THE EFFECT OF ENVIRONMENTAL FACTORS AND HUSBANDRY PRACTICES ON STRESS IN GOATS

by

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DECLARATION OF ORIGINALITY

I, Leon Paul Kruger, declare that the thesis, which I hereby submit for the degree BAgric Animal Production Management at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

11 February 2015

Leon Paul Kruger — Date

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Be sure you know the condition of your flocks, give careful attention to your herds

Proverbs 27:23

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Chapter 1
Introduction and motivation

Animals that experience thermal, physical or social stress will suffer from poor welfare. The measurement of chronic stress is very complex and context dependent. Thus, welfare assessment usually involves measurements of performance, health, physiological and behavioral responses. Farm animals are exposed to various stressors during their productive life that compromise animal welfare. Heat stress, overcrowding, and mixing are common stressors to farm animals in conventional animal production. Finding strategies to mitigate these stressors will improve both performance and welfare of farm animals so that they can supply food in an efficient and animal-friendly way (Zulkifli, 2013).
Humans and animals are in regular and at times close contact in most farming systems. The quality of human-animal interactions can have a profound impact on the productivity and welfare of farm animals. Regular pleasant contact with humans may result in desirable alterations in the physiology, behaviour, health and productivity of farm animals. On the contrary, animals that are subjected to aversive human contact will be highly fearful of humans and their growth and reproductive performance may be compromised (Zulkifli, 2013).

Farm animal welfare has become increasingly relevant in the public eye in recent years due to popular articles such as; “A Farm Boy Reflects" by Nicholas Kristof (The New York Times, July 31, 2008) and related topics discussed on talk shows such as that of Oprah Winfrey; “How we treat the animals we eat” by Lisa Ling (Oprah.com, October 14, 2008). Awareness was raised with the lay public with regard to abusive methods of farm animal handling. It is also important to note that the term “Animal Welfare" is borrowed from western countries. For traditional people (e. g. in many developing countries) the term is not easily understood due to lack of common grounds in legal, cultural and ethical issues (Lay, 2013).

Despite the controversy and merits regarding animal welfare issues or keeping of livestock for production purposes, animal wellbeing remains an important factor to ensure satisfactory production and reproduction (Silanikove, 2000; Lowe *et al.*, 2002; Hansen, 2009; Miranda-de la Lama & Mattiello, 2010). South Africa is not excluded from animal welfare issues and the Animal Protection Act no. 71 of 1962 consolidated and amended the laws relating to the prevention of cruelty to animals, including farm animals. The factor of stress from abusive methods is widely used as a measurement of the severity of the abuse. Stress can be measured by the concentration of the hormone cortisol in the body (Dwyer & Bornett, 2004; Hargreaves & Hutson, 1990). Transport, shearing, isolation and exposure to heat are topics that were extensively researched for the stress impact factor. The routine handling procedures used in general health management of a flock is not regarded as a form of abuse or being stressful, but such procedures may have the potential to elicit a stress response in the animal.

Human perception of exposure of animals to adverse environmental and husbandry conditions is also a contentious issue that is often regarded as being stressful to the animal. It is however important to realize that it cannot necessarily be considered as a cause of distress. An animal exposed to heat for instance may experience a change in its internal state from being optimal and thus experience a level of stress. The normal reaction of such an animal would be to look for ways of thermoregulation and as such look for shade, eat less and drink more water. This cannot be interpreted as the animal experiencing stress, but merely as a physiological reaction to a specific situation. Should the animal however suffer hyperthermia and dehydration due to an inability to maintain its homeostasis, in other words no opportunity exists for thermoregulation, then it can be interpreted as distress. The interpretation of normal versus abnormal goat behaviour as a result of possible stressors should therefore differentiate between physiological compensation to maintain homeostasis, eustress, stress and distress.

The goat farming industry in South Africa is a widespread and diversified industry in which various breeds are used for different enterprises. This ranges from small scale and subsistence farming in the rural areas with various breeds, but mostly unimproved indigenous goats (Masika & Mafu, 2004) to commercial and stud farming for; the production of mohair from Angora goats (Debeuf *et al.*, 2004; van der Westhuysen, 2005), meat from Boer goats (Malan, 2000) and milk from milch breeds (Olivier *et al.*, 2005). Training colleges, universities and research institutions also utilize goats for training purposes and research.

There are three important factors in any goat farming enterprise; viz. production, reproduction and health (Casey & van Niekerk, 1988). Profitable production of goats inevitably involves management practices and routine tasks to be performed on the animals. Typical routine procedures involve deworming, vaccination, weighing, selection of breeding animals and treatment for disease or injuries. During the performance of such routine procedures goats are often exposed to potential physical stressful situations for example: environmental factors such as high ambient temperatures (Silanikove, 2000; Lowe *et al.*, 2002; Marai *et al.*, 2007), handling methods (Rushen *et al.*, 1999; Miranda-de la Lama & Mattiello, 2010) and food and water deprivation (Lowe *et al.*, 2002). This is particularly of relevance in enterprises where there is no or sub-standard handling facilities available. Three specific stressors: heat, handling and food/water deprivation on its own or cumulatively may be the most common stressors that occur during execution of routine procedures. Secondary to these there are temporary stressors such as disruption of social hierarchy which is more of a psychological stressor, change of habitat and overcrowding. No published information with regards to routine handling and the cumulative effect of these potential stressors on goats is available.

There is a perception that routine handling procedures as well as the cumulative effect of handling, heat exposure and food and water deprivation may elicit a stress response in goats. Since cortisol concentration in the circulating blood is a recognized measurement tool for stress (Kannan *et al.*, 2003; Säkkinena *et al.*, 2004), the aim of the study is to evaluate the effects of routine handling-, husbandry- and environmental factors on stress in goats.

Chapter 2
Literature review

2.1 Goat farming in South Africa

According to the calculations of Meissner *et al*. (2013) South Africa has approximately 7 million goats, with the Eastern Cape boasting the highest concentration of goats (including Angora goats) in the country. The emerging and communal sectors own 67% of the goats in South Africa. The goat population consists of Angora goats kept for fibre, Boer-, Kalahari Red-and Savannah goats kept for meat, Saanen, British Alpine and Toggenburg kept for milk and unimproved indigenous goats kept mostly for meat and ceremonial slaughter purposes by the emerging and communal sectors.

2.2 Susceptibility of different goat breeds to stress

2.2.1 South African unimproved indigenous goats

There is not much information available about the susceptibility of South African (SA) unimproved indigenous goats to stress and there is a popular belief that these genotypes adapts quickly to changes in the homeostasis or being resilient to stress factors. One study by Simela *et al.* (2004) has shown that SA unimproved indigenous goats suffered both chronic and acute stress during pre-slaughter handling. The effect of heat stress on this breed has also not yet been determined and the breed is arguably well adapted to warm environmental temperatures through centuries of natural selection. In contradiction to this, Silanikove (2000) has shown the deleterious effect of hyperthermia in goats, regardless of breed and stage of adaptation. Hyperthermia is a physiological state with an elevated body temperature from causes other than infection that may have developed due to the inability of thermoregulation. According to Adogla-Bessa & Aganga (2000) Tswana goats, which is a strain/genotype of SA unimproved indigenous goats, are well adapted to semi-arid zones and can be watered once in 72 h without severe dehydration. He has however also shown that animals in the trial consumed more water in summer than in winter, and showed more signs of dehydration in summer. The argument can thus be made that the consumption of water was for purposes of thermoregulation, indicating heat as a possible stressor which may have an influence on the homeostasis of the goat.

2.2.2 Boer goat

The Boer goat is described by Malan (2000) as a hardy breed with great capacity for adaptation and an exceptional ability to withstand and resist diseases. This generalised statement is contradicted by Young *et al*. (2011) who has shown susceptibility of Boer goat kids to the pathogenic *Eimeria* species occurring after stress periods that influence the homeostasis of the goats. Silanikove (2000) also contradicts the statement on the Boer goat's ability of adaptation and has demonstrated the deleterious effect of hyperthermia, regardless of breed and stage of adaptation. This is supported by Galipalli *et al*. (2004) who reported on elevated plasma cortisol, plasma creatine kinase activities and glucose concentration in Boer goats due to transportation stress. Casey & van Niekerk *(*1988) reported on the low water turnover rates of Boer goats supporting the adaptability finding of Malan (2000). With no information to contradict this, the work done by Adogla-Bessa *et al.* (2000), on SA unimproved indigenous goats may have relevance on the Boer goats too, showing higher water consumption and signs of dehydration during the summer.

2.2.3 Angora goats

South Africa is the major mohair producer in the world with 50% of the fibre produced in the country (Dubeuf *et al.*, 2004). The breed is described as being susceptible to stress conditions, due to the possibility of insufficient adrenal cortex (Engelbrecht *et al.*, 2000). It can thus be expected that stressors such as heat and cold exposure, handling and food and water deprivation may have a detrimental effect on the breed especially due to it being adrenal insufficient.

2.2.4 Milch goats

Milch goat farming in South Africa are the smallest of the goat industries in the country. Three different breeds, Saanen, Toggenburg and British Alpine are kept mostly for milk production that are utilised for cheese making and milk for lactose intolerant people. The adverse effects of heat stress on Milch goats have been reported by Silanikove (2000); Lowe *et al.* (2002); Darcan & Guney (2008) and Salama *et al*. (2013). There is not much information available on the effect of handling on Milch goats. This might be because the animals in production are being handled twice or more per day. Handling other than the habitual handling in the milking parlour may however have a negative impact on the animal. With regard to food and water deprivation, the same argument is valid as in the case reported by Adogla-Bessa & Aganga *(*2000) where SA unimproved indigenous goats consumed more water in summer than in winter, and showed more signs of dehydration in summer.

2.3 Reaction of goats to potential stressors

As early as 1929, Cannon reported on the "fight or flight" response as a reaction to factors influencing the homeostasis of an animal. Not denying the pioneering work of Cannon, the "freeze, flight, fight, or fright" response described by Bracha (2004) is a more appropriate description of reactions to stressors especially with regard to small stock.

Sheep and goats, regardless of the breed, have a safety (flight) zone around them (Grandin, 2011). The safety zone is that space around a sheep or goat which, if entered by a perceived threat, would cause the animal to "freeze" as soon as the potential threat enters the safety zone. The natural reaction of the animals is to stop doing what it was busy doing and stare at the potential threat. If the danger persists, the natural instinct would be to flock together since there is safety in numbers and to flee in the opposite direction, in other words, "flight". Should they then be cornered into an area without any space to flee, they would try to fight off the danger. Their relative small size renders small stock defenceless to human handling. Restraint of the animal by the wrong "handles" (skin, hair or horns) or in any other way that elicits a fight response, will eventually result in the animal remembering the aversive experience (Hutson, 2007, p 155) resulting in the animal being frightened of that specific handler. Referring back to Cannon, any one of the freeze, flight, fight and fright responses could be considered an external influence on their homeostasis at that specific moment with the potential to elicit a stress response.

2.4 Hormonal response to stress

2.4.1 Corticotropin-releasing hormone (CRH)

Corticotropin-releasing hormone (CRH) is produced by parvocellular neuroendocrine cells in the paraventricular nucleus (PVN) of the hypothalamus in response to a stress stimulus and is one of at least nine substances secreted by the hypothalamus (Goodman & Gillman,. 1980). These substances are referred to as either releasing hormones, releasing factors or regulatory hormones. Since corticotropin releasing hormone has not yet been clearly characterized, it is also referred to as corticotropin releasing factor (CRF). It is released at the median eminence from neurosecretory terminals of the neurons into the primary capillary plexus of the hypothalamo-hypophyseal portal system. This portal system then carries the CRH to the pituitary where corticotropes are stimulated to secrete adrenocorticotropic hormone (ACTH). The main function of CRH is thus the stimulation of pituitary synthesis of ACTH (Goodman & Gillman, 1980

2.4.2 Adrenocorticotropic hormone (ACTH)

ACTH is a polypeptide tropic hormone produced and secreted from corticotropes in the anterior lobe of the pituitary gland in response to stimulation from CRH. The primary physiological effect of ACTH, is the stimulation of the adrenal cortex to secrete cortisol, corticosterone and aldosterone (http://en.wikipedia.org).

2.4.3 Glucocorticoid hormone (Cortisol)

Cortisol is a glucocorticoid, a class of steroid hormone. The name is derived from its involvement in the regulation of the metabolism of glucose, its synthesis in the adrenal cortex, and its steroidal structure (http://en.wikipedia.org).

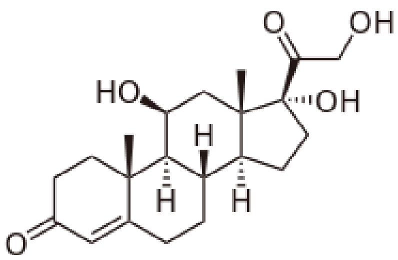

Figure 2.1 Structure of cortisol (Image: Http://en.wikipedia.org/wiki/File:Cortisol2.svg)

It is produced by the *zona fasciculata* of the adrenal cortex. The *zona fasciculata* constitutes the middle zone of the adrenal cortex, situated between the *zona glomerulosa* and the *zona reticularis*. Cortisol release is stimulated by ACTH (http://en.wikipedia.org).

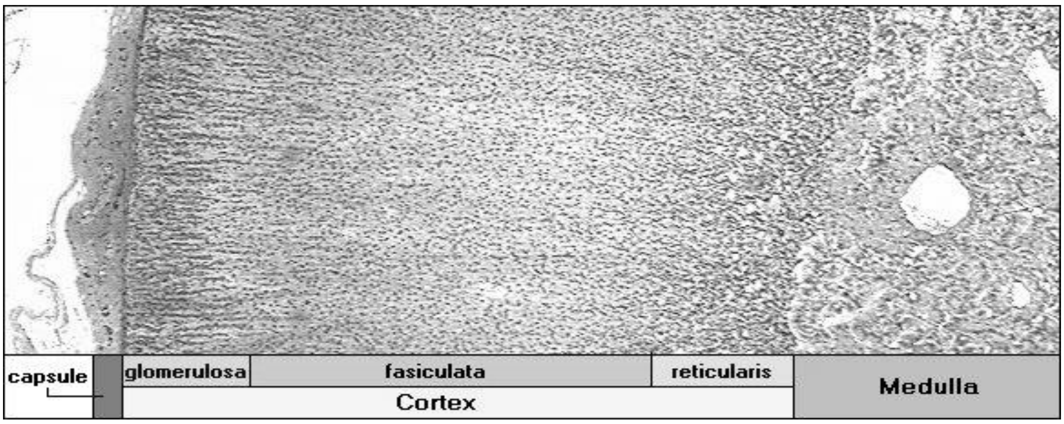

Figure 2.2 Histological image of the three cortex zones and part of the medulla (Image:http://www.vivo.colostate.edu/hbooks/pathphys/endocrine/adrenal/histo_overview.html.)

Cortisol has an effect on the body tissue to increase blood sugar through gluconeogenesis to supply energy in the form of glucose to those areas in the body that will benefit from it and enable the animal to escape from the stressor at that moment. It distributes this energy for instance to the heart and the brain and inhibits distribution of energy to the digestive and reproductive organs. The process that involves the secretion of cortisol from the interaction of the hypothalamus (H), the pituitary gland (P) and the adrenal gland (A) constitutes the HPA axis (Silanikove,2000).. Cortisol also acts in a negative feedback cycle to suppress any further release of ACTH. According to the Reference Laboratory Values for hormones the normal value for cortisol in sheep ranges between 42-82 nmol/L (Radostis *et al*., 2005).

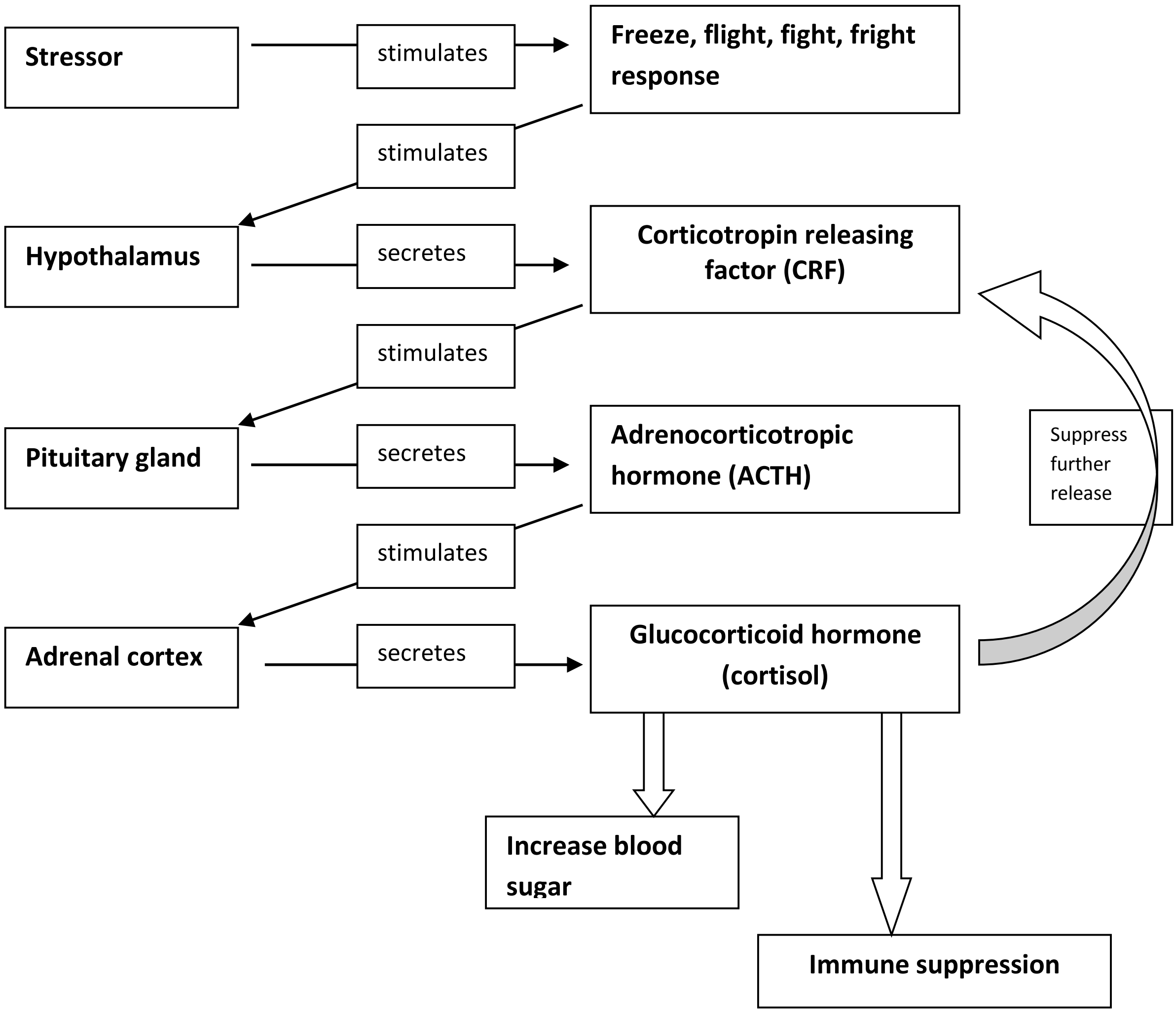

Figure 2.3 Hypothalamus, Pituitary, Adrenal (HPA) axis

Corticosteroids influence carbohydrate, protein, fat and purine metabolisms. It is involved in: electrolyte and water balance and also with the functions of the cardiovascular system, the kidney, skeletal muscle and nervous system (Goodman & Gillman, 1980).

The adrenal cortex is the one organ exclusively responsible for the freedom or adaptation that animals exhibit in a changing environment. In the absence of the adrenal cortex, animals can survive, but only under optimum conditions. Such conditions include among other the following: Food must be available. The environmental temperature must be maintained within a narrow margin. There should be physical comfort, psychological comfort, control of disease and the freedom to exercise natural behaviour. Without these optimum conditions an animal without an adrenal cortex will perish (Goodman & Gillman, 1980).

2.5 Effect of heat stress on goats

Goats are the best adapted of domesticated ruminant species to harsh warm climates with indigenous species performing better than species originating from more moderate climate (Silanikove, 2000). Silanikove (2000) also described the specific thermoregulatory behaviour of animals during heat spells. This behaviour confirms the learning process by habituation described by Broom and Johnson (1993) that involve animals resting in shade during the hot periods of the day and graze during dawn to dusk. The major adjustment that animals will make to adapt to extreme heat is to dissipate the heat into the environment by sweating and increased respiration and to reduce the production of metabolic heat by reduced feed intake (Silanikove, 2000). According to Baker (1989) this is due to warming of the pre-optic region of the hypothalamus that in turn will activate physiological and behavioural heat loss mechanisms. Among these mechanisms is the stimulation of the medial satiety centre which inhibits the lateral appetite centre with subsequent reduced feed intake. Reduced feed intake results in less rumination with subsequent decrease in buffering agents in the rumen. Silanikove (2000) regarded respiration rates above 40 breaths per minute as panting. The major reason for panting is to increase body cooling by evaporatory heat loss. Cockram (2004) describe the severity of heat stress according to panting rates: Low: 40- 60; medium: 60-80; high: 80 -200 and severely high heat stress above 200 breaths per minute. The increased respiration rate and decreased saliva entering the rumen contributes to, and make ruminants more susceptible to rumen acidosis (Bernabucci *et al.*, 2010). Despite the well-developed mechanisms of thermoregulation goats have adapted to, be it physiological or habitual, they are not always able to maintain homeothermy under heat stress (Marai, 2007). There are numerous citations on the negative effect of high temperatures and heat stress on small stock (Alamer, 2009; Hansen *et al.*, 2009; Veerasamy & Rajendra, 2010). All these concluded that animals in distress due to hyperthermia will show poor production, reproduction and increased susceptibility to disease.

2.6 Effect of handling stress on goats

Environmental factors cannot be controlled. The handling of goats is on the other hand completely under human control. Any goat production system involves handling of the animals. Goats are physically small compared to human beings who can handle them without restraining the animal in a crush crate or neck clamp. This renders the animals defenceless to wrong handling methods (Dwyer & Bornett, 2004). Wrong handling implies the handling of a sheep or goat by the wrong "handles" in the process of restraining the animal. Moving and handling often involves fear evoking stimuli (Hutson, 2007; Gonyou, 2000). According to Dwyer & Bornett, (2004) sheep have excellent memory for place and spatial learning abilities. Sheep will associate places with particular aversive experiences and can retain this information for as long as a year (Hutson, 1985 as cited in Dwyer & Bornett, 2004). There is a limited number of behavioural signs to indicate stress in sheep, viz. Increased immobility, increased locomotion, decreased resting, increased alertness, decreased eating and drinking and increased vocalisation (Cockram, 2004). These behavioural responses with the exception of vocalisation are not easily recognisable. Goats also tend to be more vocal than sheep (Lyons *et al.*, 1993) even in cases where no stressor is involved. Since the effect of handling stress is not obvious, there is a tendency to believe that it does not exist, but according to Rushen (1999) many husbandry procedures clearly do stress sheep to some degree. This is confirmed by Dwyer *et al.* (2004) indicating distress in sheep is almost implicit in their handling. Methods to quantify stress includes plasma cortisol concentrations (Kannan *et al.*, 2003; Säkkinena *et al.*, 2004), glucose, creatine kinase (Kannan *et al.*, 2003; Gallipalli *et al.*, 2004), plasma norepinephrine and epinephrine concentrations (Säkkinena *et al.*, 2004). Goats that were subjected to handling stress had higher cortisol, glucose, creatine kinase and norepinephrine concentrations (Kannan *et al.*, 2003; Galipalli *et al.,* 2004; Säkkinena *et al.,* 2004) than unstressed goats.

2.7 The effect of food and water deprivation on goats

Sheep and goats have the ability to withstand water restriction for periods of up to 2 days without causing stress to the animal (Meissner & Belonje*,* 1972; Li *et al*., 1999; Lowe *et al*., 2002; Jaber *et al.,* 2004; Alame*r,* 2009*).* Li *et al*. (1999) has however shown that water deprivation in combination with physiological stress resulted in an increase of blood cortisol levels in lactating animals. This is in accordance with the results from Parker *et al*. (2003) indicating that sheep given cortisol to simulate stress suffered from loss of body water in excess of that associated with a loss of electrolytes. This supports observations that elevated cortisol concentrations induce a diuresis in ruminants that contributes to dehydration (Parker *et al.*, 2003). According to Bernabucci *et al.* (2010)

the body fluid loss due to panting in heat stressed ruminants can increase the risk of cardiovascular dysfunction with consequent inability to maintain euthermia. The deprivation of food and water per sé might not be responsible for elevated cortisol concentrations in the goat. The cumulative effect of heat stress and handling stress might however be aggravated by food and water deprivation.

2.8 Effect of elevated cortisol concentrations in the goat

Elevated cortisol concentrations in the goat is an indication of stress, regardless the cause or stressor. This cortisol is beneficial to the animal to escape from the stressor, but it also has major detrimental consequences. It can compromises successful reproduction (Roth *et al.,* 2000; Ozawa *et al.,* 2005; Hansen,2009; Fatet *et al.,* 2011) and production (Marai *et al.*, 2007; Veerasamy *et al.,* 2010) and increases susceptibility to diseases due to compromised immunity (Leite-Browning, 2007; Abu Elzein & Housawi, 2009; Hosamani *et al.*, 2009).

2.8.1 Production and reproduction

Numerous reports on the negative effect of cortisol on blood circulation exist. This include the following: (1) increase in barren ewes due to sub-standard housing (Dwyer & Bornett, 2004), (2) blocked or delayed oestrus due to stressors such as transport, isolation or cortisol administration (Ehnert & Moberg *et al.*, 1991),(3) suppressed follicular growth and development due to restraint, confinement or transport (Dobson *et al.*, 1999),(4) decrease in lambing percentage and less multiple births (Knight *et al.,* 1988) and (5) lower milk yield (Sevi *et al.,*2001). Reports on disruptions of most aspects of reproductive functioning due to stress hormones include spermatogenesis and oocyte development, oocyte maturation, early embryonic development, foetal and placental growth and lactation (Dwyer & Bornett, 2004; Hansen, 2009; Bernabucci *et al.*, 2010). Furthermore results have shown a decrease in feed intake and utilization, disturbances in the metabolism of water, protein, energy and mineral balances, enzymatic reactions, hormonal secretions and blood metabolites due to stress (Marai *et al.*, 2007; Bernabucci *et al.,* 2010).

2.8.2 Immune suppression

Cortisol is one of the primary categories of hormones investigated during stress related research (Archer, 2005). According to Dwyer & Bornett (2008) the exact mechanism underlying the immunosuppressive effects of stress is not yet clear, but stressed sheep mount a less effective response to pathogen challenges than unstressed animals. This finding is confirmed by Rhind *et al.*

(2009) that showed large, infrequent increases in circulating cortisol to modify the cell mediated immune response in such a way that the response to a specific antigen challenge is compromised. This supports the argument that elevated cortisol levels not only act as indicators of stress in sheep and goats but are also responsible for the suppression of the immune system in the body. This has particular relevance to the small stock industry that relies to a large extent on vaccines for disease prevention in small stock. The correct handling and husbandry of animals in order to minimize stress does however not enjoy the same attention as other methods of disease prevention. This might be one explanation as to why the SA unimproved indigenous goats has such a high mortality rate (40%) in the communal areas where sub-standard husbandry practices exist (Webb & Mamabolo, 2004).

2.8.3 Disease susceptibility

The correlation between stress and immune suppression is a major cause of animals' susceptibility to disease. Poor performance and mortalities are important causes of economic loss to the South African small stock industry. Although exact numbers are not available, pre-weaning mortality percentages of 30% is not uncommon for South Africa (Sebei *et al.*, 2004; Webb & Mamabolo, 2004). Various reasons for mortalities have been described. In most cases the predisposing factors to disease are described as poor health management and lack of preventative treatments (Sebei *et al.*, 2004; Webb & Mamabolo, 2004). Although not extensively studied in South Africa, stress is considered as a predisposing factor to poor performance and mortalities for various diseases (De Wet & Bath 1994; Laval, 1994; Taylor, 1995; Leite-Browning, 2007; Abu Elzein & Housawi, 2009; Henton, 2009; Hosamani *et al.*, 2009).
Diseases typically associated with stress are: pasteurellosis, *Mannheimia haemolytica (*De Wet & Bath 1994; Henton, 2009), coccidiosis (Laval, 1994; Taylor, 1995; Young *et al*,. 2011; Lopes *et al*,. 2014), pustular dermatitis (Abu Elzein & Housawi, 2009; Hosamani *et al.*, 2009) and infectious keratoconjunctivitis (Leite-Browning, 2007). This is in accordance with the most prevalent diseases encountered in the goat flock of the Agricultural Research Council's Animal Production Institute. Most of the causative organisms are opportunistic organisms and occur under natural circumstances in sheep and goats, but is kept under control by the immune system. Immunity against these organisms develops either through vaccination or through continuous exposure to non-fatal doses. It is only when the animal's immunity is compromised due to stress (high cortisol levels) that the resistance to infection decreases to such an extent that the animal succumbs to the disease (Henton, 2009).

Mannheimia haemolytica lung infection

One of the most prevalent and important diseases that develops secondary to stress is *Mannheimia haemolytica* or *Pasteurella multocida* (pasteurellosis) lung infection (Ackermann & Brogden*,* 2000). Pasteurellosis is an important cause of economic loss in the whole of the ruminant industry. Kraabel & Miller (1997) showed susceptibility of bighorn sheep neutrophils to leukotoxin being increased by prior exposure to elevated plasma cortisol concentrations. Brogden *et al.* (1998) and Ackermann & Brogden (2000) also showed that respiratory infections often resulted from adverse physical and physiological stressors or in combination with viral and bacterial infections.

Coccidiosis

Coccidiosis in goats is caused by *Eimeria* organisms and is characterised by diarrhoea, with or without blood and mucous. Dehydration, emaciation, malaise and anorexia are typical clinical signs observed in affected animals. If left untreated the disease is often fatal. Stress plays without doubt a part in the susceptibility of lambs to coccidiosis (Laval, 1994; Taylor, 1995). Stressful situations such as overcrowding, weaning and handling compromise the immunity of the animal and make them more susceptible to clinical coccidiosis (De Wet & Bath, 1994; Laval*,* 1994; Young *et al*,. 2011; Lopes *et al*,. 2014). This supports the statement of Henderson (1994) who indicated stress factors such as bad weather or inadequate level of nutrition may precipitate an outbreak of coccidia. According to the Merck Veterinary Manual (1998) resistance to coccidian infection decrease after transport, changing rations or introduction of new animals to the flock (hierarchy disruption), of which all are potential stressors to goats.

Infectious keratoconjunctivitis

Infectious keratoconjunctivitis is characterised by blepharophthalmia, conjunctivitis, lacrimation and varying degrees of corneal opacity and ulceration (Merck Veterinary Manual, 1998). It can cause temporary blindness with subsequent weight loss and decreased performance. It is caused by *Mycoplasma conjunctivae* and / or *Chlamydophila* species. According to Leite-Browning (2007) outbreaks frequently occur after stress stimuli such as introduction of new goats to the existing herd which disturbs the hierarchy or after transportation, relocation and weather extremes.

Pustular dermatitis

Contagious pustular dermatitis (contagious ecthyma; scabby mouth; orf) is caused by a parapox virus and is one of the most widespread viral diseases of small stock worldwide (Merck Veterinary Manual, 1998). The disease is characterised by small wart like lesions around the muzzle. It begins as erythema and progresses to pustules and eventually develops into dry scabs. Affected goats suffer dysfunction of vital physiological activities. Abu Elzein & Housawi (2009) ascribe the outbreak of contagious pustular dermatitis in a goat flock to the animals being exposed to stress. This finding was confirmed by Hosamani *et al*. (2009) who showed the exposure to stress can accentuate the severity of the disease.

Chapter 3
Materials and Methods

3.1 Animals

South African unimproved indigenous goat does (*n*=36) between 10 and 12 months old and weighing between 22kg – 26kg were used.The animals were born and raised on the research facility but were purposely unaccustomed to regular handling for the purposes of the research trial. For statistical purposes the minimum number of animals per treatment must be six. Due to the fact that the trial involved exposure of the animals to potential stress factors the minimum number of animals was used for animal ethical reasons. Animal ethical clearance for the trials was obtained from the Agricultural Research Council's Animal Production Institute's Animal Ethics Committee (APIEC) with the reference number: APIEC 12/07. Prior to the start of the trial all the animals were examined and tested for internal and external parasites as well as obvious clinical symptoms to rule out the possibility of disease- or parasite related stress during the trial.

When not involved in the trial, the animals were kept in a one hectare camp with planted kikuyu pasture. Water was available *ad lib*. The grazing was supplemented with a diet of a pelleted commercial concentrate. Tin roof structures provided shelter against rain or other adverse weather conditions.

Table 3.1 Experimental design

		Basal level (Control)	Handling	Heat	Food deprivation	Water deprivation
1	6 goats	X				
2	6 goats	X	X			
3	6 goats	X		X		
4	6 goats	X			X	
5	6 goats	X				X
6	6 goats	X	X	X		
7	6 goats	X		X	X	X

3.2 Adaptation period

Before stressor treatment, a group of eight animals (six trial and two replacement) were randomly selected and placed together in an “adaptation / acclimatization camp “ for a period of two weeks to rule out the possibility of hierarchy disruption. The animals were continuously monitored, especially during feeding time, to ensure there were no incidences of victimization in the group. Victimized animals usually present with typical submissive behaviour especially during feeding time where they would not attempt to eat alongside the aggressor. No cases of victimization occurred during the trial.

3.3 Basal level determination

No untreated control (UTC) group was used. According to Meintjies (personal communication) cortisol levels cannot be measured against a set standard. The basal level (unstressed cortisol concentration) of each animal in the trial flock was established and was used as the untreated control for that animal. The basal level of all animals in the trial was determined one month prior to stressor treatments by collecting a blood sample via venipuncture. The basal level for cortisol of all the animals in the trial was within the normal value of 42-82 nmol/L for sheep according to the Reference Laboratory Values for hormones. (Radostis *et al*., 2005).

3.4 Handling stressor (HandStr)

For the handling stressor treatment a group of eight animals were randomly selected and placed together in the “adaptation / acclimatization camp” as described earlier in this chapter under paragraph 3.2: Adaptation period. The stressor procedure involved the animals being collected from the camp at 10:45 and chased at fast walking pace for 10 minutes through low density passages of the unit to the handling facility into a high density area that opened into the collection circle that in turns opened into the crush pen (chute). The animals were vaccinated subcutaneously with 1 mL sterilised water under the loose skin between the shoulder blades. The animals were then returned to the adaptation camp.

A series of blood samples were collected, stored and processed according to the techniques described under paragraph 3.10: Blood sampling and processing, later in this chapter.

3.5 Heat stressor (HtStr)

For the heat stress treatment a group of eight animals were randomly selected and placed together in the "adaptation / acclimatization camp" as described earlier in this chapter under paragraph 3.2: Adaptation period. For the stressor treatment the animals were exposed to direct sunlight in a pen with solid sides for three hours around mid-day with an atmospheric temperature of >30ºC. The temperature was measured using a minimum-maximum thermometer at 11:00 am, 12:00 pm and 14:00 pm. The time was limited to three hours as this is the time animals would more or less wait in commercial farms for processing. There was no opportunity of thermoregulation such as shade or water supply for the animals during the heat exposure. A series of blood samples were then collected, stored and processed as described under paragraph 3.10: Blood sampling and processing, later in this chapter.

3.6 Food deprivation stressor (FDS)

For the food deprivation stress treatment a group of eight animals were randomly selected and placed together in the "adaptation / acclimatization camp" as described earlier in this chapter under paragraph 3.2: Adaptation period. For the stressor treatment the animals were deprived of food and grazing but water was available *ad lib* for a period of 48 hours. After this period a series of blood samples were then collected, stored and processed as described under paragraph 3.10: Blood sampling and processing, later in this chapter.

3.7 Water deprivation stressor (WDS)

For the water deprivation stress treatment a group of eight animals were randomly selected and placed together in the "adaptation / acclimatization camp" as described earlier in this chapter under paragraph 3.2: Adaptation period. For the stressor treatment the animals were deprived of water but *ad lib* food was available for a period of 48 hours. After this period a series of blood samples were then collected, stored and processed as described under paragraph 3.10: Blood sampling and processing, later in this chapter.

3.8 Heat and handling stressor (HtHandSt)

For the cumulative effect of heat and handling stressors, a group of eight animals were randomly selected and placed together in the "adaptation / acclimatization camp" as described earlier in this chapter under paragraph 3.2: Adaptation period. After this adaptation period the animals were exposed to direct sunlight heat for three hours around mid-day with an atmospheric temperature of >30ºC. No shade or water was provided and therefore any opportunity of thermoregulation was denied. After the stressor period the animals were placed in a crush pen and dewormed orally with a placebo (water) to simulate routine handling for deworming. Immediately after the handling procedure a series of blood samples were collected, stored and processed as described under paragraph 3.10: Blood sampling and processing, later in this chapter.

3.9 Heat-, food deprivation- and water deprivation stressor (HtFdWStr)

For the cumulative effect of heat, food deprivation and water deprivation stress a group of eight animals were randomly selected and placed together in the “adaptation / acclimatization camp” as described earlier in this chapter under paragraph 3.2: Adaptation period. For the stressor treatment the animals were deprived of food and water for a period of 48 hours. After this period the animals were exposed to direct sunlight in a pen with solid sides for three hours around mid-day with an atmospheric temperature of >30ºC. There was no opportunity of thermoregulation for the animals during the heat exposure. A series of blood samples were then collected, stored and processed as described under paragraph 3.10: Blood sampling and processing, later in this chapter.

3.10 Blood sampling and processing

In accordance with recent literature on the topic blood samples were to be collected via an indwelling jugular catheter to minimize handling. The area for catheter placement was shaven and disinfected. A 18 G Jelco® I.V Catheter was inserted in the jugular vein and the port attached with superglue to the skin. This was done 48 hours before the onset of stressor treatment and flushed every 12 hours with heparinised saline. Out of 24 catheters fitted only seven were still functional after the 48 hour period. This method proved to be difficult on the goats as it either pulled out or twisted due to the activity of the animals in their natural habitat. The placement of the catheter also required some time and handling of the animal. This handling of the goats during flushing of the catheters made them apprehensive for the procedure since the area around the catheter was sensitive to handling.
After repeated attempts to place intravenous catheters and keep it functional were unsuccessful an alternative technique, venipuncture from the jugular vein, was then performed. Blood were collected with a 25G needle in a vacuum serum tube from the jugular vein after venous occlusion. This was always performed by the same experienced and skilled person. An observation was made with the animals being less anxious and vocal when blood was sampled via venipuncture than via catheters.

The venipuncture technique also required less time (5 – 15 seconds/ animal) than the catheter technique (40 – 90 seconds/ animal).

A series of blood samples were collected at 0-; 15-; 30-; 45-; 60- and 90 minutes. Blood sampling occurred only after the stressor application and not during stressor application due to the fact that the time frame of the different stressors differed; from 15 minutes for handling to 3 hours for heat and handling and up to 48 hours for food and water deprivation. Continued blood sampling during stressor application inevitably involves handling that could cause additional stress to stressors that does not involve handling. The acclimatization camp is connected with a passage to the crush pen and during series blood sampling the animals returned to the camp between sampling intervals. Blood samples were placed in a fridge (10ºC) to form a blood clot in order to prevent the presence of fibrin in the serum. After 24 hours the blood was centrifuged at 1500 rpm for 10 minutes and the serum aspirated with a disposable Pasteur pipette into a marked vial and stored at -20ºC until assayed.

3.11 Hormone assays

Analysis of the serum was done with the SIEMENS Immulite® 1000 automated Immunoassay Analyzer for quantative measurement of cortisol in serum. This was done at the Department of Companion Animal Clinical Studies of the Faculty of Veterinary Science, University of Pretoria. For the anti-cortisol antibody, Cortisol Test Units (LCO1) with one bead coated with polyclonal rabbit anti-cortisol antibody was used. Cortisol Reagent Wedge (LCO2) with 7.5mL alkaline phosphatase conjugated to cortisol in buffer, with preservative was used as reagent. For the Cortisol Adjustors (LCOL, LCOH) two vials (low and high), with 3mL each of cortisol in processed human serum with preservative was used. The cortisol concentration is expressed in nano-mol per liter (nmol/L).

3.12 Data analysis

Data analysis was performed by the Biometry unit of the Agricultural Research Council. The experimental design was a completely randomized design with stressors as treatment and animals as replication. The data was analyzed as a one-way Analysis of Variance (ANOVA) (Snedecor & Cochran, 1967) with the repeated measurements over time as a subplot factor (Little & Hills, 1972). The standardized residual were subjected to Shapiro-Wilk's test for deviation from normality (Shapiro &Wilk, 1965). In cases where deviation from normality was because of skewness outliners were removed (Glass *et al*.,1972). Means of significant source effects were compared using Student's

Least Significance Difference (t-LSD) at a 5% significance level (p=0.05). All statistical analyses were performed using SAS Statistical Software, version 9.2 (SAS,1999)

Chapter 4

Comparison between the effects of venipuncture- versus intravenous catheter blood collection on stress in goats

4.1 Introduction

The handling of the animals during blood collection, especially series blood sampling, has the potential to cause stress due to the pain sensation caused by the needle and the restraint of the animal. To overcome this an indwelling jugular catheter can be fitted to the animals. The placement and servicing of the jugular catheter does however also involve handling. The catheter needs to be inserted at least 48 hours before the onset of stressor treatment and flushed daily with heparinised saline to prevent blood clots in the catheter. The catheter can also cause infection and thus cause disease or pyrexia stress.

The animals in the trial were not accustomed to handling especially for the purpose of determining the effect of handling on cortisol release and the flushing of the catheters proved to be stressfull to the animals since they were vocal and presented with anxious behaviour during the procedure. Keeping the catheters functional also proved to be a problem with only seven out of 24 catheters being functional after the 48 hour period. The irritation the catheter caused resulted in the goats rubbing against objects and that rendered the catheter non functional. To prevent this from happening the area was closed of with bandage and plaster, but this just meant that prolonged handling was necesarry during flushing of the catheter and blood collection procedures.

An alternative collection techniquewas used and blood collected via venipuncture by an experienced person from goats who were not catherized. Blood samples were collected from catherized goats and also from goats without catheters (venipuncture). These blood samples were used to compare the plasma cortisol concentration from the venipuncture(Vp) and intravenous catheter (Ic) groups. There is a perception that venipuncture blood collection may elicit a more severe stress response than the intravenous catheter method and the aim of this investigation was to compare the effect of the two blood collection techniques on stress in goats.

4.2 Results and discussion

All the animals in this trial were subjected to the same stressor and kept in the same camp during the trial. They were accustomed to each other and were monitored to ensure that there were no incidences of victimisation. The results between blood collection via intravenous catheter,(Ic) and venipuncture (Vp) are presented in Table 4.1. Some individual animals, C12 (Ic group) and C24,(Vp group) had much higher cortisol levels in comparison with the rest of the trial animals. This can be explained due to individual temperament that influences the animal's responsiveness to stressors. Martin *et al*. (2004) make mention of the fearfulness of individual sheep and their reactivity to humans, strange, novel or threatening environments.

Table 4.1 Serum cortisol concentration (nmol/L) of goats bled via venipuncture (Vp) and intravenous catheter (Ic)

Animal number	Collection route	Basal level	0 min	15 min	30 min	45 min	60 min	90 min
C3	Vp	43.90	35.9	82.5	94.1	90.2	64.0	62.6
C4	Vp	43.30	87.2	76.7	38.4	27.4	41.9	67.6
C6	Vp	13.80	41.7	30.1	23.7	15.0	53.5	32.8
C8	Vp	50.00	59.9	55.5	43.6	31.7	105.0	49.9
C19	Vp	70.60	60.1	71.2	72.8	109.0	135.0	97.1
C24	Vp	90.20	102.0	187.0	130.0	149.0	130.0	85.8
Mean ±SD		**51.96[a]** ±26.11	**64.46[a]** ±25.69	**83.83[a]** ±53.93	**67.10[a]** ±39.94	**70.38[a]** ±53.81	**88.23[a]** ±40.38	**65.96[a]** ±23.38
C12	Ic	30.30	62.1	70.9	57.9	254.0	241.0	108.0
C20	Ic	64.60	62.4	60.7	61.0	23.2	48.0	90.5
C23	Ic	71.50	63.5	58.8	67.0	76.7	59.3	115.0
C25	Ic	83.00	40.3	39.2	47.2	53.8	43.3	27.6
C26	Ic	49.70	80.6	97.7	49.1	41.4	51.0	34.8
C27	Ic	35.00	60.4	50.2	30.6	32.6	67.6	40.0
Mean ±SD		**55.68[a]** ±20.80	**61.55[a]** ±12.81	**62.91[a]** ±20.10	**52.13[a]** ±12.88	**80.28[a]** ±87.10	**85.03[a]** ±76.89	**69.31[a]** ±39.56

[a] Means with the same superscript do not differ ($P > 0.84$)

Similar to this, the functioning of behavioural and pituitary-adrenal systems in young goats was influenced by consistent individual differences in responsiveness, according to research by Lyons *et al*. (1993). This individual temperament of goats can explain the big variation in the Standard Error and Standard Deviation seen in Figure 4.1 (15 - 45 min). Goat number C 24 in the Vp group had a cortisol level of 187 nmol/L at 15 minute blood collection time whilst the lowest cortisol level was that of C6 with 30.1 nmol/L.

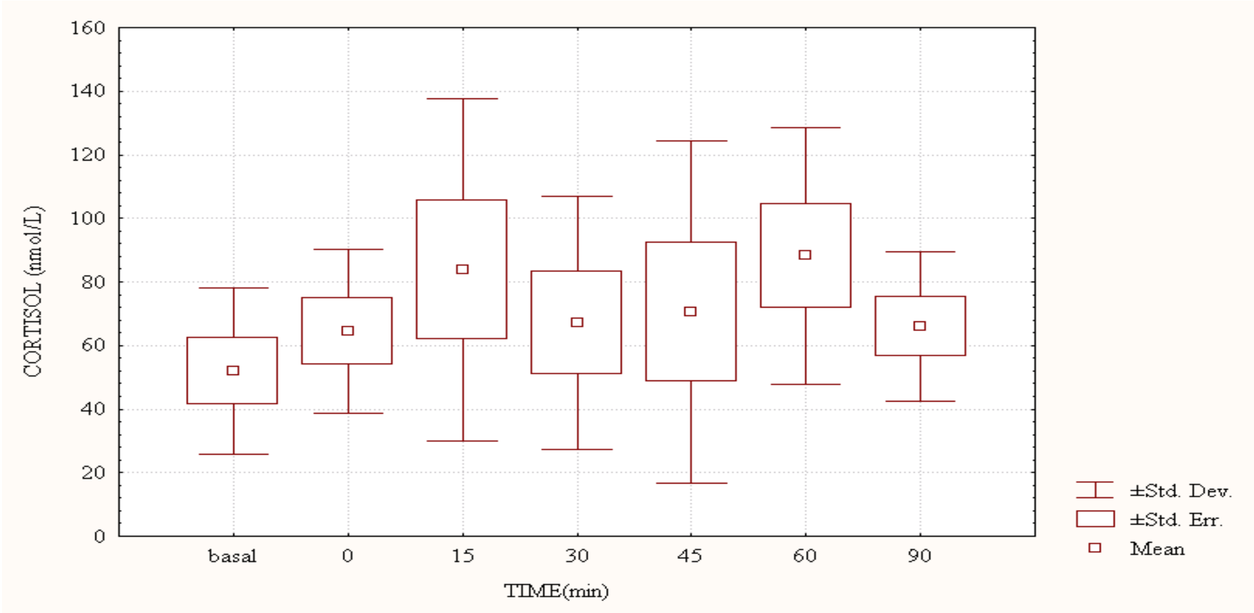

Figure 4.1 Mean, ±Standard error (±SE) and ±Standard Deviation (± SD) serum cortisol concentration(nmol/L) of goats bled via venipunture

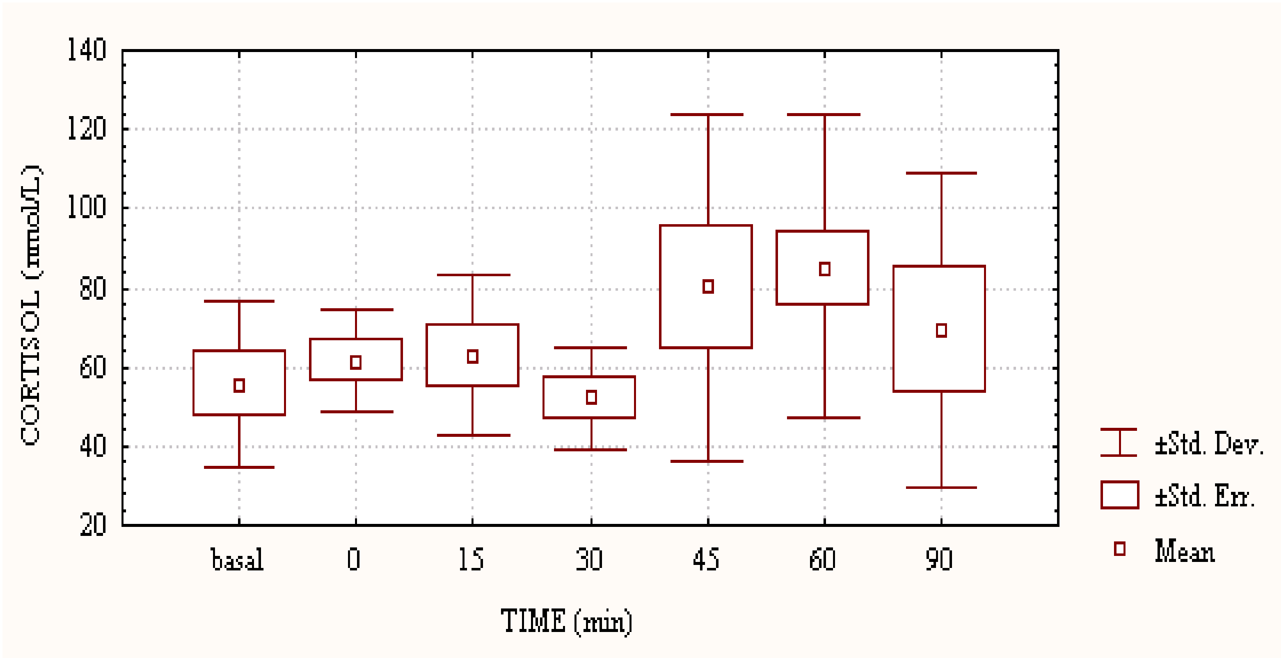

Figure 4.2 Mean, ±Standard error (±SE) and ±Standard Deviation (±SD) serum cortisol concentration (nmol/L) of goats bled via Intravenous catheter

If the mean cortisol concentration of the venipuncture (Vp) and Intravenous catheter (Ic) blood collection groups are compared (Figure 4.3), there was no difference (*P*>0.84) between the two blood sampling techniques.

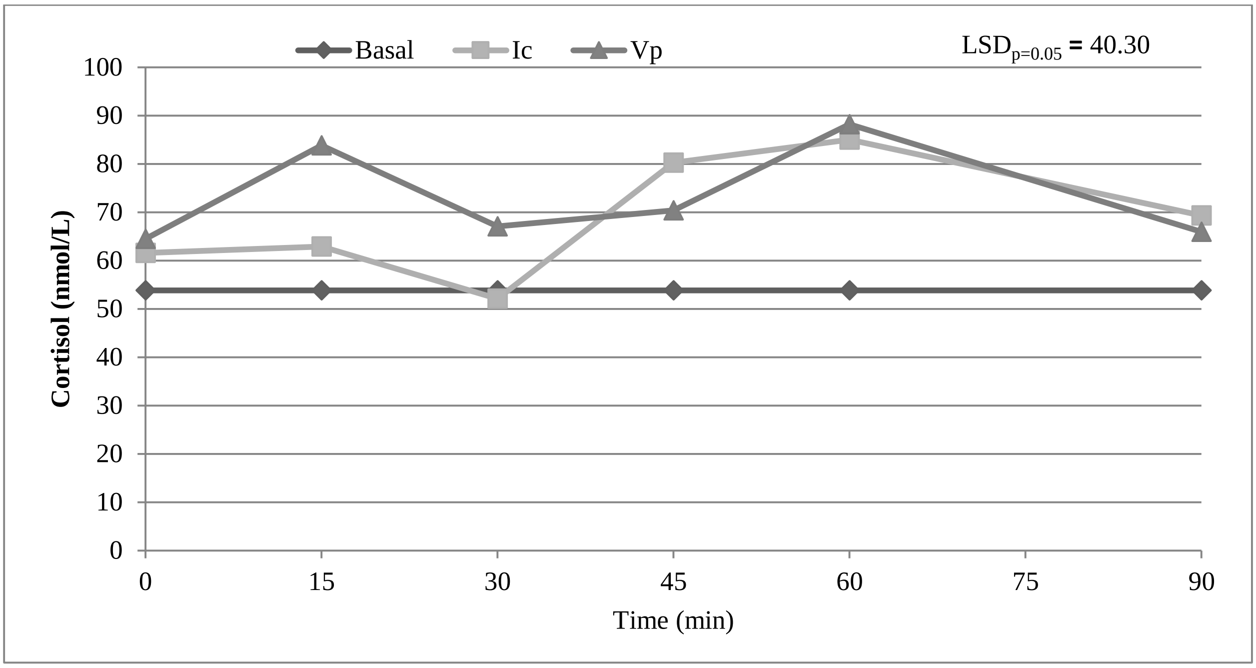

Figure 4.3 Mean serum cortisol concentration (nmol/L) of goats bled via intravenous catheter (Ic) and venipuncture (Vp)

It can be concluded that if the goats are calmed and handled correctly the venipuncture technique can be used for blood sampling without causing additional stress to the animal.

Chapter 5

The effect of management procedures alone or in combination on stress in goats

5.1 Introduction

The effects of routine handling-, husbandry- and environmental factors (e.g. heat exposure, food and water deprivation), and the combination thereof, that may elicit a stress response in goats are reported in this chapter. This is done through the measurement of cortisol concentration in the circulating blood, since it is a recognized measurement tool for stress (Kannan *et al*., 2003; Säkkinena *et al*., 2004).

5.2 Handling stress

The routine handling of goats is inevitable for the maintenance of health of the animals. Procedures such as vaccination are performed once or twice per year for the prevention of diseases such as enterotoxaemia (*Clostridium perfringens* type D), pasteurellosis (*Mannheimia haemolytica*) and Rift valley fever. These procedures involve physical handling and restraint of the animal for subcutaneous injection. During the summer months in the summer rainfall areas it is also necessary to do monthly investigation for the presence of internal parasites such as the roundworms, especially *Haemonchus contortus*, nasal bot (*Oestrus ovis*) and liver fluke (*Fasciola hepatica, Fasciola gigantica*). Such investigations can range from FAMACHA™ to faecal sample collection for faecal egg count (FEC). All of these procedures involve physical handling of the animal such as minimal restraint with handling of the head to observe the eye mucous membrane for FAMACHA™ to more physical restraint and anal penetration for rectal faeces sampling for faecal egg count. Should it be required further handling would then be necessary for deworming of the animal. This is done by injectable or oral dosing that also involve physical handling and restraint of the animal. External parasite control also involves handling by means of pour-on, plunge dipping or spraying of the animals with acaricides. No matter the reason for handling, it requires restraint of the animal. The aim of this investigation was to simulate typical routine handling of goats for the purpose of vaccination and determine the effect of this handling on the cortisol concentration in the circulating blood. The hypothesis is that procedural handling may elicit a stress response in goats.

5.2.1 Results and discussion

The cortisol concentration of the individual animals is presented in Table 5.1. The variation in cortisol concentration between individual animals is apparent with the basal concentration in some individuals (C7 and C11) being double the concentration of others, (C22 and C31). This is in accordance with observations of individual animal temperament on the small stock section of the Animal Production Institute and as described by Martin *et al*. (2004). Despite the variation at the basal level of the individual goats, the cortisol level of all the goats elevated to levels above 100 nmol/L immediately after the goats were handled (0 minutes).

Table 5.1 Individual animal data, Mean, ± Standard deviation (±SD) of serum cortisol concentration (nmol/L) of goats handled for a routine procedure

Sample no.	**Basal**	**0 min**	**15 min**	**30 min**	**45 min**	**60 min**	**90min**
C7	74.80	185.0	144.0	102.0	55.5	65.1	31.2
C11	74.20	136.0	98.5	65.9	45.8	70.1	50.8
C19	70.60	163.0	125.0	102.0	133.0	156.0	97.7
C22	30.30	152.0	98.8	53.8	57.4	91.3	36.7
C29	38.40	156.0	114.0	109.0	67.3	116.0	37.2
C31	33.90	107.0	84.4	47.5	29.0	64.3	38.9
Mean ±SD	**53.70**^{c,d} ±43.42	**149.83**^{a} ±26.38	**110.78**^{b} ±21.48	**80.03**^{b,c} ±27.39	**64.67**^{c,d} ±35.89	**93.80**^{b} ±36.40	**48.75**^{d} ±24.83

^{a,b,c,d}Means with the same superscript do not differ significantly at a 5% significance level (p=0.05)

The variation in the Standard deviation was bigger at 60 minutes than during the other collection times (Figure 5.1). This was due to an individual goat (C19) with a much higher cortisol level than the other goats in the group. The cortisol concentration of this individual, did not return below the basal concentration after 90 minutes as was the case with the other individuals. This goat can thus be considered as having an anxious temperament and would be a typical candidate susceptible to a compromised immunity and subsequent stress related diseases.

The effect of the handling on the cortisol concentration of the goats is depicted in Figure 5.2. There was a difference at a 5% significance level (*P*=0.05) between the mean cortisol concentration of the stressor treatment at 0-; 15-; 30 and 60 minutes and the mean of the basal concentration (Figure 5.2).The cortisol concentration dropped gradually up to 45 minutes after the stressor exposure. This can be explained due to cortisol in the HPA-axis that acts also in a negative feedback cycle to suppress any further release of ACTH.

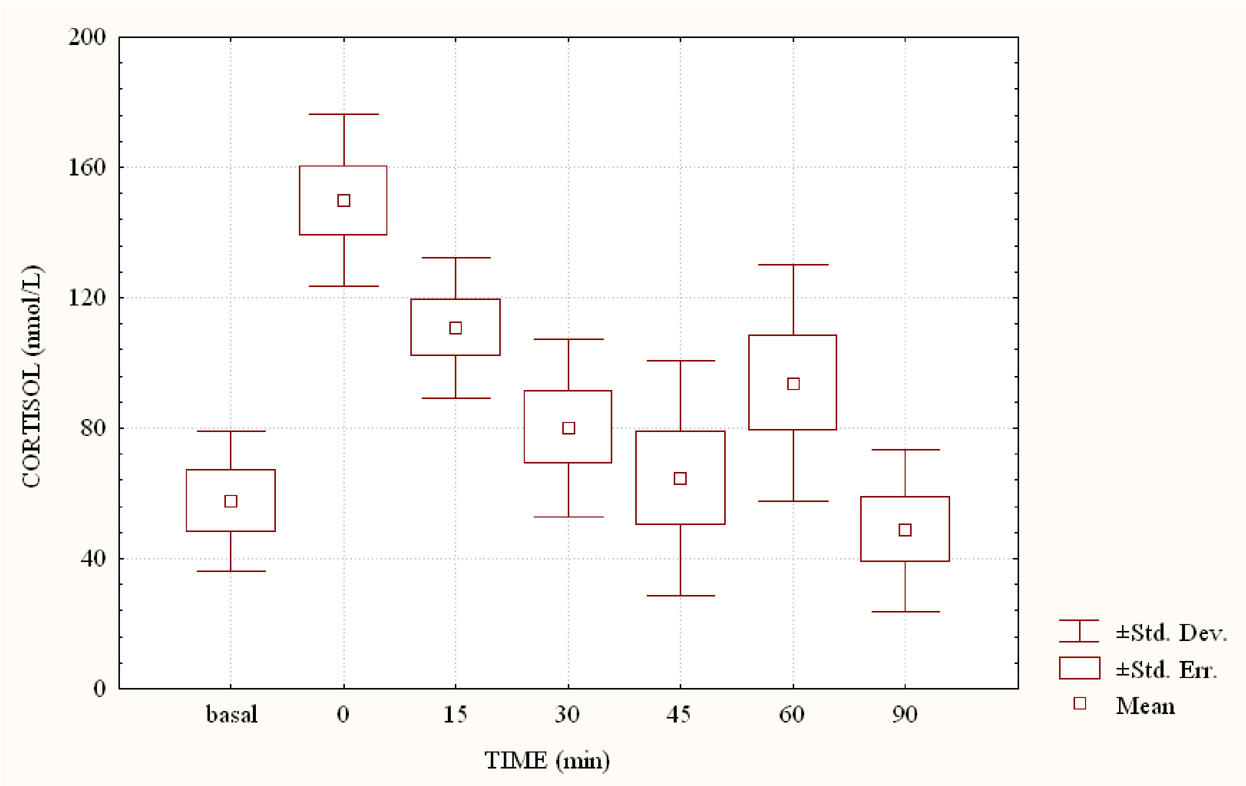

Figure 5.1 Mean, ±Standard error (±SE) and ±Standard Deviation (±SD) serum cortisol concentration (nmol/L) of goats subjected to routine handling.

The results support the statement of Martin *et al.* (2004) on the fear of goats for unknown humans, strange, novel or threatening environments. This can also explain the elevated cortisol concentration even before the animals were physically handled. The animals were accustomed to the grazing camp and when they were collected and chased to the handling facility and grouped in high density, it was against any routine that they were used to. Lyngwa (2012) has proven that high density result in more aggressive behaviour among small stock. The second rise at 60 minutes are due to individual animals, (C19 and C29) that presented with persistent elevated cortisol concentration. A rise at 60 minutes was also observed in one of the other stressors, accumulated Heat, Food and Water deprivation, (Figure 5.16) but not in any of the other stressor treatments and further investigation into this is needed. According to Rhind (2009) infrequent increases in cortisol compromise the response to a specific antigen challenge during vaccination. This is of particular importance with regards to the efficacy of vaccination procedures of animals.

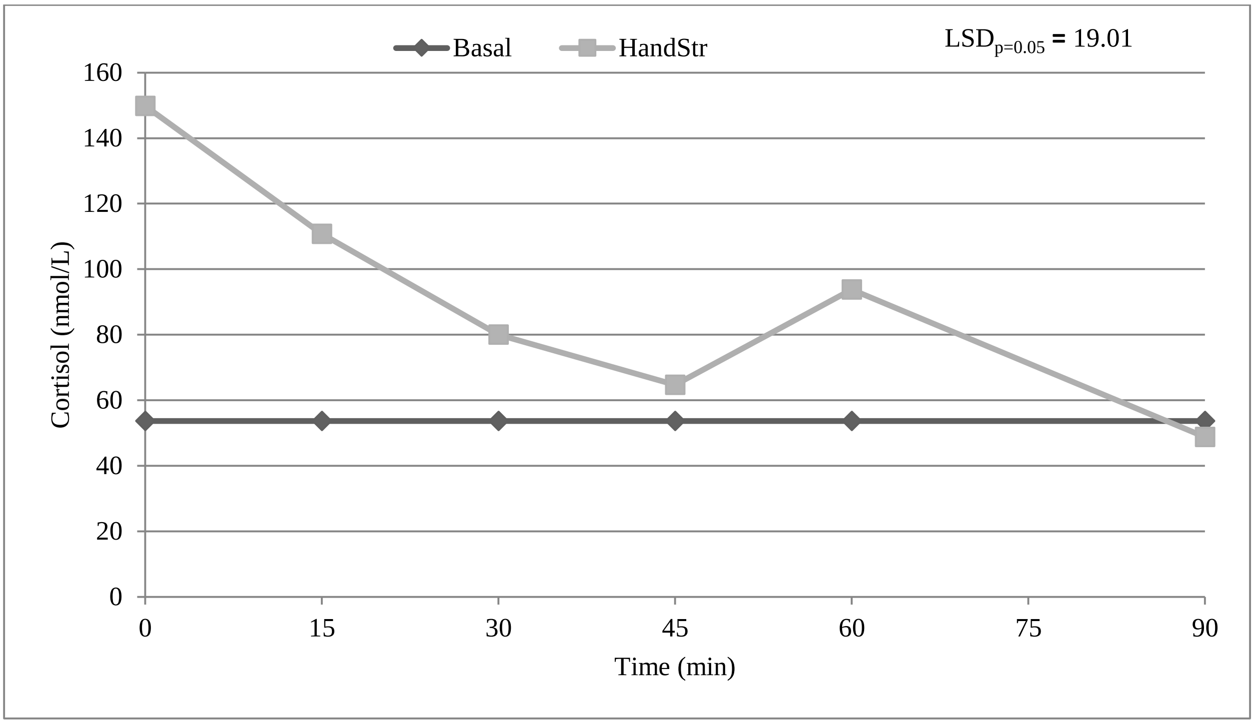

Figure 5.2 Mean serum cortisol concentration (nmol/L) of goats subjected to routine handling

The results indicate that the fear for novelty and handling is more severe when animals are outside their routine and therefore the cortisol concentration was so high at 0 minutes and declined even though the animals were handled and bled via venipuncture. It was also not just the case of individual animals that one may argue has an anxious temperament, but all the animals had elevated cortisol concentrations at 0 min.

The mean cortisol concentration during the handling procedure was higher at a 5% significance level (P=0.05) than the mean basal concentration and it can be concluded that routine handling for vaccination do elicit a significant stress response in goats.

5.3 Heat (HtStr)

Heat and sunshine is an integral part of the South African climate that man and animal has adapted to. There are numerous reports (Alamer, 2009; Hansen , 2009; Veerasamy & Rajendra, 2010) to the effect of heat as a stressor. The effect of heat as a stressor does only become of importance when the animal can no longer compensate through thermoregulation to maintain its homeostasis. In auction yards and on transport trucks goats often have to endure hours of exposure to sunlight and heat. The aim of this investigation was to simulate the effect of direct sunlight heat exposure (atmospheric temperature >30ºC) and to determine the effect thereof on the serum cortisol concentration in the goat.

5.3.1 Results and discussion

The results of the mean and individual animal's serum cortisol concentration after heat exposure (3 hours at >30ºC) are shown in Table 5.2.

Table 5.2 Individual animal data, Mean ± Standard deviation (±SD) of serum cortisol concentration (nmol/L) of goats subjected direct sunlight heat

Animal No	**Basal**	**0min**	**15min**	**30min**	**45min**	**60min**	**90min**
C5	30.30	41.9	21.1	20.4	31.2	19.9	19
C21	8.77	83	33.1	31.5	51.3	33.7	40
C22	30.3	77.5	32.6	26.4	12.9	47.5	53.5
C23	71.5	54.1	47.2	35.6	36.4	53.2	46.6
C27	35.0	26.3	30.9	21.9	20.3	12.3	48.6
C32	52.7	64.6	27.3	21.3	33.1	18.2	44.1
Mean ±SD	**39.75^{ab}** ±40.3	**53.38^{a}** ±46.07	**29.20^{b}** ±21.78	**23.14^{b}** ±19.70	**28.91^{b}** ±26.43	**29.35^{b}** ±31.58	**42.31^{ab}** ±22.21

^{a,b,c} Means with the same superscript do not differ significantly at a 5% significance level (p=0.05)

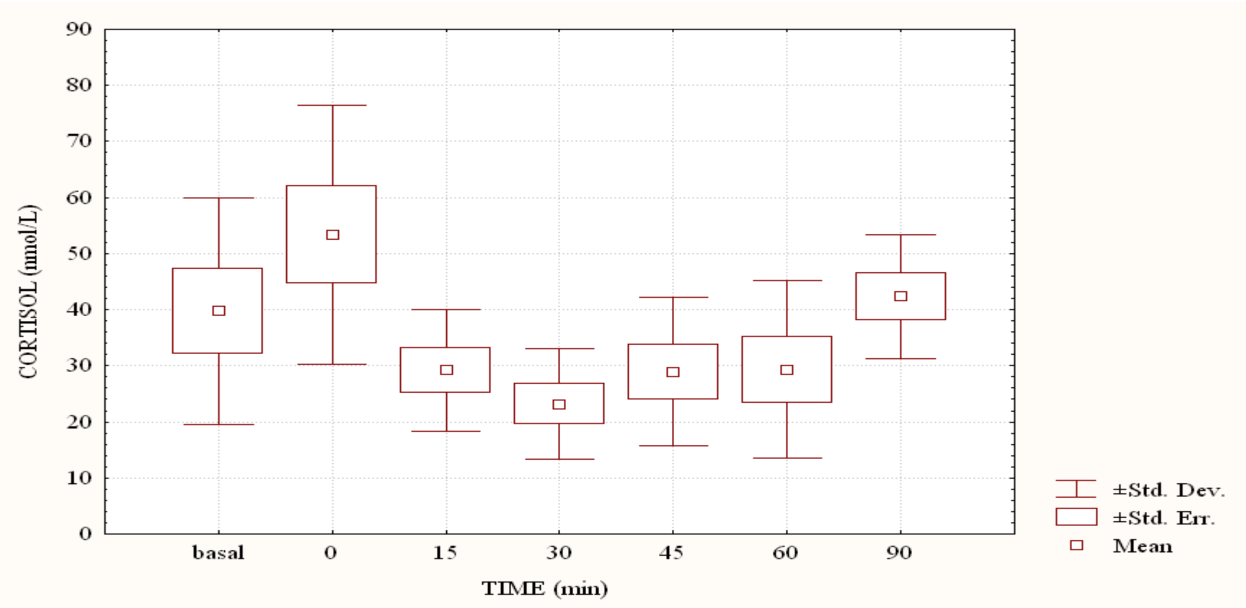

Figure 5.3 Mean, ±Standard error (±SE) and ±Standard Deviation (±SD) serum cortisol concentration (nmol/L) of goats subjected to sunlight heat (3hours)

The variation in the basal level values of individual goats (Table 5.2) is responsible for the variation in the Standard deviation seen in figure 5.3 at basal level and 0 minutes. The grouping of the goats in a small enclosed area during the stressor exposure is responsible for a disruption of the social hierarchy that in turn may be responsible for submissive goats to experience more stress than the dominant ones. The submissive goats would then have much higher cortisol levels. This result is in accordance with findings of social behaviour by Miranda-de la Lama and Mattiello, (2010). Once the goats returned to the camp the variation in the Standard deviation was less.

The mean cortisol concentration of the stressor treatment animals differed a 5% significance level (*P*=0.05) from the basal concentration only at 0 minutes, after which the stressor treatment concentration dropped below the mean basal concentration, (Figure 5.4). This concentration at 0 minutes may have been influenced by aggression and due to high density crowding as described by Lyngwa (2012) or social disruption as described by Lyons *et al*. (1993). The goats were kept in the high density camp for the duration of the 3 hours exposure to heat. In contrast to this the animals were allowed to return to the acclimatization camp between the blood collection sessions and the stressor and high density crowding was thus no longer of significance.

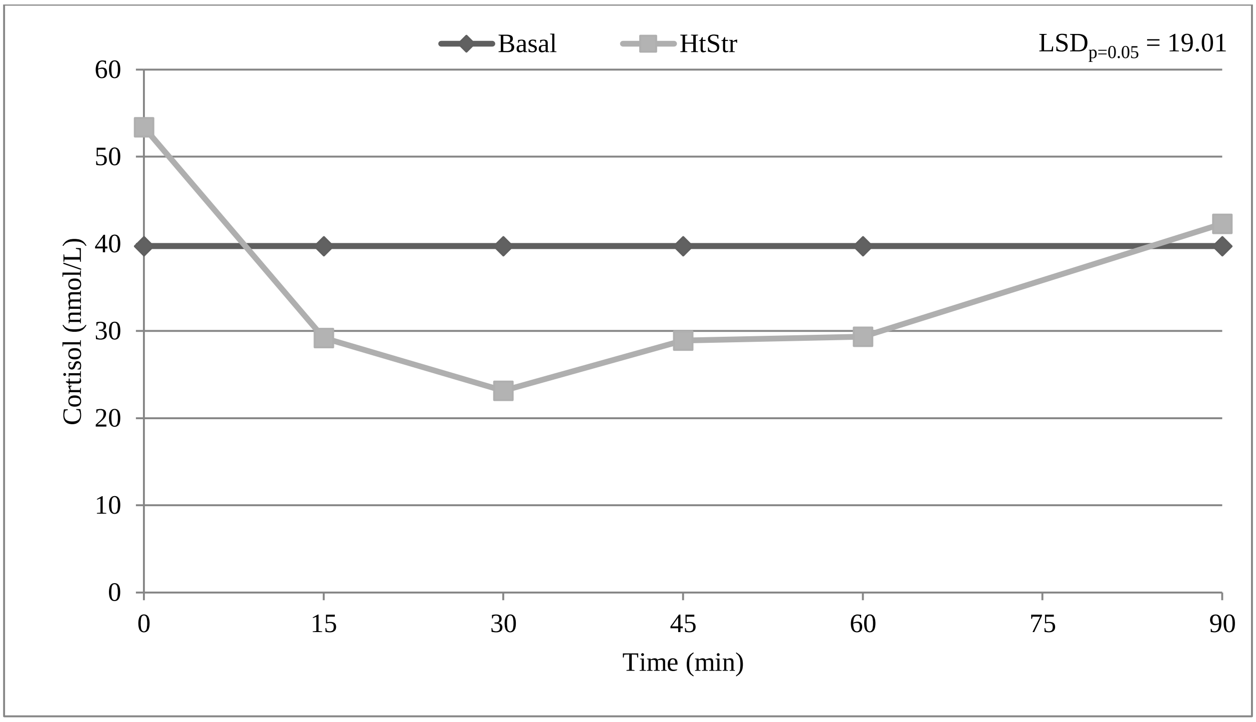

Figure 5.4 Mean serum cortisol concentration (nmol/L) of goats exposed to sunlight heat (3hours)

From the results it is evident that exposure to temperatures above 30ºC in direct sunlight for three hours does not elicit a significant stress response in goats. This is accordance with the finding of Silanikove (2000) that goats are the best adapted of the domesticated ruminant species to harsh warm climates with indigenous species performing better than species originating from a more moderate climate. Heat exposure can however aggravate a stress response when in combination with other stressors such as handling (Figure 5.5). If compared to handling alone, (Figure 5.2) and heat exposure alone, (Figure 5.4) the cumulative effect off heat and handling was significant a 5% significance level (*P*=0.05) up to 60 minutes after the stressor. The effect of heat exposure is also becoming more significant when in combination with food and water deprivation as will be demonstrated in Chapter 5.6.

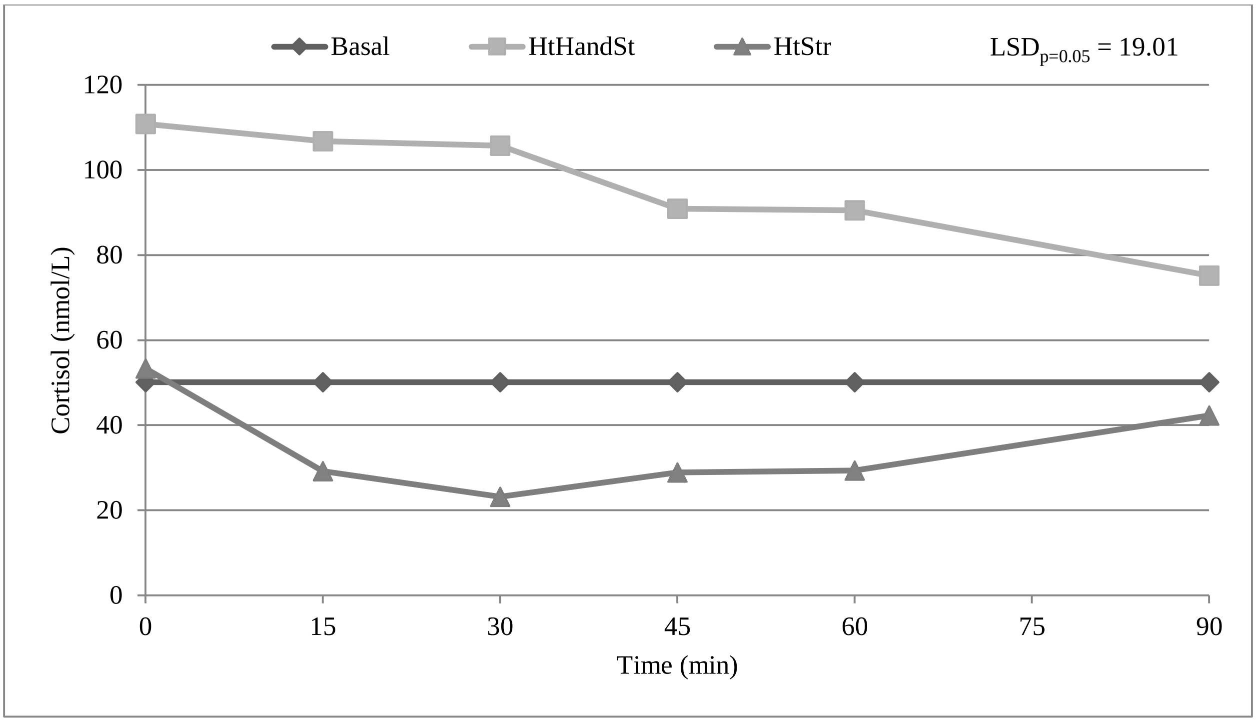

Figure 5.5 Mean cortisol concentration of goats subjected to heat (3hours) and handling versus heat exposure (3hours) alone

It can be concluded that heat exposure (>30ºC) for 3 hours to sunlight with no opportunity for thermoregulation does not elicit a significant stress response in goats with the mean cortisol concentration remaining below the mean basal concentration for up to 90 minutes after application of the stressor treatment.

5.4 Food deprivation stress (FDS)

Food deprivation is a contentious issue raised often by welfare organizations especially with regard to transport and auctioning of animals. Although there is a level of food deprivation involved when animals are being routinely handled, it is never for long periods of time. The transport and auctioning of animals can deprive them of food for 48 hours or even longer. There is a general perception that food deprivation may elicit a stress response in goats and the aim of this investigation was to deprive goats of food and grazing for 48 hours to determine the effect of this on the serum cortisol concentration in the goat. The goats had *ad lib* access to pellet feed and lucerne before the fasting period started.

5.4.1 Results and discussion

In this study the animals were not handled at all during the fasting period. The cortisol levels for the six individual goats in this trial are depicted in Table 5.3.

Table 5.3 Individual animal data, Mean ± Standard deviation (±SD) of serum cortisol concentration (nmol/L) of goats subjected to food deprivation

Animal No	Basal	0min	15min	30min	45min	60min	90min
C16	73.4	12.6	19.3	17.5	7.73	21.7	8.72
C1	3.97	19.4	26.4	23.8	19.6	46.1	25.6
C14	66.5	17.7	33.1	52.4	42.8	42.5	13.4
C15	53.8	15.4	36.7	46.6	34.8	42.2	19.8
C27	35.0	10.0	50.8	72.3	30.9	41.9	26.8
C33	81.9	53.0	40.6	60.7	43.6	44.7	29.8
Mean ±SD	**58.40^{b}** ±57.69	**21.35^{a}** ±15.87	**34.48^{a}** ±11.01	**45.55^{b}** ±21.22	**29.91^{a}** ±13.97	**39.85^{ab}** ±9.04	**20.69^{a}** ±8.26

^{a,b} Means with the same superscript do not differ significantly at a 5% significance level (p=0.05)

The variation in the Standard deviation at 0 minutes is much lower than the variation in the Standard deviation of the basal level (Figure 5.6). This can be attributed to the fact that the animals did not suffer any stress during the fasting period (48 hours) but that the variation in the basal levels differed between the individual animals from 3.97nmol/L (C1) up to 81.9nmol/L (C33).

The mean cortisol concentration was significant lower at a 5% significance level (*P*=0.05) than the mean basal concentration from 0 minutes up to 90 minutes, (Figure 5.7). The goats were vocal during the 48 hour fasting period especially when they saw the handlers which fed them daily. The results indicated clearly that the goats in this investigation tolerated 48 hours food deprivation without eliciting any stress response. This contradicts the perception that food deprivation for periods of up to 48 hours can elicit a stress response in goats. No citations to support or contradict these findings could be found and these results are thus only applicable for the goats used in this investigation that had a full stomach prior to the 48 hour fasting period.

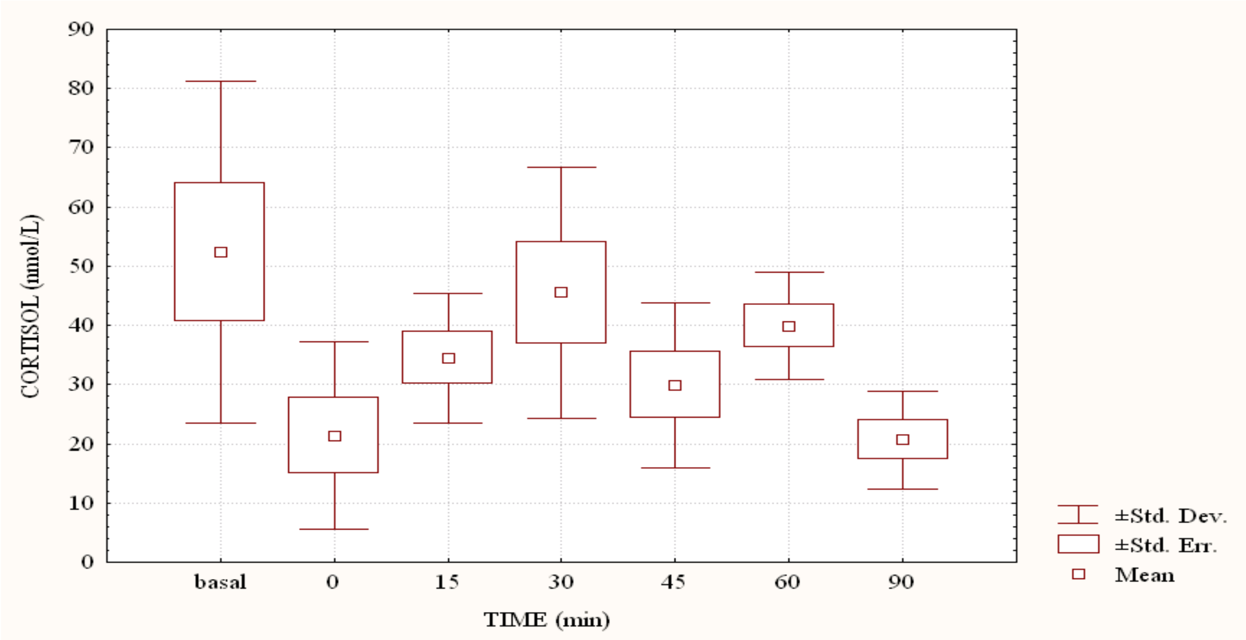

Figure 5.6 Mean, ±Standard error (±SE) and ±Standard Deviation (±SD) serum cortisol concentration (nmol/L) of goats subjected food deprivation (48hours)

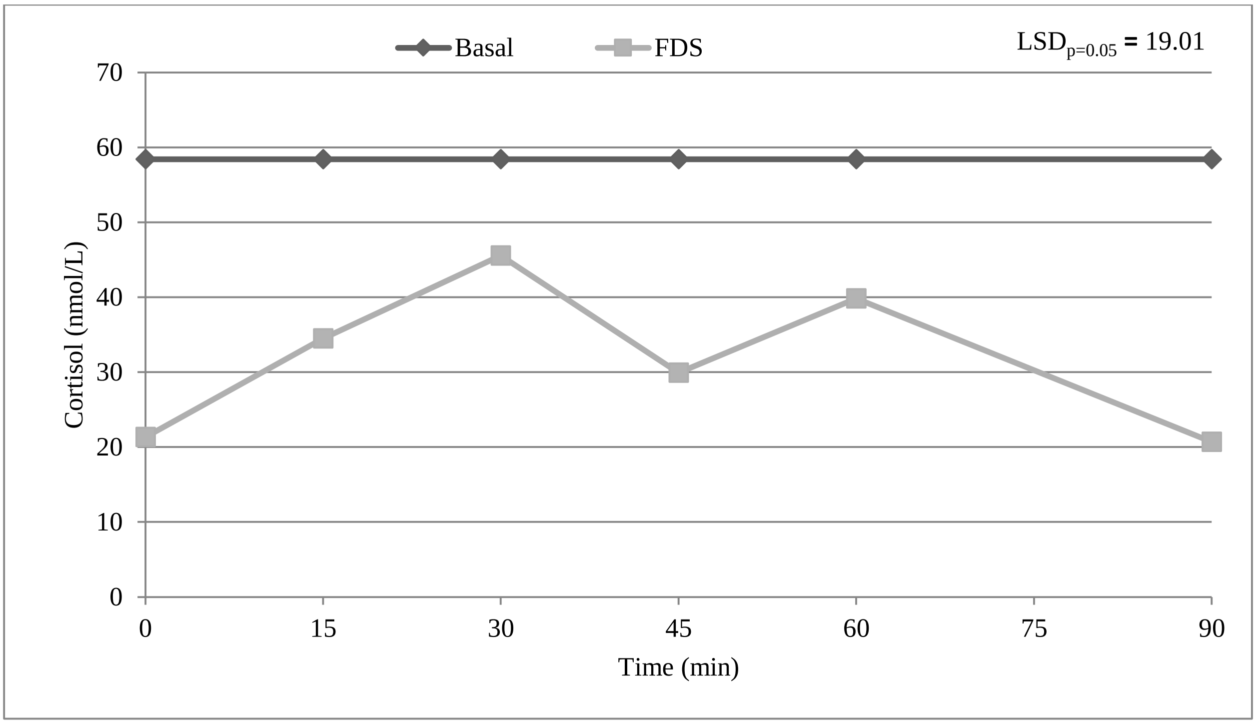

Figure 5.7 Mean cortisol concentration (nmol/L) of goats subjected to food deprivation (48hours)

The effect of food deprivation is becoming more significant when in combination with heat exposure and water deprivation as will be demonstrated in Chapter 5.6.

5.5 Water deprivation stress (WDS)

Goats are among the most efficient farm animals with regards to water utilization Alamer, (2009). Water is nevertheless very important for goats too and is essential for all metabolic processes. The water consumption of goats depends on the amount needed to maintain the normal water balance and it may vary from 100 ml to several liters. Factors such as high environmental temperature may have an influence on water intake for the purpose of thermoregulation. Typical situations of water deprivation arise when animals are being transported for long distances or at livestock auction yards and where the grazing pastures are far from the water point. It is common practice to deprive goats of water before transport with the reasoning that they urinate less and thus the surface is less slippery during the trip. The aim of this investigation was to simulate water deprivation situations and to evaluate the cortisol concentration in the water deprived goats. The perception is that water deprivation for periods up to 48 hours can elicit a stress response in the goats.

5.5.1 Results and discussion

The results of the six goats used in the water deprivation trial are depicted in Table 5.4. There are numerous citations of the ability of goats to withstand water restriction for periods of up to 2 days without causing stress to the animal (Alamer, 2009; Jaber *et al.*, 2004; Lowe *et al.,* 2002; Adogla-Bessa & Aganga, 2000; Li *et al.*, 1999). The results in this investigation confirm these findings with the mean cortisol concentration remaining below the mean basal concentration from 0 minutes through 90 minutes (Figure 5.9).

Table 5.4 Individual animal data Mean, ± Standard deviation (SD) of serum cortisol concentration (nmol/L) of goats subjected to water deprivation (48 hours)

Animal No	**Basal**	**0min**	**15min**	**30min**	**45min**	**60min**	**90min**
CB	46.9	39.5	53	71.2	39.5	34.8	30.9
CB2	47.0	25.3	27.1	29.5	24.1	27.6	9.55
C3	43.90	32.8	33.7	43.3	37.8	25.7	19.6
C5	30.30	62.1	65.9	58.5	26.1	11	14.2
C20	64.60	46.4	49.7	36.4	32.3	38.1	34.5
C26	49.70	14.7	15.8	13.2	31.2	32.6	19.8
Mean ±SD	**47.1**^{a} ±22.01	**36.8**^{a} ±16.59	**40.86**^{a} ±18.55	**42.02**^{a} ±20.70	**31.83**^{a} ±6.12	**28.3**^{a} ±9.62	**21.43**^{ab} ±9.59

^{ab} Means with the same superscript do not differ significantly at a 5% significance level (p=0.05)

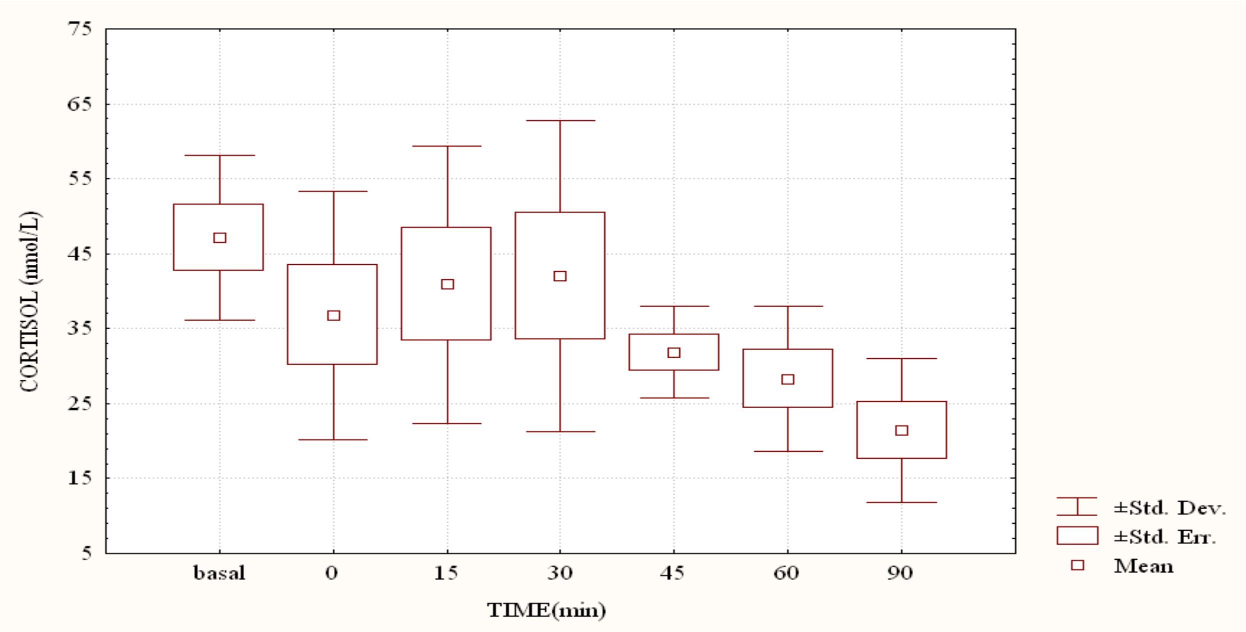

Figure 5.8 Mean, ±Standard error (±SE) and ±Standard Deviation (±SD) serum cortisol concentration (nmol/L) of goats subjected to water deprivation (48hours)

One explanation to this can be the fact that there was no handling involved and also no disruption in the social hierarchy of the flock during the stressor period.

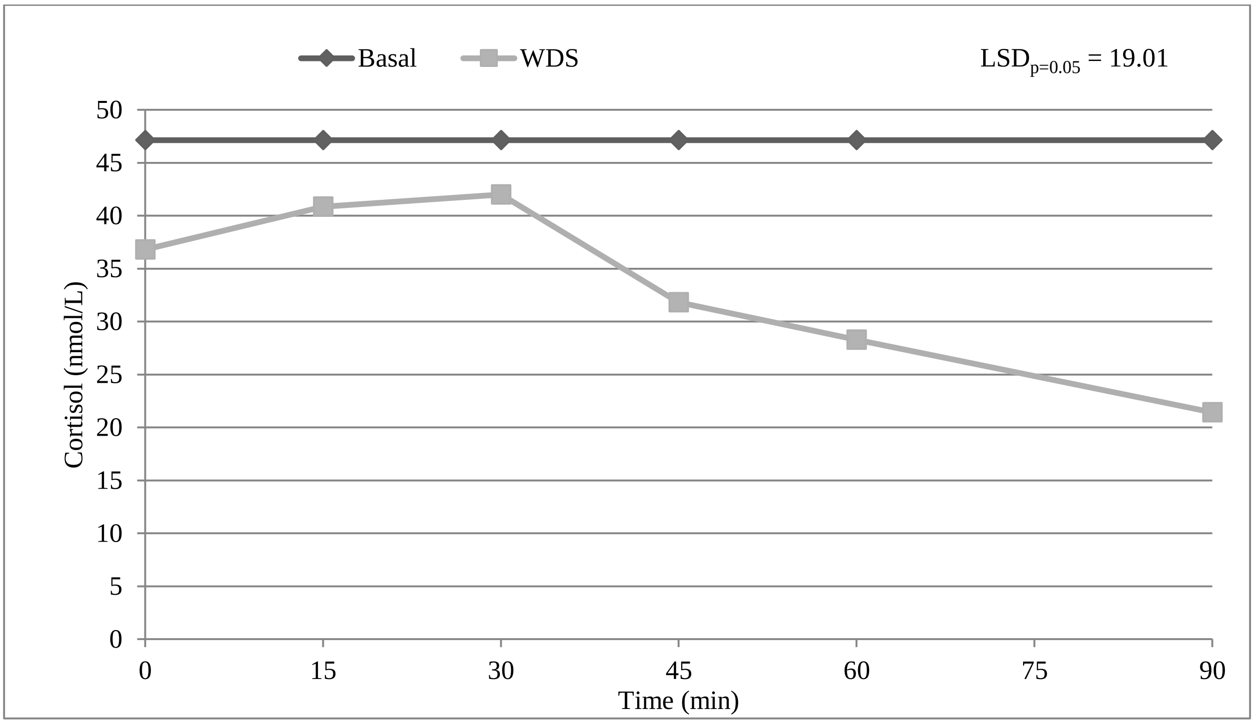

Figure 5.9: Mean cortisol concentration (nmol/L) of goats subjected to water deprivation (48 hours)

The effect of water deprivation is of more significance when in combination with heat exposure and food deprivation as will be demonstrated in Chapter 5.6. From the current results itt can be concluded that 48 hours of water deprivation does not elicit a stress response in goats. This contradicts the perception that water deprivation for 48 hours can elicit a stress response in goats.

5.6 Heat and handling stress (HtHandSt)

The purpose and reason for routine handling has been described in Chapter 5.1. In the South African climate the routine handling of animals often happens on sunny and warm days. This is especially of importance for preventative handling with regards to parasite control since internal parasites are abundant during the warm summer months. With routine handling procedures, animals are typically grouped in a waiting kraal or pen and then have to wait their turn to be treated. This implies that the animals are exposed to heat and sun. This grouping of the animals in the waiting area denies the animals the opportunity for thermoregulation but may also lead to aggressive behaviour due to overcrowding and disruption of the social hierarchy as described by Lyngwa (2012). The aim of this investigation was to simulate a typical handling procedure such as oral drenching and to determine the accumulative effect of heat exposure and routine handling on the cortisol concentration in the blood serum. The hypothesis is that the cumulative effect of heat exposure and handling may elicit a stronger stress response than handling or heat exposure alone.

5.6.1 Results and discussion

The cortisol concentration (nmol/L) of the individual animals is presented in Table 5.5.

Table 5.5 Individual animal data Mean ± Standard deviation (SD) serum cortisol concentration (nmol/L) of goats subjected to sunlight heat (3hours) and handling

Sample no.	Basal	0 min	15 min	30 min	45 min	60 min	90min
C1	39.7	112.0	86.9	58.5	43.6	43.6	39.7
C14	66.5	84.1	61.5	97.1	56.38	72.8	80.3
C15	53.8	109	114	122	105	113	107
C18	48.3	185	171	134	89.9	98.5	85.5
C33	81.9	81.7	86.1	102	117	93.3	83
C34	72.6	93.5	121	121	133	122	55.7
Mean	**60.46**^{b,c}	**110.88**^{a}	**106.75**^{a}	**105.76**^{a}	**90.88**^{a,b}	**90.53**^{a,b}	**75.2**^{b}
±SD	±31.83	±38.39	±38.03	±26.89	±34.79	±28.57	±23.85

^{a,b,c} Means with the same superscript do not differ significantly at a 5% significance level (p=0.05)

The variation in the standard deviation of the animals depicted in Figure 5.10 is fairly even with a range between ±23.85 at 90 minutes and ±38.39 at 0 minutes. This may be explained by the fear of the goats of handling and all the goats were affected by this fear. Similar results occured in Chapter 5.1 (Figure 5.1) where handling was the stressor treatment. With stressors where handling was not involved (heat exposure, food deprivation and water deprivation) the variations in the standard deviation was bigger.

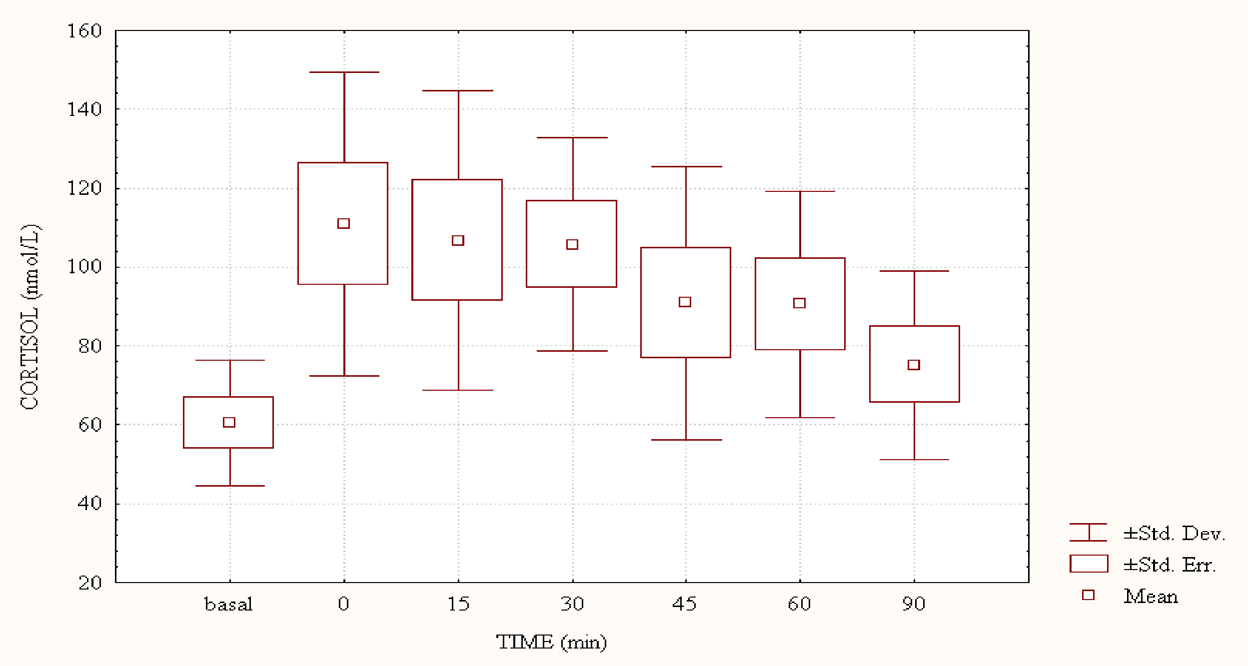

Figure 5.10 Mean, ±Standard error (±SE) and ±Standard Deviation (±SD) serum cortisol concentration (nmol/L) of goats subjected to sunlight heat (3hours) and handling

The effect of the combination of heat exposure and handling on the serum cortisol concentration of the goats is shown in Figure 5.11. The results indicate a difference at a 5% significance level (P=0.05) between the mean basal concentration and the stressor treatment from 0 minutes up to 60 minutes after the stressor was applied. The exposure to heat required that the goats had to spend 3 hours (>30ºC) in a warm and overcrowded pen and the subsequent handling (dosing) can explain the elevated mean cortisol concentration of 110.88 nmol/L already at 0 minutes. This is in accordance with results of Lyngwa (2012) which have described the influence of aggression due to high density crowding and the social disruption as described by Lyons *et al*. (1993). The handling procedure for dosing is an aggressive type handling as the mouth has to be forced open and the drench deposited on the back of the tongue with the dosing gun nozzle.

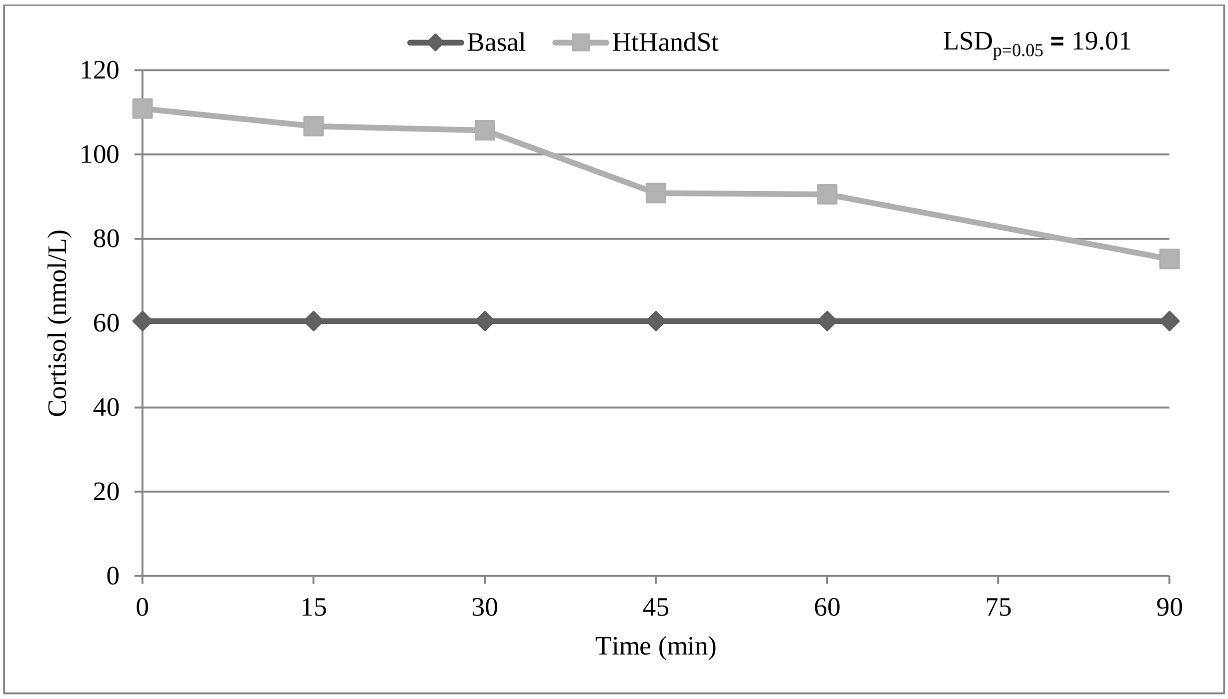

Figure 5.11 Mean cortisol concentration (nmol/L) of goats subjected to sunlight heat (3hours) and handling

The results of the cumulative heat and handling stressor support the results of the handling stressor discussed in Chapter 5.1. The cumulative effect of heat and handling is evident if the result is compared to the result of heat as a stressor on its own, (Figure 5.12).

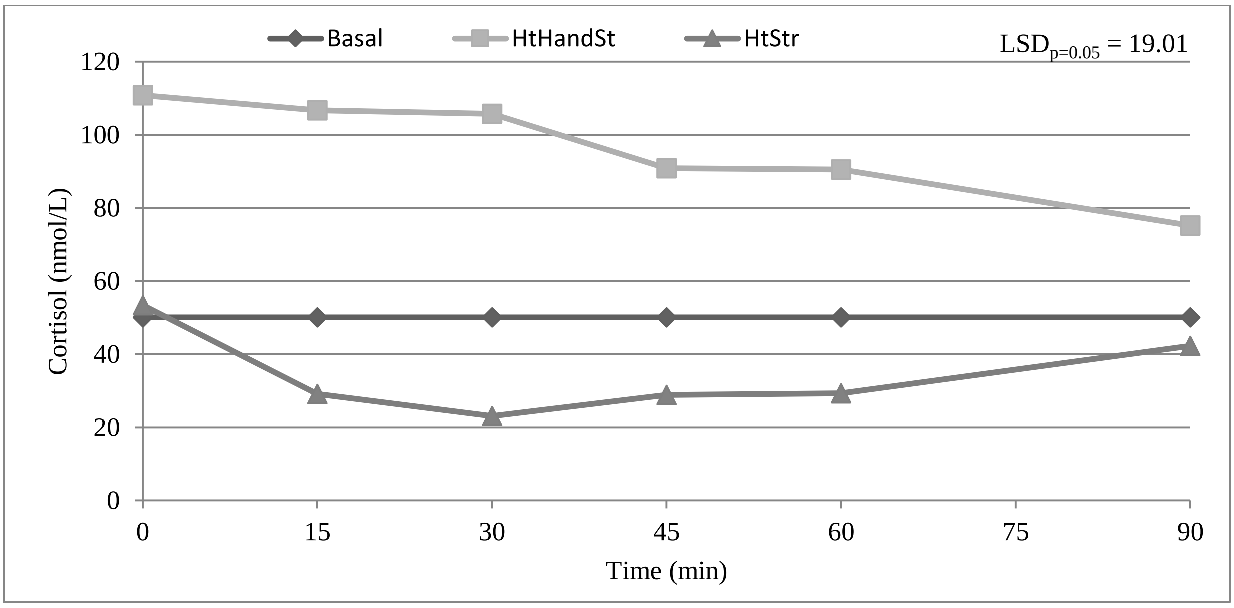

Figure 5.12 Mean cortisol concentration (nmol/L) of goats subjected to sunlight heat (3hours) and handling versus sunlight heat (3hours) exposure alone

In the heat treatment on its own the cortisol concentration never rose above the basal concentration, not even if the animals were handled for blood collection. This supports the hypothesis that routine handling procedures are capable to elicit a significant stress response with elevated cortisol concentration.

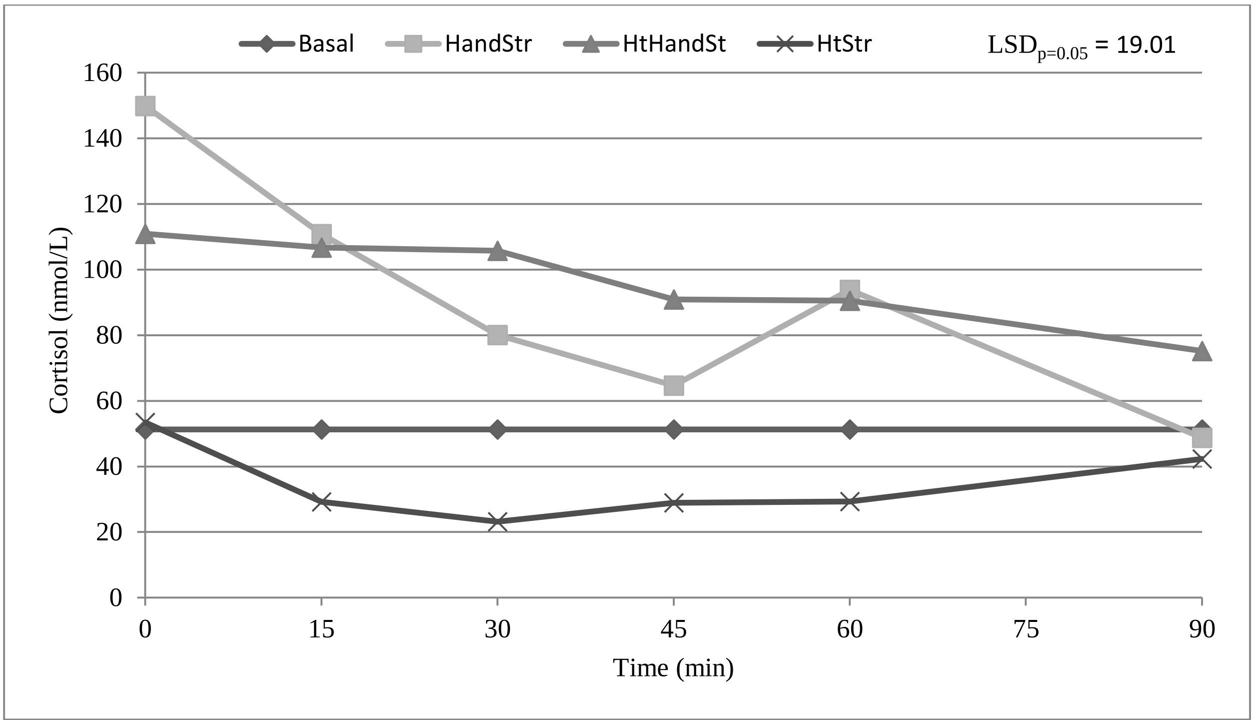

Figure 5.13 Mean cortisol concentration (nmol/L) of goats subjected to handling; sunlight heat (3 hours) and handling and sunlight heat (3hours) exposure alone

From the results it can be concluded that the cumulative effect of handling stress (dosing) and environmental stress (heat exposure), resulted in an elevated cortisol concentration at a 5% significance level (P=0.05) for up to 60 minutes, (Figure 5.13). The combination heat and handling stressor, (Figure 5.11) also had a higher mean cortisol concentration compared to the means of heat exposure alone(Figure 5.12), or routine handling procedure alone, (Figure 5.13)for up to 90 minutes after the stressor treatment.

5.7 Heat-, food deprivation- and water deprivation stress (HtFdWStr)

The keeping and trade of goats often involve the animals being transported and auctioned. Both of these actions have the potential of being stressful. Goats have the ability to withstand water restriction for periods of up to 2 days without causing stress to the animal (Li *et al*,. 1999; Lowe *et al*,. 2002; Jaber *et al*,. 2004; Alamer, 2009). There are numerous citations on the negative effect of high temperatures and heat stress of small stock (Alamer, 2009; Hansen *et al*,. 2009; Veerasamy *et al*,. 2010). The aim of this part of the study was to simulate the conditions involved with transport or auctions to determine the cumulative effect of heat exposure, food deprivation and water deprivation as potential stressors and to determine the serum cortisol concentration after the stressor treatment. The hypothesis is that the cumulative effect of the stressors would elicit a more severe stress response than the stressors alone.

5.7.1 Results and discussion

The cortisol concentration (nmol/L) of the individual animals in this investigation is presented in Table 5.6

Table 5.6 Individual animal data Mean ± Standard deviation (±SD) of serum cortisol concentration (nmol/L) of goats subjected to sunlight heat (3hours), water-and food deprivation (48hours)

Animal No	basal	0min	15min	30min	45min	60min	90min
C3	43.90	35.9	82.5	94.1	90.2	64.0	62.6
C4	43.3	87.2	76.7	38.4	27.4	41.9	67.6
C6	13.8	41.7	30.1	23.7	15.0	53.5	32.8
C8		59.9	55.5	43.6	31.7	105.0	49.9
C19	70.6	60.1	71.2	72.8	109.0	135.0	97.1
C27	35.0	60.4	50.2	30.6	32.6	67.6	40.0
Mean ±SD	**41.32^{abc}** ±41.74	**57.53.^{a}** ±19.41	**61.03^{a}** ±40.31	**50.53^{a}** ±29.35	**50.98^{a}** ±42.28	**77.83^{ab}** ±34.28	**58.33^{a}** ±31.03

^{abc} Means with the same superscript do not differ significantly at a 5% significance level (p=0.05)

The variation between individual goats is evident in with values ranging from 15.0 nmol/L up to 109 nmol/L at 45 minutes (Table 5.6). This confirms findings of Cockram (2004) who describes variation in stress responses for several reasons and at each stage of the stress response. The response of the individual goats to the cumulative stressor can also be seen in 5.14 with a big variation in the standard deviation at 45 and 60 minutes. Moberg (As cited in Cockram, 2004) describe three stages in the stress response: recognition of the stressor, biological response or defense against the stressor and the consequence of the stress response. Each individual goat can perceive stress on a different level and thus respond to it differently and that may explain the variation in cortisol values. Goats have well developed mechanisms of thermoregulation which may be either physiological or habitual (Alamer, 2009).

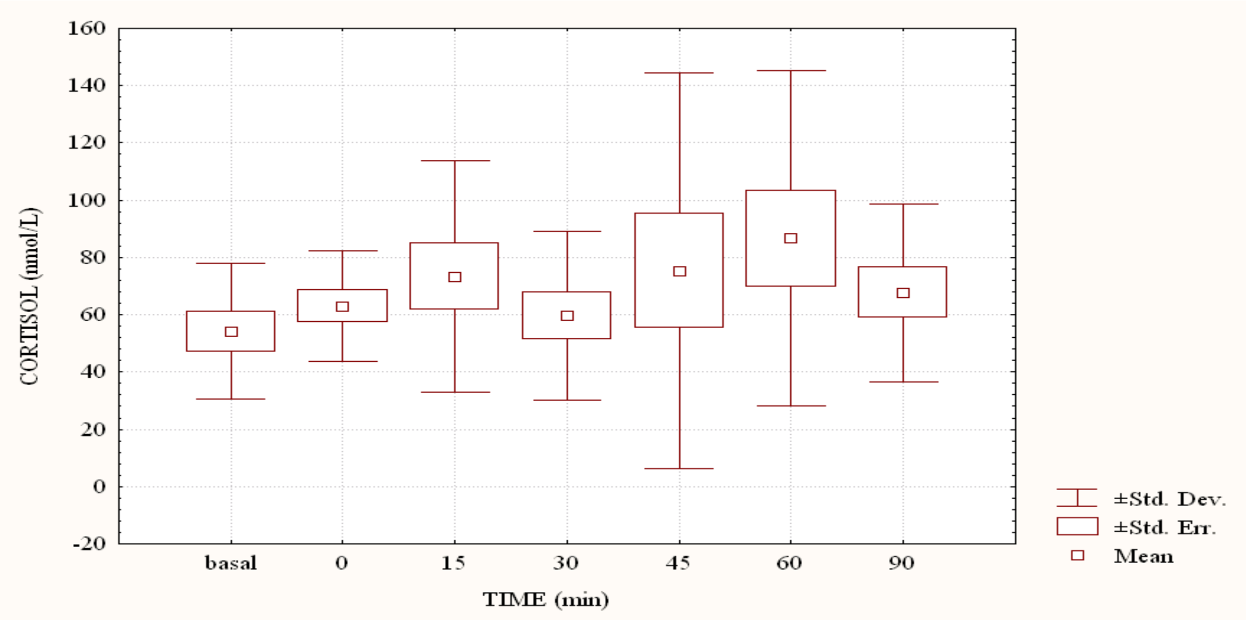

Figure 5.14 Mean, ±Standard error (±SE) and ±Standard Deviation (±SD) serum cortisol concentration (nmol/L) of goats subjected to heat (3hours) food deprivation and water deprivation (48hours)

This is evident in Figure 5.15 with the mean cortisol concentration of animals subjected to 3 hours of sunlight heat exposure (HtStr) being significantly lower at a 5% significance level (*P*=0.05) than the mean basal concentration.

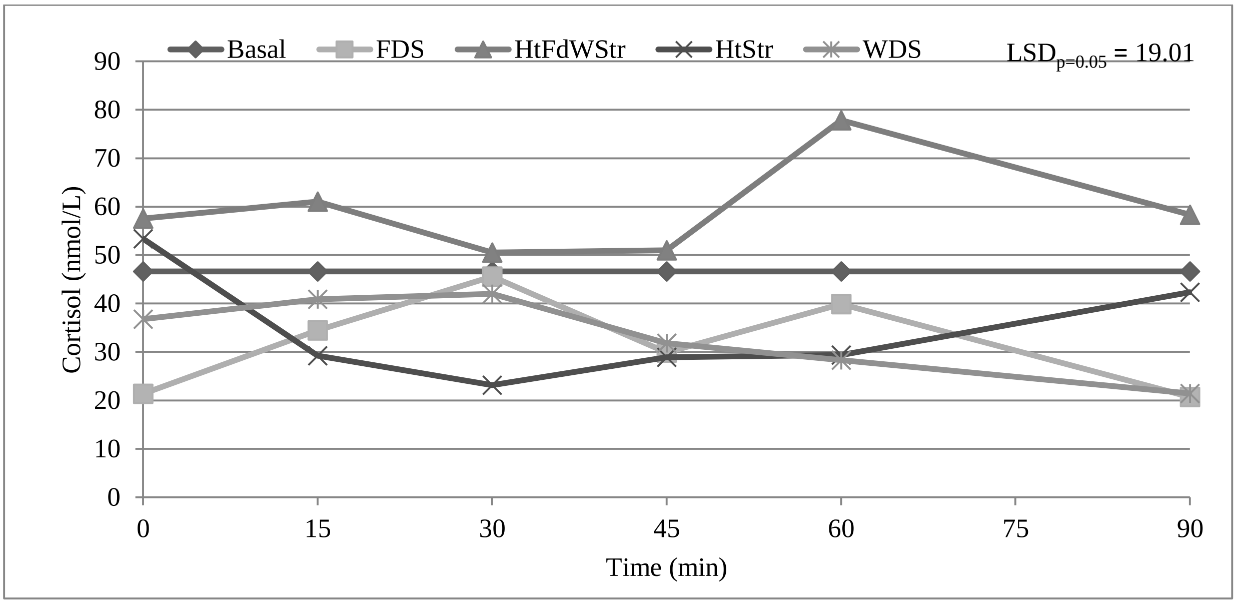

Figure 5.15 Mean serum cortisol concentration (nmol/L) of goats subjected to cumulative heat (3hours), food- and water deprivation (48hours) versus heat exposure(3hours) alone, water deprivation (48hours) alone and food deprivation (48hours) alone

Similarly the effect of food deprivation (Figure 5.15) and water deprivation (Figure 5.15) alone did not elicit any stress response with the mean cortisol concentrations of these stressor treatments lower than the mean basal concentration. Contrary to this, the cumulative effect of food and water deprivation and heat exposure (HtFdWstr) did elicit a stress response (Figure 5.16) with mean cortisol concentrations higher than the mean basal concentration with a difference at a 5% significance level (P=0.05) at 15 minutes and 60 minutes. The mean cortisol concentration reached a peak (77.83 nmol/L) at 60 minutes. If compared to the stressors where the goats were handled the peak mean cortisol concentration was higher in stressors where the goats were handled (90 nmol/L) for up to 45 minutes, (Figure 5.17) after the stressor treatment. The cortisol concentration then rose similar to the handling stressor treatment at 60 minutes to a significant level (P=0.05). In contrast to the handling stressor treatment the mean cortisol concentration did not drop below the mean basal cortisol concentration at 90 minutes and however not significant the mean cortisol concentration of combination stressor remained above the mean basal concentration 90 minutes after stressor treatment.

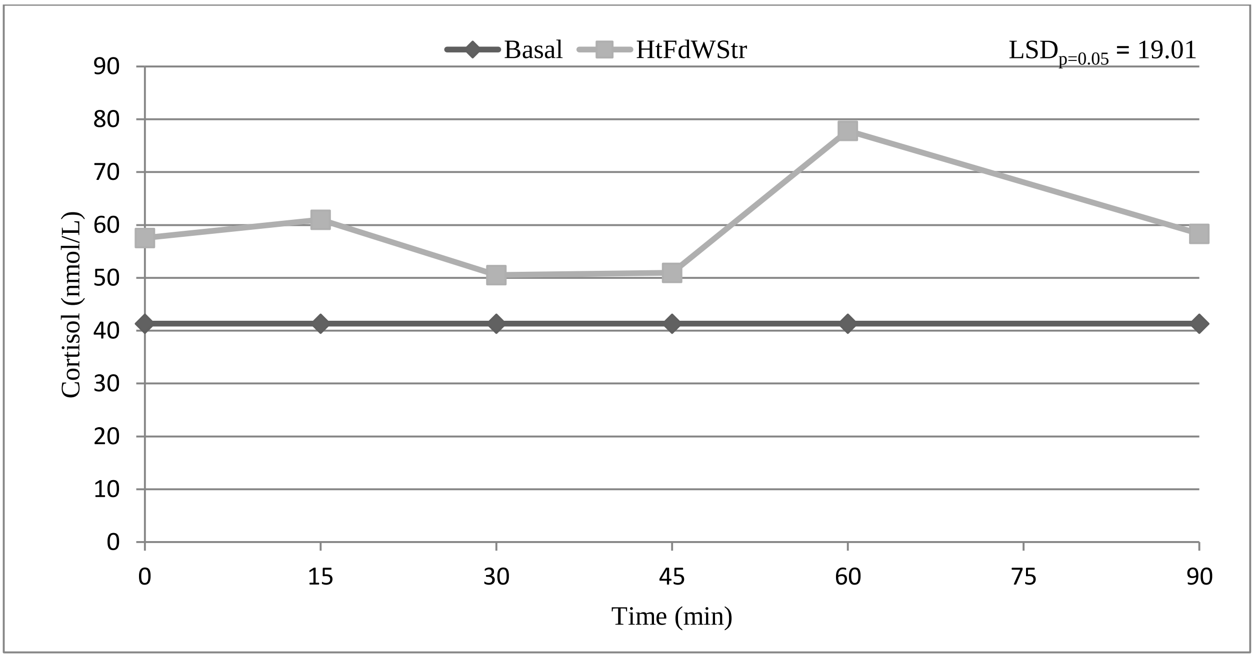

Figure 5.16 Mean serum cortisol concentration (nmol/L) of goats subjected to cumulative sunlight heat (3hours), food-and water deprivation (48hours)

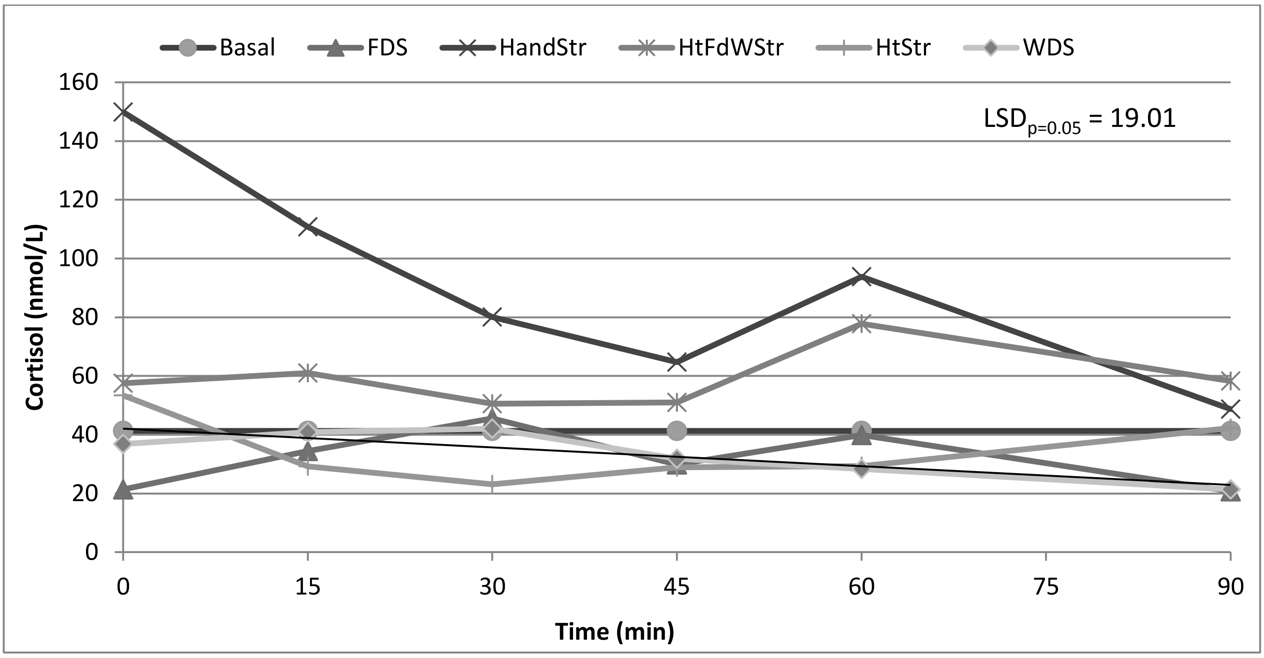

Figure 5.17 Mean serum cortisol concentration (nmol/L) of goats subjected to cumulative heat (3hours), food- and water deprivation (48hours) versus heat exposure(3hours) alone, water deprivation (48hours) alone, food deprivation (48hours) alone and handling stress

This might indicate that the cumulative effect of heat-, food- and water deprivation stress (HtFdWStr) is a more chronic stressor than handling stress. The results support the hypothesis that the cumulative effect of heat-, food- and water deprivation elicit a stronger stress response than heat exposure, food deprivation and water deprivation stressors alone. An interesting observation was made with the goats showing more interest in food after 48 hours of food and water deprivation rather than the water. This might be explained due to metabolic water production.

Chapter 6

General discussion, recommendations and conclusion

6.1 General discussion

The collection of blood samples for cortisol determination is a contentious issue with the argument that the collection technique may be a stressor on its own and thus elicit a stress response (Ghasemi-Nejad *et al.*,2014). Other samples than blood for cortisol determination has been researched with less invasive procedures and involve among others; saliva, urine, faeces and even hair. To collect any of those samples also require handling of the animal. In the current study, series sampling with 15 minute interval was required and thus faecal and urine sampling was not an option. Hair collection is the only non-invasive technique for cortisol determination. According to Russel *et al.* (2012) hair cortisol analysis presents a complementary means of monitoring stress, capturing systemic cortisol exposure over longer periods of time. If real time cortisol levels are required the hair sample would thus be insufficient. This is contradicted by Ghassemi Najed *et al.*(2013) that found wool cortisol data more precise and accurate than blood cortisol data in real time stressors such as heat and water restriction. Results of the current study indicated neither heat exposure (3 hours) nor water deprivation (48 hours) under natural conditions to elicit stress response. This may raise the question; that whether the cortisol levels in the Ghassemi Najed *et al*.(2013) trial was due to heat and water deprivation or was it because the animals were kept in metabolic crates where isolation stress and deprivation of natural behaviour may be responsible for stress as described by Russel *et al.* (2012)? The wool or hair sample for cortisol level determination does however justify further research as it is a truly non-invasive technique that requires minimal restraint.

Results from the current study (Figure 5.2) have shown that normal routine handling can elicit quite a strong stress response that lasted for more than an hour. This is in accordance with findings of Rushen *et al.* (1999) who stated that husbandry procedures do distress sheep to some degree. It must be emphasized that the goats in the trial was not accustomed to handling, specifically for the purpose of determining the effect of handling on cortisol release. Because of this the intravenous catheter method was not necessarily the best method for cortisol determination as it requires frequent handling to service the catheter by flushing it with heparinized saline. The catheter also caused discomfort to the goats and they were very sensitive to the area around the catheter when the catheters were flushed. Contrary to this, the results have also indicated that; if handled correctly, (one hand under the lower mandible and the other hand behind the head) with simultaneous calming, (massaging the area below the ear) venipuncture blood sampling can be performed without causing additional stress to the animal. This is in accordance with findings of Hopster *et al* (1999) who found that jugular puncture may induce an increase in cortisol concentration but this depends on the handling experience of the animal and on individual differences. It may not necessarily be the short term pain inflicted with venipuncture that causes stress but rather the handling itself (Figure 5.2). In stressors that did not involve procedural handling beforehand but only during serial blood sampling the cortisol levels did not rise above the basal level. This is an indication that the serial venipuncture blood collection did not cause stress which can only be attributed to the proper handling during the procedure (Figure 4.3). The high cortisol levels found during routine handling procedures such as subcutaneous vaccination and oral drenching support this findings.

Routine handling is inevitable if goats are to be kept. There is a common perception that routine handling does not cause stress to the animals. This might be true in cases such as milch breeds that are being milked regularly and is thus accustomed to the handling procedure. This perception is supported by the fact that stress does not manifest immediately with any recognizable signs (Cockram, 2004). The results of the current study have indicated that less frequent procedures such as vaccination, (one to three times per year) and oral drenching, (once per month during the internal parasite peak period) elicited a significant stress response. One possible explanation for this may be that the handler has two conflicting roles when these routine procedures are performed. One is to act as the stimulus to move the animals towards the handling facility. This is traditionally achieved by typical and repeated fear inducing stimuli such as; noise, body movements, whip cracking and even physical force as is observed on some small stock farms. The second role of the handler is to apply or administer the treatment, in which case the handler now expects the animal to stand still. Hargreaves & Huston (1989) have found elevated cortisol concentrations in sheep 10 minutes after drafting. This is in accordance with results of the current study where elevated cortisol concentrations occurred already at 0 minutes after the goats were moved to the handling facility (Figure 5.2) where the movement to the handling facility also took 10 minutes.

Stockmanship should enjoy the same emphasis to improve animal production as do genetics, nutrition and health. This is however rarely the case on commercial farms. Because fear is a potent stressor, reducing fear through positive human contact is necessary when animals are to be handled (Zulkifli, 2013). Understanding and recognition of normal behavioural signs in the goat such as safety zones and flight response when moving the animals without fear towards the handling facility will reduce stress in the animals. From the results in this study it can be concluded that infrequent handling do elicit a significant stress response in the goat. It is ironic that the specific handling to prevent disease might be responsible for compromising the immune response resulting in the animal being susceptible to stress related diseases. According to Rhind (2009) large infrequent increases in circulating cortisol can modify the cell mediated immune response in such a way that the response to a specific antigen challenge is compromised.

The results also indicated routine handling procedures such as oral drenching to be a more severe stressor than exposure to natural and environmental factors such as heat (Figure 5.13). This can be

due to the fact that natural factors of weather extremes do not interfere with the natural behaviour of the animals and where such conditions have an influence on the homeostasis of the animal it elicits a physiological response rather than a stress response to maintain the homeostasis. In cases with hyperthermia, the animals would look for ways of thermoregulation by means of panting, resting in the shade or drinking water, all of which are natural behaviour of goats. Handling on the other hand interferes directly with the natural behaviour of the animal with a subsequent stress response and increase of cortisol in the blood. The way the handler carries out his or her procedure on the goat will determine the relationship the goat will have with the handler which may vary between positive, negative or neutral. Calming of the animals during routine procedures proved to limit stress to the animals. When calming techniques were applied during the series blood collection the animals were not vocal and the cortisol levels did not rise above the basal level. This is in accordance with results of Rushen *et al.*(1999) that found gentled sheep to be more willing to approach humans. Extra efforts should be introduced for calming of the animals before the procedures are to be performed. Such animals would not have a fear of the handlers or the procedure and thus suffer less stress.
The high levels of cortisol in the blood after handling procedures in this study support the frequent occurrence of *Mannheimia haemolytica* (pasteurellosis) in the country despite efforts to prevent the disease through immunization (Henton, 2009). *Mannheimia haemolytica* (pasteurellosis) is one of the more prevalent diseases encountered on the ARC-Animal Production Institute too and this might be due to the stress the animals have to endure during research trials. This observation is supported by Zulkifli (2013) reporting on immune suppression and disease susceptibility in farm animals after challenging human contact. Further research is necessary to study the association between routine handling stress and *Mannheimia haemolytica* infection (pasteurellosis), which is also a major problem associated with weaning- and transport stress..

The exposure to heat and direct sunlight for three hours might have been too short to elicit a stress response, since goats are known to be heat tolerant and this was confirmed by the results. The aim of the study was not to determine how much heat exposure would be stressful, but rather to simulate a typical handling procedure such as dipping and dosing, where the procedure usually does not take longer than three hours. The effect of heat as a stressor cannot be denied completely since the cumulative effect of heat and handling elicited significant stress response that lasted up to 90 minutes. The cumulative effect of food- and water deprivation and heat exposure also had higher cortisol levels that food and water deprivation alone. Even though the results did not indicate a significant stress response during heat exposure, observation of natural goat behaviour indicates that, given the opportunity the animals would look for shade during extremely warm days. They would even give up grazing opportunity to stay in the shade or adjust their body orientation towards the sun..

The fact that food deprivation for 48 hours did not elicit a stress response can be explained by the fact that the animals had a full stomach prior to the food deprivation. If deprived for a longer period it might have elicited a stress response. The purpose of this study was to simulate commercial goat farming practices and it is seldom that animals will be deprived of food for periods longer than 48 hours. The welfare issue of supplying food at auctions is of relevance here. It would seem more advantageous to deprive the animals of food if within 48 hours rather than supply food to which they are not adapted to since they may develop rumen disorders.

The goats in the trial was used to water *ad lib* and thus not prepared for water deprivation. A case can be made for goats in arid areas that are accustomed to receive a once off water supply and thus drink enough for the whole day. Even the sudden water deprivation in this study did not elicit a stress response. This supports previous findings on the ability of goats to tolerate water restrictions. (Meissner & Belonje,1972; Li *et al*., 1999; Lowe *et al*., 2002; Jaber *et al*., 2004; Alamer, 2009). Once again the practical implications for commercial goat farming is relevant here, supporting the argument to rather not supply water when animals are in transit as this will lead to urination and subsequent soiling of the floor area rendering it slippery.

The cumulative effect of heat exposure and handling on circulating serum cortisol was significantly higher than the basal level at a 5% significance level (P=0.05) for more than 1 hour after stressor application and proved to be a more chronic stress than handling alone. Since animal handling and heat exposure often occur at the same time, it would require the introduction of some factors to minimize the stress. The heat exposure becomes an issue only when animals do not have any means for thermoregulation. Therefore it will be of value to allow enough space to avoid overcrowding and opportunities for thermoregulation, such as water and shade while animals are waiting in the handling facility before handling procedures are to be performed.This does however create opportunity for victimization since the dominant animals would claim these resources and prevent submissive animals to also utilize this. Multiple resources at opposite areas may solve this problem.

The cumulative effect of food- and water deprivation and heat exposure was expected to elicit a strong stress response, but this was not the case, with the circulating cortisol not significantly higher than the basal level. This supports the seriousness and significance of handling as a stressor and emphasizes the importance of proper handling during routine procedures. Since there was no handling involved, there was not a significant rise in circulating cortisol.

It was expected that the goats would immediately want to drink water after the 48 hours of food and water deprivation, but they were much more interested in the food provided than drinking water. This supports the numerous citations of goats' ability to tolerate water deprivation especially when they did not eat either.

6.2 Recommendations

General recommendations to reduce stress in goats are made below.

1. Do not move animals towards the crush pen by means of fear evoking stimuli, because they will then fear the handler when in the crush pen.
2. Proper handling with the correct "handles" would also minimize stress during routine handling procedures. The proper handling of a goat involves the goat to rest its chin in an open hand and the other hand is held behind the head while massaging the area below the ear with the fingers.
3. Calming of goats when they are in the crush pen has proven to have advantages in reducing handling stress with no increase in circulating cortisol. Regular positive interaction between the handler and goat will reduce the underlying fearfulness and stress in the goat towards the handler in future (Zulkifli, 2013).
4. Attempt to prevent any disruption of the goat's homeostatic equilibrium that requires a response to maintain its psycho-physiological integrity.
5. Any person that is to work with goats for whatever reason must have the technical skills and knowledge of animal ethology and handling.
6. Inclusion of ethology and animal handling should be included in the curriculum of students studying animal production and animal health.

6.3 Conclusion

The effect of four potential stressors; handling, exposure to heat, food deprivation and water deprivation, on the serum cortisol concentration have been investigated in this study. The results indicated a significant increase in circulating cortisol when animals were handled in contrast to environmental stressors that did not involve handling such as heat exposure and food and water deprivation, where the cortisol levels stayed below the basal level. Routine procedures such as vaccination and deworming are performed under typical commercial farming situations and are important for the maintenance of good health in the flock. It is not the handling as such that is a stressor but more so the way of handling. During this study the goats was subjected to repeated blood sampling without eliciting a stress response because the animals were gentled during the procedure. The relationship of elevated cortisol concentrations and stress related diseases need further research.

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Abstract

The welfare of animals is an emotional topic and even farm animal welfare is becoming more and more of relevance with a public consciousness on "How we treat the animals we eat". Regardless the reason why goats are being kept, their wellbeing remains an important factor to ensure satisfactory results and production. Typical stressors such as transport, shearing, isolation and heat exposure are topics that have been extensively researched for its stress impact factor. Routine handling procedures used in the general management of a goat flock is not generally regarded as a form of abuse or stressful and very little researched has been conducted on it. These routine procedures do however have the potential to cause stress and especially short term stress in the goat. The effect of stress can be determined by the concentration of the corticoid hormone, cortisol, in the body. The aim of this study was to determine the serum cortisol concentration after routine handling procedures, heat exposure, food deprivation and water deprivation as well as the cumulative effect of these potential stressors in South African unimproved indigenous goats. A series of blood samples were collected at 0-; 15-; 30-; 45-; 60- and 90 minutes into a vacuum serum tube from the jugular vein after venous occlusion. Analysis of the serum was done by chemiluminescent enzyme immunoassay with the SIEMENS Immulite® 1000 automated Immunoassay Analyzer for quantative measurement of cortisol in serum. The experimental design was a completely randomized design with stressors as treatment and animals as replication. The data was analyzed as a one-way Analysis of Variance (ANOVA) with the repeated measurements over time as a subplot factor. The effect of four potential stressors; handling, exposure to heat, food deprivation and water deprivation, on the serum cortisol concentration have been investigated in this study. The results indicated a significant increase in circulating cortisol when animals were handled in contrast to environmental stressors that did not involve handling such as heat exposure and food and water deprivation, where the cortisol levels stayed below the basal level. This large and infrequent increase in circulating cortisol can modify the cell mediated immune response in such a way that the response to a specific antigen challenge is compromised rendering the goat susceptible to stress related diseases such as pasteurellosis and coccidiosis. Routine procedures such as vaccination and deworming are performed under typical commercial farming situations and are important for the maintenance of good health in the flock. It is not the handling as such that is a stressor but more so the way of handling. During this study the goats was subjected to repeated blood sampling without eliciting a stress response because the animals were gentled during the procedure. The correct and proper handling of goats during routine procedures needs to be promoted as an important part of disease prevention and it should enjoy the same attention as deworming, vaccination and dipping do. The relationship of elevated cortisol concentrations and stress related diseases need further research.