.4	19.4	18.5
B 7	22.1	24.3	20.5
B 8	25.1	24.7	24.1
B 9	15.3	16.5	16.7
B 10	24.7	27.6	15.1
B 11	18	15	17.6
B 12	23.1	24.7	19.9
B 13	21.6	22.3	19.2
Average	**21.4**	**21.4**	**19.1**

Appendix D: Raw data. Daily feed intake of springbok. Control group (A group), Treatment or Garlic group (B).

Date	Average Intake for Boma A	Average DMI/ Animal	Average Intake for Boma B	Average DMI/ Animal
8-Mar-2017	10.88 kg (13)	837g	13.84kg(15)	923g
9-Mar-2017	10.08 kg (13)	775g	17.5kg (15)	1170g
10-Mar-2017	11.46 kg (13)	882g	15.4kg (15)	1030g
11-Mar-2017	11.14 kg(13)	857g	15.42 kg (15)	1028g
12-Mar-2017	12.02 kg(13)	925g	13.68kg (15)	912g
13-Mar-2017	9.8kg (13)	754g	13.17kg (15)	878g
GARLIUM added to Boma B				
14-Mar-17	6.52kg (12)	543g	13.02kg (13)	1002g
15-Mar-17	10.26kg(12)	855g	15.5kg(13)	1192g
16-Mar-17	10.34kg(12)	862g	15.52kg(13)	1194g
17-Mar-17	12.32kg(12)	1026g	16.31kg(13)	1255g
18-Mar-17	10.93kg(11)	994g	16.19kg(13)	1245g
19-Mar-17	7.55kg(11)	686g	11.48kg(13)	883g
20-Mar-17	8.29kg(11)	753g	13.11kg(13)	1008g
TICKS and EAR bags placed				
21-Mar-17	7.46kg(10)	746g	10.86kg(13)	835g
22-Mar-17	6.49kg(10)	649g	13.61kg (13)	1047g
23-Mar-17	6.12kg(10)	612g	11.04kg (13)	849g
24-Mar-17	8.44kg(10)	844g	13.18kg(13)	1014g
25-Mar-17	8.93kg(10)	893g	9.5kg(13)	731g
26-Mar-17	6.97kg(10)	697g	9.46kg(13)	728g
27-Mar-17	6.56kg(10)	656g	8.40kg(12)	700g
28-Mar-17	9.15kg (10)	915g	9.66kg(12)	805g
29-Mar-17	10.04kg(10)	1004g	10.40kg(12)	867g
30-Mar-17	9.68kg(10)	968g	12.47kg(12)	1039g

Appendix E: Raw data. Tick parameters in individual springbok. Control group (A group), Treatment or Garlic group (B). * boxes correspond to dead animals.

ANIMAL ID	TICKS IN EAR BAGS	TICKS IN CONTAINER	TOTAL WEIGHT (gs)	UNENGORGED TICKS (Nymphs - 8 legs)	ENGORGED TICKS (Nymphs - 8 legs)	LARVAE (6 legs)	Total ticks/ group
A 1 - Control	0	148.00	0.104	28	0	120	
A 2	0	31.00	0.062	21	5	5	
A 3	0	81.00	0.110	57	13	11	
A 4	2	38.00	0.106	22	14	2	
A 5	0	39.00	0.180	15	18	6	
A 6	0	106.00	0.262	38	60	8	
A 7	0	202.00	0.362	138	60	4	
A 8	3	184.00	0.677	58	124	2	
A 9	*	*	*	*	*	*	
A 10	0	49.00	0.132	36	13	0	
A 11	2	70.00	0.395	24	46	0	
A 12	*	*	*	*	*	*	
AVERAGE	**0.70**	**94.80**	**0.239**	**43.70**	**35.30**	**15.80**	190.54
MEDIAN	**0.00**	**75.50**	**0.156**	**32**	**16.00**	**4.50**	128.16
B 1 - Treatment	0	46.00	0.024	32	6	8	
B 2	2	38.00	0.102	24	11	3	
B 3	*	*	*	*	*	*	
B 4	3	45.00	0.122	22	10	13	
B 5	0	34.00	0.032	26	5	3	
B 6	0	42.00	0.060	10	32	0	
B 7	1	23.00	0.045	10	5	8	
B 8	0	58.00	0.107	45	6	7	
B 9	2	88.00	0.191	66	13	9	
B 10	1	64.00	0.212	21	21	22	
B 11	3	46.00	0.210	10	16	20	
B 12	0	20.00	0.041	11	0	9	
B 13	4	23.00	0.107	8	10	5	
AVERAGE	**1.33**	**43.92**	**0.104**	**23.75**	**11.25**	**8.92**	89.27
MEDIAN	**1.00**	**43.50**	**0.105**	**21.5**	**10.00**	**8.00**	84.10