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

3 **L P Kruger¹, T L Nedambale¹, M M Scholtz¹ and E C Webb²**

4 ¹ARC-Animal Production Institute, Private Bag X2, Irene, 0062, South Africa,

5 Lkruger@arc.agric.za; Lucky@arc.agric.za; Gscholtz@arc.agric.za

6 ²University of Pretoria, Private bag X20, Hatfield, 0028, South Africa,

7 Edward.Webb@up.ac.za

8
9 Corresponding author: Leon Kruger: Agricultural Research Council-Animal
10 Production Institute, Private Bag X2, Irene, 0062, South Africa,
11 Lkruger@arc.agric.za, Tel: 0027 12 6729169

12 **Abstract**

13 Routine handling procedures used in the management of a flock are not
14 generally regarded as stressful and have not been extensively researched.
15 The aim of this study was to determine the serum cortisol concentration
16 after routine handling procedures, viz. handling, heat exposure, food
17 deprivation and water deprivation as well as the cumulative effect of
18 these potential stressors in South African indigenous goats. The results
19 indicated that goats subjected to typical routine handling procedures such
20 as oral drenching and subcutaneous vaccination had significantly higher
21 serum cortisol concentration than the basal levels and can be considered
22 as acute stressors. Stressors that did not involve handling, such as heat
23 exposure, food and water deprivation did not elicit cortisol levels
24 significantly higher than the basal level in this study.

25 **Keywords**

26 Stress: Cortisol: Handling: Heat exposure: Food deprivation: Water
27 deprivation,

28 **1. Introduction**

29 Farm animal welfare has become increasingly relevant in the
30 public eye in recent years with a public consciousness on how animals
31 raised for human consumption are treated. Despite the controversy and
32 merits regarding animal welfare, animal wellbeing remains an important
33 factor to ensure satisfactory production and reproduction (Silanikove,
34 2000; Lowe et al., 2002; Hansen, 2009; Miranda-de la Lama and
35 Mattiello, 2010). Keeping of goats inevitably involves management
36 practices and routine tasks to be performed. Such routine procedures
37 often expose the goats to potential physical stressful situations, for
38 example: high ambient temperatures (Silanikove, 2000; Lowe et al.,
39 2002; Marai et al., 2007), handling methods (Rushen et al., 1999;
40 Miranda-de la Lama and Mattiello, 2010) and food and water deprivation
41 (Lowe et al., 2002). This is of particular relevance where there is no or
42 sub-standard handling facilities available. The measurement of stress is
43 very complex and context dependent (Zulkifli, 2013). Three specific
44 stressors: heat, handling and food/water deprivation on its own or
45 cumulatively may be the most common stressors that occur during
46 execution of routine procedures. Secondary to these there are
47 psychological stressors such as disruption of social hierarchy, change of
48 habitat and overcrowding. Stress can be characterized by the
49 concentration of the glucocorticoid hormone, cortisol, in the body. The
50 process that involves the secretion of cortisol is initiated by

51 corticotrophin-releasing hormone (CRH) in response to a stress stimulus
52 (Goodman et al., 1980). Corticotrophin releasing hormone stimulates
53 pituitary synthesis of adrenocorticotrophic hormone (ACTH) which in
54 turn stimulates the adrenal cortex to secrete glucocorticoid hormones
55 cortisol, corticosterone and aldosterone. Cortisol has the primary
56 function of enabling the animal to escape from the stressor and also acts
57 in a negative feedback cycle to suppress any further release of ACTH.

58 Routine handling procedures are not generally recognized as being
59 stressful and limited research has been done in this regard. Such
60 procedures may however have the potential to elicit a stress response
61 because of the disruption of the goat's homeostasis. According to
62 Zulkifli (2013) any disruption of an animal's homeostasis that requires a
63 response to maintain its psycho-physiological integrity has the potential
64 to cause stress to the animal. The cortisol concentration in the circulating
65 blood is a recognized measurement tool for stress (Kannan et al., 2003;
66 Säkkinen et al., 2004). The aim of this study was to determine serum
67 cortisol levels in order to evaluate the effects of routine handling-,
68 husbandry- and environmental factors on stress in goats.

69

70 **2. Materials and method**

71 The aim of this investigation was to simulate typical routine handling of
72 goats. The blood cortisol level was determined in goats subjected to
73 handling stress (HandStr); heat stress (HtStr); food deprivation stress
74 (FDS); water deprivation stress (WDS); cumulative heat and handling
75 stress (HtHandStr) and cumulative heat, food, water deprivation stress
76 (HtFdWStr). South African unimproved indigenous goat does (n=36)

77 between 10 and 12 months old and weighing between 22 kg – 26 kg were
78 used. Indigenous goats were used in the trial due to the perception that
79 they are less prone to stress and thus would tolerate the stressors in the
80 trial better than other goat breeds. A different group of six goats were
81 used for each of the six stressors.

82 Animal ethical approval for the use of animals was obtained from
83 the Animal Production Institute's Animal Ethics Committee (API-AEC).
84 Before stressor treatment, a group of eight animals (six trial and two
85 replacement) were placed together in an "adaptation / acclimatization
86 camp" for a period of 2 weeks to rule out the possibility of hierarchy
87 disruption. The replacement goats served the purpose to replace a trial
88 goat in the event of victimization, disease or injury. The animals were
89 continuously monitored, especially during feeding time, to ensure there
90 were no incidences of victimization of any individual within the group.
91 The basal level (unstressed cortisol concentration) of each animal in the
92 trial flock was established prior to the stressor treatments and was used
93 as the untreated control for that animal. The unstressed basal cortisol
94 levels of all the animals in the trial were within the normal range of 42-
95 82 nmol/L for sheep (Radostits et al., 2005). The mean cortisol
96 concentration was adjusted for a basal value of 100nmol/L with the
97 formula: Adjusted cortisol = (cortisol / basal) X 100.

98 For the handling stressor treatment, the procedure involved the
99 animals being collected from the grazing camp and chased at fast
100 walking pace for 10 minutes through passages of the unit to the handling
101 facility. The animals were vaccinated subcutaneously in the loose skin of
102 the neck.

103 For the heat stress treatment, the animals were exposed to direct
104 sunlight in a pen with solid sides for three hours around mid-day with an
105 atmospheric temperature of $>30^{\circ}\text{C}$. The time was limited to 3 hours as
106 this is the time animals would more or less wait in commercial farms for
107 processing. There was no opportunity of thermoregulation such as shade
108 or water supply for the animals during the heat exposure.

109 For the food deprivation stress treatment, the animals were
110 deprived of food and grazing for 48 hours. Water was available ad
111 libitum during the period of food deprivation.

112 For the water deprivation stress treatment, the animals were
113 deprived of water for a period of 48 hours. Ad libitum food was available
114 during the period of water deprivation. For the cumulative effect of heat
115 and handling stressors the animals were exposed to direct sunlight heat
116 for 3 hours around mid-day with an atmospheric temperature of $>30^{\circ}\text{C}$.
117 No shade or water was provided and therefore any opportunity of
118 thermoregulation was denied. After the stressor period the animals were
119 placed in a handling chute and dewormed orally with a placebo (water)
120 to simulate routine handling for deworming.

121 For the cumulative effect of heat, food deprivation and water
122 deprivation stress treatment the animals were deprived of food and water
123 for a period of 48 hours. After this period the animals were exposed to
124 direct sunlight in a pen with solid sides for 3 hours around mid-day with
125 an atmospheric temperature of $>30^{\circ}\text{C}$. There was no opportunity of
126 thermoregulation for the animals during the heat exposure. The
127 hypothesis was that the cumulative effect of heat exposure and handling

128 may elicit a stronger stress response than handling or heat exposure
129 alone.

130 A series of blood samples were collected at 0, 15, 30, 45, 60 and
131 90 minutes after exposure to stress treatments. Blood was collected with
132 an 18-gauge needle in a vacuum serum tube from the jugular vein after
133 venous occlusion. This was always performed by the same experienced
134 and skilled person. Blood samples were placed in a refrigerator (10°C) to
135 form a blood clot in order to prevent the presence of fibrin in the serum.
136 After 24 hours the blood was centrifuged at 500 x g for 10 minutes and
137 the serum was aspirated with a disposable Pasteur pipette into a marked
138 vial and stored at -20°C until assayed.

139 Cortisol in serum was analyzed by means of the SIEMENS
140 Immulite® 1000 automated Immunoassay Analyzer for quantitative
141 measurement of cortisol in serum. For the anti-cortisol antibody, Cortisol
142 Test Units (LCO1) with one bead coated with polyclonal rabbit anti-
143 cortisol antibody was used. Cortisol Reagent Wedge (LCO2) with 7.5mL
144 alkaline phosphatase conjugated to cortisol in buffer, with preservative
145 was used as reagent. For the Cortisol Adjustors (LCOL, LCOH) two
146 vials (low and high), with 3mL each of cortisol in processed human
147 serum with preservative was used. The cortisol concentration is
148 expressed in nano-mol per liter (nmol/L).

149 The experimental design was a completely randomized design
150 with stressors as treatment and animal as the experimental unit. The data
151 were analyzed as a one-way Analysis of Variance (ANOVA) (Snedecor
152 and Cochran, 1967) with the repeated measurements over time as a
153 subplot factor (Little and Hills, 1972). The standardized residual was

subjected to Shapiro-Wilk's test for deviation from normality (Shapiro and Wilk, 1965). 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)

3. Results

Table 1 Adjusted cortisol means (nmol/L) for stressor x time interaction

Time (min)	HandStr	HtStr	FDS	WDS	HtHandSt	HtFdWst
0	314.2 ^a	133.4 ^{b,c}	35.0 ^{c,d}	86.5 ^{b,c}	203.8 ^b	168.6 ^{b,c}
15	229.0 ^b	76.66 ^{c,d}	67.8 ^{c,d}	95.6 ^{b,c}	191.5 ^{b,c}	165.5 ^{b,c}
30	161.9 ^b	61.5 ^{c,d}	94.0 ^{b,c,d}	98.2 ^{b,c}	181.5 ^{b,c}	133.1 ^{b,c}
45	129.1 ^{b,c}	63.5 ^{c,d}	56.2 ^{c,d}	70.1 ^{c,d}	150.3 ^{b,c}	125.0 ^{b,c}
60	199.3 ^b	73.3 ^{c,d}	69.2 ^{c,d}	58.7 ^{c,d}	152.5 ^{b,c}	202.9 ^b
90	96.9 ^{b,c,d}	105.4 ^{b,c,d}	36.4 ^{c,d}	45.2 ^d	129.1 ^{b,c}	157.6 ^{b,c}

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

LSD_{p = 0.05} = 79.035

Standard Error of Means (SEM) = 27.81

HandStr (handling stress)

HtStr (heat stress)

FDS (food deprivation stress)

WDS (water deprivation stress)

HtHandSt (heat and handling stress)

HtFdWst (Heat, food deprivation and water deprivation stress)

3.1 Handling stress (HandStr)

The goat's fear for novelty and handling when outside their routine has been described by Martin et al. (2004). The results of this study are in accordance with this. There was a difference ($P=0.05$) between the adjusted mean cortisol concentration at 0, 15, and 60 minutes post treatment and the adjusted basal level (i.e., 100nmol/L).

3.2 Heat stress (HtStr)

179 The effect of heat as a stressor in animals is well documented
180 (Alamer, 2009; Hansen et al., 2009; Veerasamy et al., 2010). The
181 adjusted mean cortisol concentration of the stressor treated animals did
182 not differ significantly ($P=0.05$) from the basal concentration. The
183 results indicate that exposure to temperatures above 30°C in direct
184 sunlight for three hours did not elicit a significant stress response in goats
185 in this trial. This is in accordance with the review article of Silanikove
186 (2000) that goats are the best adapted of the domesticated ruminant
187 species to harsh warm climates.

188 **3.3 Food deprivation stress (FDS)**

189 Food deprivation is a contentious issue often raised by welfare
190 organizations especially with regard to animals in transit and sale of
191 animals at auction yards. The transport and auctioning of animals can
192 deprive them of food for 48 hours or longer. Blood was collected after
193 the 48 hour fasting period. The adjusted mean cortisol concentration was
194 not significantly higher ($P=0.05$) than the adjusted basal concentration
195 post exposure to the stressor. The results indicated that the goats in this
196 study tolerated 48 hours of feed deprivation without eliciting a chronic
197 stress response. This contradicts the perception that feed deprivation for
198 periods of up to 48 hours can elicit a stress response in goats. No
199 citations to support or contradict these findings could be found and these
200 results are thus only applicable for the goats used in this investigation
201 that had a full stomach prior to the 48 hour fasting period.

202 **3.4 Water deprivation stress (WDS)**

203 Goats are among the most efficient farm animals with regards to
204 water utilization and the ability to withstand water restriction for periods
205 of up to 2 days without causing stress to the animal (Alamer, 2009; Jaber
206 et al., 2004; Lowe et al., 2002; Adogla-Bessa and Aganga, 2000; Li et
207 al., 1999). The results in this study confirm the findings of Meissner and
208 Belonje (1972) and Jaber et al., (2004) of a goat's ability to withstand
209 water restriction. The adjusted mean cortisol concentration remained
210 below the adjusted mean basal cortisol concentration from 0 minutes
211 through 90 minutes after exposure to 48 hours of water deprivation.

212 **3.5 Heat and handling stress (HtHandSt)**

213 In the South African climate, the routine handling of animals
214 often happens on sunny and warm days. The aim of this part of the study
215 was to simulate a typical handling procedure such as oral drenching and
216 to determine the accumulative effect of heat exposure and routine
217 handling on the cortisol concentration in the goats. The adjusted mean
218 cortisol concentration during the stressor treatment was higher than the
219 adjusted basal level ($P = 0.05$) at 0, 15 and 30 min. As in the handling
220 stress treatment part of this study the results confirm results of Martin et
221 al. (2004) on the fear of goats for unknown humans, strange, novel or
222 threatening environments.

223 **3.6 Heat-, food deprivation- and water deprivation stress** 224 **(HtFdWStr)**

225 The ability of goats to withstand water restriction have already
226 been mentioned earlier. The aim of this part of the study was to
227 determine the cumulative effect of heat exposure, food deprivation and
228 water deprivation as potential stressors. The hypothesis was that the

229 cumulative effect of the stressors would elicit a more severe stress
230 response than the stressors alone.

231 The cumulative effect of food and water deprivation and heat
232 exposure (HtFdWstr) did elicit a stress response with adjusted mean
233 cortisol concentrations higher than the mean basal concentration with a
234 difference ($P=0.05$) at 60 minutes only. The results did not support the
235 hypothesis that the cumulative effect of heat-, food- and water
236 deprivation elicit a stronger stress response than heat exposure, food
237 deprivation and water deprivation stressors alone. The adjusted cortisol
238 concentration was higher than the stressors alone, but not significantly
239 so.

240 **4. Discussion**

241 There is a common perception that routine handling does not cause
242 stress to the animals. This perception is supported by the fact that stress
243 does not manifest immediately with any recognizable signs (Cockram,
244 2004). The results of this study demonstrated that less frequent
245 procedures such as vaccination and oral drenching elicited a significant
246 short term stress response for up to 30 minutes in goats. The two stressor
247 treatments that involved handling (HandStr and HtHandStr) had elevated
248 cortisol concentrations at 0 minutes already. This is in accordance with
249 findings of Hargreaves and Hutson (1989) that found elevated cortisol
250 concentrations in sheep 10 minutes after drafting. One possible
251 explanation for this may be that the handler has two conflicting roles
252 when routine procedures are performed. One is to act as the stimulus to
253 move the animals towards the handling facility. This is traditionally
254 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. 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. Zulkifli, (2013) further described the effect of fear as a potent stressor and advised reducing fear through positive human contact when animals are to be handled. Infrequent handling interferes with the homeostasis of the animals. They are moved from the grazing camp, grouped together, the social hierarchy disrupted and handled. All of these have the potential to elicit a stress response. 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. Gentling of the animals during routine procedures have the potential for the animals to be more receptive to human handling. The effect of gentling has been described by Rushen et al., (1999) that found gentled sheep to be more willing to approach humans. Gentling of the goat in a handling chute involves that the handler stands on the balance point (more or less in line of the shoulder blades of the goat). The goat is handled with the lower mandible resting in one hand and the other hand is held behind the head and lightly massages the area around the ear with the fingers. The gentling technique described was applied during the series blood collection of the trial and the animals were not vocal. The cortisol levels of the procedures that did not involve routine handling procedures (i.e. heat stress, water deprivation stress and food deprivation

280 stress) did not rise above the basal level. One explanation for this may be
281 the gentling of the animals prior to blood collection.

282 The exposure to heat and direct sunlight for three hours might
283 have been too short to elicit a stress response, since goats are known to
284 be heat tolerant (Silanikove, 2000) and this was confirmed by the results.
285 The aim of the study was not to determine how much heat exposure
286 would be stressful, but rather to simulate a typical handling procedure
287 such as vaccination and dosing. Such procedures usually do not take
288 longer than three hours.

289 The fact that food deprivation for 48 hours did not elicit a long
290 term stress response can be explained by the fact that the animals had a
291 full stomach prior to the food deprivation. If deprived for a longer period,
292 it might have elicited a stress response. The purpose of this study was to
293 simulate commercial goat farming practices and it is seldom that animals
294 will be deprived of food for periods longer than 48 hours.

295 The goats in the trial were used to ad libitum water and thus not
296 prepared for the water deprivation period. The water deprivation did not
297 elicit a long term stress response that supports findings of the ability of
298 goats to tolerate water restrictions (Meissner et al., 1972).

299 The stress response that occurred in the cumulative heat and
300 handling stressor can be attributed to the handling of the animals since
301 the heat stressor alone did not elicit a stress response.

302 The cumulative effect of food- and water deprivation and heat
303 exposure was expected to elicit a strong stress response, but this was not
304 the case.

305 **5. Conclusion**

306 The results of this study indicated a significant short term increase in
307 circulating cortisol when animals were handled for vaccination and
308 dosing procedures. This was in contrast to environmental stressors such
309 as heat exposure and food and water deprivation with cortisol levels not
310 significantly higher than the basal level. The relationship of routine
311 handling and the effect thereof on stress needs further research.

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