The effect of environmental factors and husbandry practices on stress in goats

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Highlights

- The effect of normal procedural handling on stress in goats was evaluated.
- The effect of husbandry and environmental factors on stress in goats was evaluated.
- Cortisol levels significantly higher than basal levels was seen during routine handling procedures.
- Food and water deprivation and heat exposure did not cause significant stress in goats.

Abstract

Routine handling procedures used in the management of a flock is not generally regarded as a form of abuse or stressful and has not being extensively researched. The aim of this study was to determine the serum cortisol concentration after routine handling procedures, viz. handling, heat exposure, food deprivation and water deprivation as well as the cumulative effect of these potential stressors in South African 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 chemi-luminescent enzyme immunoassay with the SIEMENS Immulite® 1000 automated Immunoassay analyser for quantitative measurement of cortisol in serum. The experimental design was a completely randomized design with stressors as treatment and animals as replication. The data was analysed as a one-way Analysis of Variance (ANOVA) with the repeated measurements over time as a subplot factor. The results indicated that goats subjected to typical routine handling procedures such as oral drenching and subcutaneous vaccination had significantly higher serum cortisol concentration than the basal levels. The cortisol concentration in animals subjected to handling was significantly higher than in animals exposed to heat (30°C for 3 hours), food and water deprivation(48 hours). From the results it can be concluded that handling of goats for routine procedures such as vaccination and deworming can be considered an acute stressor capable to elicit elevated cortisol concentration in goats. Stressors without handling such as heat exposure food- and water deprivation did not elicit cortisol levels above the basal level in this study.

Keywords

stress, cortisol, handling, heat exposure, food deprivation, water deprivation

Introduction

Farm animal welfare has become increasingly relevant in the public eye in recent years with a public consciousness on "how animals raised for human consumption are treated". Despite the controversy and merits regarding animal welfare, 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). In any farming enterprise there is regular human-animal interaction. Keeping of goats inevitably involves management practices and routine tasks such as drenching, vaccination, weighing and selection to be performed. Such routine procedures often expose the goats to potential physical stressful situations, for example: 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 where there is no or sub-standard handling facilities available. The measurement of stress is very complex and context dependent (Zulkifli, 2013). 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 psychological stressors such as disruption of social hierarchy, change of habitat and overcrowding. Stress can be measured by the concentration of the glucocorticoid hormone, cortisol, in the body. The process that involves the secretion of cortisol is initiated by corticotrophin-releasing hormone (CRH) in response to a stress stimulus (Goodman *et al*. 1980). The main function of CRH is the stimulation of pituitary synthesis of adrenocorticotropic hormone (ACTH). The primary physiological effect of ACTH, is the stimulation of the adrenal cortex to secrete glucocorticoid hormones cortisol, corticosterone and aldosterone. Cortisol has the primary function of enabling the animal to escape from the stressor at that moment. Cortisol also acts in a negative feedback cycle to suppress any further release of ACTH.

Transport, shearing, isolation and exposure to heat are topics that were extensively researched in terms of their effects on stress in livestock. Routine handling procedures are not generally recognized as being stressful and limited research has been done in this regard. Such procedures may however have the potential to elicit a stress response because of the disruption of the goat's homeostasis. According to Zulkifli (2013) any disruption of an animal's homeostasis that requires a response to maintain its psycho-physiological integrity has the potential to cause stress to the animal.

Stress and the subsequent release of cortisol affects the goat negatively in various ways. Rhind *et al*. (2009) showed that 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. This has particular relevance to the small stock industry that relies to a large extent on vaccines for disease prevention. The correct handling and husbandry of animals in order to minimize stress does not enjoy the same attention as other methods of disease prevention. The correlation between stress and immune suppression is a major cause of animals' susceptibility to disease. 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). Stress also affects the reproductive ability of goats such as an increase in barren ewes (Dwyer *et al*, 2004), blocked or delayed oestrus (Ehnert *et al*. 1991); suppressed follicular growth and development (Dobson *et al*. 1999); decreased lambing percentage and less multiple births (Knight *et al*, 1988) and lower milk yield (Sevi *et al*. 1999).

The cortisol concentration in the circulating blood is a recognized measurement tool for stress (Kannan *et al*., 2003; Säkkinen *et al*. 2004). The aim of this study was to determine serum cortisol levels in order to evaluate the effects of routine handling-, husbandry- and environmental factors on stress in goats.

Materials and method

The aim of this investigation was to simulate typical routine handling of goats. Blood cortisol levels was determined in goats subjected to handling stress (HandStr); heat stress (HtStr); Food deprivation stress (FDS); water deprivation stress (WDS); cumulative heat and handling stress (HtHandStr) and cumulative heat, food, water deprivation stress (HtFdWStr). South African unimproved indigenous goat does (n=36) between 10 and 12 months old and weighing between 22kg – 26kg were used. 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)

Animal ethical clearance for the trial was obtained from the Agricultural Research Council's Animal Production Institute's Animal Ethics Committee (API-AEC). Before stressor treatment, a group of eight animals (six trial and two replacement) were 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. The basal level (unstressed cortisol concentration) of each animal in the trial flock was established prior to the stressor treatments and was used as the untreated control for that animal. The unstressed basal cortisol levels of all the animals in the trial were within the normal range of 42-82 nmol/L for sheep according to Reference Laboratory Values for hormones (Western College of Veterinary Medicine of the University of Saskatchewan and College of Veterinary Medicine of the Ohio State University).

For the handling stressor treatment, the stressor procedure involved the animals being collected from the camp and chased at fast walking pace for 10 minutes through low density passages of the unit to the handling facility. The animals were vaccinated subcutaneously under the skin between the shoulder blades. For the heat stress 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 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. For the food deprivation stress treatment the animals were deprived of food and grazing but water was available *ad lib* for a period of 48 hours. For the water deprivation stress treatment the animals were deprived of water but food was available *ad lib* for a period of 48 hours. For the cumulative effect of heat and handling stressors 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 handling chute and dewormed orally with a placebo (water) to simulate routine handling for deworming. For the cumulative effect of heat, food deprivation and water deprivation stress 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. The hypothesis was that the cumulative effect of heat exposure and handling may elicit a stronger stress response than handling or heat exposure alone.

A series of blood samples were collected at 0-; 15-; 30-; 45-; 60- and 90 minutes after exposure to stress treatments. Blood were collected with an 18G needle in a vacuum serum tube from the jugular vein after venous occlusion. This was always performed by the same experienced and skilled person. Blood samples were placed in a refrigerator (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 was aspirated with a disposable Pasteur pipette into a marked vial and stored at -20ºC until assayed.

Cortisol in serum was analyzed by means of the SIEMENS Immulite® 1000 automated Immunoassay Analyzer for quantative measurement of cortisol in serum. 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).

Results

Handling stress

The aim of this investigation was to simulate typical routine handling of goats for the purpose of vaccination. The cortisol concentration of the individual animals is presented in Table 1. 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). The effect of the handling on the cortisol concentration of the goats is depicted in Figure 1. There was a difference (P=0.05) between the mean cortisol concentration of the stressor treatment at 0-; 15-; 30-, 60 and 90 minutes post treatment and the mean of the basal concentrations.

Table 1 Individual animal data, Mean ± Standard deviation (±SD) of serum cortisol concentration (nmol/L), post handling, 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 results support the findings 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.

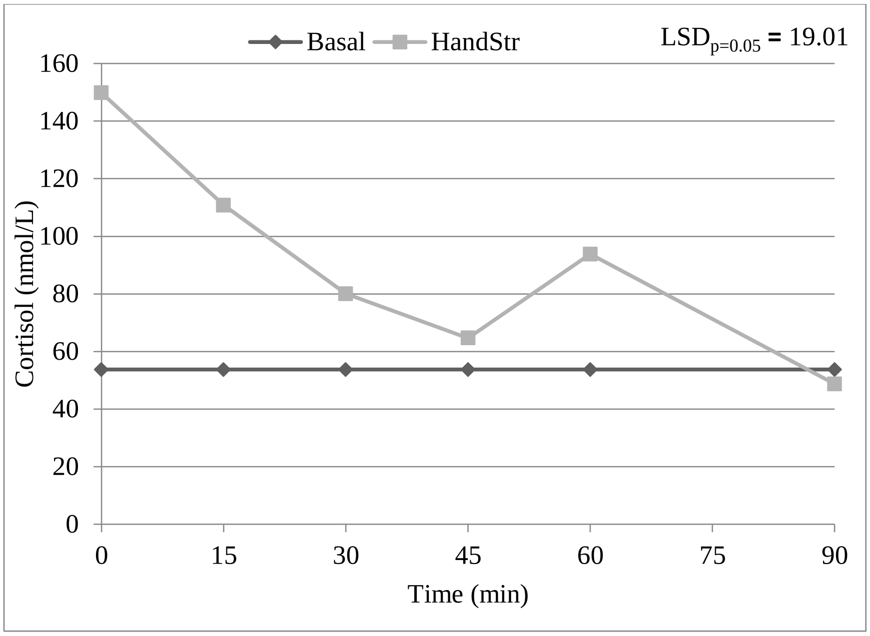

Figure 1 Mean serum cortisol concentration (nmol/L) of goats subjected to routine handling (HandStr)

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. 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.

Heat stress (HtStr)

The effect of heat as a stressor have been cited (Alamer, 2009; Hansen *et al.*, 2009; Veerasamy *et al.*, 2010). The results of the mean and individual animal's serum cortisol concentration after heat exposure (3 hours at >30ºC) are shown in Table 2.

Table 2 Individual animal data, Mean ± Standard deviation (±SD) of serum cortisol concentration (nmol/L), post exposure, of goats subjected to direct sunlight heat

Animal No	Basal cortisol concentration	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)

The mean cortisol concentration of the stressor treated animals differed (*P*=0.05) from the basal concentration only at 0 minutes post treatment, after which the concentration of cortisol levels in the stressor treatment decreased below the mean basal concentration, (Figure 2). This concentration at 0 minutes may have been influenced by aggression and may be due to high density crowding as described by Lyngwa (2012) or social disruption as described by Lyons *et al*. (1993). From the results it is evident that exposure to temperatures above 30ºC in direct sunlight for three hours did not elicit a significant stress response in goats in this trial. This is in 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 3). If compared to handling alone, (Figure 1) and heat exposure alone, (Figure 2) the cumulative effect off heat and handling was significant a 5% significance level (P=0.05) up to 60 minutes after the stressor.

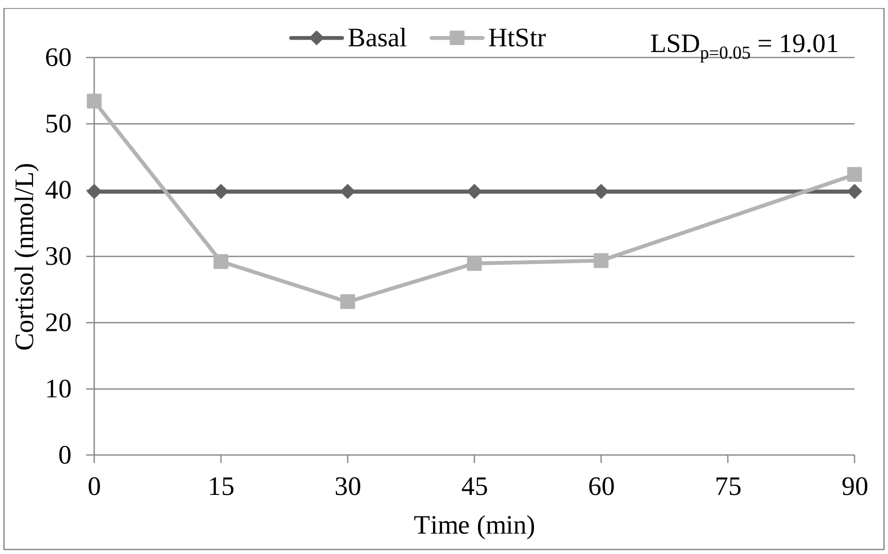

Figure 2 Mean serum cortisol concentration (nmol/L) of goats exposed to heat (HtStr) for 3hours

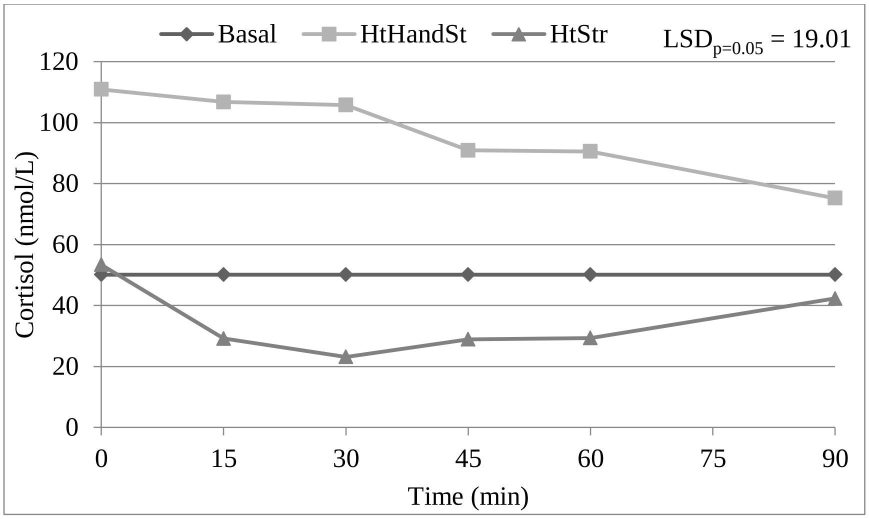

Figure 3 Mean cortisol concentration of goats subjected to heat and handling (HtHandSt) versus heat (HtStr) for 3 hours alone

From the results it can be concluded that heat exposure (>30ºC) for 3 hours to sunlight with no opportunity for thermoregulation did not elicit a significant stress response in goats in this trial. The mean cortisol concentration remained below the mean basal concentration for up to 90 minutes after exposure to the stressor treatment.

Food deprivation stress (FDS)

Food deprivation is a contentious issue often raised by welfare organizations especially with regard to transport and auction sale of animals. The transport and auctioning of animals can deprive them of food for 48 hours or longer. 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 presented in Table 3.

Table 3 Individual animal data, Mean ± Standard deviation (±SD) of serum cortisol concentration (nmol/L), post deprivation, of goats subjected to food deprivation

Animal No	Basal cortisol concentration	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 mean cortisol concentration was significantly lower (*P*=0.05) than the mean basal concentration from 0 minutes up to 90 minutes post exposure to the stressor, (Figure 4).

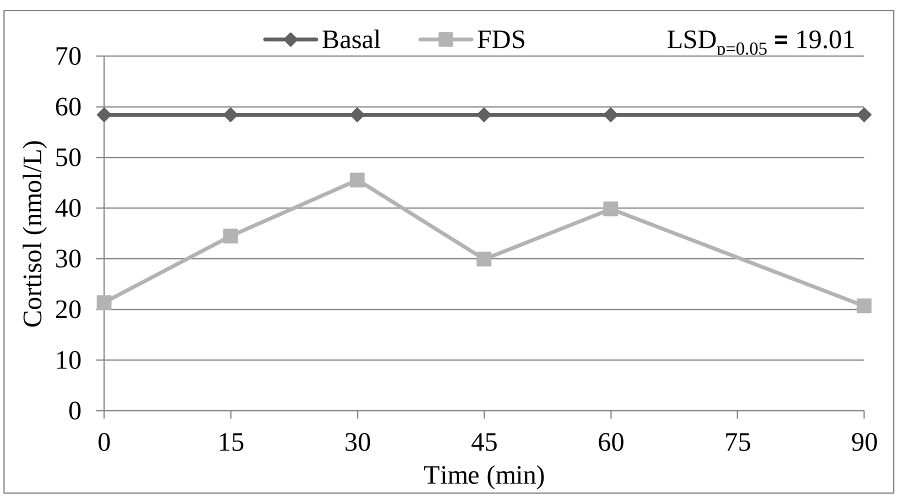

Figure 4 Mean cortisol concentration (nmol/L) of goats subjected to food deprivation (FDS) for 48 hours

The results indicated that the goats in this study tolerated 48 hours of feed deprivation without eliciting a stress response. This contradicts the perception that feed 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.

Water deprivation stress (WDS)

Goats are among the most efficient farm animals with regards to water utilization and the ability 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 aim of this part of the investigation was to simulate water deprivation situations and to evaluate the cortisol concentration in the water deprived goats. The perception is that water deprivation of goats for periods up to 48 hours can elicit a stress response. The post deprivation cortisol concentration of the six goats used in the water deprivation trial are presented in Table 4.

Table 4 Individual animal data, Mean ± Standard deviation (SD) of serum cortisol concentration (nmol/L), post deprivation, of goats subjected to water deprivation (48 hours)

Animal No	**Basal cortisol concentration**	**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

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

The results in this study confirm the findings of Meissner and Belonje (1972) and Jaber *et al* (2004) of a goat's ability to withstand water restriction with the mean cortisol concentration remaining below the mean basal cortisol concentration from 0 minutes through 90 minutes after exposure to 48 hours of water deprivation (Figure 5). From the results it 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.

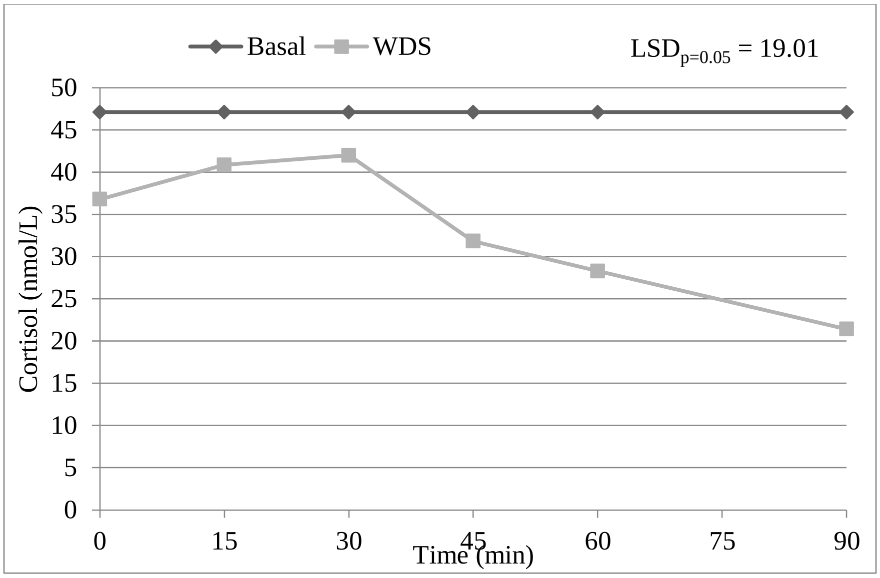

Figure 5 Mean cortisol concentration (nmol/L) of goats subjected to water deprivation (WDS) for 48 hours

Heat and handling stress (HtHandSt)

Routine handling of goats is inevitable for maintenance of the flock. In the South African climate the routine handling of animals often happens on sunny and warm days. The aim of this study 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 post exposure cortisol concentration in blood serum of experimental animals. The cortisol concentration (nmol/L), post exposure and handling, of the individual animals is presented in Table 5.

Table 5 Individual animal data, Mean ± Standard deviation (SD), post exposure and handling, 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 ±SD	**60.46[b,c]** ±31.83	**110.88[a]** ±38.39	**106.75[a]** ±38.03	**105.76[a]** ±26.89	**90.88[a,b]** ±34.79	**90.53[a,b]** ±28.57	**75.2[b]** ±23.85

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

The effect of the combination of heat exposure and handling on the serum cortisol concentration of the goats is shown in Figure 6. 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.

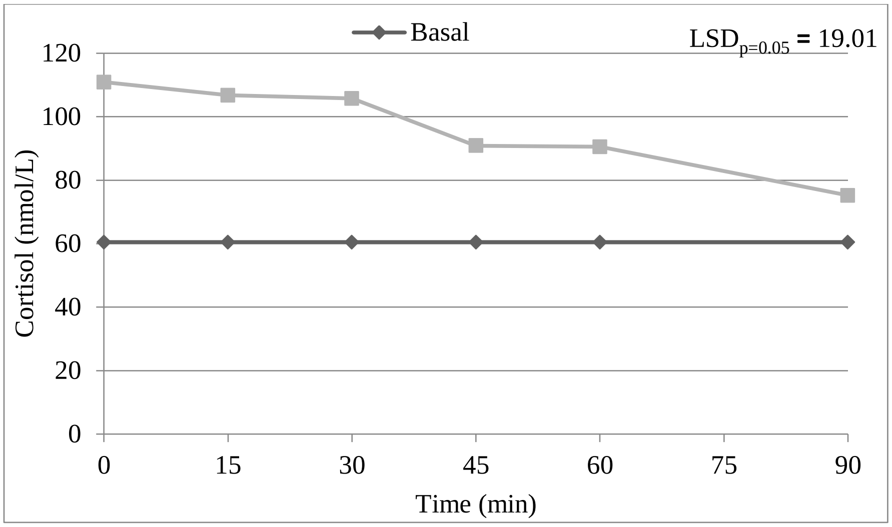

Figure 6 Mean cortisol concentration (nmol/L) of goats subjected to heat and handling (HtHandSt)

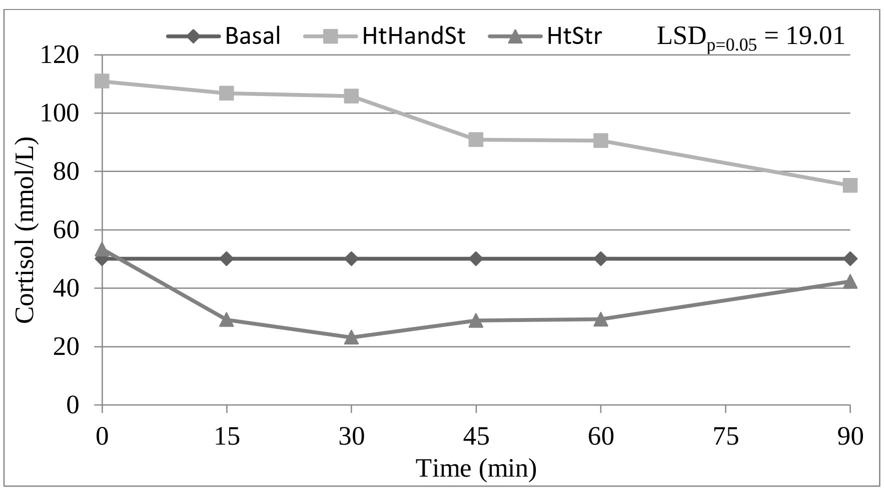

Figure 7 Mean cortisol concentration (nmol/L) of goats subjected to heat and handling (HtHandSt) versus heat (HtStr) for 3hours alone.

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 7). 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.

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

The ability of goats to withstand water restriction have already been mentioned earlier. The negative effect of high temperatures and heat stress of small stock are well documented (Alamer, 2009; Hansen *et al*,. 2009; Veerasamy *et al*,. 2010). The aim of this part of the study was to determine the cumulative effect of heat exposure, food deprivation and water deprivation as potential stressors. The hypothesis is that the cumulative effect of the stressors would elicit a more severe stress response than the stressors alone. The cortisol concentration (nmol/L), post stressor exposure, of the individual animals in this investigation is presented in Table 6.

Table 6 Individual animal data, Mean ± Standard deviation (±SD) of serum cortisol concentration (nmol/L), post stressor exposure, 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

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

The cumulative effect of food and water deprivation and heat exposure (HtFdWstr) did elicit a stress response (Figure 8) with mean cortisol concentrations higher than the mean basal concentration with a difference (*P*=0.05) at 15 minutes and 60 minutes. The mean cortisol concentration reached a peak (77.83 nmol/L) at 60 minutes. 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.

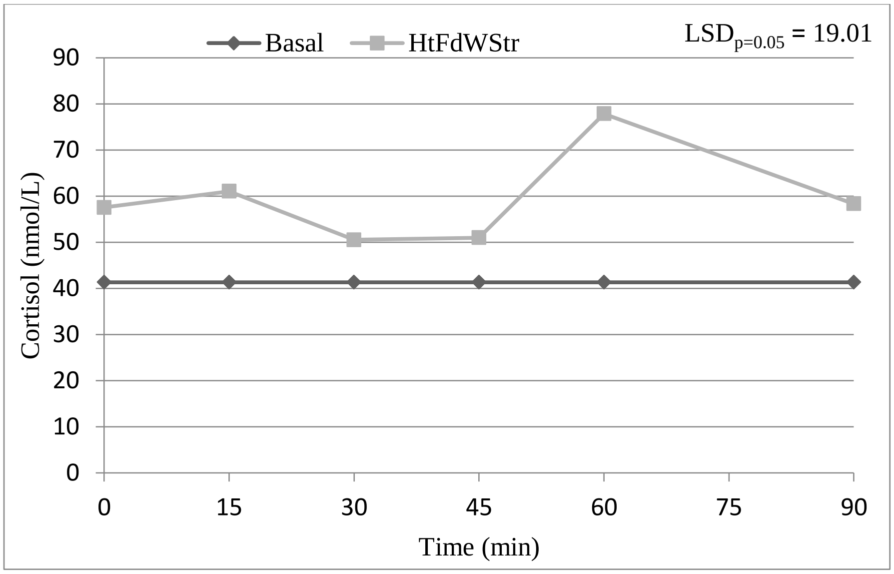

Figure 8 Mean serum cortisol concentration (nmol/L) of goats subjected to cumulative heat, food-and water deprivation (HtFdWStr)

Discussion

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 perception is supported by the fact that stress does not manifest immediately with any recognizable signs (Cockram, 2004). The study demonstrated that less frequent procedures such as vaccination and oral drenching 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, and even physical force. 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 & Hutson (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 1), where the movement to the handling facility also took 10 minutes.

Stockman ship 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 evoking stimuli 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 and result in 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 2). This can be due to the fact that natural factors of weather extremes do not interfere with the natural behaviour of the animals. Where such conditions influences 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 the procedure 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. Animal expose to calming effects 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 goats despite efforts to prevent the disease through immunization (Henton, 2009). 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 relationship between routine handling stress and *Mannheimia haemolytica* infection (pasteurellosis).

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 vaccination and dosing. Such procedures 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 goats would look for shade during extremely warm days which is normal behaviour as a means for thermoregulation.

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 as this may lead to rumen disorders.

The goats in the trial was used to *ad lib* water and thus not prepared for water deprivation. An argument 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 et al.,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.

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. The circulating cortisol was 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.

Conclusion

It was concluded that there was a significant increase in circulating cortisol when animals were handled. This was in contrast to environmental stressors such as heat exposure and food and water deprivation with cortisol levels 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. According to

Zulkifli (2013) there is a lack of information on the effect of human contact on the immune response and disease resistance in farm animals, with the exception of poultry. The relationship of handling on circulating cortisol and stress related diseases need further research, especially with regard to feedlot systems where there is a drastic disruption of the homeostasis in the animals.

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Reference list

Abu Elzein,E.M.E. & Housawi, F.M.T. (2009) Drastic cutaneous multi-focal orf infection in goats, causing severe dysfunctioning. **Revue scientifique et technique**. 28 (3) 1025 – 1029.

Adogla-Bessa, T. & Aganga, A.A. (2000) Responses of Tswana goats to various lengths of water deprivation. **S A Journal of Animal Science** 2000: 30: 87-91.

Alamer, M. (2009) Effect of water restriction on lactation performance of Aardi goats under heat stress conditions. **Small Ruminant Research** 84: 76 – 81.

Cockram, M.S. (2004) A review of behavioural and physiological responses of sheep to stressors to identify potential behavioural signs of distress. **Animal Welfare** 13:: 283 -291

De Wet, J. & Bath, G. (1994) Kleinveesiektes. Tafelberg Uitgewers. Kaapstad

Dobson, H., Tebble, J.E., Phogat, J.B. & Smith, R.F. (1999) Effect of transport on on pulsatile and surge secretion of LH in ewes in the breeding season. **Journal of Reproduction and Fertility** 49: 451 -461

Dwyer, C.M. & Bornett, H.L.I. (2004) Chronic stress in sheep: Assessment tools and their use in different management conditions. **Animal Welfare** 13: 293 – 304

Ehnert, K. & Moberg,G.P. (1991) Disruption of oestrus behaviour in ewes by dexamethasone or management related stress. **Journal of Animal Science** 69: 2988 – 2994

Glass, G. V., Peckham, P. D., & Sanders, J. R. (1972). Consequences of failure to meet assumptions underlying the fixed effects analyses of variance and covariance. **Review of educational research**, 42(3), 237-288.

Goodman and Gilman's The Pharmacological Basis of Therapeutics. Sixth edition. Macmillan Publishing Co. New York

Hansen, P.J. (2009) Effects of heat stress on mammalian reproduction. **Philosophical Transactions of the Royal Society** B. 364: 3341-3350

Hargreaves, A.L. & Hutson, G.D. 1989. Some effects of repeated handling on stress responses in sheep. Applied Animal Behaviour Science 26: 253–265

Henton, M. (2009) Pasteurella vrektes by lammers. Vra vir Faffa column. Lanbou.com online.

Hosamani M, Scagliarini A, Bhanuprakash V, McInnes CJ, & Singh RK. (2009) Orf: an update on current research and future perspectives. **Expert Review of Anti Infective Therapy**. 7(7):879-93.

Jaber, L.S., Habre, A., Rawda, N.,Abi Said, M., Barbour, E.K. & Hamadeh, S. (2004) The effect of water restriction on certain physiological parameters in Awassi sheep. **Small Ruminant Research** 54: 115-120

Kannan, G., Terrill, T.H., Kouakou, B. Gazal, O.S.,Gelaye, S., Amoah, E. A. & Samake, S. (2000) Transportation of goats: effects on physiological stress responses and live weight loss. **Journal of Animal Science** 78:1450-1457.

Knight, T.W., Hall, D.R.H., Lynch, P.R. & Hockey, H.U.P. (1988) Effects of pre-joining shearing, stress, pasture allowance and haemoglobin type on reproductive performance of Romney and Marshall Romney ewes. **New Zealand Journal of Agricultural Research**. 31: 249-258

Laval, A. (1994) decoquinate: Practical utilization in the control of coccidiosis in mammals. **Requeil de Medicine Vetrinaire.** 170: 811 – 821

Leite-Browning, M. (2007) Keratoconjunctivitis in goats. Alabama cooperative Extension system. UNP-88

Li, B.T., Christopherson, R.J. & Cosgrove, S.J. (1999) Effect of water restriction and environmental temperatures on metabolic rate and physiological parameters in sheep. Canadian **Journal of Animal Science**. 80: 97 – 104

Little, T. M., & Hills, F. J. "Transformations." Statistical Methods in Agricultural Research. Univ. California, Davis, CA (1972): 103-120.

Lopes, W.D.Z., Carvalho, R.S., Pereira, V., Martinez, A.C., Cruz, B.C., Texeira, W.F., Maciel, W.G., da Costa, A.J., Soares, V.E., Borges, D.G.L, Rodriguez, F de S. & Borges, F de A. (2014) Efficacy of sulfadoxine + trimethoprim compared to management measures for the control of Eimeria parasitism in naturally infected and clinically asymptomatic sheep that were maintained in a feedlot. **Small Ruminant Research**. 116: 37 – 43.

Lowe, E.T., Gregory, N.G., Fisher, A.D. & Payne, S.R. (2002) The effects of temperature elevation and water deprivation on lamb physiology, welfare and meat quality. **Australian Journal for Agricultural Research**. 53: 707-714

Lyngwa,C. (2012) Fear response and social interactions in dairy goat housed in three different densities during pregnancy. Master Theses. NorwegianC:\norwegian University of Life Sciences.

Lyons, D.M. (1989) Individual differences in temperament of dairy goats and the inhibition of milk ejection. **Applied Animal Behaviour Science**.22: 262-282

Marai, I.F.M., El-Darawany, A.A., Fadiel, A. & Abdel-Hafez, M.A.M. (2007) Physiological traits as affected by heat stress in sheep – A review. **Small Ruminant Research**. 71: 1-12

Martin, G.B., Milton, J.T.B., Davidson, R.H., BAnchero Hunzicker, G.E., Lindsay, D.R. & Blache,D. (2004) Natural methods for increasing reproductive efficiency in small ruminants. **Animal Reproduction Science**. 82: 231-246

Meissner, H.H. & Belonje, P.C. (1972) Preliminary study on water and electrolyte metabolism during thermal and dehydrational stress in two breeds of sheep. **South African Journal of Animal Science** 2: 97 – 100.

Miranda-de la Lama, G.C. & Mattiello,S. (2010) The importance of social behaviour for goat welfare in livestock farming. **Small Ruminant Research**.90: 1–10

Rhind, S.M., Reid, H.W. & McMillen, S.R. (2009) Effects of pulsed or continuous infusion of cortisol on immune function in sheep. **Domestic Animal Endocrinology**.16:1-9

Rushen, J., Taylor, A.A. & dePassillé, A. (1999) Domestic animals' fear of humans and its effect on their welfare. **Applied Animal Behaviour Science** 65: 285–303

Säkkinen, H., Tornbeg, J., Goddard, P.J., Eloranta, E., Ropstad, E. & Saarela, S. (2004) The effect of blood sampling method on indicators of physiological stress in reindeer (*Rangifer tarandus tarandus*) **Domestic Animal Endocrinology** 26: 87–98

Sevi, A., Albenzio, M., Annicchiarico, G., Dell'Aquila, S., Muscio, A. & Taibi, L. (2001) Effects of solar radiation and feeding time on behaviour, immune response and production of lactating ewes under high ambient temperature. **Journal of Dairy Research** 84: 629 – 640

Shapiro, S.S. & Wilk, M.B. (1965) An analysis of variance test for normality. **Biometrika**. 52

Silanikove, N. (2000) Effects of heat stress on the welfare of extensively managed domestic ruminants. Review Article. **Livestock Production Science**. 67:1-18

Snedecor, G. W., & Cochran, W.G. (1967) "Statistical Methods. Iowa State University Press."

Taylor, M. (1995) Diagnosis and control of coccidiosis in sheep. **In Practice**17: 172-177

Veerasamy, S. & Rajendra, S.S. (2010) Effects of Melatonin on Adrenal Cortical Functions of Indian Goats under Thermal Stress. **Veterinary Medicine International**.

Young, G., Alley, M.L., Foster, D.M. & Smith, G.W. (2011). Efficacy of amprolium for the treatment of pathogenic *Eimeria* species in Boer goat kids. **Veterinary Parasitology** 178: 346 – 349

Zulkifli,I. (2013). Review of Human-animal interactions and their impact on animal productivity and welfare. **Journal of Animal Science and Biotechnology** 4 (1): 2