THE INFLUENCE OF SLAUGHTER ON BLOOD COMPOSITION IN CATTLE

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Abstract

In this study the effects of blood sampling, transportation and slaughter on certain blood variables (sodium, potasium, chloride, glucose, lactates, lipids, proteins, colloidal osmotic pressure, cortisol, ACTH, T3, TSH, osmolality etc.) of cattle were investigated.

The experimental animals, crossbreed (Brahman, Afrikander and Hereford) heifers and oxen obtained from Kanhym Estates, Middelburg, were unaccustomed to handling. One group of animals was subjected to the stress of sampling with restraint, a second group was sampled with restraint after transportation and a third group had blood taken after slaughter at the Kanhym abattoir at Balfour. Control blood samples were obtained from Friesland dairy cows accustomed to handling and blood sampling and from crossbreed oxen unaware of the sampling procedure. Both control and experiment groups were studied in the same season. (April and May, 1984).

Results were analysed and compared statistically. The blood cortisol, ACTH, lactates and glucose were significantly higher in the experimental groups than in the control group. Cortisol and ACTH values were significantly higher, and the lactate and glucose values significantly lower in the group subjected to the stress of sampling with restraint than in the other two experimental groups.

Therefore, if the variables measured are an assessment of stress and change because of stress, then the animals investigated experienced stress. In addition, cattle unaccustomed to handling perceive the slaughtering process as less stressful than blood sampling in a crush (based on cortisol and ACTH results).

Declaration

I declare that this dissertation is my own, unaided work. It is being submitted for the degree of Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.

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1st day of April, 1986.

Dedication

I dedicate this dissertation to my wife, Marlene who assisted and encouraged me throughout this study.

Preface

In this study the influence of slaughter on certain blood variables which could possibly be indicative of stress in cattle was determined.

The stress of slaughter was also compared to other stressors that could be experienced by cattle in normal transport and handling routine.

I would like to thank Professor J. Hattingh for his help and guidance throughout this study and Professors P. Wright, G. Mitchell, Miss M. Ganhao and Kanhym Estate, Middelburg for the assistance given with the collection of data. I here would like to mention that sections of this study have been published.

I would also like to thank Mrs N. Strahlendorf for the final typing of the dissertation and Mrs I. Hurst for the proof reading.

List of Abreviations

A/G	Albumin Globulin Ratio
ACTH	Adrenocorticotrophic Hormone
ANOVA	Analysis of Variance
BSA	Bovine Serum Albumin
COP	Colloidal Osmotic Pressure
ETR	Effective Thyroxine Ratio
GAS	General Adaptation Syndrome
PBI	Protein Bound Iodine
SDC	Succinyldicholine (muscle relaxant)
T3	Triiodothyronine
TSH	Thyroid-Stimulating Hormone

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CHAPTER 1

1.1 Scientific Problem

The slaughter of cattle by conventional means is a well established and generally accepted practice. Yet certain methods used in game culling (buffalo and elephants) raise objections (Pharmacology of Succinylcholine, 1980). It would be of interest to compare the stress buffalo undergo during culling to the stress cattle undergo with slaughter. To date work has been done on the physiological changes occuring in buffalo and elephants culled with the use of succinyldicholine (SDC) (Hattingh et al, 1984).

In this dissertation one aspect of the above-mentioned problem is examined, namely the stress cattle undergo during handling, transportation and slaughter. Certain of the results obtained have been published. (Hattingh et al, 1985).

1.2 Objectives

The first objective of this study was to determine possible changes in blood composition of cattle (specifically those variables affected by stress, i.e. blood electrolytes, glucose, lactates, lipids, proteins, colloidal osmotic pressure, haematocrit, hormones (cortisol, ACTH, T3 and TSH) and osmolality) which may or may not occur during handling, transportation and slaughter.

The second objective was to determine if slaughter was more stressfull than transportation and/or restraint by comparing the changes occuring in blood under these different stress conditions.

1.3 Description of Terms

1.3.1 General Adaptation Syndrome (GAS)

The General Adaptation Syndrome (GAS) is the nonspecific response of the body to stress. This response can be divided into three phases: the alarm reaction, the phase of resistance and the phase of exhaustion. (Jordaan, Jordaan & Nieuwoudt, 1975; Selye 1976)

1.3.2 Stress

According to Hardy (1981) stress is a term which, in the physiological sense, embraces a variety of circumstances in which an animal's internal or external environment becomes disturbed to an extent where normal homeostatic or behavioural responses are insufficient to correct the disturbances.

1.3.3 Stressors

Any factor causing stress is called a stressor. In this text, in accordance with Ganong (1977) and Hardy (1981), a stressor refers to any stimulus that activates the pituitary-adrenal axis and this will always result in the increased secretion of ACTH.

1.4 Changes which may be expected in the blood composition during stress

A stressor will result in the activation of the hypothalomo-pituitary adrenal axis. The activation of this axis will result in the increased secretion of ACTH (Ganong, 1977; Guyton, 1976: Bell, et al 1980; Hardy, 1981) and stress could possibly influence other hormones via the above-mentioned system. These hormones include growth hormones, plasma corticoids, TSH and others.

In turn these hormones influence other variables; for example

- an increase in growth hormone secretion could result in an increase of blood glucose.
- an increase in catecholamine secretion could result in the increase of blood glucose and blood fatty acid concentration.
- an increase in glucocorticoid secretion could result in an

increased blood glucose concentration, increased blood fatty acid concentration, increased blood protein concentration, an increase in red blood cell count.

Therefore, when examining the influence of possible stressors on the blood composition, it is necessary that the blood variables mentioned above be taken into consideration, as well as other variables.

1.5 Literature Review

The aspects dealt with in this review are limited to the literature dealing with the influence of possible stressors, diurnal variation, breed differences, sex differences and slaughter (related to the present study) on the blood composition of cattle.

1.5.1 Factors affecting adreno-corticoid activity in cattle

In cattle the administration of ACTH has been found to increase the blood 17-OH-CS (Robertson & Mixner, 1956; Saroff & Turner, 1956; Brush, 1960) and cortisol (Venkataseshu & Estergreen, 1970) concentrations significantly. These findings are important as the secretion of ACTH is increased by stressors.

Wagner (1970) found significant diurnal variation in blood-corticoid concentrations and Doornenbal (1977) found significant yearly variations; variations between breeds and variations between sexes. Friend, Gwazadauskas and Polan (1979) found that glucocorticoids increased with free stall competition.

Non-significant diurnal variation (Khan, Dickson & Meyers, 1970), significant seasonal variations (Paterson, 1957) and variations between breeds (Venkataseshu & Estergreen, 1970; Van der Westhuysen, 1973; Erasmus & Krause, 1982) were observed in blood cortisol concentration in cattle. No circardian, ultradian or diurnal rhythm was recorded for cortisol by Ganhao et al (1986).

These findings are important as cortisol is one of the principle circulating corticoids in cattle (Brush, 1960; Estergreen & Venkataseshu, 1967) and has been found to have a negative effect on growth rate and meat tenderness (Hafs, Purchas & Pearson, 1971).

The normal values for circulating cortisol concentration as well as the factors influencing its circulating concentration are given in Table 1.

Table 1: "NORMAL" VALUES FOR CIRCULATING CORTISOL CONCENTRATION AND FACTORS AFFECTING IT IN CATTLE

Date	Authors	Normal Value $\bar{X}$	Bleeding	ACTH Administration	Diurnal[1] Seasonal[2]	Breed Differences	
1964	Saba	0,5ug/100mℓ * 14nmol/ℓ			△[1] N.S		
1967	Estergreen & Venkataseshu	7,2ug/100mℓ * 201nmol/ℓ					
1969	Steyn	0,86-2,42mcg/100mℓ * 24-67nmol/ℓ				△ S	anaesthesia caused a significant increase in blood cortisol concentration
1970	Khan, Dickson & Meyers	3,88ug/100mℓ 108,64nmol/ℓ	↑ ?		△[1] N.S		cold stress (-18°C) caused a significant increase in blood cortisol concentration
1970	Venkataseshu & Estergreen	7,2ug/100mℓ * 201nmol/ℓ	↑ ?	↑ S	△[1]	△	
1971	Hafs, Purchas & Pearson	9ug/100mℓ *252nmol/ℓ				△	
1972	Willet & Erb	5,9-14,7ng/100ml					Psychological stimulation (manupulation of head & neck anticipation of feeding, removal of companion) increased cortisol levels

Date	Authors	Normal Value $\bar{X}$	Bleeding	ACTH Administration	Diurnal' Seasonal[2]	Breed Differences	
1973	Van der Westhuysen	76-154ng/100mℓ				△	
1980	Fulkerson, Sawyer & Gow	25ng/100mℓ				△	
1980	Purchas, Barton & Kirton	123-183nmol/ℓ				△	
1980	Thun, Eggenberger, Zerobin, Lüscher, Vetter	(0,4-9,7ng/100mℓ)					
1982	Erasmus & Krause	123-183nmol/ℓ				△	
1986	Ganhao <u>et al</u>	12 - 10nmol/ℓ					

↑ : Increase in cortisol levels resulted
△ : Differences where found
S : Significant
N.S : Not Significant
* : Values converted to nmol/ℓ
? : Possible cause of variation

1.5.2 Factors affecting thyroid activity in cattle

Robertson et al (1958) found the mean plasma levels of PBI in cattle under stress non-significantly (P 0,05) lower than the mean of normal animals. Post (1963) found variations in PBI concentration among the different breeds and between the different sexes and suggested that heat might depress thyroid activity.

Cowley, Gutierrez, Warnick, Hentges and Feaster (1971) found that the thyroid hormone concentration varied between the different breeds and different sexes.

Doornenbal (1977) reported variations in ETR among the breeds and between the different sexes.

Erasmus and Krause (1983) noted that with the removal of heifers from veld to pen conditions in winter, the thyroxine values rose significantly (P 0,05) (from 33,9 to 52,4 ng/mℓ in Shorthorn, 33,3 to 109,9 ng/mℓ in Afrikander and 39,7 to 105,3 ng/mℓ in Bonsmara). They also found significant differences in thyroxine values between the different breeds.

1.5.3 Factors affecting glucose concentration in cattle

The administration of ACTH as well as stress (electric prod) was found to cause a significant increase in blood glucose concentration (Saroff and Turner, 1956). Khan, Dickson and Meyers (1970) reported that cold stress caused a significant increase in the blood glucose concentration.

1.5.4 Factors affecting haematocrit values in cattle

Van der Westhuysen (1973) and Doornenbal (1977) found that differences in the haematocrit value existed among the different breeds of cattle. Doornenbal (1977) also found the highest haematocrit values in heifers (54,8%) and the lowest in bulls (51,9%).

Steyn (1969) reported decreased haematocrit values in cattle exposed to the stress of anaesthesia and Khan et al (1970) reported decreased haematocrit value in newborn calves exposed to cold stress.

1.5.5 Factors affecting blood electrolyte values in cattle

Steyn (1969) noted that no changes in chloride, sodium and potassium values were induced by the stress of anaesthesia.

Doornenbal (1977) found significant differences in sodium and potassium values between the different sexes and among breeds.

1.5.6 Effects of slaughter on the blood composition of cattle

Very little if any literature exists concerning the influence of slaughter on the blood composition of cattle. Doornenbal (1977) determined the blood parameters (haematocrit, corticoids, ETR Na^+ and K^+)of blood obtained from cattle (bulls, heifers and steers) after slaughter over a period of four years. (1971-1974) Purchas, Barton and Kirton (1980) sampled cattle after slaughter and determined the plasma cortisol levels. Neither Doornenbal (1977) nor Purchas et al (1980) determined the effect of slaughter as such on the blood composition of cattle.

Therefore this study will be a contribution to the study of stress in slaughter, as very little is known concerning the effect of slaughter as such on the blood composition of cattle.

CHAPTER 2

2 Experimental design and materials (Fig. 1, page 13)

2.1 Experimental design

The experimental animals used were crossbreed (Brahman, Afrikander and Hereford) heifers and oxen of similar age, obtained from the feedlot on the Kanhym Estate, Middelburg, Transvaal. These animals were unaccustomed to handling. Before transport, 25 heifers and 25 oxen were subjected to the stress of blood sampling with restraint. This involved leading the animal into a crush, where its head was clamped and pulled to one side to allow blood sampling from a jugular vein. This took approximately 45 seconds per animal. On the same afternoon the animals were transported in two trucks (about 50 animals per truck) from Kanhym Estates, Middelburg to Kanhym abattoir at Balfour, a distance of about 180km.* After transportation, blood samples were taken from 10 heifers and 10 oxen, of which 5 oxen and 5 heifers had been sampled that morning. The animals were again led into a restrainer where the head was held to allow blood sampling from a jugular vein.

The animals were then kept overnight in pens with no food, but water ad lib. The next morning, about 18 hours after transportation and 20 hours after the first sampling, the animals were slaughtered. At slaughter the animals were stunned using a captive bolt, suspended by their hind legs and exsanguinated. Blood samples were then obtained from 32 oxen and 31 heifers (of which 25 oxen and 25 heifers had been bled before) from cut blood vessels in the neck within 30 seconds of stunning. The blood samples were heparinised (1000 U/mℓ) centrifuged and the haematocrit determined. Plasma was separated, frozen (-50°C) and transported to the laboratory for analysis. Glucose, lactate, lipid and protein were analysed within 72 hours of

* Duration of the trip was approximately 3 hours.

The handling of the experimental animals between Kanhyn feedlots and the abbatoir was restricted to the loading of the animals onto the trucks at the feedlot, the transport of the animals, the off-loading of the animals from the truck into the overnight pens at the abbatoir. (Ramps were used to load and off-load the animals.)

Before the animals were slaughtered they were led from the overnight pens *via* a crush into the stunning room.

sampling. The remaining samples were kept frozen until required.

The animals used as controls were Friesland dairy cows accustomed to handling and blood sampling, and crossbreed mature oxen, from which blood was drawn via an indwelling catheter in the jugular vein with the animals unaware of samples being taken. Control animals were housed under stress free conditions at the Animal and Dairy Science Research Institute, Irene (Kay and Grobbelaar, 1985). Blood samples of the control animals were treated in the same way as the blood samples of the experimental animals. Both experimental and control animals were studied in the same season. (April/May 1984)

2.2 Methods

The following blood analyses were done.

2.2.1 Albumin / Globulin (A/G) ratio

Protein was separated into different protein fractions using Gelman electrophoresis equipment. (Cellulose acetate strips with Ponceau S solution as stain and a high resolution microzone buffer (TRIS pH8,6)). The percentage of each fraction was then determined using a Beckman R-112 densitometer.

2.2.2 Colloidal Osmotic Pressure (COP)

Plasma colloidal osmotic pressure was determined in an open chamber osmometer, according to the method of Prather et al (1968) using BSA as standard.

2.2.3 Electrolytes

Sodium (Na^+) and potassium (K^+) concentrations in plasma were determined using a flame photometer (Flm 3a, Radiometer, Copenhagen).

Chloride (Cl^-) concentration in the plasma was determined using a chloride titrator (CMT 10, Radiometer Copenhagen).

2.2.4 Glucose

The GOD - PAP colorimetric assay (Boehringer Mannheim) was used to determine glucose concentration in the plasma. This is a method which does not require deproteinization and uses only small sample volumes (0.02mℓ)

2.2.5 Hormones

The hormonal measurements were all made using radioimmunoassay kits designed for human hormones. The degree of cross-reactivity of cattle material with anti-human antibodies is unknown, so that the results can only be used to indicate relative differences between groups; they may not indicate absolute hormonal levels in this species. (This only applies to the protein hormones.)

2.2.5.1 ACTH

Double antibody liquid phase ^{125}I radio-immunoassay kit, DPC, was used to determine ACTH concentration in the plasma.

2.2.5.2 Cortisol

Coat-a-count solid phase ^{125}I radio-immunoassay kit, DPC, was used to determine cortisol concentration in the plasma.

2.2.5.3 T3

Coat-a-count solid phase ^{125}I radio-immunoassay kit, DPC, designed for quantative measurements of free T3 levels in plasma was used.

2.2.5.4 TSH

Solid phase ^{125}I radio-immunoassay kit, Amersham, designed for the quantitative measurements of TSH was used to determine the plasma concentration of TSH.

2.2.6 Lactate

Flouride-EDTA, enzymatic UV-method (Boehringer and Mannheim) was used to determine the lactate concentration in the plasma. This is a method requiring small sample volumes (0,05mℓ)

2.2.7 Lipids

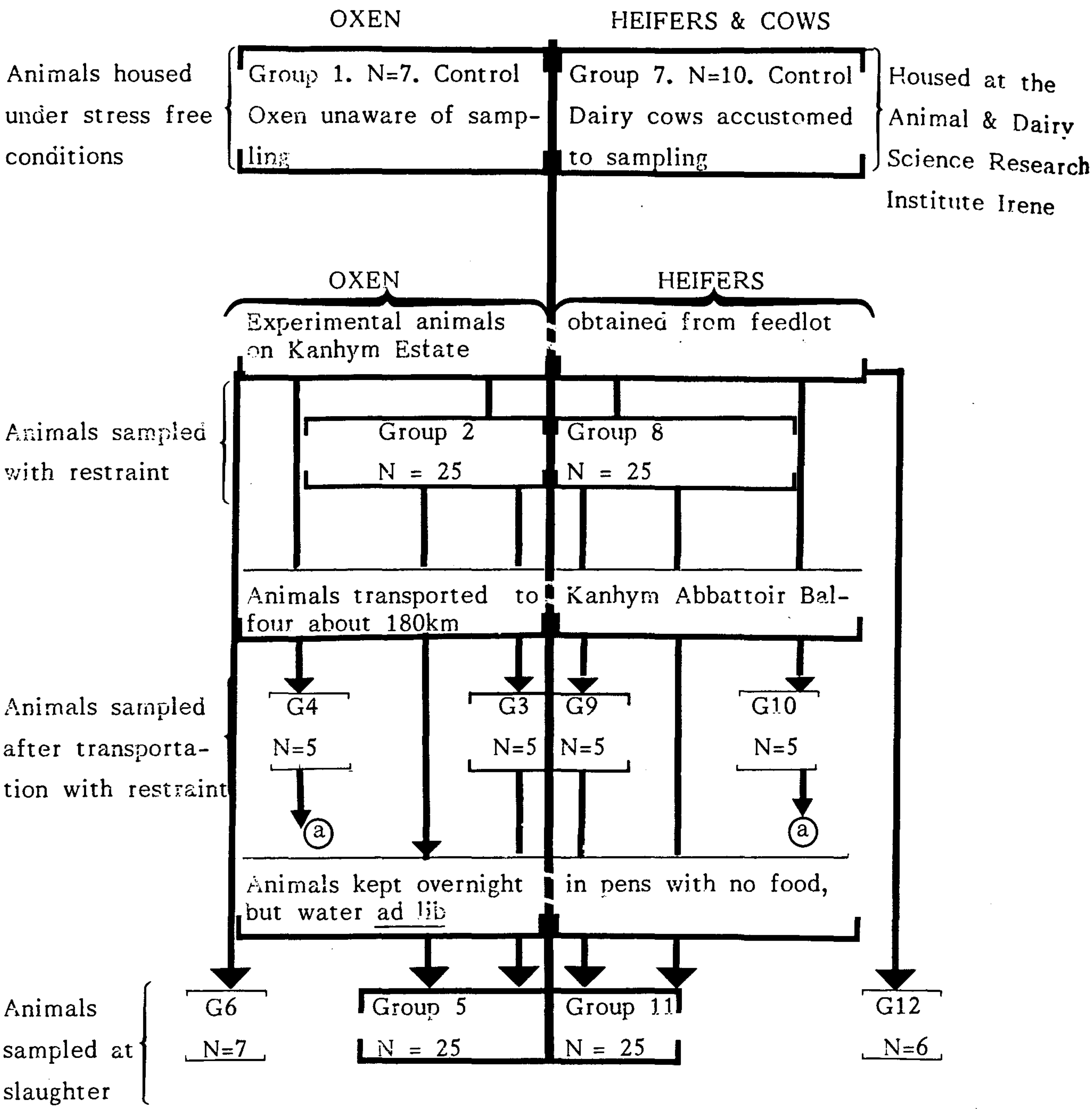
Lipid concentration in the plasma was determined using a colorimetric assay. (Boehringer and Mannheim)

2.2.8 Plasma Proteins

Total protein was determined according to the method of Lowry et al (1951) using BSA as standard.

2.3 Statistical Evaluation of Data

Data were compared statistically using the t-Test for a difference between two independent means, the t-Test for related measures and ANOVA (Repeated measures design) (Brunning and Kintz, 1968; Byron et al 1977)



Pooled Data

Group 13 = Groups 1 & 7

Group 14 = Groups 2 & 8

Group 15 = Groups 3 & 4 & 9 & 10

Group 16 = Groups 5 & 6 & 11 & 12

Figure I: Experimental and Control Groups

CHAPTER 3

3.1 Experimental and Control Groups (Fig. 1)

The animals were grouped according to the conditions they were exposed to prior to or at sampling. These conditions were duplicated for oxen and heifers (see Fig. 1). Oxen and heifers of the experimental groups were of the same breeds.

Group 1 Seven mature crossbreed oxen (Brahman, Afrikander and Hereford) from which blood samples were drawn *via* an indwelling catheter in the jugular vein with the animal unaware that samples were being taken. (Group 7. Ten dairy cows (Friesland), accustomed to handling and sampling).
These two groups served as control groups.

Group 2 Twenty five crossbreed oxen (Brahman, Afrikander and Hereford) unaccustomed to handling which were sampled by restraining them in a crash.
(Group 8, twenty five crossbreed heifers exposed to the same conditions as the oxen of Group 2).

Group 3 Five oxen from Group (2) which were sampled by restraining them after transportation over a distance of about 180km.
(Group 9, five crossbreed heifers exposed to the same conditions as the oxen of Group 3).

Group 4 Five oxen of similar age and type as those in Group (2) which were only sampled by restraint after transportation over a distance of about 180km.
(Group 10, five crossbreed heifers exposed to the same conditions as the oxen of Group 4).

<u>Group 5</u> The twenty-five oxen of Group (2) which were slaughtered after being kept overnight in a pen without food, but with water *ad lib*. In this group blood samples were obtained from cut neck blood vessels within 30 seconds of stunning.
(Group 11, twenty-five heifers exposed to the same conditions as the oxen of Group 5).

<u>Group 6</u> Seven oxen of similar age and type as those in Group 5, from which blood was collected for the first time at slaughter. In this group, which had not been sampled before or after transportation, blood samples were obtained from cut blood vessels within 30 seconds of stunning.
(Group 12, six heifers exposed to the same conditions as the oxen of Group 6).

To determine the effects, if any, of sampling before transportation Groups 3 and 9, Groups 4 and 10 were used for comparison.

To determine the effects, if any, of sampling before slaughter on Groups 5 and 11, Groups 6 and 12 were used for comparison.

3.2 <u>Pooled Data</u>

<u>Group 13</u> Seventeen control animals (*i.e.* Groups 1 and 7)

<u>Group 14</u> Fifty cattle sampled with restraint in the crush before transportation (*i.e.* Groups 2 and 8)

<u>Group 15</u> Twenty cattle sampled with restraint after transportation (*i.e.* Groups 3, 4, 9 and 10)

<u>Groups 16</u> All the cattle sampled after slaughter (*i.e.* Groups 5, 6, 11 and 12)

CHAPTER 4

4. Results

The blood variables recorded for various groups of cattle are displayed in Tables (2.1 - 4.3). These tables include the number of samples (N), mean $\pm$ SD and the range for each variable.

Table 2.1: ELECTROLYTE, OSMOLALITY, COLLOIDAL OSMOTIC PRESSURE AND HAEMATOCRIT VALUES FOR OXEN

Groups		Na^+ mmol/ℓ	K^+ mmol/ℓ	Cl^- mmol/ℓ	Osmolality mosm/kg	C.O.P. mm Hg	Haematocrit %
1. Control	$\overline{X}$	146	3.4	104	294	20	-
Oxen	SD	5	0.2	7	3	1	-
unaware of	N	7	7.	7	7	7	-
sampling	Range	(139-153)	(3.1-3.4)	(96-114)	(291-298)	(19-22)	-
2. Oxen	$\overline{X}$	141	4.8	106	301	19	46
sampled with	SD	4	0.5	6	15	2	5
restraint	N	25	25	25	25	6	
before transit	Range	(137-157)	(3.9-6.1)	(100-127)	(284-320)	(17-24)	(41-54)
3. Oxen (a)	$\overline{X}$	149	5.6	105	300	18	46
sampled with	SD	10	1.0	9	17	2	4
restraint	N	5	5	5	5	4	5
after transit	Range	(135-156)	(4.7-6.8)	(96-119)	(280-315)	(17-24)	(41-51)
4. Oxen (b)	$\overline{X}$	114	6.3	107	302	21	47
sampled with	SD	4	0.7	4	6	4	4
restraint	N	5	5	5	5	3	4
after transit	Range	(140-146)	(5.8-7.5)	(103-113)	(296-312)	(17-24)	(41-48)
5. Oxen (a)	$\overline{X}$	142	5.7	108	296	18.5	47
sampled at	SD	5	1.0	4	9	2	4
slaughter	N	23	23	22	24	4	13
	Range	(131-152)	(4.2-7.0)	(103-119)	(280-315)	(17-21)	(42-52)
6. Oxen (b)	$\overline{X}$	143	5.3	105	306	19	46
sampled at	SD	3	0.8	3	24	0	5
slaughter	N	7	7	7	6	2	4
	Range	(137-148)	(4.0-6.1)	(100-108)	(280-337)	(19)	(40-50)

a = Animals sampled previously

b = Animals not sampled previously

Table 2.2: PROTEIN, A/G RATIO, LIPID, LACTATE AND GLUCOSE VALUES FOR OXEN

Groups		Total proteins g/ℓ	A/G Ratio	Lipids mg/100mℓ	Lactates mmol/ℓ	Glucose mmol/ℓ
1. Control	$\overline{X}$	85.0	0.740	4.37	-	3.75
Oxen	SD	6.6	0.154	0.67	-	0.87
unaware of	N	7	5	7	-	7
sampling	Range	(78.0-96.0)	(0.573-1.000)	(3.73-5.81)	-	(2.82-5.26)
2. Oxen	$\overline{X}$	90.0	0.757	7.32	2.79	4.88
sampled with	SD	10.0	0.135	1.07	1.66	0.57
restraint	N	25	15	25	25	25
before transit	Range	(71.0-113.0)	(0.504-1.087)	(5.40-9.30)	(0.82-6.34)	(3.40-5.85)
3. Oxen (a)	$\overline{X}$	86.0	0.724	6.37	2.69	5.60
sampled with	SD	16.0	0.223	1.35	1.51	0.57
restraint	N	5	4	5	4	5
after transit	Range	(69.0-112.0)	(0.489-1.08)	(5.08-8.61)	(1.30-4.56)	(4.79-6.18)
4. Oxen (b)	$\overline{X}$	78.4	0.698	5.61	4.50	5.21
sampled with	SD	14.1	0.163	0.79	0.54	0.30
restraint	N	5	4	5	4	5
after transit	Range	(61.8-94.0)	(0.428-0.826)	(4.60-6.30)	(3.91-5.05)	(4.87-5.57)
5. Oxen (a)	$\overline{X}$	85.0	0.713	5.56	8.27	6.28
sampled at	SD	13.0	0.170	0.9	2.92	1.41
slaughter	N	24	15	25	25	25
	Range	(58.0-110.0)	(0.415-1.032)	(3.08-7.42)	(4.24-16.95)	84.60-10.8)
6. Oxen (b)	$\overline{X}$	72.8	0.739	5.50	6.06	5.30
sampled	SD	9.0	0.142	1.28	2.00	1.50
at slaughter	N	6	6	7	5	7
	Range	(61.5-83.0)	(0.562-1.000)	(4.03-7.25)	(3.59-10.50)	(3.40-7.73)

a = Animals sampled previously

b = Animals not sampled previously

Table 2.3: HORMONAL COMPOSITION OF BLOOD OF OXEN

Groups		ACTH (uIU/mℓ)	Cortisol nmol/ℓ	T3 pmol/ℓ	TSH (uU/mℓ)
1 Control Oxen unaware of sampling	$\overline{X}$	-	31	1.49	1.45
	SD	-	42	0.44	0.29
	N	-	7	7	7
	Range	-	(14-39)	(0.40-2.38)	(1.12-1.84)
2 Oxen sampled with restraint before transit	$\overline{X}$	317	175	2.67	1.05
	SD	162	51	0.81	0.34
	N	25	25	24	25
	Range	(86-665)	(53-250)	(1.30-4.30)	(0.48-1.80)
3 Oxen (a) sampled with restraint after transit	$\overline{X}$	59	62	2.40	1.00
	SD	30	56	0.60	0.23
	N	4	5	5	5
	Range	(14-76)	(12-158)	(1.40-2.90)	(0.58-1.20)
4 Oxen (b) sampled with restraint after transit	$\overline{X}$	144	44	3.46	1.32
	SD	89	33	0.92	0.36
	N	4	5	5	5
	Range	(50-255)	(10-86)	(27.0-4.60)	(0.96-1.80)
5 Oxen (a) sampled at slaughter	$\overline{X}$	84	73	1.77	0.88
	SD	48	46	0.90	0.28
	N	25	25	25	25
	Range	(19-199)	(12-170)	(0.40-3.30)	(0.48-1.50)
6 Oxen (b) sampled at slaughter	$\overline{X}$	76	89	2.53	1.4
	SD	25	40	1.34	0.53
	N	7	7	7	7
	Range	(46-115)	(42-138)	(1.10-4.40)	(0.58-2.15)

a = Animals sampled previously

b = Animals not sampled previously

Table 3.1: ELECTROLYTE, OSMOLALITY, COLLOIDAL, OSMOTIC PRESSURE AND HAEMATOCRIT VALUES FOR HEIFERS & COWS

Groups		Na^+ mmol/ℓ	K^+ mmol/ℓ	Cl^- mmol/ℓ	Osmolality mosm/kg	C.O.P. mm Hg	Haema-tocrit %
7 Control	$\bar{X}$	136	4.20	105	290	18	-
Dairy cows	SD	2	0.36	4	5	1	-
accustomed	N	9	9	9	8	10	-
to sampling	Range	(132-140)	(3.40-4.60)	(102-113)	(284-297)	(17-19)	-
8 Heifers	$\bar{X}$	144	4.50	107	303	20	46
sampled with	SD	4	0.50	4	9	3	3
restraint	N	25	25	25	25	5	20
before transit	Range	(137-149)	(3.70-5.20)	(99-112)	(290-315)	(17-25)	(40-51)
9 Heifers (a)	$\bar{X}$	146	6.00	105	308	21	47
sampled with	SD	7	1.50	5	9	3	1
restraint	N	5	5	5	5	5	2
after transit	Range	(137-154)	(4.60-8.30)	(97-111)	(296-320)	(19-22)	(46-48)
10 Heifers (b)	$\bar{X}$	140	5.00	106	299	21	44
sampled with	SD	6	0.70	2	5	1	2
restraint	N	5	5	5	5	3	5
after transit	Range	(134-151)	(4.20-6.10)	(103-108)	(295-305)	(19-22)	(42-47)
11 Heifers (a)	$\bar{X}$	142	5.30	106	301	20	43
sampled at	SD	6	0.80	3	9	23	3
slaughter	N	25	25	25	23	3	20
	Range	(132-153)	(4.20-7.60)	(102-112)	(285-312)	(19-23)	(38-49)
12 Heifers (b)	$\bar{X}$	137	6.00	107	304	19	38
sampled at	SD	6	1.20	2	9	0	4
slaughter	N	5	6	6	6	1	6
	Range	(130-145)	(4.80-8.10)	(104-109)	(297-320)	(19)	(35-43)

a = Animals sampled previously

b = Animals not sampled previously

Table 3.2: PROTEIN, A/G RATIO, LIPID, LACTATE AND GLUCOSE VALUES FOR HEIFERS AND COWS

Groups		Total proteins g/ℓ	A/G Ratio	Lipids mg/100mℓ	Lactates mmol/ℓ	Glucose mmol/ℓ
7 Control	$\bar{X}$	96	0.730	7.34	0.43	2.82
Dairy cows	SD	8	0.164	1.60	0.24	0.11
accustomed	N	9	5	10	10	10
to sampling	Range	(80-106)	(0.553-1.000)	(5.40-9.60)	(0.16-0.98)	(2.65-30)
8 Heifers	$\bar{X}$	79	0.701	6.27	4.55	5.79
sampled with	SD	16	0.198	1.35	3.10	1.59
restraint	N	25	18	25	25	25
before transit	Range	(49-104)	(0.530-1.110)	(4.50-10.30)	(0.16-12.55)	(4.02-11.60)
9 Heifers (a)	$\bar{X}$	96	0.716	7.13	5.95	5.80
sampled with	SD	20	0.421	0.87	1.08	0.81
restraint	N	5	5	5	5	5
after transit	Range	(71-121)	(0.307-1.306)	(6.10-8.18)	(5.22-7.50)	(4.95-6.65)
10 Heifers (b)	$\bar{X}$	83	0.702	7.37	6.97	5.72
sampled with	SD	15	0.091	1.51	3.76	1.51
restraint	N	5	5	5	5	5
after transit	Range	(67-106)	(0.632-0.881)	(4.81-8.61)	(4.08-6.96)	(4.64-6.96)
11 Heifers (a)	$\bar{X}$	82	0.715	5.77	6.66	6.09
sampled at	SD	12	0.293	1.04	3.00	1.11
slaughter	N	25	18	25	25	25
	Range	(57-96)	(0.598-1.155)	(4.27-7.38)	(2.61-13.09)	(4.79-9.43)
12 Heifers (b)	$\bar{X}$	102	0.723	5.52	7.91	5.40
sampled at	SD	17	0.108	1.27	2.24	0.40
slaughter	N	6	6	6	6	6
	Range	(79-122)	(0.505-0.922)	(4.12-7.35)	(24-11.08)	(4.79-5.89)

a = Animals sampled previously

b = Animals not sampled previously

Table 3.3: HORMONAL COMPOSITION OF BLOOD OF HEIFERS AND COWS

Groups		ACTH (uIU/mℓ)	Cortisol nmol/ℓ	T3 pmol/ℓ	TSH (uU/mℓ)
7. Control	$\bar{X}$	19	30	3.06	1.68
Dairy cows	SD	7	15	0.73	0.60
accustomed	N	10	10	10	10
to sampling	Range	(<-28)	(12-51)	(1.80-4.00)	(0.80-2.70)
8 Heifers	$\bar{X}$	292	175	3.64	1.23
sampled with	SD	82	65	1.16	0.55
restraint	N	25	24	25	25
before transit	Range	(67-580)	(68-320)	(1.80-5.80)	(0.66-3.50)
9 Heifers (a)	$\bar{X}$	193	52	5.26	1.42
sampled with	SD	115	11	1.59	0.25
restraint	N	5	5	5	5
after transit	Range	(58-330)	(38-65)	(3.90-7.80)	(1.15-1.65)
10 Heifers (b)	$\bar{X}$	119	110	3.04	1.00
sampled with	SD	41	77	0.67	0.38
restraint	N	5	5	5	5
after transit	Range	(72-165)	(10-220)	(2.20-3.9)	(0.38-1.30)
11 Heifers (a)	$\bar{X}$	89	112	2.61	1.35
sampled at	SD	57	56	1.04	1.00
slaughter	N	24	24	25	25
	Range	(22-280)	(42-220)	(1.10-5.20)	(0.48-5.40)
12 Heifers (b)	$\bar{X}$	70	76	3.07	0.83
sampled at	SD	54	26	1.18	0.21
slaughter	N	6	6	6	6
	Range	(14-170)	(42-100)	(1.80-4.90)	(0.48-0.96)

a = Animals sampled previously

b = Animals not sampled previously

Table 4.1: ELECTROLYTE, OSMOLALITY, COLLOIDAL OSMOTIC PRESSURE AND HAEMATOCRIT VALUES FOR THE POOLED GROUP

Groups		Na^+ mmol/ℓ	K^+ mmol/ℓ	Cl^- mmol/ℓ	Osmolality mosm/kg	C.O.P. mm Hg	Haema-tocrit %
13 Samples from all the control animals	$\overline{X}$	140	4.0	105	292	19	-
	SD	16	0.5	5	5	2	-
	N	16	16	15	15	17	-
	Range	(132-153)	(3.1-4.6)	(96-114)	(284-298)	(17-22)	-
14 Pooled samples from all animals sampled before transit	$\overline{X}$	142	5.0	107	302	19	46
	SD	3	0.5	5	12	45	5
	N	50	50	50	44	11	38
	Range	(137-157)	(3.7-6.1)	(99-127)	(284-320)	(17-25)	(40-54)
15 Pooled samples from all animals sampled after transit	$\overline{X}$	143	6 .0	106	302	21	46
	SD	5	1 .0	5	10	3	3
	N	20	20	20	20	12	15
	Range	(134-156)	(4.2-8.3)	(96-119)	(280-320)	(17-24)	(41-51)
16 Pooled samples from all animals sampled at slaughter	$\overline{X}$	142	5.5	107	300	19	44
	SD	6	1.0	3	11	2	4
	N	63	63	63	63	14	48
	Range	(131-153)	(4.2-8.1)	(100-119)	(280-337)	(17-21)	(35-52)

Table 4.2: PROTEIN, A/G RATIO, LIPID, LACTATE AND GLUCOSE VALUES FOR THE POOLED GROUPS

Groups		Total proteins g/ℓ	A/G Ratio	Lipids mg/100mℓ	Lactates mmol/ℓ	Glucose mmol/ℓ
13 Samples from all the control animals	$\overline{X}$	91	0.741	6.11	0.43	3.11
	SD	9	0.151	1.97	0.24	0.60
	N	16	10	17	10	17
	Range	(78-106)	(0.553-1.00)	(3.73-9.61)	(0.08-0.98)	(2.65-5.26)
14 Pooled samples from all animals sampled be-before transit	$\overline{X}$	84	0.754	6.80	3.69	5.34
	SD	14	0.255	1.32	2.61	1.27
	N	50	33	50	50	50
	Range	(48-113)	(0.504-1.110)	(4.50-10.30)	(0.16-12.55)	(3.40-11.60)
15 Pooled samples from all animals sampled after transit	$\overline{X}$	86	0.705	6.64	5.13	5.58
	SD	16	0.326	1.29	2.65	0.66
	N	20	18	20	17	20
	Range	(62-112)	(0.415-1.155)	(4.60-8.61)	(1.30-7.50)	(4.64-6.96)
16 Pooled samples from animals sampled at slaughter	$\overline{X}$	82.8	0.718	5.59	7.45	6.01
	SD	14	0.196	1.04	2.89	1.23
	N	63	47	63	63	63
	Range	(58-122)	(0.415-1.155)	(4.08-7.38)	(2.61-16.95)	(3.40-10.82)

Table 4.3: HORMONAL COMPOSITION OF BLOOD OF THE POOLED GROUPS

Groups		ACTH (uIU/mℓ)	Cortisol nmol/ℓ	T3 pmol/ℓ	TSH (uU/mℓ)
13 Samples from all the control animals	$\bar{X}$	19	26	2.41	1.59
	SD	7	14	1.05	0.50
	N	10	16	17	17
	Range	(<-28)	(12-51)	(0.40-4.00)	(0.80-2.70)
14 Pooled samples from all animals sampled before transit	$\bar{X}$	310	175	2.82	1.41
	SD	145	58	1.30	0.27
	N	50	49	49	50
	Range	(60-665)	(53-320)	(1.30-5.80)	(0.48-3.50)
15 Pooled samples from all animals sampled after transit	$\bar{X}$	255	66.95	3.50	1.21
	SD	77	53	1.44	0.29
	N	18	20	20	20
	Range	(14-330)	(10-220)	(1.40-7.80)	(0.38-1.65)
16 Pooled samples from all animals sampled at slaughter	$\bar{X}$	85	89	2.32	1.09
	SD	54	50	1.08	0.68
	N	6	6	6	6
	Range	(14-280)	(12-220)	(0.40-5.20)	(0.48-5.40)

CHAPTER 5

Analysis of Data

Table 5: STATISTICAL COMPARISON OF THE BLOOD VALUES OF CONTROL OXEN (GROUP 1) AND COWS (GROUP 7)

Blood Variable	Comparison between control cows (Group 7) and oxen (Group 1)	
	t	▲
Na^+	5.236	$P<0.001$ ↑
K^+	5.500	$P<0.001$ ↓
Cl^-	1.000	N.S.
Osmolality	0.954	N.S.
C.O.P.	4.906	$P<0.001$ ↑
Total proteins	2.987	$P<0.01$ ↓
Lipids	4.724	$P<0.001$ ↓
Glucose	3.600	$P<0.01$ ↑
Cortisol	1.080	N.S.
TSH	0.960	N.S.
T3	5.220	$P<0.001$ ↓

t = t–Test for the difference between independent means

N.S. = Not Significant

↑ = Higher in oxen

↓ = Lower in oxen

5.1 Statistical comparison between Group 1 and Group 7 (Table 5)

No significant statistical differences in Cl^-, osmolality, cortisol and TSH values were evident between the samples of the control oxen (Group 1) and control cows (Group 7).

Significantly higher Na^+, C.O.P. and glucose values were evident in the control oxen (Group 1).

Significantly higher K^+, total proteins, lipids and T3 values were evident in the control cows (Group 7).

Table 6: STATISTICAL COMPARISON OF THE BLOOD VALUES OF THE CONTROL OXEN (GROUP 1) WITH THE OXEN EXPOSED TO DIFFERENT STRESS CONDITIONS (SAMPLING WITH RESTRAINT BEFORE TRANSPORTATION, GROUP 2, SAMPLING WITH RESTRAINT AFTER TRANSPORTATION GROUP 3 AND GROUP 4)

Blood Variables	Comparison between Group 1 & Group 2		Comparison between Group 1 & Group 3		Comparison between Group 1 & Group 4	
	P<	△	P<	△	P<	△
Na^+	N.S.		N.S.		N.S.	
K^+	0.001	↑	0.001	↑	0.001	↑
Cl^-	N.S.		N.S.		N.S.	
Osmolality	N.S.		N.S.		N.S.	
Total Proteins	N.S.		N.S.		N.S.	
Lipids	0.001	↑	0.01	↑	0.001	↑
Glucose	0.010	↑	0.001	↑	0.001	↑
Cortisol	0.001	↑	0.500	↑	0.001	↑
TSH	N.S.		N.S.		N.S.	
T3	0.01	↑	0.01	↑	N.S.	↑
A/G Ratio	N.S.		N.S.		N.S.	

N.S. = Not Significant

↑ = Values higher than the values of the control group.

Statistical test ANOVA

5.2 Statistical comparison of the blood values between control oxen Group 1 and oxen exposed to stressors (Groups 2, 3 and 4). Table 6.

No significant statistical differences in Na^+, Cl^-, A/G ratio, osmolality, total proteins and TSH values were evident between the control oxen (Group 1) and oxen exposed to stressors (Groups 2, 3 and 4).

The values for K^+, lipids, glucose, cortisol and T3 were significantly higher in the oxen exposed to stressors (Groups 2, 3 and 4) than in the control group (Group 1).

Table 7: STATISTICAL COMPARISON OF BLOOD VALUES BETWEEN THE CONTROL COWS (GROUP 7) WITH THE HEIFERS EXPOSED TO DIFFERENT STRESS CONDITIONS (SAMPLING WITH RESTRAINT BEFORE TRANSPORTATION GROUP 8, SAMPLING WITH RESTRAINT AFTER TRANSPORTATION GROUPS 9 AND 10)

Blood Variables	Comparison between Group 7 & Group 8		Comparison between Group 7 & Group 9		Comparison between Group 7 & Group 10	
	P	△	P	△	P	△
Na^+	N.S.		N.S.		N.S.	
K^+	N.S.		0.001	↑	0.001	↑
Cl^-	N.S.		N.S.		N.S.	
Osmolality	0.001	↑	0.001	↑	0.001	↑
C.O.P.	N.S.		N.S.		N.S.	
Total Proteins	0.02	↓	N.S.		N.S.	
Lipids	0.01	↓	N.S.		N.S.	
Lactates	0.001	↑	0.001	↑	0.001	↑
Glucose	0.001	↑	0.001	↑	0.001	↑
Cortisol	0.001	↑	0.05	↑	0.001	↑
TSH	N.S.		N.S.		N.S.	
T3	N.S.		N.S.		N.S.	
ACTH	0.001	↑	0.001	↑	0.001	↑
A/G Ratio	N.S.		N.S.		N.S.	

N.S. = Not Significant

↓ = Values lower than ccntrol group values.

↑ = Values higher than control group values.

Statistical test ANOVA

5.3 Statistical comparison between the blood values of control cows (Group 7) and heifers exposed to stressors (Groups 8, 9 and 10) Table 7.

No significant statistical differences in Na^+, Cl^-, A/G ratio, C.O.P., TSH and T3 values were evident between the control cows (Group 7) and the heifers exposed to stressors.

In all the heifers exposed to stressors (Groups 8, 9 and 10) there were significantly higher lactates, glucose, cortisol, ACTH and osmolality values than in the control group (Group 7).

In Groups 9 and 10 the K^+ values were significantly higher than the control group (Group 7).

In Group 8 the lipid and protein values were significantly lower than the control group (Group 7) .

Table 8: STATISTICAL COMPARISON OF THE BLOOD VALUES OF CONTROL OXEN (GROUP 1) AND OXEN SAMPLED AT SLAUGHTER (GROUP 5)

Blood Variable	Comparison between Group 1 & Group 5	
Na^+	N.S.	
K^+	0.001 *	↑
Cl^-	N.S.	
Osmolality	N.S.	
C.O.P.	N.S.	
Total Proteins	N.S.	
Lipids	0.01 *	↑
Glucose	0.001 *	↑
Cortisol	0.01 *	↑
TSH	N.S.	
T3	N.S.	
A/G Ratio	N.S.	

N.S. = Not Significant
↑ = Higher values than control group.
Statistical test ANOVA
* = Level of Significance (P)

5.4 Statistical comparison of the blood values between the control oxen (Group 1) and the oxen sampled at slaughter (Group 5) Table 8.

No significant differences in Na^+, Cl^-, osmolality, A/G ratio, C.O.P., total proteins, TSH and T3 were evident between Group 1 and Group 5.

In Group 5 (oxen sampled at slaughter) there were significantly higher K^+, lipids, glucose and cortisol values than in the control group (Group 1).

Group 6 and Group 5 were statistically compared using ANOVA and no significant differences in the blood variables were found. Therefore only Group 5 was used.

Table 9: STATISTICAL COMPARISON OF THE BLOOD VALUES OF CONTROL COWS (GROUP 7) AND HEIFERS SAMPLED AT SLAUGHTER (GROUP 11)

Blood Variables	Comparison between Group 7 & Group 11	
Na^+	N.S.	
K^+	0.001 *	↑
Cl^-	N.S.	
Osmolality	0.001 *	↑
C.O.P.	N.S.	
Total Proteins	0.05 *	↓
Lipids	0.001 *	↓
Lactates	0.001 *	↑
Glucose	0.001 *	↑
Cortisol	0.001 *	↑
TSH	N.S.	
T3	N.S.	
ACTH	0.5 *	↑
A/G Ratio	N.S.	

N.S. = Not Significant
↑ = Higher values than control group values.
↓ = Lower values than control group values.
Statistical test ANOVA
* = Level of Significance (P)

5.5 Statistical comparison of the blood values between the control heifers (Group 7) and the heifers sampled at slaughter (Group 11) Table 9.

No significant differences in Na^+, Cl^- A/G ratio, C.O.P., TSH and T3 values were evident between the control cows (Group 7) and the heifers sampled at slaughter (Group 11).

In Group 11 (heifers sampled at slaughter) there were significantly higher K^+, osmolality, lactates, glucose, cortisol and ACTH values while the total proteins and lipids were significantly lower than those of the control group (Group 7).

Group 11 and 12 were statistically compared using ANOVA and no significant differences in the blood variables were found. Therefore only Group 11 was used.

Table 10: STATISTICAL COMPARISON OF BLOOD VALUES BETWEEN OXEN SAMPLED BEFORE TRANSPORTATION (GROUP 2) AND AFTER TRANSPORTATION (GROUP 3).

Blood Variables	Comparison between Group 2 & Group 3	
	T	P
Na^+	0.164	N.S.
K^+	1.0860	N.S.
Cl^-	1.415	N.S.
Osmolality	0.745	N.S.
C.O.P.	0.745	N.S.
Lipids	2.0	N.S.
Lactates	0.279	N.S.
Glucose	1.975	N.S.
Total Proteins	1.9	N.S.
Cortisol	4.927	0.1 ↓
TSH	0.395	N.S.
T3	0.686	N.S.
ACTH	5.263	.01 ↓
Haematocrit	0.889	N.S.
A/G Ratio		N.S.

N.S. = Not Significant

↓ = Values lower than Group 2 values.

Statistical test used, t - Test for related measures.

5.6 Statistical comparison of the blood values between oxen sampled before transportation (Group 2) and oxen sampled after transportation (Group 3) Table 10.

No significant differences in Na^+, K^+, Cl^-, A/G ratio, osmolality, C.O.P., lipids, lactates, glucose, total proteins, TSH, T3 and haematocrit values were evident between the oxen of Group 2 and the oxen of Group 3.

In Group 3 (oxen sampled after transportation) there were significantly lower cortisol and ACTH values than in Group 2 (oxen sampled before transportation).

Group 3 and Group 4 were statistically compared, using ANOVA and no significant differences in the blood variables were found. Therefore only Group 3 was used.

Table 11: STATISTICAL COMPARISON OF BLOOD VALUES BETWEEN HEIFERS SAMPLED BEFORE TRANSPORTATION (GROUP 8) AND HEIFERS SAMPLED AFTER TRANSPORTATION (GROUP 9).

Blood Variables	Comparison between Group 8 & Group 9	
	t	P
Na^+	0.689	N.S.
K^+	2.20	N.S.
Cl^-	1.411	N.S.
Osmolality	0.8	N.S.
C.O.P.	0.9	N.S.
Lipids	2.317	N.S.
Lactates	0.14	N.S.
Glucose	0.995	N.S.
Total Proteins	1.00	N.S.
Cortisol	7.07	0.01 ↓
TSH	1.105	N.S.
T3	1.65	N.S.
ACTH	1.725	N.S. ↓
Haematocrit	1.9	N.S.
A/G Ratio		N.S.

N.S. = Not Significant

↓ = Values lower than Group 8 values.

Statistical test used, t - Test for related measures.

5.7 Statistical comparison of the blood values between heifers sampled before transportation (Group 8) and heifers sampled after transportation (Group 9) Table 11.

No significant differences in Na^+, K^+, Cl^-, A/G ratio, osmolality, C.O.P., lipids, lactates, glucose, total proteins, TSH, T3, ACTH and haematocrit values were evident between the heifers of Group 8 and heifers of Group 9.

In Group 9 (heifers sampled after transportation) there were significantly lower cortisol values than in Group 8 (heifers sampled before transportation. (ACTH values were insignificantly lower in Group 9 than Group 8)

Group 9 and 10 were statistically compared using ANOVA and no significant differences in the blood variables were found therefore only Group 9 was used.

Table 12: STATISTICAL COMPARISON OF BLOOD VALUES BETWEEN OXEN SAMPLED BEFORE TRANSPORTATION (GROUP 2) AND OXEN SAMPLED AT SLAUGHTER (GROUP 5).

Blood Variables	Comparison between Group 2 & Group 5	
	F	P
Na^+	0.08	N.S.
K^+	21.49	0.001 ↑
Cl^-	11.4	N.S.
Osmolality	0.86	N.S.
C.O.P.	0.00	N.S.
Lipids	101	0.001 ↓
Lactates	85.9	0.001 ↑
Glucose	21.48	0.001 ↑
Total Proteins	1.60	N.S.
Cortisol	72	0.001 ↓
TSH	3.34	N.S.
T3	1.8	N.S.
ACTH	56.1	0.001 ↓
Haematocrit	0.657	N.S.
A/G Ratio		N.S.

N.S. = Not Significant
↑ = Higher values than Group 2 values.
↓ = Lower values than Group 2 values.
Statistical test used, t - Test for related measures.

5.8 Statistical comparison of the blood values between oxen sampled before transportation (Group 2) and the oxen sampled at slaughter (Group 5) Table 12.

No significant statistical differences in Na^+, Cl^-, A/G ratio, osmolality, C.O.P., total protein, TSH, T3 and haematocrit values were evident between the oxen of Group 2 and the oxen sampled at slaughter (Group 5).

In Group 5 (oxen sampled at slaughter) there were significantly higher K^+, lactate and glucose values than in Group 2 (oxen sampled before transportation).

In Group 5 there were significantly lower lipid, cortisol and ACTH values than in Group 2.

Group 5 and Group 6 were statistically compared using ANOVA and no significant differences in the blood variables were found, therefore only Group 5 was used.

Table 13: STATISTICAL COMPARISON OF BLOOD VALUES BETWEEN HEIFERS SAMPLED BEFORE TRANSPORTATION (GROUP 8) AND HEIFERS SAMPLED AT SLAUGHTER (GROUP 11).

Blood Variables	Comparison between Group 8 & Group 11	
	F	P
Na^+	0.5	N.S.
K^+	4.5	0.25 ↑
Cl^-	0.72	N.S.
Osmolality	0.18	N.S.
C.O.P.	0.46	N.S.
Lipids	4.236	0.05 ↓
Lactates	11.72	0.005 ↑
Glucose	1.2719	N.S.
Total Proteins	0.574	N.S.
Cortisol	20	0.001 ↓
TSH	0.460	N.S.
T3	24	0.001 ↓
ACTH	50.94	0.001 ↓
Haematocrit	0.81	N.S.
A/G Ratio		N.S.

N.S. = Not Significant

↑ = Higher values than Group 8 values

↓ = Lower values than Group 8 values

Statistical test used, t - Test for related measures.

5.9 Statistical comparison of the blood values between heifers sampled before transportation (Group 8) and the heifers sampled at slaughter (Group 11) Table 13.

No significant differences in Na^+, Cl^-, osmolality, C.O.P., glucose, total protein, TSH and haematocrit values were evident between the heifers of Group 8 and the heifers of Group 11.

In Group 11 (heifers sampled at slaughter) there were significantly higher K^+ and lactate values while the lipids, cortisol, T3 and ACTH values were significantly lower than the values in Group 8.

Table 14: STATISTICAL COMPARISON OF THE BLOOD VALUES OF THE POOLED DATA OF CONTROL CATTLE (GROUP 13) AND THE POOLED DATA OF THE CATTLE EXPOSED TO DIFFERENT STRESSORS (SAMPLING WITH RESTRAINT BEFORE TRANSPORTATION GROUP 14, SAMPLING WITH RESTRAINT AFTER TRANSPORTATION GROUP 15 AND SAMPLING AT SLAUGHTER GROUP 16).

Blood Variables	Comparison between Group 13 & Group 14			Comparison between Group 13 & Group15			Comparison between Group 13 & Group 16		
Na^+	N.S.			N.S.			N.S.		
K^+	.001 *	↑		.001 *	↑		.001 *	↑	
Cl^-	N.S.			N.S.			N.S.		
Osmolality	.02 *	↑		.01 *	↑		.01 *	↑	
C.O.P.	N.S.			N.S.			N.S.		
Total Proteins	.05 *		↓	N.S.		↓	.02 *		↓
A/G Ratio	N.S.			N.S.			N.S.		
Lipids	N.S.			N.S.			N.S.		
Lactates	.001 *	↑		.001 *	↑		.001 *	↑	
Glucose	.001 *	↑		.001 *	↑		.001 *	↑	
Cortisol	.001 *	↑		.001 *	↑		.001 *	↑	
TSH	N.S.			N.S.			N.S.		
T3	.02 *	↑		.01 *	↑		N.S.		↓
ACTH	.001 *	↑		.02 *	↑		.05 *	↑	

N.S. = Not Significant

Test = ANOVA

↑ = Values higher than control values (Group 13).

↓ = Values lower than control values (Group 13).

* = Level of Significance (P)

5.10 Statistical comparison of the blood values between the control cattle (Group 13) and the cattle exposed to different stressors (Group 14, Group 15 and Group 16) Table 14.

No significant differences in Na^+, Cl^-, C.O.P., lipids and TSH values were evident between the cattle of Group 13 and the cattle exposed to different stressors (Group 14, 15 and 16).

In all the cattle exposed to different stressors (Group 14, 15 and 16) there were significantly higher K^+, osmolality, lactates, glucose, cortisol and ACTH values while the total protein values were significantly lower than those of the control group (Group 13).

Table 15: STATISTICAL COMPARISON OF THE BLOOD VALUES OF THE POOLED DATA OF CATTLE SAMPLED WITH RESTRAINT BEFORE TRANSPORTATION (GROUP 14) AND THE POOLED DATA OF GROUP 15 AND GROUP 16.

Blood Variables	Comparison between Group 14 & Group 15		Comparison between Group 14 & Group 16	
Na^+	N.S.		N.S.	
K^+	.001 *	↑	.001 *	↑
Cl^-	N.S.		N.S.	
Osmolality	N.S.		N.S.	
C.O.P.	N.S.		N.S.	
Total Proteins	N.S.		N.S.	
Lipids	N.S.	↑	.001 *	↑
Lactates	N.S.	↑	.001 *	↑
Glucose	N.S.	↑	.01 *	↑
Cortisol	.001 *	↓	.001 *	↓
TSH	N.S.		N.S.	
T3	0.001 *	↑	N.S.	
ACTH	.001 *	↓	.001 *	↓
Haematocrit	N.S.		N.S.	
A/G Ratio	N.S.		N.S.	

N.S. = Not Significant

Statistical test ANOVA

↑ = Values higher than Group 14 values.

↓ = Values lower than Group 14 values.

* = Level of Significance (P)

5.11 Statistical comparison of the blood values between the cattle sampled before transportation (Group 14) and cattle sampled after transportation (Group 15) Table 15.

No significant differences in Na^+, Cl^-, osmolality, C.O.P., total proteins, TSH, T3 and haematocrit values were evident between the cattle of Group 14 and Group 15.

In Group 15 the K^+, T3, lactates and glucose values were significantly higher while the cortisol, lipid and ACTH values were significantly lower than in the cattle of Group 14.

5.12 Statistical comparison of the blood values between the cattle sampled before transportation (Group 14) and cattle sampled at slaughter (Group 16) Table 15.

No significant differences in Na^+, Cl^-, osmolality, A/G ratio, C.O.P., total proteins, TSH and haematocrit values were evident between the cattle of Group 14 and Group 16.

In Group 16 the K^+, lactates and glucose values were significantly higher while the cortisol, lipids and ACTH values were significantly lower than the values in the cattle of Group 14.

Table 16: STATISTICAL COMPARISON OF THE BLOOD VALUES OF THE POOLED DATA OF CATTLE SAMPLED WITH RESTRAINT AFTER TRANSPORTATION (GROUP 15) AND THE POOLED DATA OF GROUP 16.

Blood Variables	Comparison between Group 15 & Group 16	
Na^+	N.S.	
K^+	N.S.	
Cl^-	N.S.	
Osmolality	N.S.	
C.O.P.	N.S.	
Total Proteins	N.S.	
Lipids	.01 *	↑
Lactates	.01 *	↓
Glucose	N.S.	
Cortisol	N.S.	
TSH	N.S.	
T3	.001 *	↑
ACTH	N.S.	
Haematocrit	N.S.	
A/G Ratio	N.S.	

N.S. = Not Significant

↑ = Values higher than Group 16 values.

↓ = Values lower than Group 16 values.

Statistical test ANOVA

* = Level of Significance (P)

5.13 Statistical comparison of the blood values between the cattle sampled after transportation (Group 15) and cattle sampled at slaughter (Group 16) Table 16.

No significant differences in Na^+, K^+, Cl^-, osmolality, C.O.P., total proteins, glucose, cortisol, TSH, ACTH, haematocrit and A/G ratio values were evident between Group 15 and Group 16.

In Group 16 the lactate values were significantly higher while the lipids and T3 values were significantly lower than the values in cattle of Group 15.

Table 17: SUMMARY OF DIFFERENCES FOUND IN CERTAIN VARIABLES BETWEEN THE DIFFERENT GROUPS OF POOLED DATA

		Group 14 Before Transport	Group 15 After Transport	Group 16 After Slaughter
Group 13 Control	K^+	(0.001) ↑	(0.001) ↑	(0.001) ↑
	Osmolality	(0.02) ↑	(0.01) ↑	(0.01) ↑
	Total Proteins	(0.05) ↓	(N.S.) ↓	(0.02) ↓
	Lactates	(0.001) ↑	(0.001) ↑	(0.001) ↑
	Glucose	(0.001) ↑	(0.001) ↑	(0.001) ↑
	Cortisol	(0.001) ↑	(0.001) ↑	(0.001) ↑
	T3	(0.02) ↑	(0.01) ↑	(N.S.)
	ACTH	(0.001) ↑	(0.02) ↑	(0.05) ↑
Group 14 Before Transport	K^+		(0.001) ↑	(0.001) ↑
	Lipids		(N.S.) ↑	(0.001) ↑
	Lactates		(N.S.) ↑	(0.01) ↑
	Glucose		(N.S.) ↑	(0.001) ↑
	Cortisol		(0.001) ↓	(0.001) ↓
	T3		(N.S.) ↑	(0.001) ↓
	ACTH		(0.001) ↓	(0.001) ↓
Group 15 After Transport	K^+			(N.S.)
	Lipids			(0.01) ↓
	Lactates			(0.01) ↑
	Glucose			(N.S.) ↑
	Cortisol			(N.S.) ↓
	T3			(0.001) ↓
	ACTH			(N.S.)

↑ = Values higher than group in the horizontal column.

↓ = Values lower than group in the horizontal column.

CHAPTER 6

6 Summary (Table 17)

6.1 Introduction

Only the results of the pooled data are discussed as the changes evident in the individual group analyses, with the exception of plasma proteins (see 6.5) and lipids (see 6.7) agree with the changes evident in the pooled data analyses.

A summary of differences found in certain variables between the different groups of pooled data is given in Table 17.

6.2 Sodium and Potassium

No significant differences were evident in the Na^+ values between the cattle exposed to different stressors and the control groups (Table 14). The Na^+ values measured agree with those reported in literature concerning cattle exposed to the stress of anaesthesia (Steyn, 1969) and with those obtained from cattle sampled after slaughter (Doornenbal, 1977).

Significantly higher K^+ values were evident in the cattle exposed to different stressors than in the control cattle (Table 4.1 and Table 14). The values measured after slaughter agree with the values reported in the literature concerning cattle sampled after slaughter (Doornenbal, 1977)

6.3 Chloride

No significant differences were evident in Cl^- values between the cattle exposed to different stress conditions and the control group. (Table 4.1 and Table 14)

The Cl^- values measured agree with those values reported by Steyn (1969) and Doornenbal (1977), but are lower than the values recorded by Ganhao et al (1986).

6.4 Colloidal Osmotic Pressure and Osmolality

No significant differences were evident in C.O.P. values between the cattle exposed to the different stress conditions and the control group of cattle (Table 4.1 and Table 14).

Significantly higher osmolality values were evident in the cattle exposed to different stress conditions than in the control group of cattle (Table 4.1 and Table 14).

No published data on C.O.P. and osmolality for cattle exposed to stress could be found. The control osmolality values were higher than the osmolality values recorded by Ganhao et al (1986).

The osmolality values are higher than those found in buffalo while the C.O.P. values are lower. (Hattingh et al, 1984)

6.5 Plasma Proteins

The plasma protein values in the control milking cows were significantly higher than those of the control oxen (Table 5) and higher than those of the experimental groups (Groups 14, 15 and 16, Table 7). The protein values of the control milking cows were higher than the values recorded by Ganhao et al (1986). This makes the comparison of plasma protein between the pooled control groups and the pooled experimental groups questionable.

The protein values are higher than reported values (Irfan, 1967) but are lower than the protein values reported in buffalo (Hattingh et al, 1984).

No significant differences in A/G ratio were evident between the cattle exposed to the different stressors and the control groups. The mean A/G values are higher than the values reported by Deutsch et al (1945), (A/G ratio 0.7); Putnam (1960), (A/G ratio 0.666) and lower than the values reported by Irfan (1967) (A/G, 0.757).

6.6 Haematocrit values

No changes in the haematocrit values between the different experimental groups were evident (Table 4.1 and Table 14). The mean haematocrit values are lower than the values reported by Doornenbal (1977), higher than the values recorded by Ganhao et al (1986) and agree with the values reported by Van der Westhuysen (1973)

6.7 Lipids

The lipid values of the control milking cows were significantly higher than those of the control oxen (Table 5) and higher than those of the experimental groups. The control lipid values for both oxen and cows were much higher (6.11 mg/100mℓ compared to 1.7 mg/100mℓ) than the values recorded by Ganhao et al (1986). This makes the comparison of lipids between the pooled control and pooled experimental groups questionable.

The lipid values were significantly lower in the group that was slaughtered than in the group that was restrained before transportation (Table 4.2 and Table 16). An increase in lipid value during stress was reported in buffalo (Hattingh et al, 1984).

6.8 Lactates and Glucose

The lactate and glucose values of the animals exposed to stress were significantly higher than the values of the control animals (Table 4.2 and Table 15). The lactate and glucose values were the highest in the slaughtered animals (Table 4.2, Table 15 and Table 16).

Increase in blood glucose values have been reported for cattle exposed to stress (Saroff & Turner, 1956; Khan, Dickson & Meyer, 1970; Steyn 1969). The control glucose values agree with the values reported by Saroff et al (1956), Steyn (1969), Khan et al (1970), and the values recorded by Ganhao et al (1985).

6.9 Cortisol, also known as hydrocortisone, compound F, 17 Hydroxy-corticosterone - (Holcombe, 1957)

Significantly higher cortisol values were evident in the cattle exposed to stress than the control group of cattle (Table 4.3 and Table 14). The cortisol values were significantly higher in animals restrained before transportation than the groups sampled after transportation (Table 4.3 and Table 15). The lower cortisol values in the group sampled after slaughter in comparison to the group sampled before transportation could possibly be ascribed to adaptation. (see 7.3)

The cortisol values measured after slaughter agree with the only results reported in the literature (Purchas et al, 1980). Increase in cortisol values have been reported for cattle exposed to stress (Steyn, 1969; Khan et al, 1970; Venkataseshu & Estergreen, 1970; Willet & Erb, 1972).

The control cortisol values agree with the values reported in the literature (Robertson et al, 1957; Steyn, 1969; Willet & Erb, 1972) and are higher than the values recorded by Ganhao et al (1985).

6.10 ACTH

Significantly higher ACTH values were evident in the cattle exposed to different stressors than in the control cattle. (Table 4.3 and Table 14). The ACTH values were significantly higher in the animals restrained before transportation than in the groups sampled after transportation and slaughter. (Table 4.3 and Table 15). The lower ACTH values in the groups sampled after transportation may possibly be ascribed to adaptation (see 7.3).

The mean ACTH values of the control groups are lower than the values recorded by Ganhao et al (1985).

6.11 T3 and TSH

Significantly higher T3 values were evident in the groups of cattle sampled before transportation and after transportation than in the control group and the group of cattle sampled after slaughter (Table 5.10, Table 5.12, Table 5.13, Table 14, Table 15 and Table 16). The T3 values of the control animals are higher than the values recorded by Ganhao et al (1985).

No significant differences were evident in TSH values between the cattle exposed to the different stress conditions and the control group of cattle. The TSH values of the control animals are higher than the values recorded by Ganhao et al (1985).

6.12 Conclusion

To conclude: the results indicate that the following variables were significantly higher in concentration in cattle exposed to different stressors than in the control groups: (Table 17)

- K^+ ($P < 0,001$, Groups 14, 15 & 16)
- Osmotality ($P < 0,01$, Groups 14, 15 & 16)
- Lactates ($P < 0,001$, Groups 14, 15 & 16)
- Glucose ($P < 0,001$, Groups 14, 15 & 16)
- Cortisol ($P < 0,001$ Groups 14, 15 & 16)
- ACTH ($P < 0,001$, Groups 14 & 15, $P < 0,05$ Group 16)
- T3 ($P < 0,01$ Group 15, $P < 0,02$ Groups 14)

Within the experimental groups, the following variables were significantly different in concentration in the group (Group 16) sampled at slaughter than in the groups (Groups 14 & 15) exposed to other stressors (all animals sampled with restraint before transportation and all animals sampled with restraint after transportation):

- K^+ (Higher ($P < 0{,}001$) than in Group 14)
- Lipids (Higher ($P < 0{,}001$) than in Group 14, and lower ($P < 0{,}01$) than Group 15)
- Lactates (Higher ($P < 0{,}01$) than in Groups 14 & 15)
- Glucose (Higher ($P < 0{,}01$) than in Group 14)
- Cortisol (Lower ($P < 0{,}001$) than in Group 14)
- T3 (Lower ($P < 0{,}001$) than in Groups 14 & 15)
- ACTH (Lower ($P < 0{,}001$) than in Group 14)

CHAPTER 7

7 Discussion

7.1 Control Group

The control group were cattle that were undisturbed as far as possible when sampled. These were used for obtaining control values of the variables (Na^+, K^+, Cl^- etc.) for comparison with those obtained from the animals in the experimental groups. In this study the control animals were not of the same stock (breed and location) as the experimental groups.

The use of the same animals in both the control and experimental groups would have been ideal. However, the sampling took place under normal transportation and slaughter routine, so that the results obtained would, as far as possible, be a reflection of the changes that could be expected in certain variables during this routine. The use of tame animals in the experimental group could have been difficult and would have created an unnatural situation making comparison between groups questionable. Anaesthesia could not be used as this might also be stressful (Steyn, 1969).

The fact that the control animals were not of the same group as the experimental animals is taken into consideration in this study. The control groups were statistically compared and variable values (lipid proteins) that were questionable were omitted from the comparison between the experimental and control groups in the discussion.

7.2 Quantifying stress

According to Stott (1981) the magnitude of environmental stress can only be measured indirectly through the response of the animal. The acute response may be a measurement of strain due to stress. After consistently long periods of stress the measurable response is propably adaptation.

Therefore stress itself is not measured directly, but the possible response to it. The acute response to a stressor referred to by Stott (1981) could be interpreted as the alarm reaction of GAS. During this phase (alarm reaction), there is stimulation of the pituitary adrenal axis. (Jordaan, et al 1975; Selye, 1976; Fox 1984) and mass sympathetic discharge. Therefore the variables primarily influenced by stress are those directly influenced by the pituitary adrenal axis and mass sympathetic discharge and include (Ganong, 1977; Guyton, 1976; Bell, 1980; Hardy, 1981)

- ACTH
- plasma corticoids (cortisol)
- and the catecholamines

These primary variables themselves will influence other variables Such secondary variables including:

- glucose
- lipids
- lactates

  and others

The measuring of the change in the primary and secondary variables could possibly lead towards a quantification of stress, although stress cannot yet be accurately quantified.

Adaptation as referred to by Stott (1981) could be interpreted as the resistance phase of GAS. This phase follows the alarm reaction and is marked by a depression in adrenal steroid concentration (Jordaan et al, 1975; Selye, 1976; Stott, 1981). As this phase may follow a few hours after exposure to acute stress it is taken into consideration in this study.

7.3 Summary

In this study cattle were exposed to possible stressors. (Sampling with restraint, before and after transportation and slaughter). If these conditions are stressful, they should cause a non-specific response referred to as GAS (Selye, 1976). The cattle were exposed to these possible stressors for a short period and were sampled immediately, so that the changes expected in the blood (if these conditions are stressors) are those that occur during the alarm reaction phase of GAS. These changes are associated with the stimulation of the pituitary adrenal axis and mass sympathetic discharge (Jordaan et al, 1975; Selye, 1976: Ganong, 1977).

The plasma K^+ values were significantly higher in the animals exposed to the different stressors (Groups 14, 15 & 16) than in the control group (Group 13). The higher K^+ values could be as a result of K^+ liberation from disintergrating cell bodies at the time of the catabolic impulse (Selye, 1950) and/or the effect of catecholamines on membrane permeability for K^+ (Metivier, 1968) or it could be artefactual due to haemolysis after the collection of samples (McKechnie et al, 1982).

The osmolality values were significantly higher in the animals exposed to the different stressors (Groups 14, 15 & 16) than in the control group (Group 16). This might be explained on the basis of fluid loss or a greater ingress of osmotically active substances during stress.

It has been found that stressful agents (Robertson et al, 1957) and high temperature (Ganong, 1977) have a depressing effect on the activity of the thyroid gland, while exsposure to cold (Ganong, 1977) the activation of the sympathetic innervation to the thyroid and the pressence of catecholamines (Hardy, 1981) could stimulate thyroid activity. Thyroxine is also increased by excercise (Shepard, 1982). The significantly higher T3 values in the animals exposed to the stress of sampling with restraint (Group 14) and sampling after transportation (Group 15) could be as a result of temperature differences (Ganong, 1977) and/or muscle activity (Shephard, 1982), and/or the release of catecholamines (Hardy, 1981).

Within the experimental groups (Groups 14 & 16) the lactate and glucose values were higher and the lipid values lower in the group sampled at slaughter (Group 16). The higher glucose values could be as a result of secondary hyperglycemia caused by the release of glucocorticoids which follows the initial hyperglycemia caused by the release of adrenalin (Steyn, 1969 and Hardy, 1981). The lower lipid values in the group sampled at slaughter could be attributed to an increase of lipase caused by the higher levels of circulating catecholamines and glucocorticoids during the stress of sampling with restraint (Ganong, 1977). The high lactate values in the group sampled at slaughter (Group 16) could be as a result of increased muscle activity.

The possibility of adaptation in the experimental groups (Group 14, 15 & 16) cannot be ignored as some of the animals exposed to the stress of slaughter were also exposed to the stress of sampling before transportation (Group 14) and the stress of sampling after transportation (Group 15). The lower cortisol and ACTH values could be ascribed to adaptation, although this is unlikely as the time laps between the first sampling (sampling in a crush - Group 14) and the second sampling (sampling after transportation - Group 15) was about 4 hours and there was no significant differences in the variable values of the groups (Groups 5 and 11) that had been sampled previously before slaughter and the groups (Groups 6 and 12) that had not been sampled before.

Therefore if the variables measured are an assesment of stress and changes induced by stress, then slaughter is stressful and cattle unaccustomed to handling probably perceive the slaughter process as less stressful than blood sampling in a crush.

CHAPTER 8

8 Conclusion

1. In this study the cattle exposed to the different stressors (sampling in a crush, transportation and slaughter) had significantly higher K^+, osmolality, lactate, glucose cortisol, T3 and ACTH values than the control groups. The most noteworthy changes been ACTH (4-15X), cortisol (3-6X), glucose (2X) and lactates (8-18x).

2. If the variables (ACTH, cortisol, glucose and lactates) measured are an assessment of stress and of changes induced by stress then slaughter is stressful.

3. The groups that were sampled at slaughter had significantly higher K^+, lactate, lipid and glucose values and significantly lower cortisol, ACTH and T3 values than the group sampled with restraint before transportation.

4. No significant differences in the variables were observed between the group sampled at slaughter and the group that was sampled with restraint after transportation.

5. Cattle unaccustomed to handling probably perceive the slaughtering process as less stressful than blood sampling in a crush.

6. The results in this study could also be of importance to the meat industry as stress may have an effect detrimental on meat quality. (Thornton & Gracy, 1974).

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