

"A study of the Interactions between Plasma Cortisol
levels, Estrous cycles, Rectal Temperatures
and Respiratory Rates in Heifers."

by

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Dedication

To Adeyelu and Gabriel Adeyemo

My Loving Mother and Father.

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Abstract

The need to improve the animal protein diet of the Nigerian population has underlined the importation of temperate-evolved cattle into Nigeria. How these animals adapt to the new environment should be of economic and scientific interest. Under the natural hot/humid sub-equatorial climate of Ibadan, some physiologic, adrenocortical and reproductive functions were investigated in 6 German Brown, 5 Holstein and 6 White Fulani heifers.

The Brown, Holstein and Fulani heifers attained puberty at the average ages, with the standard deviations of 17.8 ± 1.6 , 16.7 ± 1.8 and 23.7 ± 1.9 months respectively. Observations on estrous cycles showed that estrous period ranged between 7 and 31 hours in the three breeds, the mean values, with the standard errors, being 16.2 ± 0.7 , 15.8 ± 0.7 and 14.6 ± 0.8 hours in the Brown, Holstein and Fulani heifers respectively. The difference between the *Bos taurus* and *Bos indicus* cattle was slight but significant. Most estrus commenced during the day with a greater concentration in the morning time. The intensity of estrus was high in both species though, occasionally, a few Fulani heifers showed weaker estrual signs.

Average estrous cycle length was slightly longer in the Brown and Fulani than in the Holstein heifers, the values, with the standard errors, being 21.0 ± 0.3 , 21.4 ± 0.2 and 20.1 ± 0.2 days respectively. Ovulations occurred mostly within a day after estrus, and this as well

as the duration of estrus and estrous cycle length showed no seasonal variations. There was no indication that *Bos taurus* and *Bos indicus* cattle under the semi-intensive management preferred any particular season for increased sexual activity or breeding in the sub-equatorial climate.

Marked shifts occurred in the respiratory rates during four selected quarters of the year. Increases occurring in the dry hot season from the values in the relatively cooler season were highly significant. Both mean morning and mean afternoon values (14 - 44 and 17 - 75 breaths per minute respectively) were highest in the Holstein and lowest in the Fulani heifers.

Rectal temperatures showed slight but significant seasonal changes. Mean values were 101.3°F (38.5°C) and 102.2°F (39.0°C) in the morning and afternoon respectively for all the heifers together through the year. The lowest afternoon values occurred in the wettest and coolest months. The Fulani and Holstein heifers showed the lowest and highest mean values respectively.

Diurnal and circadian shifts in the respiratory rates and rectal temperatures in the heifers were most exaggerated in the sun and in the Holsteins, the latter particularly exhibited polypnea and hyperthermia. Unlike the Zebu, the *Bos taurus* cattle sought shade in the sun. The Holstein heifers sought shade more frequently and stayed there longer than the Brown heifers.

Basal plasma cortisol concentrations at 07 - 08.00 hours, determined by radioimmunoassay during four quarters of the year was low,

ranging between 1 - 10 ng/ml with occasional mid-cycle and more frequent proestrous and/or estrous elevations. Mean values showed slight but significant seasonal changes. The levels in the dry season were slightly lower than in the wet season. Breed differences were not significant. Diurnal and circadian plasma cortisol concentrations in heifers in the shade and in the sun showed no rhythmical pattern.

Exogenous corticotrophin at and after mid-cycle stage elicited marked and prolonged adrenocortical response which varied between heifers, and did not alter estrous cycle rhythmicity. A high adrenal reserve in the heifers was indicated.

Bos taurus cattle have been found to be adaptable to the southern Nigerian climate represented by the Ibadan condition. Management practices should, however, ensure all-year-round provision of shade and adequate nutrition including the adoption of night grazing.

The Brown cattle are recommended over the Holsteins because the former are more comfortable. A mixed herd of the two breeds should be discouraged because socially the Browns dominate over the Holsteins.

The Fulani cattle are more adaptable to the subequatorial climate than the temperate-evolved cattle as evidenced by the physiological responses. Artificial breeding should be suitable for the Fulani cattle as it is for the *Bos taurus* cattle. The need to adopt better management practices than hitherto existing range system for the Fulani cattle, so that their reproductive and productive attributes may be well manifested, is indicated.

List of Abbreviations

The following abbreviations were used in this study:

ng	=	nanogram
µg	=	microgram
mg	=	milligram
g	=	gram
pg	=	picogram
%	=	per cent
°F	=	degree Fahrenheit
°C	=	degree centigrade
ml	=	millilitre
µl	=	microlitre
H ⁺	=	hydrogen ion
Kg	=	kilogram
mMol	=	millimole
Ci	=	curie
µCi	=	micro Curie
h.	=	hour
min.	=	minutes
BDH	=	British Drug House
UK	=	United Kingdom
USA	=	United States of America
et al	=	and others
M.W.	=	molecular weight
max	=	maximum
T _{re}	=	rectal temperature
ed	=	edition
cpm	=	counts per minute
pH	=	hydrogen ion concentration
S.D.	=	standard deviation
SE	=	standard error

\bar{x}	=	mean
im.	=	intramuscularly
S	=	Significant
NS	=	Not significant
P.C.V.	=	packed cell volume
Hb	=	Haemoglobin
T_a	=	environmental temperature [ambient temperature]
RH%	=	Relative Humidity
mm	=	millimeter
ins	=	inches
L.S.D.	=	least significant difference
P_{O_4}	=	phosphate radical
Na^+	=	Sodium ion
LH	=	Luteinising hormone
H_2O	=	Water
CL	=	corpus luteum
Cl	=	chloride radical
K^+	=	Potassium ion
ACTH	=	Adrenocorticotrophic hormone
CV	=	coefficient of variation
CNS	=	central nervous system
RIA	=	radioimmunoassay
CPB	=	competitive protein binding
Fig(s)	=	figure(s)
CRF	=	corticotrophin-releasing factor
B	=	German Brown (as used in identifying the heifers)
H	=	Holstein-Friesian (as used in identifying the heifers)
F	=	White Fulani (as used in identifying the heifers)

Steroid Nomenclature

Trivial names	systematic name
cortisol, hydrocortisone	11 α , 17 β , 21-trihydroxy-pregn-4-ene 3, 20-dione
corticosterone	11 α , 21-dihydroxy-pren-4-ene 3, 20-dione
Progesterone	Pregn-4-ene 3, 20-dione

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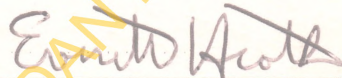
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Certification By Supervisor

This is to certify that the work recorded in this thesis was wholly carried out by Dr. O. Adeyemo under the supervision of Dr. Everett Heath.



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Table of Contents

	<u>Page</u>
Title page	i
Dedication	ii
Abstract	iii
List of Abbreviations	vi
Acknowledgements	ix
Certification by Supervisor	xi
Table of Contents	xii
List of Figures	xvi
List of Tables	xviii
 INTRODUCTION	 1
LITERATURE REVIEW	
Adrenocortical Physiology	8
Plasma cortisol	8
Control of secretion of cortisol	10
Effect of stress	12
Effect of exogenous ACTH	16
Role of cortisol in reproduction	17
Methods of assaying plasma cortisol	20
 Estrous Cycles	
Length of estrous cycle	24
Duration of estrus	25

	Page
Time of onset of estrus	26
Estrual behaviour and ovulation	27
Effect of high environmental temperature on estrous cycle	29
Relationship of photoperiod and estrous cycles ..	32
Relationship of nutrition and reproduction	33
Physiology of Body Temperatures	34
- Body temperature measurement	34
- Temperature regulation	35
- Heat tolerance	43
- Influence of shade on thermoregulation	48
- Climatic heat stress factors	48
 MATERIALS AND METHOD	
Experimental animals	53
Climate of Ibadan	54
Experimental design	58
Radioimmunoassay of bovine plasma cortisol	74
- Introduction	74
- Glassware and solutions	74
- Preparation of samples	78
- The assay	78
- Calculations	80

RESULTS AND DISCUSSIONS

I	Physiological responses					
	Results:					
	Respiratory rates					81
	Rectal temperature					82
	Shade seeking					83
	Discussion:					85
II	Estrous cycles in heifers					
	Results:					
	Pubertal age					100
	Estrual signs					100
	Time of onset of estrus					104
	Duration of estrus					105
	Ovulation					107
	Rhythmicity of estrous cycles					107
	Discussion:					110
III	Plasma cortisol concentration during estrous cycles and different quarters of the year.					
	Results:					122
	Discussion:					124
IV	Diurnal and circadian changes in physiological responses and plasma cortisol concentration					
	Respiratory rate and rectal temperatures ..					133
	Responses in heifers in the sun					133

	Page
Physiological responses in heifers under shade	135
Circadian rectal temperature and respiratory rate in the shade	137
Plasma cortisol	137
Discussion:	140
V Effect of exogenous adrenocorticotrophin on adrenocortical functions and estrous cycle	
Results	149
Discussion:	151
SUMMARY AND CONCLUSIONS	156
REFERENCES	214
APPENDIX	248
Test of reliability of radioimmunoassay of cortisol	248

(See also List of Appendix Tables)

<u>List of Figures</u>			<u>Page</u>
Figure 1:	A German-Brown heifer		186
Figure 2:	A Holstein-Friesian heifer		187
Figure 3:	A White Fulani heifer		188
Figure 4:	Percentage monthly weight changes: composite of four heifers/breed		189
Figure 5:	Weather conditions of the University of Ibadan Campus from December 1975 to December 1976		190
Figure 6:	Mean diurnal environmental temperature and humidity during periods when physiological studies on heifers were conducted ..		191
Figure 7:	Mean respiratory rates and rectal temperatures in heifers		192
Figure 8:	Rectal temperature: Composite of 5 - 7 cycles/breed		193
Figure 9:	Shade seeking habit of heifers ..		194
Figure 10:	Panting Holstein heifer		195
Figure 11:	Percent distribution of time of onset of estrus		196
Figure 12:	Percent distribution of duration of estrus		197
Figure 13:	Percent distribution of estrous cycle length		198
Figure 14:	Plasma cortisol concentration during estrous cycles: composite of 4 heifers/ breed (December - January)		199
Figure 15:	Plasma cortisol concentration during estrous cycles: composite of 4 heifers/breed (March - April)		200

	Page
Figure 16: Plasma cortisol concentration during estrous cycles: composite of 4 heifers/breed (July - August).	201
Figure 17: Plasma cortisol concentration during estrous cycles: composite of 4 heifers/breed (November)
Figure 18: Mean plasma cortisol concentration in heifers during four periods of the year.	203
Figure 19: Diurnal changes in rectal temperatures and respiratory rates in heifers (Unshaded)	204
Figure 20: Diurnal changes in rectal temperatures and respiratory rates in heifers (under shade)	205
Figure 21: Circadian changes in rectal temperature and respiration rates in heifers (under shade)	206
Figure 22: Diurnal plasma cortisol concentration in heifers	207
Figure 23: Diurnal plasma cortisol concentration in heifers	208
Figure 24: Circadian plasma cortisol concentration: composite of three heifers/breed.	209
Figure 25: Plasma cortisol concentration after saline and ACTH injections	210
Figure 26: Mean plasma cortisol concentration after saline and ACTH injections: composite of six heifers	211
Figure 27: Standing-for-mounting estrual behaviour	212
Figure 28: Estrual sign (tail raising)	213

List of Tables

In Text:	Page
Table I: Mean respiratory rates in heifers during different periods of the year.	165
Table II: Mean percent change in respiratory rate at 15.00 from values at 07.00 hours.	166
Table III: Mean morning (07.00 hour) and afternoon (07.00 hour) rectal temperatures, along with the standard deviations, in heifers during four quarters of the year.	167
Table IV: Mean age of commencement of estrous cycles in Brown, Holstein and Fulani heifers.	168
Table V: Percentage distribution of time of onset of estrus in heifers	169
Table VI: Duration of estrus in heifers through one calendar year.	170
Table VII: Breed comparisons of the duration of estrus in heifers.	171
Table VIII: Comparison of the duration of estrus in heifers during the wet and dry seasons ..	171
Table IX: Ovulation interval (in hours)...	172
Table X: Comparison of ovulation interval after the end of estrus in heifers.	172
Table XI: Ovulation interval after the end of estrus in Brown, Holstein, and Fulani heifers: observations in the wet and dry seasons compared.	173
Table XII: The character of the frequency of estrous cycle lengths in heifers.	174
Table XIII: Estrous cycle lengths in heifers through the wet and dry seasons.	175
Table XIV: Comparison of estrous cycle length in the wet and dry seasons	176

	Page
Table XV: Comparison of the estrous cycle lengths in the Brown, Holstein and White Fulani heifers	177
Table XVI: Mean plasma cortisol concentration in heifers during different quarters of the year	178
Table XVII: Mean rectal temperature and respiratory rates in heifers kept outdoors in the sun throughout the day time.	179
Table XVIII: Diurnal rectal temperatures and respiratory rates of heifers in the shade	180
Table XIX: Circadian rectal temperature and respiratory rates in heifers in the shade	181
Table XX: Diurnal plasma cortisol concentration in heifers (Experiment 1)	182
Table XXI: Plasma cortisol concentration in heifers separated from the usual group (Experiment 2)	183
Table XXII: Mean, with the standard error, diurnal plasma cortisol concentration in heifers (Experiment 3)	184
Table XXIII: Diurnal plasma cortisol, with the standard error, in heifers of the Brown, Holstein and Fulani breeds of cattle (Experiment 4)	185

In Appendix

Table A 1: Assay of cortisol in increasing volumes of plasma	251
Table A 2: Recovery of cortisol in water in a single assay	252
Table A 3: Recovery of cortisol in aqueous solutions in 20 duplicate assays	253

	Page
Table A 4: Duplication of samples in the same assay ..	254
Table A 5: Duplication in two different assays ..	255
Table A 6: Test of accuracy of assay	256
Table A 7: World Health Organisation (W.H.O.) quality control of radioimmunoassay	257
Table A 8: Standard curve of cortisol radiomunoassay	258
Table A 9: Summary of monthly weather observations for the University of Ibadan Campus from December 1975 through December 1976	259
Table A 10: Mean haemoglobin concentration and packed cell volume during cool wet and dry hot seasons	260
Table A 11: PCV (%) and Hb (g/100ml): Cool wet and dry hot seasons compared	261
Table A 12: Mean respiratory rates (breaths/minute) of individual Brown, Holstein and Fulani heifers at 07.00 and 15.00 hours during 42 consecutive days at different quarters of the year	262
Table A 13: Analysis of variance of respiratory rates in heifers of three breeds of cattle during four periods of the year.	263
Table A 14: Contribution to total variation that occurred in the respiratory rates	263
Table A 15: Mean rectal temperature ($^{\circ}$ F) of individual Brown, Holstein and Fulani heifers at 07.00 and 15.00 hours during 42 consecutive days at different periods of the year	264
Table A 16: Analysis of variance of rectal temperatures in heifers of three breeds of cattle during four different periods of the year. ..	265

	Page
Table A 17: Contribution to total variations that occurred in rectal temperatures	265
Table A 18: Analysis of variance of shade seeking scores in heifers of Brown and Holstein cattle grazed in the sun.	266
Table A 19: Mean percentage scores of shade seeking in the heifers during different periods of the year; observations were taken on 10 randomly chosen days of each quarter.	266
Table A 20: Average scores of estrous intensity in heifers.	267
Table A 21: Distribution of the time of commencement of estrus through the 24-hour day in heifers: observations were made over one calendar year.	268
Table A 22: Analysis of variance of time of onset of estrus in heifers through the year.	269
Table A 23: Duration of estrus in Brown Holstein and Fulani heifers through the year.	270
Table A 24: Interval of ovulation from end and onset of estrus in Brown, Holstein and Fulani heifers	271
Table A 25: Mean plasma cortisol concentration in individual heifers (observations made on alternate days).	272
Table A 26: Mean plasma cortisol concentration in individual heifers.	273
Table A 27: Analysis of variance of plasma cortisol levels in heifers: levels at diestrus, estrus and the mean during four quarters were compared.	274
Table A 28a: Analysis of variance of plasma cortisol concentration in Brown, Holstein and Fulani heifers through four different quarters of the year.	275
Table A 28b: Contribution to total variation	275

	Page
Table A 29: Rectal temperature and respiratory responses of heifers in the sun.	276
Table A 30: Analysis of variance of respiratory rates of heifers in the sun.	277
Table A 31: Analysis of variance of rectal temperature of heifers in the sun.	278
Table A 32: Correlations between rectal temperature, respiratory rate and environmental temperature in the sun	279
Table A 33: Analysis of variance of readings of circadian rectal temperature of heifers in the shade.	280
Table A 34: Analysis of variance of respiratory rates of heifers kept in the shade and in the sun.	281
Table A 35: Analysis of variance of values of rectal temperatures in heifers in the shade and in the sun.	282
Table A 36: Plasma cortisol concentration and rectal temperatures of heifers kept in the sun throughout the day (Experiment 1). ..	283
Table A 37: Diurnal plasma cortisol concentration in heifers (Experiment 3)	284
Table A 38: Analysis of variance of diurnal plasma cortisol concentration in heifer (Experiment 1). ..	285
Table A 39: Correlation between plasma cortisol and rectal temperatures of heifers kept in the sun (Experiment 1)	286
Table A 40: Analysis of variance of diurnal plasma cortisol concentration in heifers (Experiment 3).	287
Table A 41: Circadian plasma cortisol concentration in heifers (Experiment 4)	288

	Page
Table A 42: Analysis of variance of circadian plasma cortisol in heifers (Experiment 4) ..	289
Table A 43: Mean rectal temperatures of non-lactating pregnant cows and non-lactating non-pregnant nulliparous heifers in the sun, unshaded and shaded.	290
Table A 44: Plasma cortisol concentration at different time intervals in heifers injected with saline and ACTH (200 IU).	291
Table A 45: Analysis of variance of plasma cortisol concentration in heifers injected with saline and ACTH.	292
Table A 46: Analysis of variance of plasma cortisol values in heifers injected with ACTH.	293
Table A 47: Analysis of variance of plasma cortisol values in heifers injected with saline	294

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INTRODUCTION

The shortage of animal protein is a nutritional problem among the human population in most developing tropical countries including Nigeria. Development plans call for increased production of milk as well as beef. About thirty percent of the beef supply to Nigerian population has been from neighbouring countries of Chad and Niger as live animals, and recently from more distant countries in South America, Europe and Australia as frozen meat (David-West 1977). The supply of milk is solely through importations from Europe and America. The national cattle herd of Nigeria (about 8.5 million) is largely in the hands of nomadic landless traditional grazers. The level of productivity of these animals with respect to beef is far below market demand, and with respect to milk, is so low as to be unnoticed by the population.

To improve upon the quantity and quality of locally supplied beef, ranches stocked with African beef breeds of cattle have been established at Mokwa, Upper Ogun, Ado-Ekiti, Akoko, Obudu, Bauchi and a few other locations in Nigeria. Nomadic herdsmen have also been encouraged to settle in different states.

The increasing demand for high quality animal products has also underlined the urgent need for providing animals of enhanced efficiency in Nigeria (Oyenuga and Olubajo 1966). As in many hot countries of the world, improvement has been sought by the introduction

of animals from the temperate zones for the establishment of dairy units and for upgrading the local breeds through cross breeding. Other objectives have been to increase the earning capacity of farmers and promote national self-sufficiency in food supply. There now exist small dairy units stocked with exotic breeds and, in some cases, the crosses with zebu breeds at Agege, Ibadan, Vom, Obudu and other government farms.

Generally the performance of European cattle introduced to the tropics and sub-tropics have been poor. This has been blamed on both direct and indirect climatic factors. The indirect climatic factors include irregular feed supply, inadequate water supply and disease (Bonsma 1949; Johnson 1975). The combination of the direct factors such as environmental temperature, solar radiation, relative humidity, precipitation and wind movement is believed to affect the body heat balance of cattle (Lee and Phillip 1948; Bonsma and Louw 1963; Ojo 1971). Responses are therefore stimulated in the animal and these include changes in reproduction and in endocrine and physiological functions (Gaalaas 1945; Rhoad 1936; Stott and Thomas 1972; Thatcher 1974).

In response to exposure to hot environment, cattle tend to increase body heat loss by their respiratory and sweating activities. Marked elevation of body temperature and respiratory rate are considered indicative of heat stress in cattle (Rhoad 1940;

Findlay 1954; McDowell et al 1955; Wolff and Monty, Jr. 1974).

Rectal temperature changes form the basis of heat tolerance tests and have been used to determine adaptability of different breeds of cattle to hot environment (Berman 1968; Bianca 1962). Cattle also exhibit behavioural responses to exposure to heat among which are panting and shade seeking (Hafez 1964). It is, therefore, desirable to determine the adaptability of both exotic and local breeds of cattle in a tropical environment by observing their physiological and behavioural responses under different weather conditions.

The maintenance of reproductive capacity is an essential aspect of cattle production. Bonsma and Louw (1963) stressed the need to study the physiology of reproduction of exotic breeds of cattle in a tropical region "because of the great economic importance of fertility, adversely affected by hot environment in determining the turnover of any animal production". Many literature reviews have also stressed the deliterious effect of tropical and sub-tropical environment on reproductive performance of European breeds of cattle (Ulberg 1958; Bianca 1965; Vincent 1972; Thompson 1973; Moberg 1976; Waites 1976).

Seasonal depression in bovine fertility in a subtropical region has been attributed to heat stress affecting the cow (Stott 1961). Heat is, however, also known to depress reproductive

efficiency of bulls (Johnston et al 1963). The two periods of the bovine reproductive cycle vulnerable to heat stress are the periods of estrus to ovulation and of implantation and early embryogenesis (Plasse et al 1970; Stott 1972; Moberg 1976; Stott and Williams 1962). Various reports have shown that heat stress can alter the estrous cycle of *Bos taurus* (Bond & McDowell 1972; Gangwar et al 1965; Hall et al 1959; Labhsetwar et al 1963; Stott and Williams 1962). Despite the relatively more uniform daylight and ambient temperatures throughout the year in areas near the equator, zebu and some other African types of cattle have shown seasonal pattern in breeding (Wilson 1946; Steinbach & Balogun 1971; Jochle 1971).

The reproductive physiology of temperate-evolved cattle introduced into tropical Nigeria has not been well studied and much of the knowledge about them is based on observations made in other parts of the world. Detailed study of the reproductive cycles of the local zebu breeds of cattle is also still lacking; and as Jochle (1972) pointed out, most literature reviews overlook this aspect in zebu cattle. A study of the estrous cycles of both local and exotic breeds of cattle may elucidate how their breeding is influenced by environmental conditions of the hot climate.

Adrenocortical function is increased in cattle under acute thermal stress (Stott & Robinson 1970; Christison & Johnson 1972; Alvarez & Johnson 1973). It however decreases under prolonged heat exposure (Christison & Johnson 1972, Rhynes & Ewing 1973). Severe

seasonal heat exposure is also known to depress cortisol secretion in cattle (Stott and Wiersma 1971). Abilay, Johnson and Madan (1975) suggested this latter change to be a thermoregulatory mechanism to reduce body heat load, since hydrocortisone is thermogenic (Yousef & Johnson 1967). Increased circulating cortisol levels would be undesirable in a hot environment not only because it can be associated with increased metabolic heat, but also because of the catabolic effects of glucocorticoids by causing increased mobilisation of tissue amino-acids for gluconeogenesis and increased blood glucose (Ray 1968; Burke 1973). Most of the studies on adrenocortical functions in cattle have been done in the climatic laboratory with the result that little is known regarding the influences of natural weather.

In 1971, Ojo reported a study of heat balance in Jersey and Shortorn using energy budget on the skin surface at different locations in Nigeria at different periods of the year. He concluded that the area around Ibadan is the next most suitable place after the Jos Plateau area in Nigeria for the establishment of temperate-evolved cattle.

Amakiri (1974), in a study of the skin of both exotic and local breeds of cattle in the Ibadan area, found that the Friesian Cattle had developed sweat gland characteristics suitable to a hot environment. Informations on the physiological and hormonal responses of these animals were, however, lacking.

Generally, equatorial zone (sea level) is believed to present severe limitations to the performance of temperate-evolved cattle (Johnson 1975). Rollinson (1962) has summarised the general agreement that temperate-evolved cattle are most suited to geographical areas with temperatures ranging between 30 and 65°F. High altitudes in the equatorial zone and places with temperatures ranging between 70 and 75°F might be tolerable. Zebu cattle are most suited to temperature ranges of 50°F to 80°F. By these standards, Ibadan, the area of the present study, is suitable for zebu cattle but not for temperate-evolved cattle. How the latter type of cattle, translated to the tropics, adapts to a potentially unsuitable climate would be of economic and scientific interest.

The objectives of the present study were:

- (1) to characterise estrous cycles in temperate-evolved and zebu heifers with regard to the length of the cycles, time of onset of estrus, duration of estrus and ovulation time, in a hot/humid subequatorial climate,
- (2) to investigate the relationship of adrenocortical functions and related physiological functions (respiratory rate and rectal temperature) with the phases of estrous cycles at different quarters of the year,
- (3) to investigate the variations in adrenocortical functions and the physiological responses during the diurnal and/or circadian period in the shaded and unshaded environments,

and (4) to examine adrenocortical responsiveness to exogenous adrenocorticotrophic hormone (ACTH), as well as the effect of the latter on normal estrous cycles.

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LITERATURE REVIEWAdrenocortical physiologyPlasma cortisol

Cortisol and corticosterone have been identified as the principal corticosteroids in bovine plasma (Estergreen and Venkateshu 1967). Of these two glucocorticoids, cortisol is both qualitatively and quantitatively the more important (Estergreen and Venkateshu 1967; Rhynes and Ewing 1973; Venkateshu and Estergreen 1970). The ratio of cortisol to corticosterone in adrenal effluent is 5:1 (Whipp et al 1967). In jugular blood, the ratio has been found to be 2.4:1 and 24:1 by Venkateshu and Estergreen (1970) and Swanson et al (1972) respectively.

Other important adrenal cortical hormones, aldosterone (mineralocorticoid) and androgens, have been identified in cattle as well as limited quantities of other steroids with sex hormone activity whose roles are not fully known (Gorski et al. 1958, Melampy et al. 1954; McDonald 1969; Turner and Bagnara 1971).

The structure, biosynthesis and metabolism of adrenal corticoids have been well studied (Gray et al. 1961; Loraine and Bell 1966; Turner and Bagnara 1971). Cortisol, like other adrenal

corticoids, is synthesised in the cortex of the gland from cholesterol; Δ -5 pregnenolone and progesterone are important in the series of intermediaries.

Ninety-nine percent of circulatory cortisol is metabolised in the liver and less than one percent appears in urine unchanged (Gray et. al 1961). The carbonyl group at position 3 is utilised in conjugation to glucuronic acid, or possibly sulphuric acid. The renal clearance of reduced and conjugated metabolites is so rapid that the unmetabolised hormone constitutes the preponderant part of plasma corticoids (Migeon et. al 1956).

Eighty percent of circulatory cortisol is bound in plasma to the cortisol binding globulin (CBG), transcortin; and the remainder is loosely bound to albumin (Sandberg and Slaunwhite, Jr. 1959). The level of plasma cortisol is dependent on the binding capacity of transcortin in a given species (Linder 1964). High levels occur in man and rat; and low levels occur in sheep and cattle. The binding affinity of transcortin is reduced at increasing environmental temperatures (4-37°C) (Linder 1964).

The amount of cortisol bound in blood of man is increased by estrogen (Sandberg and Slaunwhite Jr. 1959). Estrogen, however, has no such effect in the ewe. There is evidence to indicate that is the unbound (free) fraction of cortisol which is physiologically active (Sandberg and Slaunwhite 1959 ; Slaunwhite, Jr. et. al 1962). An increase in the circulating level of cortisol therefore

does not necessarily imply an increased availability of biologically active hormone. For example, in man, the elevated levels during pregnancy is due to an increase in the concentration of plasma transcortin and the bound fraction of cortisol whereas the amount of free cortisol is not markedly changed (Booth et al. 1961).

Control of secretion of cortisol

The rate of biosynthesis and secretion of adrenal steroids is controlled by adrenocorticotrophic hormones (ACTH), an anterior pituitary hormone (Fevold 1967; Salmenpera and Kahri 1976). The release of ACTH is also under the control of corticotrophin-releasing factor (CRF), a neural hormone from nuclei in the median eminence of the hypothalamus (Hiroshige et al. 1969). CRF is passed via the hypothalamic-hypophyseal portal system. The secretion of CRF, in turn, is affected by neural impulses from higher centres. It is believed that impulses from the reticular formation inhibit CRF while impulses from other areas resulting from trauma, fever or noise, for example, decrease reticular inhibition (James and Landon 1976).

Rising levels of cortisol suppress the production of ACTH in the adenohypophysis and CRF production in the median eminence of the hypothalamus (Brodish and Lang 1962; Chowers et al. 1967). This is referred to as the "long feedback loop." ACTH also has a direct inhibitory effect on the production of CRF in the median eminence, constituting the "short feedback loop."

Because of these interrelationships, rhythmical changes in the release of CRF have been associated with corresponding variations in the release of adrenocorticoids. For example, synchronous peaks of ACTH and corticosteroids have been demonstrated in man (Krieger et al. 1971). There is also evidence that the daily rhythm of CRF is associated with variations in plasma corticosteroid concentration in rats (Takebe et al. 1970, Seiden and Brodish 1972). Circadian rhythm in the plasma levels of cortisol has been described in man, with peaks occurring before the period of awakening and decreasing gradually subsequently through the 24-hour period (Perkoff et al. 1959). A similar rhythm has been described in rats, with the peak occurring in the evening when these animals become active (Guillemin et al. 1959). Initial animal and human studies delineating corticosteroid circadian periodicity were based on a sampling frequency of every 4 - 6 hours over 24-hour period with resultant curves describing a smooth rise and fall over this time period (Migeon et al. 1956). Krieger et al. (1969) and Hellman et al. (1970) demonstrated episodic peaks in plasma corticosteroids (and ACTH) throughout the day by sampling man more frequently at every 30 and 20 minutes respectively.

Evidence for circadian rhythmicity in circulatory plasma corticosteroid in cattle was first given by MacAdam and Eberhart (1972) and Wagner and Oxenreider (1972) but was contradicted in other reports (Paape et al. 1973; Hudson et al. 1975).

Effect of stress

Studies in psychrometric chambers have shown that acute thermal exposure of cattle caused increases in their adrenocortical functions. Under this condition, levels of plasma cortisol (or glucocorticoid) rose (Stott and Robinson 1970; Christison and Johnson 1972; Alvarez and Johnson 1973; Abilay, Mitra and Johnson 1975). The turnover rate of plasma glucocorticoid or cortisol in particular also rose (Stott and Robinson 1970; Christison and Johnson 1972). Such elevations in plasma cortisol level have been found to be shortlived, lasting only 1 hour and declining to original basal levels in 4 - 5 hours (Alvarez and Johnson 1973). Impulses set up at the thermoreceptors in the skin by acute thermal stimuli are capable of stimulating increased ACTH release and a corresponding adrenocortical secretion (Chowers et al. 1966).

Adrenocortical functions in cattle under chronic thermal stress is dissimilar to that under acute thermal stress. Plasma cortisol concentration has been found to be reduced in cows exposed chronically to a warm environment of 29°C for 9 consecutive weeks (Bergman and Johnson 1963). In addition to the reduction in plasma levels, the turnover rate of cortisol was found to be reduced in cattle exposed to 35°C (RH 50%) for 7 - 10 weeks (Christison and Johnson 1972). Similarly, bulls exposed chronically to constant environmental temperature of 35°C had low plasma glucocorticoid

levels (Rhynes and Ewing (1973). Heat stress affects plasma cortisol level more than corticosterone levels, the ratio at 21°C and 35°C being 6:1 and 4:1 respectively (Rhynes and Ewing 1973).

Yousef and Johnson (1967) found that administration of hydrocortisone acetate (1.25g/cow intravenously) caused heat production in cows to increase. The decline in plasma levels of glucocorticoids which occurs during chronic exposure to a hot environment has therefore been suggested to be a regulatory protective mechanism to reduce the animal's metabolic heat production.

The mechanism by which heat causes a reduction in adrenal corticoid secretion in cattle is not known. Yates et al. (1961) suggested that the threshold of the adrenal cortex may shift during prolonged heat exposure, so that even when plasma ACTH level is high, little glucocorticoid is produced. Marple et al. (1972) working on gilts also supported this suggestion. Abilay, Johnson and Madan (1975) proposed that the 17-hydroxylating enzyme responsible for the synthesis of cortisol from progesterone may be inhibited by chronic exposure to heat. This may therefore be responsible for observed increases in adrenal content and circulating level of progesterone in cattle chronically exposed to heat (Wiersma and Stott 1969; Abilay, Johnson and Madan 1975).

As regards the effect of natural weather, the excretion of 17-ketogenic steroids in sheep has been found to be reduced in summer (Robinson and Morris 1960). It has been reported that levels obtained during lactation remain stable and have no association with season or mean environmental temperature (Koprowski and Tucker 1973; Shayanfar et al. 1975). Contrarily, Lee et. al (1976) reported lowered plasma glucocorticoid levels in dairy cattle during hot weather. This supports an earlier observation that summer heat depressed adrenocortical function in cattle (Stott and Wiersma 1971). Adrenocortical function has not been examined in either local or imported temperate-evolved cattle in the tropical Nigerian environment.

Christison and Johnson (1972) suggested that the initial brief increase in adrenocortical response in cattle exposed to heat was a result of stress reaction not specific to heat exposure. Accordingly, the effect of many other stressful conditions have been observed by various workers. Elevated corticoid levels were obtained in "untrained" sheep bled by venipuncture (Linder 1964; Basset and Hinks 1969). Stress to the method of bleeding may be responsible for the large variations obtained in bovine plasma corticoid (Venkateseshu & Estergreen 1970; Garverick et al. 1971; Sprague 1969). Other causes may be the removal of large quantities of blood and the assay method (Venkateseshu and Estergreen 1970; Robertson and Mixner 1956; Paterson 1957). The time interval for collecting samples could also affect the plasma level of

cortisol. Shaw and Nichols (1963) found no elevation of 17-hydroxycorticosteroid in cows sampled at 30 to 60-minute intervals, but sampling at intervals of 10 minutes caused an elevation within 30 to 110 minutes which could be further increased by ACTH administration.

Animals, however, can get used to handling and blood sampling as was demonstrated in trained, untrained and even herded sheep (Bassett and Hinks 1969; Linder 1964; McNarthy and Thurley 1973). It has also been possible to obtain physiological levels of plasma glucocorticoid in trained heifers (Gimenez et al. 1974). Similarly to the report on sheep quoted above, Rhymes and Ewing (1973) observed that steers were apprehensive on first handling and confinement and their plasma cortisol level was high; the level however dropped by about 40% when (after 5 weeks) the heifers got used to the procedure and acclimatized.

"Training" or adaptation by the animal to handling or experimental procedure is suggested to be controlled by the central nervous system (McNarthy and Thurley 1973). Studies in rat also show that adaptation to stress is associated with normalising of the activity of the tropic cells of adenohypophysis which had been hyperactive initially (Pollar et al 1976).

Effect of exogenous ACTH

Stress-like response of the adrenal cortex has been elicited on administration of exogenous ACTH to cattle with pronounced secretions of adrenal glucocorticoids (Shaw and Nichols 1963; Venkateseshu and Estergreen 1970; Gwazdauskas et al 1972; Paape et al. 1973; Paape et al. 1974; Shayanfar et al. 1975; Satterlee et al. 1977). Plasma corticosterone values did not change significantly after ACTH administration compared to the changes in plasma cortisol concentration (Venkateseshu and Estergreen 1970).

Peak corticosteroid response occurred 60 minutes post-ACTH treatment (Venkateseshu and Estergreen 1970; Wagner et al 1972). These reports also show that plasma corticosteroid levels returned to normal within 24 hours post-ACTH treatment. Accumulation of adrenocortical responses to method of blood collection and ACTH administration has been demonstrated in calves (Shaw and Nichols 1963).

A group of workers found no differences in either magnitude or duration of plasma corticosteroid responses of lactating cows to ACTH (200IU) among controlled temperature periods of 21°C, 65% RH and fluctuating 12-hour cycles of 32°C, 65% RH during the day and 21°C, 65% RH at night (Paape et al. 1973). In another study in the climatic laboratory in which more prolonged acclimatisation periods than in the former report were allowed, heifers under cold (5°C, 30% RH), thermoneutral (18°C, 50% RH) and hot (35°C, 80% RH) conditions showed similar plasma glucocorticoid responses to ACTH treatment (Satterlee et al. 1977).

These results did not agree with the report of Shayanfar et al (1975) in the field that lower adrenal responsiveness to ACTH may exist in lactating cows exposed to high environmental temperatures ranging from 21.1 to 26.9°C than in cows exposed to low environmental temperatures (6.4 to 21°C). It is not known what the adrenocortical responsiveness to exogenous ACTH in heifers raised under natural hot/humid climatic condition would be like.

Role of cortisol in reproduction

The direct role of glucocorticoids in reproductive activity and with particular regard to different stages of the estrous cycle of cattle, has not been fully established. Plasma levels of cortisol fluctuate throughout the menstrual cycle of women (Abraham et al 1972). Many reports also show that plasma levels of glucocorticoid vary during estrous cycle of cattle with no significant differences between the days (Sprague 1969; Randel et al. 1971; Garverick et al., 1971). Other reports have shown that despite the fluctuations, a significant elevation on the day of estrus or around estrus was noticeable (Shaw et al, 1960; Arije et al, 1971; Miller and Alliston 1974; Gimenez et al., 1974). Contrarily, the result of Abilay, Johnson and Madan (1975) showed that levels on the day of estrus were lower than other days of estrous cycles in heifers in the climatic laboratory.

Either ACTH or glucocorticoid administration has delayed onset of estrus in the sow (Liptrap 1970). ACTH administration has also delayed onset of estrus in mice (Christian 1964). Administration of glucocorticoid or stress-induced increases in the hormone had increased embryonic mortality in rats, rabbits and sheep (Euiker and Riegle 1973; Kendall and Liggins 1972; Howarth and Hawk 1968). Hormone of adrenal-pituitary axis can, therefore, affect those aspects of reproduction which are vulnerable to stress.

In rats, administration of synthetic glucocorticoid, dexamethasone, has altered estrous cycle length, blocked ovulatory surge of luteinising hormone (LH), apparently by inhibiting the synthesis and release of pituitary LH (Baldwin and Sawyer 1974). A similar treatment has also inhibited pregnant mare serum-induced ovulation in rat (Hagino et al. 1969). It has therefore been suggested that stress prior to ovulation in r may be capable of blocking ovulatory release of LH and thereby change the natural temporal pattern of estrual behaviour and the time of ovulation, which can cause a wrong timing of breeding in cattle and, therefore, adversely affect conception rate (Moberg 1976).

Administration of dexamethasone to cows at the beginning of estrous cycles during summer in Arizona did not improve fertility. It was therefore, suggested that low circulating plasma cortisol level under hot conditions may not be a cause of infertility (Monty Jr. and Wolff 1974).

Only very few attempts have been made to investigate the influence of environmental conditions on the association between plasma cortisol and different stages of estrous cycles. In the psychrometric chamber, Abilay, Johnson and Madan (1975) investigated the effect of constant high temperature (35.5°C) while Miller and Alliston (1974) utilised programmed circadian environmental temperatures varying between 21°C by night and 34°C by day. Thermal treatment caused a depression in the levels of circulating cortisol at all stages of the estrous cycle except on the day of estrus, that is, the first day of exposure to heat in the former study, or except during the first week of exposure including the day of estrus in the latter study. Reports are still lacking on the influence of changing seasons on the association between plasma cortisol and the different stages of estrous cycles in cattle in the field.

In sheep, parturition is associated with increased fetal adrenal secretion of corticoids near the end of gestation (Liggins 1968; Basset and Thorburn 1969). Elevated plasma glucocorticoid 1-4 days prepartum has been demonstrated in cows (Adams and Wagner 1970). The elevation might occur 0-15 days prepartum (Heitzman et al. 1970). Parturition has been induced in cattle and sheep by administration of synthetic corticoid (Adams and Wagner 1970; Liggins 1969; Lauderdale 1972). It has been suggested that the abrupt rise in plasma glucocorticoid near term may cause a decline in corpus luteal (CL) function and thus initiate parturition (Adams and Wagner, 1970). This suggestion

is supported by the report of Brunner et. al (1969) that exogenous ACTH administered on days 2 through 8 of estrous cycle in cycling heifers suppressed corpus luteal development during the period of treatment. The effect of this treatment is most probably due to elevated endogenous plasma glucocorticoid.

Adrenocortical suppression with betamethasone has resulted in prolonged estrous cycle length (Kanchev et. al 1976). Although these reports show that the CL of normal estrous cycle of cattle may be influenced by glucocorticoids, exogenous glucocorticoid administered to heifers from day 10 of estrous cycle could not produce luteolysis (Gimenez et. al 1974). These last quoted workers therefore suggested that the luteolytic effect of glucocorticoid at the end of pregnancy is probably aided by the presence of some other factors present at that time in blood but absent during normal estrous cycles. It is yet to be investigated whether increasing the levels of endogenous glucocorticoid by administering ACTH from midcycle stage, and later, can initiate the luteolytic process.

Methods of assaying plasma cortisol

Various methods have been used to quantitate plasma levels of corticoids (Loraine and Bell 1966; Sandberg and Slaunwhite Jr. 1975).

Assay techniques based on biological activities are unsatisfactory from the quantitative standpoint, and have given little information regarding the compound being measured (Vogt. 1943; Paschkis et. al 1950). Chemical methods of colorimetry lack specificity (Nelson and Samuels 1952; Robertson and Mixner 1956). The physico-chemical methods including double-isotope labelling, though specific, are laborious and require initial purification of extracts (Frazer and James 1968). Also, the fluorometric technique developed by Mattingly (1962), though requiring smaller plasma volume and faster than colorimetric procedures suffers the disadvantage of interference by other fluorescing contaminants, and, therefore, requires scrupulous cleaning of glassware (Sandberg and Slaunwhite, Jr., 1975).

In the nineteen sixties assay of cortisol which depends upon the principle of displacement analysis involving the use of very small volumes of plasma were developed. These have the advantages of rapidity, simplicity and specificity. Large numbers of samples can also be treated at the same time. These methods have employed iodine 125- or tritium-labelled cortisol as a binding agent. The competitive protein binding (CPB) method involves the use of transcortin as the

binding agent- (Murphy 1967). The binding agent in radioimmunoassay (RIA) is an antibody raised against cortisol (Abraham et. al 1972; Farmer and Pierce 1974). Because of the specificity of the antibody, the problem of interference due to other hormones or other compounds in plasma is considerably reduced, thus improving the sensitivity of the assay when RIA is employed (Abraham et. al 1972).

The technical details of RIA and the preparation of antibodies have been well studied (Abraham 1974; Ekins 1974; Nieschlag and Wickings 1975). The principle may be described as competition between labelled and unlabelled antigen (hormone) for binding sites on the common antibody resulting in the formation of labelled antigen-antibody and unlabelled antigen-antibody complexes. After the separation of the bound and free fractions, the concentration in the unknown (sample) is obtained by a comparison of its inhibition of binding of labelled antigen to antibody with the inhibition produced by a set of known standards introduced into the system.

Various levels of basal plasma cortisol in cattle have been reported, depending on the method used. Saba (1964) using a method of soda fluorescence on paper chromatography obtained a cortisol concentration of 0.5 ug/100 ml in bovine plasma. Some other workers using the

chemical method to determine 17-hydroxycorticosteroids in bovine plasma obtained a range of 1.2 to 11.9 ug/100 ml (Shaw et. al 1960; Robertson and Mixner 1956; Shaw and Nichols 1963). Using the competitive protein binding (CPB) method basal levels of plasma cortisol ranging between 1-10 ng/ml had been obtained in heifers and cows (Whipp and Lyon 1970; Swanson et. al 1972; Gimenez et. al 1974; Shayanfar et. al 1975; Satterlee 1977; Garverick, 1971). Lower levels occur in cows than in calves (Whipp and Lyon 1970; Saba 1964). Using the RIA, Dobson and Kanchev (1977) found a level of 3.5-10 ng/ml in the plasma of heifers.

From evidences showing a normal relative high proportion of cortisol to the other glucocorticoids in bovine plasma including after ACTH or during acute thermal treatment, it is obvious that results from assaying cortisol alone in bovine blood would produce the same information about adrenocortical function as assaying total glucocorticoid. This observation was also made by Wagner and Oxenreider (1972), Venkateseshu and Estergreen (1970) and Swanson et. al (1972).

Estrous Cycle

The average age of puberty in *Bos taurus* has been found to be between 6 - 15 months (Hammond, 1927). Studies in India indicated that pubertal age of the *Bos indicus* population was between 33-47 months (Amble et. al. 1958; Johari and Talapatra, 1957). Jochle (1971) observed that 28.4% of purebred Brahmans (American zebu) in Mexico had first conceptions between the ages of 15 and 24 months while 44.6% conceived between 25 and 30 months. The average age of puberty in Brahman heifers in a subtropical climate was found to be 19.4 months with a range of 14 to 24 months (Plasse, Warnick and Koger 1968). Reynolds et. al. (1963) found that *Bos indicus* reached pubertal age later than *Bos taurus* and crossbreds.

Length of estrous cycle

In *Bos taurus*, average estrous cycle lengths varying between 20 and 30 days have been recorded by several workers (Champman and Casida, 1937); Olds and Seath, 1951; Trimberger, 1956; Hall et al 1959). The cycle is reported to be slightly longer in the cow than in the heifers, the average in days being 21.28 and 20.23 days respectively (Thibault and Lavesseur, 1975). Rollinson (1962) indicated that 2.2 - 6.8% of estrous cycles could fall below 18 days and about 13% above 24 days. About 16% of the cycles have been found to fall above 18 - 24 days in *Bos taurus* cows (Asdell, et al 1949).

Generally the reports on estrous cycle length in zebu cattle are few and inconsistent. An average estrous cycle length of 22 and 23 days have been reported in *Bos taurus* x *Bos indicus* and purebred *Bos indicus* cattle respectively (Anderson 1944). Rakha et al (1970) recorded average estrous cycle lengths of between 21 - 24 days in zebu and African types of cattle in Central Africa. Their result was similar to some previous reports in this respect (Anderson, 1936; Clamohoy, 1952; Nazareno, 1954). The average in Brahman heifers was found to be 28.8 days, with a standard deviation of 27.7 days, and a significant difference between heifers (Plasse et al 1970). Quinlan and Roux (1936) also reported similarly long estrous cycles in Africander x Sussex cattle.

Duration of estrus

Widely varied values have been reported for the duration of estrus in cattle. A range of 6 - 30 with a mean of 17 hours had been recorded in *Bos taurus*, being longer in the cow than in the heifer with an average of 19.3 and 16.1 hours respectively (Hammond 1927). Values varying between 4 - 48 hours with means from 12.5 to 17 hours have been reported by several other workers (Brewster and Cole, 1941; Asdel, 1946; Hansel and Trimberger, 1952; Hough et al 1955; Trimberger and Hansel 1955; Ivankov, 1956; Gersimova, 1940; Esslemont and Bryant 1976). Wiltbank et. al. (1957) reported a mean length of estrous of 21.1 hours in beef breeds of *Bos taurus*.

Relatively short estrous periods have been recorded in *Bos indicus* by several workers, and have been believed to be the cause of low

reproductive performance in this species. Anderson (1936) reported an average length of 1 hour and 20 minutes for 74 estrous periods in zebu cattle. Again in 1944, Anderson reported an average of 4.8 hours in zebu, and 7.4 hours in *Bos taurus* X Zebu cross, with a standard deviation of 2.2 and 2.4 respectively. The mean duration of estrus in Brahman heifers has been found to be 6.7 hours (Plasse et al 1970). Contrary to these reports, estrus lasting between 13.4 and 24 hours have been observed in Zebu cattle by other workers (Rollinson, 1955; Villacorta 1960). In the Central Africa, Rakha et al, 1970 recorded a mean of 16.3, 17.4 and 14.8 hours with standard deviations of 1.08, 1.18, and 0.53 respectively in Angoni, Barotse and Boran, all African and Zebu types of cattle; their heifers also exhibited longer estrus than the cows (Rakha and Igboeli, 1971). Their report was similar to that of Villacorta (1960). Quinlan et al. 1941 reported estrous duration ranging between 7 and 40 hours in Afrikander cattle in South Africa.

Time of onset of estrus

Estrus can commence at any time of the day. Esslemont and Bryant (1976) reported a distribution of onset of estrus through the 24 hours of the day in *Bos taurus* dairy cows, with the greatest mounting activity occurring between 02.00 and 05.00 hours. Plasse et al (1970) found that 82.9% of estrus recorded in Brahman heifers occurred between 8 a.m. and 8 p.m., with peaks between 4 a.m. and 6 a.m. 10 a.m. and 12 noon, and 6 p.m. and 8 p.m.

In Zebu and African breeds in Central Africa the onset of heat has been found to concentrate around sunset and sunrise hours (Rakha et al. 1970). Various reports tend to show that the night is well favoured by the zebu. Rollinson (1955) in a report from Uganda, stated that 40% of cows came on heat at night; while cows coming on heat between 24.00 and 05.00 constituted 45.3 percent of the herd in another report from Kenya (Anderson 1944).

Estrual behaviour and ovulation

Estrual behaviour has been described by various authors (Hammond 1927; Salisbury and Vandermark 1961; Williamson et al. 1972; Esslemont and Bryant 1976). Commonly described as estrual signs are the swelling and reddening of the vulva, clear mucous vaginal discharge, and raising and switching of the tail. Observable changes in behaviour include restlessness, mounting of other animals and standing to be mounted. Mounting by a cow at estrus is similar to that of the bull (Hafez et al. 1969). The animal at estrus is also anorexic, and might roam over wide areas on the field doing little grazing (Hafez 1969). When estrual signs and behaviour are not intense and the animal does not stand to be mounted, the condition may be subestrus. "Silent ovulation" or "silent heat" is ovulation not accompanied by estrual signs and behaviour. Anovulatory estrus may also occur. Anestrus is the stage when ovaries are quiescent and there are no estrual signs (Roberts 1971).

Some early reports stated that estrual signs and behaviour could not be observed in the zebu (Anderson 1936; Richard 1946). Contrary to these reports estrual signs have been observed in zebu and African breeds though the estrual behaviour was weak (Rakha et al. 1970). It is also believed that the zebu cow accepts the bull after long intervals while on heat (Rollinson 1962).

Ovulation occurs at about 24 to 30 hours after the onset of estrus in the cow (Thibault and Levasseur 1975; Wolff and Monty Jr. 1974). The range of this interval has been found to be about 16 to 42 hours (Swanson and Hafs 1971). Salisbury and Vandermark (1961) summarised reports showing that cows and heifers ovulate at about 12.5 and 11 hours respectively after the end of estrus, with a range of 2 hours before the end of estrus to 20 hours after estrus for cows, and 2.2 to 22 hours after estrus for the heifers. The interval between the end of estrus and ovulation which is an average of 10.5 hours as determined by Trimberger (1948) has, therefore been confirmed in many later studies. In 1974, van der Westhuysen and Venter, summarised reports showing that "even when estrus and ovulation occur regularly in cows, abnormal time relationships may cause infertility".

In addition to visual observation of estrual signs and behaviour, aids such as estrous-detection devices and vasectomised bulls with or without markers have been employed with varying degrees of success to detect estrus (Farris 1954; Donaldson 1968; Wagnon 1972; Williamson et al. 1972). The incidence of missed estrus decreases with increase in the

frequency of heat checks (Hall et al. 1959). Some reports of failure of occurrence of estrus in cattle are due to failure of observation (Lauderdale 1974; Appleyard and Cook 1976). The value of detection of estrus resides in its association with pregnancy; and if estrus is not detected by the observer, the cow does not have an opportunity to be bred, and the calving interval is prolonged, thus decreasing productivity. Clinically, ovulation is determined by periodic rectal palpation of the ovaries to locate the Graafian follicle and the ovulation depression (Trimberger 1948; Erb et al. 1971; Wolff and Monty Jr. 1974).

Effect of high temperature on estrous cycle

Specific aspects of the estrous cycle reported to be affected by heat stress include estrous cycle length, duration of estrus and intensity of estrual behaviour. High temperature in the climatic laboratory has increased estrous cycle length in *Bos taurus* cows and heifers (Bond et al. 1960; Bond and McDowell 1972; Gangwar et al. 1965). It is believed that estrous cycles are shorter in *Bos taurus* when introduced into the tropics (Hafez 1969; Robert 1971). Hot weather in the subtropical climate has also caused a shortening of estrus cycle length in dairy cattle (Branton et al. 1957). Contrary to this, Hall et al. (1959) and Wolff and Monty Jr. (1974) observed that estrous cycle lengths were normal during both cool and hot seasons.

Anestrous incidence has been observed in cattle under heat stress in the climatic laboratory or natural weather conditions (Bond et al.

1960; Gangwar et al. 1965; Bond and McDowell, 1972). **Anestrus** might be temporary and estrus might reappear some weeks after the animal had acclimated to the hot environment (Gangwar et al., 1965; Bond et al. 1960). The latter authors found that 5 out of 6 cold-acclimated heifers exposed to 32°C ceased cycling after 5 weeks of treatment, but by 21 weeks all heifers were cycling. Bond and McDowell (1972) found that summer-acclimated heifers (24.4°C) did not stop cycling when exposed to 32°C 60% RH but became anestrus at 38°C while cold-acclimated animals ceased to cycle at 32°C but reestablished the cycles after 16 weeks.

Heat exposure has caused a reduction in the estrous intensity of *Bos taurus* heifers (Bond et al 1960; Madan and Johnson 1972). Estrus has therefore been difficult to detect in heat stressed cattle (Poston et al. 1962). The occurrence of silent ovulations, that is, ovulations not accompanied by estrual signs, is increased in cattle under hot conditions (Labhsetwar et al. 1963). Silent ovulation is an important cause of observed irregularity of estrus in cattle (Trimberger 1956). It has been suggested that anestrus associated with heat stress may actually be unobserved estrous periods, because of the difficulties associated with detection of estrus during hot weather.

The duration of estrus was found to be shortened in cows and heifers under heat stress (Branton et al. 1957; Gangwar et al. 1965; Bond et al, 1960). Wolff and Monty Jr. (1974) reported that estrous

period was shortened in lactating cows but not in nonlactating cows in the hot season.

Hot conditions have been associated with reduced release of luteinising hormone (LH) corresponding with observed reduction in intensity of and frequency of estrus (Madam and Johnson 1973). In other studies on cows exposed to hot environment, progesterone concentration was found to be elevated in plasma (Wiersma and Stott 1969; Abilay, Johnson and Madan 1975). An increase in adrenocortical but not corpus luteal content of progesterone had also been observed in heat stressed cattle (Stott et al. 1967). Exogenous ACTH administration has caused elevated plasma progesterone concentration confirming, among other things, the adrenocortical source of increased plasma progesterone in heat-stressed cattle (Gwazdauskas et al. 1972; Wagner et al. 1972). Increased plasma concentration of progesterone under thermal stress has been suggested as one of the causes of infertility in cows (Wiersma and Stott 1969). Marshal (1942) stated that seasonal changes in sexual rhythms are less marked in the lower latitudes due to the less pronounced variation in the daylight. Contrarily, Anderson (1948) has associated high temperature and sunlight with increased sexual function in cattle in Kenya at very low latitudes, suggesting seasonality in sexual activity of the African cattle. Brody (1956) also stated that *Bos indicus* and the crosses have higher fertility in spring and summer months in countries near the equator.

The highest conception rates and perhaps sexual activity have occurred in zebu and African types of cattle in South West Nigeria during the hot months of the year from March through June (Steinbach and Balogun 1971). Similarly, seasonal variation in fertility had been observed in zebu under extensive systems of management in other parts of Africa, India and Pakistan as well as in South America (Wilson 1946; Kale 1963, Majeed 1966; Kohli and Suri 1960; Caneiro 1950; Jochle 1972). Because highest fertility was recorded in the hot period of the year, it has been suggested that heat may be triggering off in the *Bos indicus* an endocrine response completely different from that in the *Bos taurus* thus imposing a peculiar evolutionary advantage on the former (Thompson 1973).

Relationship of photoperiod and estrous cycles

An improved ovulation rate in spring in South African and North American cattle has been attributed to increasing day light (Van Rensburg and de Vos 1962; Kidder et al 1952). Spring time has been associated with increased sexual activity in Brahman cattle (Plasse et al 1968). Anderson (1948) also suggested association between increasing daylight and sexual activity. Thibault et al (1966) believed that photoperiod has a regulatory effect on estrous cycles. It has been suggested that the rapid increase in daylight and not the actual day length per se may be stimulatory to sexual activity (Farner, 1961; Steinbach and Balogun 1971).

Relationship of nutrition and reproduction

High levels of nutrition has quickened the onset of puberty in cattle and prevented adverse winter effects on estrus and sexual activity (Joubert 1954; Wiltbank et. al. 1962). Feed restriction has been found to cause a reduction in LH in rats suggesting effects on the pituitary gland (Howland 1972). The deficiency of phosphorus has been suggested as the cause of delayed puberty in Indian zebu cattle on all-roughage feeding (Johari and Talapatra, 1957). Seasonal availability and quality of pasture has been associated with seasonal fertility patterns in zebu cattle (Carneiro, 1952; Jochle 1972). Increases in the plane of nutrition has been found to prevent the occurrence of long estrous cycles and subsequent anestrus in zebu cattle in Central Africa during the dry season when nutritional supply was low (Rakha and Igboeli 1971). When adequate nutrition was supplied the effect of stress on estrous cycle due to the season was sufficiently contained.

The wide-spread belief that adverse dry season or winter may depress fertility may therefore, to some extent, be explained by the nutritional deficiency prevalent during these periods. To avoid confusing the effect of nutrition with that of the ambient conditions, the nutritional level of cattle observed from season to season should not be excessively altered during the period of study.

Fertility is reduced if the normal sequence of events during the estrous cycle (particularly the expression of intense estrus and occurrence of ovulation) is disturbed (van der Westhuysen and Venter 1974). Sufficiently prolonged estrus increases the chance of the cow to be recognised at estrus, and to be bred artificially or by the bull. A knowledge of the time of onset of estrual behaviour is a useful guide for observation of estrus. An understanding of the pattern of sexual cycles and of ovulation through the seasons is essential for planning breeding programmes (Rollinson 1962; Van Rensburg and de Vos 1962).

Physiology of Body Temperature

Body temperature measurement

Various locations on the body surface have been used to estimate the temperature of the body under different environmental and physiological conditions (Bianca 1965; Bianca 1964). The skin temperature has been found to vary in proportion to the rectal temperature. The tympanic membrane temperature, however, is known to be more sensitive than rectal temperature and to change more rapidly when environmental or ruminal temperature is suddenly changed (Guidry and McDowell 1966).

The temperatures of the rectum and vagina have been used to monitor deep body temperature in cattle (Kriss 1921; Wren et al 1958; Bane and Rajakoski 1960). The temperature of the rumen had similarly

been monitored (Dale et al 1954). Some workers measured jugular, pulmonary and carotid blood temperature (Bligh 1957; Ingraham and Whittow 1961). The second group of the last quoted authors also measured the hypothalamic temperature.

Most frequently the means of describing body temperature is the rectal temperature. The advantages of the rectum over other locations of the body for monitoring body temperature, include the ease of approach to the rectum, and the facts that rectal temperature responds to abrupt changes in environmental temperature only after a short delay, and that the changes in rectal temperature parallel the change in deep body temperature (Bligh 1955; Bianca 1965).

Rectal temperature may be measured by ordinary clinical thermometer, a resistant thermometer or a thermocouple. The demerit of a simple mercury rectal thermometer is said to be the possibility of hyperthermy being produced in the animal by excessive muscular activity due to struggling during the time of measurement; whereas by using radio telemetry, the animal can be unrestrained and not conscious of being under observation (Wilson et al 1971).

Temperature regulation

In cattle, the amount of metabolic heat produced increases with increase in body weight, the level of feeding and productivity (Worstell and Brody 1953; Kibler 1960; Thompson 1973). Heat production in lactating dairy Holstein cows is, therefore, higher (30%) than in the heifers. (Johnson 1978). Metabolic heat production increases in

cattle exposed for a short period to severe heat; but it decreases if the animal is exposed to either a milder or a more intense heat for a prolonged period (Johnston et al 1958; Worstell and Brody 1953; Kibler 1960; McLean 1963).

The heat produced by metabolism is carried to the skin surface by the circulatory system. At the skin surface some of the heat is lost by conduction, radiation and convection and the rest is used in moisture evaporation (Thompson 1973).

Thermoreceptors found in the skin of some domestic animals have not been demonstrated in the skin of cattle. Infrared irradiation of the skin has been found to cause a rise in body temperature and respiratory rate in cattle (Findlay and Ingraham 1961). These thermoreceptors are therefore believed to set up impulses under thermal stimulation which reflexly initiate hyperpnea. The presence of heat sensory receptors in the hypothalamus has been reported (Findlay and Ingraham 1961; Hales and Whittow 1966). These are also believed to be present in the spinal cord (Hales and Jessen 1969). Locally heating these places had caused excessive heat loss leading to hypothermia. Sensory nerves synapse with motor nerves in the brain which stimulate the different organs of the body concerned with heat loss. The neurotransmitter here has been suggested to be 5-hydroxytryptamine (Findlay & Robertshaw 1967; Findlay and Thompson 1968).

Reduction in the sympathetic nervous activity causes peripheral vasodilation which allows more heat to flow to the skin. Vasodilation would occur in thermoneutral environment hence it is not an important way of losing heat (Thompson 1973). Some of the body heat is conducted to the surface of the coat. At the coat-air interphase the heat is lost to the environment by physical means, that is, radiation, convection or conduction.

Heat loss by conduction to the environment is not important in cattle in a hot environment. Dairy Holstein cows have, however, been observed to lie in mud and/or wet places in hot sub-tropical weather, showing heat sensitive cattle might desire to lose heat by conduction (Seath & Miller 1947; Romanance-Pounce et al 1977).

Heat loss by convection from the coat surface to the surrounding air is influenced by wind velocity, direction of air movement and shape of the animal's body (Kibler and Brody 1954a; Thompson 1973). The texture of the coat is also important: the short stiff zebu hair allows better air movement than the long curly hair of *Bos taurus* (Dowling 1959). Loss of heat through the skin surface by radiation depends on coat colours: the light or cream coloured coats are known to reflect infrared radiations more than darker coat (Rhoad 1940a; Bonsma and Low 1963; Reimerschmidt 1943).

Cutaneous evaporation is due to moisture normally diffused through the skin and also produced by sweat glands. The population density and volume of bovine sweat glands vary slightly between different breeds

(Nay and Hayman 1963). They have been found to be higher in *Bos indicus* than in *Bos taurus* (Nay 1959; Taneja 1960).

Climatic factors influence sweating activity in cattle.

Increasing environmental temperatures have been found to stimulate sweating in cattle from about 18°C (Kibler and Brody 1950). The depressing effect of high humidity on sweating has been noticed only at very high environmental temperatures (35°C) (McDowell et al. 1961). At high environmental temperatures, ventilation has increased evaporative heat loss (Ragsdale et al. 1950). Solar radiation increases sweating by increasing the skin temperature. It has, therefore, been shown to be responsible for the higher rate of sweating under field conditions than in hot climatic laboratories (Murray 1966). Cutaneous evaporation was found to rise when cows were exposed to radiation at low environmental temperatures (Kibler and Brody 1954).

Cutaneous evaporation has been found to be higher in *Bos indicus* calves than in *Bos taurus* under conditions of heat stress (Kibler and Yeck 1958; Kibler and Yeck 1959). Friesian cattle have been found to develop a greater number of sweat glands than African types of cattle when raised under Nigerian and Egyptian conditions (Amakiri 1974; Shafie and El-Tannikhy 1970). The ability of *Bos taurus* for greater cutaneous evaporation improved when the animals were raised at high temperature environment (Kibler and Yeck 1959). It is not known if the increased sweat gland population in the Friesian cattle acclimatised

to tropical Nigerian condition is associated with high sweat production which should reflect in lower respiratory rates.

Some quantity of body heat is utilized in evaporating moisture at the respiratory surfaces. Respiratory moisture loss, therefore, is directly related to respiratory heat loss. From the works of Ingraham and Whittow (1962) and Bligh (1957), it has been suggested that the main cooling effect of respiratory evaporation takes place in the upper respiratory passages and not in the lungs. There is, however, a limit to the importance of the respiratory activity in losing body heat since respiratory evaporation is low compared to cutaneous evaporation, the ratio of the contributions of the latter and former to total surface moisture loss being 6:1 (Bianca 1965).

Under conditions of heat stress in the psychrometric chamber, cattle exhibit polypnea to a degree which depends on the magnitude and duration of the heat stress. Heat sensitive types of cattle are particularly more affected (Worstell and Brody 1953; Kibler et al 1965; Bond and McDowell 1972; Johnson et al. 1967). In this respect, cattle are unlike man, but are similar to a few mammals including swine, sheep, mice, rats, dogs and cats (Findlay 1954). Increases in respiratory rate occur if demands for cooling continues (Bianca 1958). Respiratory activity in cattle under conditions of heat stress has been suggested to be a measure of the inadequacy of the more important cutaneous evaporation to maintain a temperature balance (Riek & Lee 1948).

It has been shown that polypnea does not prevent a rise in body temperature in calves and heat sensitive cows (Bianca 1962; McDowell et al 1953). This is probably because the respiratory muscles generate more heat during fast breathing or panting in a hot environment. It has, therefore, been shown that oxygen consumption is increased in cattle under heat stress (Hales and Findlay 1968). Under similar conditions, blood lactic acid is increased, and respiratory alkalosis can develop (Bianca and Findlay 1962). The respiratory response of cattle to progressively severe thermal stress occurs in two stages, the first which is panting, is followed by slow deep breathing which has no significant metabolic cost (Hales and Findlay 1968).

Reports from field studies also show that respiratory responses of cattle vary with the weather conditions, increases in respiratory rate occurring in hot weather (Berman and Morag 1971; Rainey et al 1967; Wolff and Monty Jr 1974; McDowell et al. 1955; Berman 1967).

The capacity of cattle to dissipate heat through evaporation is low thus making them particularly susceptible to thermal stress. When the body of heat sensitive cattle gains heat, the deep body temperatures of cattle do not rise at environmental temperatures below $70 - 80^{\circ}\text{F}$ ($21 - 26^{\circ}\text{C}$) Kibler and Brody, 1950). Many studies in the climatic laboratory have demonstrated the response of body temperature in cattle to increasing environmental heat (Worstell and Brody 1953; Kibler et al 1965; Johnston et al 1963). Studies in the field have also demonstrated

similar phenomenon in cattle. This is more pronounced in animals under stress of lactation than in non-lactating cows (Wolff and Monty Jr., 1974). Non-lactating cows have been found to show little or no seasonal differences in their rectal temperatures (Wolff & Monty Jr., 1974).

Normal body temperatures may be affected by the nutritional level (Robinson & Lee 1947; Rogerson 1960). The temperature of the jugular vein blood has been found to increase as a result of feeding cattle. (Ingraham and Whittow 1961).

Periodic fluctuations may occur in the deep body temperature without compensatory reactions being brought to play. For example, diurnal patterns in body temperature with an early morning minimum and early evening peak has been observed in cattle (Gaalaas 1945; Kriss 1921). When body temperatures of cattle were recorded continuously 67% were diphasic having 2 peaks a day, 23% were polyphasic and 3% were aphasic. Ovariectomy and pregnancy did not affect the incidence of these patterns (Wrenn et al 1961). This circadian shift in thermoregulatory functions has been demonstrated in **both** the *Bos taurus* and *Bos indicus* types of cattle under natural environment (Rainey et al. 1967; Berman and Morag 1971; Moran 1970; Bligh and Lampkin 1965).

When Holstein cows were exposed continuously to the sun, the highest body temperatures occurred in the afternoons and the lowest in the mornings (Rainey et al 1967). However, no significant difference occurred in the circadian variation in deep body temperature of *Bos indicus* and *Bos taurus* breeds grazing under moderate atmospheric

temperatures (Bligh & Lampkin 1965). It has been suggested that the ability of cattle to thrive in the tropical region could be better indicated by the diurnal variation in the body temperature rather than a particular body temperature (Kendal 1948). Brown et al. 1969 also suggested that the rate of rise in the body temperature can be used to identify heat-sensitive cattle.

Hutchinson and Mabon (1954) found more decreases in morning rectal temperatures in zebu cattle in the hot season than in the cold season in Tanganyika. Initial rectal temperatures were also lower in heat-acclimatised calves than in non-acclimatised calves on exposure to hot humid environment (Bianca 1959 a, b). Non-lactating Holstein-Friesian cows were found to show lower morning rectal temperatures during the hot summer months than in the cooler season in Arizona (Wolff and Monty, Jr. 1974). Bianca (1959b) believed that the lower body temperature before heat exposure are beneficial because they signify a low starting point for the animal in its defence against hyperthermia. Seasonal depression in the morning rectal temperature coinciding with the hottest months of the year is suggested to be of a similar thermoregulatory advantage by preparing the animal for the heat of the day and delaying, but not necessarily preventing, hyperthermia (Wolff & Monty Jr. 1974).

Cyclic changes in the body temperature of cattle in relation to estrous cycle have been studied (Wren et al 1958; Bane and Rajakoski 1960). Marked depression in the body temperature occurred two to three days before estrus. The body temperature was elevated on the day of estrus, depressed on the day after, coinciding with ovulation, and remained depressed till the 7th day of the cycle when an elevation occurred until the approach of the next estrus. In a field study, no consistent relationship was found between the rectal temperature and the ovulation time (Howes, et al. 1960).

As regards breed effect, Worstell and Brody (1953) reported that no significant differences could be observed among breeds of *Bos taurus*. The study by Bligh and Lampkin (1965), quoted above, also showed that species differences between *Bos indicus* and *Bos taurus* in rectal temperature was insignificant under moderate climatic environment.

Heat tolerance

Heat tolerance is a measure of the extent to which the animal can maintain normal body temperature and other physiological functions on exposure to a hot environment. Various methods including body temperature changes, respiratory evaporation, skin evaporation, productivity, skin and hair coat characteristics have been used to determine heat tolerance in both field and laboratory studies; and the relative usefulness of these had been well assessed (Bianca 1961; McDowell 1966). Body temperature activity has been more generally preferred and various

field tests make use of this parameter in assessing adaptability of cattle to a hot environment. Comparisons have been made between *Bos indicus* and *Bos taurus* types of cattle both in the field and in the climatic laboratory regarding the effect of air temperature on body temperature. It has been shown, for example, that under hot conditions the Brahman (zebu) maintained its body temperature best, the Aberdeen Angus (*Bos taurus*) least well, and crossbreds gave results which lay between those for the other two (Rhoad 1938; Rhoad 1940a).

On the basis of experiments similar to this, Rhoad (1944) devised the Iberia Heat Tolerance Test by which a heat tolerance coefficient of cattle is calculated from the increase of rectal temperature which occurs during a day in an unshaded environment of 90°F in the sun. The formula employed is: $A^{\circ} = 100 - 10 (BT^{\circ} - 101)$, where A° is the heat tolerance coefficient and BT° is the body temperature. Perfect efficiency in maintaining body temperature at 101°F, the average rectal temperature of cattle, holds when the adaptability coefficient is 100. On this basis, adaptability of a number of cattle types had been worked out and *Bos indicus* and half-breds have been shown to possess higher heat tolerance than pure *Bos taurus* (Rhoad 1944). The same method had been used to test different breeds in the field in Greece (Phillip 1949). The adaptability coefficient for the White Fulani cattle has been shown to be 90 (Hill 1960). The differences in the respiratory rate under hot conditions have also been used to determine differences in heat tolerance in animals with similar rectal temperatures.

Some laboratory methods also make use of the rectal temperature for assessing heat tolerance in cattle (Lee and Phillips 1948; Bianca 1962; Bianca 1963). Bianca (1962) stressed that the rate of rectal temperature rise could be used as an indicator of heat tolerance. It has been emphasised that rectal temperature if properly used is the single most reliable indication of deep body temperature and the best physiological criterion for heat tolerance (Bianca 1961; Bianca 1965). Factors that influence heat tolerance include age, levels of nutrition and production, breed and species genetic differences, coat colour and hair type.

Between the ages of 2 to 24 months, the heat tolerance of cattle has been found to be low in *Bos taurus* (Riek and Lee 1948; Schein et al 1957). A similar report has been given for African types of cattle (Walker 1957). Heat tolerance increases as the animal matures (Bonsma 1949; Gaalas 1947). Lactating cows show greater rise in respiratory rate and rectal temperature than non-lactating cows (Worstell and Brody 1953; Schein et al 1957; Wolff and Monty Jr. 1974; Johnson et al 1962; Kibler et al 1965). Increasing milk production is associated with a decline in heat tolerance (Brown et al 1969). Sudden increases in the level of feeding have been found to depress the heat tolerance of heifers (Yeates 1956).

Various studies have obtained results similar to the report by Rhoad (1944) showing the species differences in heat tolerance (McDowell et al 1953; Bonsma 1949; Schein et al 1957; Johnston et al 1963). Johnston et al (1963) found that rectal temperatures of Red

Sindhi (Bos indicus) bulls were significantly lower than those of the Holstein and Brown Swiss at both temperatures of 82 and 104^oF. The level of tolerance has been shown to increase with the amount of the Bos indicus blood in the crosses between Bos indicus and Bos taurus types of cattle (Rhoad 1944; McDowell et al 1953). The superiority of the Brahman to Holstein and Jersey in heat tolerance has been attributed to the surface structures such as the dewlap, navel fold, brisket and the hump which tend to increase surface area/body weight ratio, and to a superior radiating coat, slower rate of hair growth and greater ability to sweat (Phillip 1949). In a field study, slight breed differences in heat tolerance were observed between Holstein and Jersey, being slightly higher in the latter (Seath and Miller 1947). Similarly, Romance-Pounce et al (1977) found that rectal temperatures differed between Holstein, Guernsey, Jersey and Brown cows exposed to natural hot climate, with values decreasing in the order of the breeds.

The hair and hide colours influence the heat tolerance of the animal by determining the amount of solar radiation absorbed or reflected. Red, yellow or redish-brown hair reflects heat or infra-red rays; yellow, redish-brown or black hair reflects ultra-violet (short-wave) rays. Cream or light-coloured coats reflect more visible radiation than dark-coloured coats (Riemerschmid 1943; Bonsma 1943). It is believed that white, yellow or red coat with dark hide is the best combination to render the animal resistant to high temperature and

intense radiation of the long-wave (heat) and short-wave rays which are most prevalent in the tropical and sub-tropical regions. This combination of hair and hide colours is possessed by tropical breeds of cattle (Riemerschmid and Elder 1945; Bonsma 1949). Bos indicus cattle generally have shorter and lighter coats than Bos taurus (Hayman and Nay 1961).

Other hair characteristics aid heat resistance. The thick, stiff and short medullated hair fibres of the zebu type enhance air movement at the skin surface, thus aiding vaporisation of moisture, and are highly correlated with heat tolerance (Dowling 1959). The white coat of the Fulani cattle is 100% medullated, short thick stiff and glossy (Hill 1960). That of most temperate-evolved cattle is naturally long, curly and wooly in texture. The hair of the Holstein cattle reared in Nigeria has been modified into relatively shorter form by the long exposure to tropical environment, though it has not attained the shortness of the coat of the zebu types (Amakiri 1974). Wooly animals have been found to show greater increases in body temperature than glossy coated animals (Bonsma 1943). Berman and Volcani (1961) showed that the thickness of hair coat varies with the season, minimum values occurring in spring and summer. Such seasonal variations may also cause seasonal changes in the effect of hair coat on heat tolerance.

Influence of shade on thermoregulation

Rainey et al (1967) found that the body temperature and respiratory rates of cows in the shade were lower than in animals in the sun. In another more recent field study involving Holstein, Guernsey, Jersey and Brown Swiss cattle, it has also been shown that shade provides a micro-environment for the animals and modifies their physiological responses (Romance-Pounce et al 1977). Heat intolerant animals have been reported to seek shade while in the field (Seath and Miller 1947; Romance-Pounce et al 1977).

Kelley et al (1950), proposed that shade structures with high roofs, referred to as high shade, would provide more comfort than structures with low roof (low shade). The higher efficiency of high shade has been attributed to the fact that the animal can lose more heat by radiation to the clear sky (Kelly et al 1957).

Besides the fact that the physiological responses are ameliorated, the reproductive efficiency has been found to be improved in shaded animals compared to animals exposed to the sun (Wiersma and Stott 1969; Romance-Pounce et al 1977). Milk yield is also improved when cows are reared in the shade (Romance-Pounce et al 1977).

Climatic heat stress factors

The principal climatic heat stress factors include the environmental temperature, radiation, humidity and wind. The wind is a stressful factor only when the environmental temperature exceeds body temperature.

Under such conditions, the inhaled air is cooled as it passes through the respiratory tract and the animal gains heat, (Hales and Findlay 1968).

The combined effect of high temperature and humidity have been regarded by various workers as heat stress causing increases in the physiological responses (Worstell and Brody 1953; Beakley and Findlay 1955; Cargill et al 1962; Johnson et al 1963; Kibler 1964). The relationship of temperature-humidity index to production and certain aspects of reproduction have been examined in cattle (Johnson et al 1963; Thatcher 1974). "High environmental temperature without its relation to humidity of the air does not express the severity of the environmental condition" (Johnson et al 1963).

Bianca (1963) found no difference in the order of heat tolerance of calves in each of 10 environments having different combinations of relative humidity and temperature. Crossbreeds ($\frac{1}{2}$ Jersey x $\frac{1}{2}$ Brahman) were also reported to be more affected by changes in air humidity than pure Holsteins and Jersey cattle; and this was attributed to the higher sweat production in the crossbreeds (Quazi and Shrode 1954). High humidity causes a reduction in the vapour pressure gradient between the skin and air, and therefore inhibits evaporative moisture loss.

High humidity may not always considerably affect cutaneous moisture loss by the animal. Mclean and Calvert (1972) found that at an environmental temperature of 35°C a reduction of vapour pressure by increasing the relative humidity of the air from 32 to 72% had only a

slight effect on skin evaporative moisture loss. This was explained by assuming that the rate of sweat secretion was unchanged and an increase in air humidity caused a temporary reduction in evaporation and a build up of moisture on the skin. As a result, the vapour pressure of the skin surface increased automatically re-establishing the vapour pressure gradient between the skin surface and air until evaporation rate was in equilibrium with the rate of sweat secretion. When extremely high levels of humidity was applied, these workers obtained marked reduction in evaporation. In the context of the Southern Nigerian situation, relatively high humidities occurring during the wet season may not necessarily be high enough to disturb cutaneous evaporation in cattle in the field. McDowell et al (1961) reported that humidity-induced decrease in cutaneous evaporation in Holstein can only occur at a temperature as high as 35°C . Ojo (1973) suggested that about July in the wet season, high temperatures combined with high humidities in areas including the middle belt of Nigeria could impose heat load on cattle; Ibadan area was excluded because of the relatively lower air temperatures occurring there at that time of the year.

High humidity enhances the effect of high air temperature on respiratory activities. Air temperatures of 30° and 35°C at high humidity have been found to be equivalent in their effect on respiratory rates in calves to air temperatures of 33°C and 40° respectively at a low humidity (Beakley and Findlay 1955). Expired air is almost

saturated with moisture at body temperature (McLean 1963). It would be expected that if the humidity of inspired air is high, it would directly reduce respiratory evaporation and respiratory evaporative heat loss for a given respiratory minute volume, McLean and Calvert (1972) however found that increasing the relative humidity of atmospheric air from 32 to 72% at an environmental temperature of 35°C, while causing an increase in respiratory frequency, only slightly reduced respiratory evaporation. The increased respiratory frequency was suggested to be reflecting an effort towards a compensatory increase in respiratory minute volume which would tend to resolve respiratory evaporation to the normal level. Under field conditions, therefore, moderately high humidity may not necessarily cause a reduction in respiratory evaporation or be stressful in cattle.

Solar radiation is effective as a strong heat stress factor in the field, causing rectal temperature and respiratory rate to rise in cattle (William et al 1960). Solar radiation was found to influence body temperature even at environmental temperatures below 32°C. Buffaloes are particularly very sensitive to solar radiation exhibiting greater responses than Harina (Indian zebu) cattle in the sun in India (Badreldin & Ghany 1952, Mullick 1960). The potential influence of solar radiation in imposing heat load on cattle at different locations and during different periods of the year in Nigeria had been studied (Ojo 1971). The author showed that solar heat load increases inland from the coast and is lower during the wet season. This is suggested to be probably due to higher cloudiness in the south and in the wet

season which reduces the amount of incoming solar radiation.

Among the climatological variables, air temperature has been found to be the most important with respect to environmental heat stress in cattle under conditions prevailing in Texas. The same changes in respiratory rate, rectal temperature and heart rate were obtained when air temperature alone or a combination of all the climatological variables was applied (Shrode et al 1960). Environmental temperature is said to be the "most important climatic factor" that may modify the ability of cattle to secrete milk (Johnson 1965). It has been said that "dry and wet bulb temperatures, solar radiation or hours of sunshine, air movement, and precipitation patterns for an area near the animal will suffice for assessment of climatic stress" under field conditions (McDowell and Johnson 1971).

Climatic stress, therefore, results from effects of these factors all of which act either singly or in combination to influence the physiological system of the animal (Rhoad 1940; Lee and Phillip 1948; Kibler and Brody 1954a). However, generally under hot conditions, wind or ventilation, especially with relatively high velocities of 5-9 mph, widens the comfort zone of the animal by increasing the convective and evaporative heat exchange between the animal and the environment (Kibler and Brody 1954b). It has been found that such effects of the wind is not felt by the Brahman cattle until air temperature of 95°F is reached or exceeded (Kibler and Brody 1954b; Findlay 1954).

MATERIALS AND METHODExperimental animals

The two breeds of *Bos taurus*, Holstein-Friesian and German Brown were imported to the University of Ibadan Teaching and Research farm between 1969 and 1973 from Western Germany. A White Fulani (*Bos indicus*) dairy herd on this same farm had been established over ~~twenty~~ years ago under an experiment to select trypanosomiasis resistant animals of this breed. White Fulani cattle are naturally accustomed to the hot tropical climate of the savanna zone of West Africa with seasonal rainfall and prolonged dry season. See figures 1, 2 and 3.

Six, five and six heifers of the Holstein, Brown and Fulani respectively with ages ranging from 16 to 24 months were used for the present investigation. They had a mean initial weight of 210.4 (\pm 29.9 SD) kg. The heifers of the Holsteins and Browns were selected from the first generation heifers of imported cows. In selecting the experimental animals, the maturing heifers were observed for expression of estrus; and ovaries were examined by palpation per rectum for changes (presence of corpus luteum, or follicles). The ages of the animals when ovaries showed changes were noted. The heifers were all cycling normally before the commencement of the investigation.

The Brown and the Holstein cattle evolved in the temperate regions of the world and are not adapted to tropical climate. In the strict sense, neither the experimental *Bos taurus* nor *Bos indicus* (zebu) cattle belong to the forested subequatorial zone of Southern Nigeria.

Climate of Ibadan

Ibadan lies at latitude $7^{\circ} 26'$ North and $3^{\circ} 54'$ East at an elevation of about 227 meters above sea level (Church 1974); it has a lowland rainforest vegetation (Keay 1959). It is about 144 kilometers inland from the Western Coast of Nigeria.

The climate of Ibadan has been described as seasonal-equatorial which is characterised by slight seasonal changes in temperature and daylight period. There are two main seasons: the dry and the wet seasons (Church 1974).

At the University of Ibadan, the mean monthly minimum and maximum temperatures for the last 20 years were 70°F (21.1°C) and 80°F (26.6°C), and for the year 1976, the values were 69°F (20.5°C) and 80°F (26.6°C) respectively. The two rainfall maxima occur in June - July and September coinciding with the equinoxes. The total precipitation for the year 1976 was 877.2 milliliters.

The dry season, extending from November through early March, is characterised by high afternoon temperatures (maxima temperatures varying between 82 and 98°F) with high diurnal variations

(about 23 - 26°F), low afternoon humidities (15 - 77 RH %), and low precipitations (0 - 125mm/month). The wet season extends from mid or late March to September and is characterized by high temperatures which are relatively lowered in the wettest months (maxima temperatures varying between 76 - 95°F), high afternoon humidities (52 - 94 RH %), high precipitation (63 - 218 mm/month) and light to heavy cloud cover. Diurnal variation of temperatures is low (about 10 - 13°F). The highest humidities and lowest dry bulb temperatures occur at 07.00 hour; and the lowest humidities and highest dry bulb temperature occur at 16.00 hours in the two seasons. Total sunshine hours decrease from December in the dry season to August in the wet season (237 to 69.8 hours/month in 1975).

Overlapping of the two main seasons may occur sometimes, so that the months of March and October may be quite wet in some years and, in other years, may be dry with little precipitation.

The year may be divided into four quarters.

- (1) December through February is dry and hot. The dry hazy and cold 'harmattan' winds blow in December and January making the daily minimum temperatures relatively low and resulting in cold nights and mornings.

- (2) March through May represents the transition to the wet season and is hot and wet with lower diurnal temperature range than in quarter (1). Rainfall may range from medium to heavy.
- (3) June to September represents the wettest period, hot especially in June, and humid, but relatively cool in July and August. The cloud cover is highest at that time with low diurnal temperatures and high humidities.
- (4) Late October through November represents the end of the rainy season and the beginning of the dry season. Precipitation gradually diminishes through October to very low or nothing in November; temperatures are high with marked diurnal ranges, and cloud cover and relative humidities are low.

The changing seasons are dependent upon the movement northwards during the wet season and southwards during the dry season of the Intertropical Convergence Zone (ITCZ) which is determined by the interplay of two major winds in West Africa. The dry north easterly winds are predominant in the dry season causing dry hazy weather with cold nights and mornings. The south westerly winds are predominant during the wet season and are associated with much cloud cover and rain. For the purpose of the present investigations, meteorological records from November 1975 through February 1977 were obtained from the

Department of Geography, University of Ibadan. All recordings were made at the meteorological station located within the campus and at a point less than 1 kilometer from the University farm.

The weather conditions from November 1975 through December 1976 are tabulated (table A9) and represented graphically (Fig. 5). The relationship of air temperature to the relative humidity during the period when blood samples were collected are also shown in a histogram (Fig. 6).

Air temperatures were high from late October through April ranging from 86.5 - 97.1°F (30-36.2°C). The highest air temperatures occurred in March with average maximum temperature of 92.4°F (33.6°C). July to August period was cooler with lower air temperatures; average maximum temperature was 81°F (27.3°C). Average minimum temperatures were 64.5°F (18.1°C), 71.6°F (22.0°C), 68.1°F (20.0°C), and 69.0°F (20.6°C) for December - January, March - April, July - August and late October through November periods respectively. More rapid rise in air temperatures occurred in the mornings during March - April period than in other periods of the year. Relative humidities at 16.00 hours when maximum temperatures were recorded, were 42.6, 54.4, 76.0 and 55.9 percent for December - January, March - April, July - August and late October through November periods respectively and were, therefore, highest during July - August

period. Air was thus driest during December - January period but nonetheless cold in the evenings through the night and morning due to the prevalent cold winds blowing till the middle of January.

Rainfall occurred from March through the middle of October; there was a short "August break" in the later half of August till early September when precipitation was very low. Other months had little or no rain.

Sunshine hours were longest during the drier months and lowest in July - August period. A very important feature was the intense sunshine in the afternoon, especially when the sky was cloudless.

Experimental design

Blood samples were collected during periods selected from the four quarters of the year described above. Breathing rates (respiratory rates) and rectal temperatures were recorded. Behavioural responses to heat particularly shade seeking, were also observed.

The periods selected were early December 1975 through the middle of January 1976, early March through the middle of April 1976, early July through the middle of August 1976, and late October through November 1976. Each period lasted 42 days.

The character of estrous cycles was observed throughout the year. Ovarian changes were observed by palpation per rectum.

The heifers on experiment were kept in two adjacent pens. The length, breadth and height of each pen were 96, 63.5 and 90cm respectively. The pens were open on the sides and were only separated by metal crossbars and had a high corrugated roof and concrete floors which were covered every morning with wood shavings after the pens had been cleaned.

One Fulani bull was selected, vasectomised and made to join the heifers. An already vasectomised Brown x Ndama (BN) bull also joined the heifers. These two bulls were used as teasers for detecting estrus in the heifers.

Heifers stayed in the pen from about noon till the next morning (06 - 07.00 hours) when they were released to graze on the improved pastures mostly of giant star grass (*Cynodon* sp.) in the paddocks adjoining the pen. While in the pen, they were fed with grass and concentrate: the latter was composed of guinea corn or maize 65%, palm kernel 15%, groundnut cake 20%, and served at the rate of 1kg / 123kg body weight/day. Grass (mainly giant star species) was served at the rate of 1 kg/13.0kg body weight/day.

During the dry season when grass was minimal in the paddocks, the amount of grass supplied in the pen had to be increased to 1 kg/ 11 kg body weight/day. As grass supply dwindled, a day's supply had to be supplemented with brewers' grain. Alternatively, corn

silage was served at the rate of 1 kg/7 kg body weight/day.

Water was supplied ad libitum in concrete troughs that run the breadths of the pens; salt licks were also available.

The routine health programme for the animals included faecal examination, deworming, spraying with insecticide, samorin prophylaxis against trypanosomiasis and inoculations against anthrax, heart water disease, foot and mouth disease, rinderpest and haemorrhagic septicaemia. Wet preparations of peripheral blood from all heifers were examined regularly for trypanosomes; but no positive case was observed throughout the period of this study although tsetse flies were caught regularly on the farm. Packed cell volume determined in the conventional way and haemoglobin concentration by cyanmethaemoglobin method were within normal ranges (Saror and Coles 1973; Olusanya 1976) (tables A10 and A11).

The heifers were also weighed every month (Fig. 6). The growth rate in the heifers through the year was not of the steady and uniform fashion observed in the heifers raised in the temperate region (Brody et al. 1947; Bachner 1960). Marked weight increases coincided with the two rainfall peaks in April to June and September. A slight drop either in absolute body weight or weight gain occurred in the dry season. The lack of uniform growth rate throughout the year may be attributed to the effects of climate and

some nutritional inadequacies.

Generally, the coat of the *Bos taurus* heifers was less hairy during the dry season. The coat colour of the Brown heifers became lighter in that season than in the wet season when it was of a deeper brown lustre.

Other pens in the house were occupied by dry cows which were either pregnant or yet to be bred, and heifers growing to reach mature age and breeding weight. Animal attendants worked on shift basis. Morning shift started at 6.00 hours and ended at 14.00. Afternoon workers started at 13.00 hours and closed at 18.00 hours. Their services and those of the night-watchmen were employed for most procedures carried out on the animals. Apart from these workers, an assistant was permanently assigned to take care of the experimental animals. He assisted in the general management and handling of the animals and in recording observations. His duty hours had to be adjusted from time to time to suit the needs to carry out observations and some procedures demanded by the study.

Since the animals were to be exposed to the routine on the farm during the time of the study, it was decided not to catheterise the heifers but to train them adequately enough to get used to handling and blood collection by venipuncture.

Every morning by 07.00 hours, the animals were passed through the fenced passage leading to the crush located in a paddock close to the animal house. They were stopped one by one in the crush; some blood, about 2 - 5 ml, was collected by jugular venipuncture. Ovaries were frequently examined per rectum. After all the animals had passed through the crush, they were led into one of the paddocks close by to graze till about 11.00 hours when they were returned into the pens.

During this training period, the animals established a hierarchy of dominance, and there was no real fighting since animals lower in the hierarchy tried to avoid animals higher in the order. They also formed a leadership - followership order of entering the passage and the crush, as some heifers entered the crush first and easily before others could be made to follow. The very docile BN bull, however, facilitated the entry of the heifers into the passage since he entered the passage and the crush easily. There was a tendency for a more dominant animal to stay behind the animal lower in the established order, which gave rise to rear butting and pushing. The animals had to be reorganised and were made to follow the BN bull in the descending order of the established hierarchy. The arrangement was permanently followed and there was no disturbance among the animals. Because the Fulanis

tended to stay together most of the time, they were made to enter the crush last, the most docile among them first and led by the Fulani bull. The training period lasted five weeks.

The routine of passing the heifers through the crush before grazing in the paddock was continued everyday throughout the year. During the periods when blood samples were not collected, the recording of the rectal temperatures of the heifers was continued while they passed through the passage and crush and ovarian examination per rectum was done when there was a need for it.

During the marked four quarters of the year, the rectal temperatures were recorded with a veterinary thermometer inserted into the rectum, and the breathing rate by counting flank movements. Normally the heifers were used to the procedure of rectal temperature recording every morning and afternoon. If approached carefully and slowly, the temperature could be recorded in the pen without any excitement.

For the purposes of this investigation, the breathing rates were recorded in the pen between 06.30 and 07.20 hours in the morning. Immediately after that, the animals were moved out gradually and lined in the weighing race approaching the crush. The rectal temperatures were recorded by an assistant while the animals were being bled one after the other. The afternoon readings of the rectal

temperatures and respiratory rates were recorded in the pen between 14.00 and 16.00 hours. The results were tested by analysis of variance, the sources of variation being breed, season, and time of the day (Snedecor and Cochran 1967).

Ten milliliters of blood were collected by jugular venipuncture between 07.10 and 8.00 hours into heparinised tubes which were then cooled in iced water. The tubes were centrifuged at 3000 rpm for 10 minutes and plasma was separated and stored frozen at -20°C until the time for the assay.

Restraint and blood collection were carried out very rapidly and noise was strictly avoided. When the collection time was unduly prolonged or an unusual excitement was displayed either before getting into the crush or at the time of bleeding, the animal was not bled, so as to avoid excessive variation in plasma cortisol levels.

Plasma cortisol concentration was determined by radioimmunoassay. The mean values of four heifers of each breed that showed the best growth rate and were most amenable to the procedure of blood collection were used in constructing the composite graphs of the values through the estrous cycles.

Values of plasma cortisol obtained in the heifers during each quarter representing uneven numbers of observations were subjected to analysis of variance. Values obtained on alternate days,

representing fairly even numbers of observations were similarly treated because of the missing values on some days and because during October - November period, plasma cortisol was determined on alternate days except around estrus. Plasma cortisol levels on the day of estrus, the middle of estrus cycle and mean for the cycle were also subjected to analysis of variance, the sources of variation being breed, season and stage of the cycle.

In order to observe the behavioural responses to heat while in the field, the heifers were grazed at least 10 times during each quarter in paddocks which had trees to provide shade. The animals under shade at 12.00 hours were noted. The total number of days when an animal was found to seek shade was scored against her as a percentage of the total number of days of observation, and the result was treated by analysis of variance, sources of variation being breed and season. The grazing behaviour of the heifers was also observed.

The diurnal and circadian variations in the physiological responses were investigated in the heifers. Respiration and rectal temperatures were recorded in the heifers while in the paddock and unshaded on three hot days in March 1977. The heifers were kept grazing in the paddocks adjoining the weighing crush throughout the day. They were carefully mustered and passed into the passage approaching the crush each time the rectal temperatures were to be

taken, although many of them would stay and allow the temperatures to be taken while grazing. Breaths per minute were counted before the animals were passed through the crush and before grazing. Readings were taken at 07-8.00, 10.30-11.00, 15.30-16.00 and 18.00 hours. On another two consecutive days, the animals were kept in the pen and both breaths per minute and rectal temperatures were recorded at 7.00, 10.00-11.00, 14.00, 17.00, 20.00 and 23.00 hours. In addition, readings were taken at the times given above and at 01.00 and 04.30 hours in the pen on one occasion. The readings were all subjected to analysis of variance. Correlations between the responses were tested as well as between the responses and the ambient temperatures.

In investigating the diurnal and circadian variations in plasma cortisol in the heifers, blood samples were collected at various times and under different conditions during the year. The investigations were grouped as four experiments.

1: In the first experiment, blood samples were collected in the morning, afternoon and evening in heifers kept outdoors throughout the sunny day. For a week in April 1976, before the end of the second quarter (marked for morning blood sample collection), the heifers were taken through the routine of passing through the crush when returning from the field into the pen between 11 and 12.00 hours. For four days after the end of the

quarter, the frequency of passing the heifers through the crush in the afternoon was increased. The heifers were kept in the paddock adjacent to the crush from 7.00 till 17.00 hours daily. Cut grass was supplied to supplement the low amount of grazing that was possible in that paddock. Water was supplied ad libitum. On the fifth day (24th April), 12 heifers (4 Brown, 4 Holstein and 4 Fulani) were bled by jugular venipuncture at 7.00, 12.00 and 17.00 hours as they passed through the crush. Their rectal temperatures were recorded.

2: The aim of the second experiment was to see if more frequent blood sampling could be done by using few animals. In early June, all the experimental heifers were again conditioned as described above ^{to} cross the weighing race and crush frequently during the day for about a week, after which (June 9) three heifers (Brown 84, Holstein 145 and Fulani 21) were separated from the other heifers and bled at 7.30, 11.00, 16.00 and 18.30 hours of the day.

3: In the third experiment, the same procedure as above was repeated in late August (25th) when 13 heifers (4 Brown, 5 Holstein and 4 Fulani) were bled at 07.00, 11.00, 15.00 and 18.30-19.00 hours. Thus, more blood samples were collected in the heifers kept in the sun throughout the day than in experiment (1).

4: The aim of the fourth experiment was to determine the circadian levels of plasma cortisol in shaded heifers. Three heifers were restricted by tying to the stanchion in the pen for a week during which time they were visited at about 3 - 4 hours intervals during the day and twice in the night, and restrained briefly in the posture for jugular blood collection. Blood (2 ml) was collected only in the morning (7.30 hours) as part of the conditioning, and was discarded. The heifers were bled by jugular venipuncture by 07.30, 15.00, 18.00, 23.00, 01.00 and 04.30 hours on the 16th of May. The experiment was repeated in the middle of September and early October. However, in order to diminish the possible effect of restraint on plasma cortisol levels, blood was collected from the tail vein on these two occasions, making use of heparinised evacuated venoject tubes (Terumo Corporation, Tokyo, Japan) (Brown 1963; Ghoshal and Getty 1967). Different heifers were used on each occasion and they were tied to the stanchion for a week prior to blood collection.

Rectal temperatures were not taken during the subsequent investigations after experiment 1 so as to avoid prolonged handling of the animals, and, to decrease the interval of bleeding between the heifers. Heifers were bled under 1 minute

of approach or restraint in the crush or pen. Only 5 mls of blood were collected each time, and this was kept cool in iced water until the plasma was separated. Plasma samples were stored at -20°C till the time they were analysed for cortisol concentration by radioimmunoassay. The results were treated by analysis of variance, using 'breed' and 'time of the day' as sources of variation.

The adrenocortical response to ACTH was investigated in early January 1977 after all the quarterly blood samples had been collected from the heifers. Two heifers of each breed in the diestrous phase of estrous cycle (between days 7 and 10) were weighed and used for the investigation. Led by the BN teaser bull, they were made to pass the weighing crush at 2 hourly intervals from 07.00 till 16.00 hours daily for 5 days. After each round, the heifers and the bull were kept in a space on one side of the passage and crush. This space was compact enough to allow driving them out again easily. They were supplied with their normal feed in that space.

On the sixth day, ^{the} heifers were bled by jugular venipuncture at 08.30 hours, that is, zero time before the intramuscular injection of 5 ml of 0.9% physiological saline. After the blood collection, the same animal was immediately led back to queue

behind the other animals in the passage for the second and third blood collections. A time interval of about 60 minutes was allowed before the 4th to 6th blood collections; thereafter the intervals of 2 and 3 hours were allowed for the last two blood collections. At the end, it was found that the intervals rounded up to 0, 30, 30, 80, 65, 60, 120 and 180 minutes. The heifers were again bled by 07.00 hours on the following morning, and the routine of passing through the crush every 2 hours was continued. Three days after the saline injection, the heifers were injected intramuscularly with 200 I.U ACTH (Porcine, Sigma Chemical Company, St. Louis, Mo. U.S.A.) dissolved in 5 ml physiological saline, and were bled as described above. Blood was also collected on the following morning by 07.00 hours. Another 100 I.U. of ACTH was then injected but blood samples were no longer collected.

The same heifer served as her own control. The procedure was carried out as quietly and as rapidly as possible with the aid of a number of assistants. Five milliliters of blood were collected each time into heparinised tubes which were kept in iced water until centrifuged. The plasma was stored at -20°C until the time of assay. The result was treated by analysis of variance; time after injection, type of treatment and breed were considered as separate sources of variation.

Observation for estrus and per rectal examination of ovaries of experimental heifers were carried out during a period of investigation lasting 14 calendar months. The attendant assisting in the project was taught how to detect estrus. Other attendants also reported cases of mounting by the bulls. The BN bull was made to wear a heat detector halter filled with a dye on some nights and some week-ends when it was expected that regular observations might not be possible. A teaser bull was always in each of the two pens occupied by the heifers. All the experimental animals stayed together in the same paddock while in the field in the mornings, but they were kept far from the other cows and heifers not on experiment so that the teaser bulls might not be distracted. Observations for estrual signs normally began at 05.0 hours by the author or any of the attendants. The special assistant allocated to the project continued the observation until 15.30-16.00 hours.

Afternoon workers reported cases of mounting among the heifers or by the bulls between 16.00 and 18.00 hours. The author mandatorily observed the animals twice daily in the morning and in the evening apart from the need to be around for various procedures at some other times. Observations for estrus by the author in the night between 22.00 and 04.00 hours was often necessary when heifers showed proestrual signs during the day or

when the end of an estrual period was to be recorded.

Estrus was recorded as standing heat (staying to be mounted) and subestrus as slight estrual signs without standing to be mounted. The time when a heifer first stayed to be mounted was regarded as the onset of estrus. During the four quarters, marks were awarded for each animal for the intensity of expressed estrus. Intensity was graded as high, medium and low, marked (+++), (++) and (+) respectively. In accordance with the method of Hafez (1969), estrual intensity was judged by the occurrence of vaginal discharge, the expression of certain behaviour including the frequency of mounting by the animal, standing to be mounted and restlessness.

To determine the duration of estrus, the heifer on heat was allowed to graze with the group in the paddock in the morning, and upon returning from the field she was separated into another pen away from the teaser bull. The BN bull, or occasionally the Fulani bull, was introduced into the pen at intervals of 1 hour. If estrual signs had commenced at night, the heifer was similarly teased, but the interval of teasing was longer (2-3 hours). At times, the heifer on heat was not allowed to graze but was kept in the pen and teased as long as estrus lasted. The time when estrus stopped was taken to be the mid-point between the last time the heifer stood for mounting by the bull and the next and last teasing when the heifer would not stand for mounting.

Ovarian examination by rectal palpation was done while the heifer was stall-tied or restrained in the crush. Ovaries were palpated once at estrus to locate the Graafian follicle. Ovarian examination was then repeated at intervals of 6 hours. The time when ovulation occurred was estimated as midway between the time the Graafian follicle was last observed and the time when an ovulation depression was found. In some cases where an ovulation depression was palpated during the first examination after the end of estrus, the time of ovulation was assumed to be the time of examination.

Histograms were drawn to show the percentage distribution of the time of onset of estrus through the 24 hours of the day, the duration of estrus and the length of estrous cycles in the different breeds (Figs. 11, 12, 13). The time of onset of estrus through the 24-hour day was tested by chi square analysis, and the analysis of variance was used to test the effect of the month of the year. The significance of the breed and seasonal differences in the length of estrous cycle, duration of estrus and ovulation time were determined by employing the student t-test.

Radioimmunoassay of bovine plasma cortisol

Introduction

Very few studies involving displacement analysis in estimating bovine plasma cortisol have employed specific antibody (Dobson and Kanchev, 1977). This is probably so because of the relatively low cost of the "binding protein" employed in competitive protein binding assays which have been more commonly used, or in some places, the low reproducibility of RIA of cortisol.

RIA ensures greater sensitivity because of the specificity of the antibody. Early work on the RIA of plasma cortisol involved extraction and chromatographic purification (Ruder et al. 1972). Other assays have been performed on crude extracts after plasma protein precipitation with ethanol because of the greater specificity of the antiserum, (Abraham et al. 1972; Farmer and Pierce 1974; Viscei 1974). Highly specific antibody to cortisol can also be used to assay cortisol directly in very small volumes of plasma.

Materials and Solutions

Assay tubes were made of pyrex glass (Joblin and Co.) and had a dimension of 12 x 75 mm and ^{were}/rimless. Round bottomed tubes had a dimension of 100 x 14 mm. The tubes were soaked for at

least 24 hours either in pyroneg (Cockfosters, Barnet, Herts, U.K.) or dichromate solution. They were then washed with tap water and distilled water. Counting vials were soaked for at least 24 hours before washing. Other glasswares were similarly treated. Oxford pipettes (Oxford Laboratories, U.K.) with disposable tips were used.

Phosphate buffer stock solution was made up of 17.4 gm of disodium hydrogen orthophosphate anhydrous (M.W. 142), (Na_2HPO_4), 10.8 gm of sodium phosphate (NaH_2PO_4) (M.W. 138), 2.0 gm of sodium azide (NaN_3), 18.0 gm of sodium chloride (NaCl) in 2 litres. The buffer had a pH of 7 and was stored at room temperature. The assay buffer contained an additional 0.1% gelatin and was stored at 4°C .

Non-radioactive cortisol standard (Sigma Chemical Company) obtained in vials containing 50 $\mu\text{g}/\text{vial}$ was diluted without further purification with absolute ethanol to a concentration of 20 $\mu\text{g}/\text{ml}$ which was stored at -15°C as stock solution I. From this solution, stock solution II containing 1 μg of cortisol/ml ethanol was prepared and stored at -15°C . Different volumes of stock solution II, that is, 100 μl , 50 μl , 20 μl , and 10 μl were dried down in vials labelled A, B, C, D, respectively. The residues were dissolved in 10 ml phosphate buffer (pH 7) by placing the vials in a 30°C water bath for 10 minutes and

subsequently mixed on the vortex mixer (Vortex-Genie, Scientific Industries Inc. Springfield, Mass.) for 15 seconds. 5.0 ml of solution in vial D was transferred to another vial E to which 5 ml of phosphate buffer was added. In this way, standard solutions A to E were prepared containing, 5,000, 2,500, 1,000, and 500 and 250 pg per 0.5 ml phosphate buffer respectively.

(1, 2, 6, 7(n) - ^3H)-cortisol with specific activity of 85 Ci/m mol (Radiochemical Centre, Amersham, England) was purchased in vials containing 250 μCi each. The content of each vial was diluted to 5 ml with benzene-methanol (9:1) solution and stored at 4°C . About 20 μCi was purified each time by Sephadex LH-20 column chromatography (30 cm x 1 cm) using solvent system chloroform-ethanol (98:2). The purified radioactive cortisol was stored in benzene-methanol (9:1) and stored at 4°C . Working solution was made up in phosphate buffer just before the assay and contained approximately 5,000 cpm/100 μl .

Lyophilised antiserum to cortisol (Batch S-6#3) was obtained from Dr. G. E. Abraham, Harbour General Hospital, Torrance, California 90509, U.S.A. The antiserum had been raised in rabbit against Cortisol-21-hemisuccinate-Human serum albumin. (The cross reactivity of the antiserum with some plasma steroids are as follows:

Corticosterone 31%, progesterone 5%, 17-hydroxyprogesterone 6%, aldosterone 0.7%, estradiol-17 β 0.12%, estradiol-17 α 0.12% and testosterone 2%). Aliquots of the antiserum were diluted with distilled deionised water, and kept frozen at -20°C . An aliquot of the stored dilution was thawed before use and diluted with phosphate buffer solution to the working dilution. The final dilution chosen was that which bound between 30-35% of 5,000 cpm of (^3H)-Cortisol, and this was between 1:46,000 and 1:48,000.

The fine particles in Norit A (activated charcoal) were removed by soaking in methanol overnight and filtering with ordinary filter paper to which the particles adhered. 0.625 gm of dry charcoal with 0.0625 gm Dextran T.70 made up to 100 ml in assay buffer was prepared to last 2 to 3 days.

The scintillation fluid was made up of toluene (Fisher Scientific Company, New Jersey, U.S.) containing 5 gm per litre of 2-5-Diphenyl oxazole (PPO) (BDH Chemicals Ltd. Poole, England), 0.1 gm/litre of 1-4-bis(2(4-methyl-5-phenyl-oxazolyl) benzene (POPOP) (Sigman Chemical Company, St. Louis, Missouri, U.S.). This was mixed in a ratio of 2:1 with triton X-100 (BDH Poole, England). The fluid dissolved aqueous solutions satisfactorily.

Preparation of Samples

0.1 to 0.2 ml of plasma was taken in duplicate round-bottomed tubes, and 3 ml diethyl ether was added and mixed on the vortex mixer for 1 minute. The aqueous layer was frozen by keeping the tubes at -60°C for 10 minutes; the ether layer was quickly decanted into labelled assay tubes and evaporated at 40°C or at room temperature overnight. To each tube was then added 0.5ml of the assay buffer. All the assay tubes were then heated in the water bath at 60°C for 10 minutes and then mixed for 30 seconds on the vortex mixer. Deionised distilled water served as a blank (BK) in duplicate tubes. Two solutions of non-radioactive cortisol containing 10 ng and 5 ng/ml of deionised distilled water in duplicate tubes labelled 'R10' and 'R5' respectively, and bovine pooled plasma in duplicate tubes labelled 'P' were also treated like the plasma samples.

The Assay

For the standard curve, assay tubes were arranged in triplicates and labelled N, B⁰, T, A, B, C, D, E. "N" represented 'charcoal residual', that is, the amount of (^3H)-cortisol not removed by charcoal during adsorption process. 'B⁰' represented maximal binding by the antiserum at zero concentration of non-radioactive hormones; 'T' represented total radioactivity added to tube 'T'

and to all other tubes, A to E are the decreasing concentrations of standard non-radioactive steroid.

The working solutions of antiserum and (^3H)-cortisol were prepared just before the assay was performed and equal volumes of the two solutions were mixed. 200 μl of the mixture was added to tubes 'Bo', 'A' to 'E', the 'sample' tubes, and tubes 'BK', 'R₁₀', 'R₅' and 'P'. 100 μl of the working solution of (^3H)-cortisol and 600 μl of distilled deionised water were added to tubes 'N' and 'T' only. 500 μl of the non-radioactive cortisol standard preparations in vials A to E were added to the corresponding assay tubes 'A' to 'E'. To tube 'Bo' was also added 500 μl of the assay buffer.

The tubes were incubated at 60°C for 10 minutes and then at 30°C for 2-3 hours. They were then cooled in ice water (4°C) for a minimum of 10 minutes. 0.5 ml of the charcoal suspension was added to all tubes except 'T'. The racks were shaken from time to time during the charcoal adsorption process to make the suspension homogenous. After 10 minutes, the tubes were centrifuged at 4°C and at 3000 rpm for 10 minutes. The supernatants were collected in vials to which were added 5 ml of scintillation fluid. Counting was for 2 minutes per vial in a Packard β -scintillation automatic counter model 3390, the efficiency of which was 60% for tritium.

Calculations

The 'charcoal residual' (N) count was subtracted from all counts except 'T'. The binding percentage was calculated as $B_0/T \times 100$. This gave values ranging from 25-37% throughout the assays. The binding in tube 'Bo' was taken as maximal or 100% binding and the binding (B) in all other tubes (Standards A-E, Bk, R5, R10, P and samples) were calculated as percentages of 'Bo' (i.e. $B/B_0 \times 100$). For the standards, the logit transformation of the percent bound was plotted against the decadic logarithm of concentration as proposed by Rodbard et al (1968); and a linearised curve was obtained. The logits for the percent bound in the samples were found and the *cortisol* concentrations read from the graph. A hand calculator, Hewlett Packard HP-25, was programmed for regression analysis; the logarithm of concentration was plotted against the logit. A fast calculation was obtained by using this device (Cekan 1976). Assay values were corrected for recovery by multiplying with the yield factor and were expressed in nanograms/ml. Experiments to determine the reliability of the assay procedures are reported in the appendix.

RESULTS AND DISCUSSIONSI. Physiological responsesResult:Respiratory rates

Mean respiratory rates in the heifers during each quarter are shown in tables 1 and A12. The result of analysis of variance (tables A 13 and A 14) shows that the effect of season, breed and time of the day were individually significant ($P < 0.05$, $P < 0.05$ and $P < 0.01$ respectively). There was no significant ($P > 0.05$) interaction between the season, breed and time of the day effects. From the analysis of variance, season, breed and time of the day contributed well to the total variations that occurred (table A 14). The respiratory rates both in the morning (7 - 7.30 hr) and the afternoon (13 - 15.00 hrs) were significantly ($P < 0.05$) higher in the Holstein than in the Brown which also has significantly higher ($P < 0.05$) readings than in the Fulani.

Mean respiratory rates varied between the different periods of the year with highest afternoon readings occurring in the dry hot months of November through April and the lowest in the wet months of July and August. Lowest afternoon readings during the dry season were recorded in December and early January. Higher morning respiratory rates were obtained during November through April than in July - August; the values in the mornings in March - April were higher than other periods in the dry season. See figure 7.

The percentage change of respiratory rates in the afternoon over the morning low values is shown in table II. There was a significant ($P < 0.05$) difference between the morning and afternoon respiratory rates as shown by the analysis of variance. The difference was less marked in July - August. Panting was exhibited by the Holstein heifers on hot afternoons most especially in the sun before they were brought into the pen.

Rectal temperature

The mean rectal temperatures of the heifers are shown in tables III and A 15. The analysis of variance (table A 16) shows that the main effects, season, breed and time were individually significant ($P < 0.01$). Significant ($P < 0.01$) interactions that occurred were between season and breed and between season and time of day. Time of the day effect contributed most significantly to the total variation that occurred (Table A 17).

Seasonal differences were more noticeable in the afternoon rectal temperatures the values being significantly lower in July - August than in other periods (Table III). There was a small relative rise in the morning rectal temperature in March/April over the values in December/January. See Fig 7 for seasonal values in the physiological responses.

Diurnal changes in rectal temperature were most impressive than seasonal changes. Differences between morning and afternoon

temperatures were higher in November through April than in July - August. Highest diurnal changes were obtained in November. On many days in the July - August period there was little difference ($< 1^{\circ}\text{F}$) between the morning and afternoon rectal temperatures. Both morning and afternoon rectal temperatures were generally lower in the Fulani than in the temperate breeds. Mean afternoon rectal temperatures were generally higher in the Holstein than in the Brown heifers though the difference was not significant ($P > 0.05$). On some hot afternoons fairly high shade temperature $103 - 104^{\circ}\text{F}$ ($39.4 - 40^{\circ}\text{C}$), 103.5°F (39.5°C), and 103°F (39.4°C) were recorded in the Holstein, Brown and Fulani respectively recurring most often in the Holstein than in the Brown or Fulani heifers and very infrequently in the Fulani.

Rectal temperature did not show any rhythmicity through the estrous cycle. Occasionally in some animals, rectal temperatures in the morning of the day of estrus resembled the usual afternoon values, that is, about 102°F or more; this may not be obvious in a composite graph since at other times the values were low (Fig. 8). Elevations were more frequent in the afternoon of the day of estrus when the values might be as high ^{as} 103°F . The value on the day of ovulation was not depressed below usual levels.

Shade seeking

The Fulani heifers did not seek shade throughout the periods of observation (Fig. 9). The result of analysis of variance (table

A 18) of the scores of shade seeking in the Holstein and Brown heifers (table A 19) shows separate significant breed ($P < 0.01$) and season ($P < 0.05$) effects. Heifers of both temperate breeds sought shade during all periods of the year but the frequency was higher in the Holstein. The percentage scores ranged between 80 and 100 and between 20 and 60 in the Holstein and Brown heifers respectively during the hotter more sunny periods (November - April), and between 30 and 50, and 10 and 20 in the same order of breeds in July - August period. The established hierarchical order was brought to play in occupying shaded areas and animals with higher rank often displaced those with lower rank. Generally the Brown heifers easily displaced the Holstein heifers.

The grazing of the heifers was not disturbed and there was no breed difference in the cool weather and especially when cloud cover was considerable. On sunny cloudless hot days, when grazing in the paddock having tree shade, the grazing time of the Holstein heifers lasted about 10 - 20 minutes at a stretch and about 20 - 35 minutes for the Brown.

The animals might move into or out of the shade depending on whether the sunshine was still intense or had reduced due to a temporary cloud cover. When no shade was available in the paddock, the reaction of a Holstein heifer might just be to stop grazing and to stand on one spot, panting. Individual differences occurred in grazing behaviour as some heifers of the same breed stayed longer

than others. Generally the shade seekers did not graze far from the shade. The grazing behaviour of the Fulani heifers was apparently not affected by solar radiation, as they grazed continuously in the sun, covering wide areas.

Only the Holstein heifers exhibited panting behaviour among the breeds. This behaviour was shown mostly in the field either in the sun or in the shade. The typical panting heifers (Fig. 10) stopped grazing, the head was lowered, the mouth opened and there was profuse loss of saliva as well as some nasal discharge. Polypnea was about 120 breaths per minute and above. The rectal temperature of panting heifer was always abnormal, ranging from 104 - 107°F (40 - 41.6°C). Upon getting into the pen polypnea and hyperthermia subsided after about two to four hours.

Discussion

Despite the significant differences in the values obtained in the different periods of observation, the mean rectal temperature responses were within the normal range in the heifers. Both respiratory and rectal temperature responses suggest that the heifers were more comfortable during July - August period than during the rest of the year.

The physiological responses are a reflection of the magnitude of heat load suffered by the animals during the different seasons. Factors which contribute to the amount of heat load experienced by cattle in Nigeria include solar radiation, environmental temperature

and humidity, the geographical and seasonal distribution of which are, in turn, influenced by the south westerly winds (Ojo 1973).

The amount of solar radiation reaching the ground surface is dependent on the amount of cloud cover during different periods of the year. During the July - August period and some other wet months, because the inter-tropical convergence zone (ITCZ) has moved far up over West Africa, much of Nigeria is under the southwesterly winds with the associated heavy cloud cover which limits incoming radiation. Lower direct and indirect solar radiation, therefore, reach the animals. The reverse situation occurring in the dry season when ITCZ moves down to the coastal area brings the country under the dry north easterly winds and the influence of the cloud cover is absent. Since the heifers were outdoors till 11.00 hours or just after, the influence of solar radiation in evoking physiological responses and imposing heat load can not be overlooked. Generally, less sunshine occurred before 11:00 hour in July - August or during the wet cooler months compared to other periods of the year, especially, November through April. A recent report (Romance-Pounce et al 1977) and a part of this present investigation show that responses are greater in unshaded than shaded cattle. Sufficient heat load might have been gained by the heifers outdoors before they were moved into the pen and this might have influenced their afternoon rectal temperatures.

The relatively low environmental temperature occurring in July - August (maximum ET° ranging between 76 and 85 $^{\circ}$ F) (24.4 - 29.4 $^{\circ}$ C) possibly increased the temperature difference between the body and the environment. More heat was, therefore, lost to the environment through radiation and convection. Hence less heat load might have occurred during July - August (Ojo 1973). By the same token, higher environmental temperature in the hot dry season would mean generally more heat load and higher physiological responses. The air temperature was however not higher than the body temperature; and there was, therefore, probably no convectational heating from the wind.

The higher humidity prevalent in July - August would be expected to interfere with the evaporative heat loss and thus impose some heat load and evoke high physiological responses during that period. The observed responses in the present study show that the effect of high humidity was not as stressful as expected. This can be explained by the work of McLean and Calvert (1972). They showed that surface evaporative moisture loss was not disturbed by moderately high humidities at high temperatures because of a continuous process of reestablishment of new vapour pressure gradients between the skin surface and the surrounding air when the vapour pressure of the air was increased. Moreover, in the field, the influence of the wind in the micro-climate, in disturbing continuously the vapour pressure gradient can further cause increased evaporative moisture and, therefore, heat loss.

Ojo (1973) in his report on the possible effect of the environment on cattle in Nigeria, showed that evaporative power of air and consequently evaporative heat loss is reduced in July in areas where high humidities combine with environmental temperatures higher than $80 - 85^{\circ}\text{F}$ ($26 - 29.4^{\circ}\text{C}$) especially in the middle belt of the country. The Ibadan area to the south is not so affected, the maximum air temperature during July - August period $76 - 85^{\circ}\text{F}$ ($24.4 - 29.4^{\circ}\text{C}$) being relatively low. Kibler and Brody (1954) reported that rectal temperatures of cattle do not rise at environmental temperatures below $70 - 80^{\circ}\text{F}$ ($21.1 - 26.6^{\circ}\text{C}$). The closeness of this range to the values obtained in Ibadan area during July - August may also explain the lower diurnal changes in rectal temperatures in the heifers at that time. The influence of exercise in the field and at times in the pen might, however, exaggerate the rectal temperature changes.

Seasonal variation in rectal temperature reported in the heifers in this study is not in agreement with the report by Wolff and Monty Jr. (1974) who found no such seasonal differences in the rectal temperature in non-lactating cows. In that report, only the lowest morning and highest afternoon temperatures were considered. On the other hand, the rectal temperatures considered in this present report were obtained at definite or specific times of the day, (at 07 - 08.00 and at 14 - 16.00 hours) and not necessarily the lowest or highest possible, respectively, in the day. There is a greater

tendency to obtain uniform results when only the minimum or maximum values of the morning and afternoon respectively are considered than when values at a specific time of day are considered for very many days.

The seasonal differences in rectal temperature were, however, not great. The depression in the mean values in July - August was small. The only significant feature was that lower afternoon rectal temperatures were recorded during that period of the year than in the dry hot season. The fact that the animals were not under lactational stress must have contributed to the relatively low seasonal variation in the rectal temperatures, since milking cows have been found to respond markedly to seasonal weather conditions (Berman & Morag 1970; Wolff & Monty, Jr. 1974). Secondly, the fact that the heifers were under shade (in the pen) for a greater period of the 24 hour-day (between 11.00 till 07:300 hours) must have caused the relatively little seasonal differences in the rectal temperatures. Cows in the shade have been found to show lower rectal temperatures than those not in ^{the} shade (Rainey et al 1967; Romance-Pounce et al 1977).

The higher morning rectal temperature in the rainy season may reflect an increase in body heat production due to increased metabolic rate. The commencement of the rains and the associated increase in available pasture and therefore ingested material might have caused an increase in metabolic rate. Increased feeding has been associated

with higher metabolic rate (Robinson & Lee 1947; Rogerson 1966; McDowell et al 1955; Ingraham and Whittow 1961).

It is also possible that the lower rectal temperatures in December/January were partly due to the cold mornings. Moran (1970) found positive correlation between the rectal temperatures and dry bulb temperatures and believed that the varying morning air temperatures influenced the rectal temperatures. Howes et al (1960) also found that cold mornings depressed vaginal temperatures of cattle.

Breed differences in rectal temperature observed in the heifers also agree with some previous reports (Romance-Pounce et al 1976; Sheath and Miller 1947). The lower mean morning rectal temperatures in the Fulani as against the Brown and Holstein could be of thermoregulatory advantage permitting the Fulani to experience greater increases in body temperatures without becoming hyperthermic. Previous studies have also discussed the thermoregulatory advantage of low morning temperatures (Bianca 1959a, 1959b). Bianca (1959b) reported that rectal temperatures were lower in acclimatized than in non-acclimatized zebu calves. Hutchinson and Mabon (1954) also found that zebu cattle reared under temperate conditions had lower rectal temperatures in summer than in winter months. Similarly non-lactating dairy cows had been found to show lower morning rectal temperatures during the hot season than during the cool season (Wolff and Monty Jr. 1974).

The generally low morning rectal temperatures in all the heifers in December - January including November in the Fulani and the Brown may be of similar thermoregulatory advantage by delaying the onset of hyperthermia as also suggested by Bianca (1959b). It may also be signifying a reduction in the metabolic rate in the animals during the dry season.

The amplitudes of the respiratory responses were more obvious and pronounced than rectal temperatures both diurnally and seasonally. Earlier reports have also shown that respiratory rates in calves and cows were more sensitive to heat stress than the rectal temperature (Bligh 1957; Rjek & Lee 1948). The high respiratory responses in November through April is probably due to the generally poor ability of the body to eliminate heat through the skin.

The sensitivity of the respiratory responses to environmental temperature changes was demonstrated by the distinct values obtained during the different quarters, thus indicating the severity of thermal stress during each period similar to the report on non-lactating and lactating cows (Wolff and Monty Jr. 1974; Berman & Morag 1970). The generally higher morning respiratory rates in March - April might be associated with higher morning environmental temperatures which increased more rapidly during that time than at any other period of the year.

The high sensitivity of the respiratory responses to environmental heat is due to the sensitivity of the peripheral and central thermoreceptors which set up impulses that initiate polypnea (Findlay and Ingraham 1961; Ingraham & Whittow 1962).

Because of its high sensitivity to environmental heat, respiratory response has been described as an anticipatory defence mechanism preventing the rise in core temperature and delaying the onset of hyperthermia (Bligh 1957). The high respiratory responses in the Holstein heifers under shade (in the pen) were not necessarily associated with abnormal rectal temperatures. The heifers were therefore maintaining relatively normal to low hyperthermia in the hot weather at the expense of great respiratory effort.

The breed differences in respiratory responses may be reflecting the differences in cutaneous evaporative heat loss including the extent to which the coat characteristics prevented heat gain or interfered with heat loss. The advantage of a light coat in aiding heat tolerance is derived from its being able to absorb less heat and reflect more visible radiation than brown and black coats respectively (Riemerschmid 1943; Bonsma 1943; Bonsma and Louw 1963). The texture of the short and medullated zebu hair further aids evaporative heat loss and has therefore been associated with increased heat tolerance (Hayman & Nay 1961). This attribute of the zebu coat has also been described in the Fulani cattle (Hill 1960; Amakiri 1974). The coat type advantage must have

contributed to the greater heat tolerance in the Fulani over the Brown and Holstein. A greater inadequacy of the sweating mechanism in the Holsteins as against the Brown and the Fulani is also suggested.

The breed differences in shade seeking habit signify obvious differences in heat tolerance. It also suggests that the efficiencies of the evaporative heat loss mechanism and of the coat type in preventing heat load differed between the Fulani, Brown and Holstein heifers decreasing in that order of breeds. Sheath and Miller (1947) and Romance-Pounce et al (1977) have also described the shade-seeking habit in Holstein cattle which, in addition, frequented and lay in wet places. This has been related to low heat tolerance in this breed (Sheath and Miller 1947). The species difference in shade seeking shows the superiority of the Fulani over the temperate types of cattle in heat tolerance.

Hafez (1964) described shade seeking habit as a physical voluntary behaviour of thermoregulation in homeothermic animals seeking micro-climate in the ambient temperature. In this present observation, the animals sought shade from direct solar radiation and not from ambient temperature. The behaviour of the shade seeking heifers shows that the direct solar radiation is capable of causing rapid increases in body temperature. Voluntary behavioural responses to heat might be controlled by the central nervous system (CNS) (Johnson et al 1962; Hammel et al 1960). It is possible that impulses set up

at the thermoreceptors in the skin by the solar radiation as well as at the central thermoreceptors in the anterior hypothalamus due to increased blood temperature initiate motor responses in the CNS causing a withdrawal from the sun into the shade.

The lower frequency of shade seeking during the July - August cooler wet period was due to less sunshine as a result of a more extensive cloud cover. The grazing behaviour was closely related to the shade seeking behaviour. Since shade seeking behaviour commenced at any time from 10.00 hours depending on the severity of incoming insolation, the grazing time of the Holsteins was markedly affected and the total amount of feed consumed could be highly reduced by the long periods spent in the shade. Seath and Miller (1946) similarly found that solar radiation with high ambient temperature depressed grazing time. Since the animals moved out of the shade to graze when clouds temporarily prevented direct solar radiation, the appetite of the heifers was probably not depressed by the environmental heat, although there are reports from studies in the laboratory that feed intake is depressed by high environmental temperatures up to 29 or 30°C in both non-heat tolerant and heat tolerant cattle (Johnson et al 1967; Holder 1960).

The Fulani cattle in their natural environment, are grazed over wide areas in the savannah bush. The dairy stock of this breed from which the experimental heifers for this study were obtained

are also normally grazed over wide areas on the university campus and farm from morning till about 15:30 hours after which they were left outdoors in the paddock close to the milking barn. The ability of the Fulani heifers involved in the present study, to graze for long periods in the sun is therefore typical of the breed.

The ineffectiveness of panting in reducing body temperature is evidenced by the fact that panting heifers showed hyperthermia in the field. Panting might in fact increase body heat production (Thompson 1973). The fact that the Brown and Fulani heifers did not pant shows that they possess greater heat tolerance than the Holstein heifers. There are indications that peripheral thermoreceptors in response to ambient temperature, infra-red irradiation, and heating cause marked increases in respiratory rate (Bligh 1957; Findlay & Ingraham 1961). In the present study, the combined effect of high ambient temperature and solar radiation can be implicated as the cause of panting. A probable poorer thermolytic ability of the Holstein might have caused the observed high body temperature while grazing on the field and this hyperthermia might have further exacerbated panting by stimulating the hypothalamic thermoreceptors. The opened mouth during panting is probably due to increased respiratory tidal volume. The loss of saliva was due to the normal continuous parotid gland secretion, finding an outlet in

the opened mouth. This fluid loss may play some role in heat loss, the magnitude of which is however not known.

Amakiri (1974) found that Holsteins in the Ibadan environment have developed sweat gland characteristics (including increased sweat gland population) suggestive of increased heat tolerance. The physiological responses observed in this present study do not suggest greater heat tolerance in the Holstein than in the Fulani heifers. It is however possible that the large number of sweat glands developed in the adapted Holsteins are not as efficient as those in the Fulani cattle. If the greater surface area per unit weight which Kibler and Brody (1950) found for the Brahman (zebu) cattle over the Holstein is true for the Fulani over the Holstein, the Holstein heifers would be suffering the disadvantage of a relatively lower cutaneous evaporative surface area. This, coupled with the poorer influence of the coat type in preventing heat gain and aiding heat loss, may be overshadowing whatever advantage may be conferred by the improved sweat gland characteristics.

Generally, however, the *Bos taurus* breeds seem to be able to adjust to the changing seasonal weather conditions. The major indication of breed differences in heat tolerance or in the effort of adjustment to the seasons, when the animals were shaded, was the respiratory rate. The large seasonal shifts in respiratory rates in the temperate breeds as well as the relatively normal body temperatures

under high environmental temperatures shows that to a large extent these animals have large capacity to withstand heat under natural tropical climate, especially if shade is provided. The occurrence of occasional hyperthermia in the shade particularly in some Holstein heifers is a poor prognosis for the heat tolerance of these animals at lactational age.

As regards the relationship of rectal temperatures to the different stages of the estrous cycle, the elevation of body temperature on some mornings of the day of estrus is not well reflected in the composite graph, because of the more commonly recorded normal temperatures at that time. This is probably so because animals at estrus can exercise less in the pen than in the paddock. The more commonly recorded elevated temperature in the afternoon of the day of estrus was, therefore, probably due to the increased activity associated with estrus while the animal was in the field.

The present results disagree with some previous reports showing an occurrence of definite cyclic pattern in the body temperatures (Vollan & Vollan 1942; Bane & Rajakoski 1961; Wrenn et al 1958). The distinct depression in the body temperature on day 2 of the cycle coinciding with ovulation, reported by these workers and by Smirnova (1954) was not observed in the present study. The elevation in rectal temperature on the day of estrus observed in the present study agrees with previous reports. It has been suggested that the cyclic pattern

in vaginal or rectal temperature during estrous cycle is due to the effect of circulating progesterone and therefore the activity of the corpus luteum (Bane & Rajakoski 1961; Wrenn et al 1958).

Howes et al (1960) found little evidence of diphasic shift in vaginal temperature of cattle and suggested that the effect of environmental temperature can alter the pattern of the cyclic vaginal temperature changes, since they found that cold mornings also depressed vaginal temperatures particularly in Indian cattle (Zebu). The environmental background for the study conducted by Wrenn et al (1958) was not given. They obtained a diphasic pattern in 90 per cent of the dairy cattle they investigated. To obtain this percentage, the animals must have possibly been maintained under uniform conditions in a closed barn, and not exposed to the field as has been done in the present study and in that conducted by Howes et al (1960).

This test, as applied in humans, perhaps because of the better controlled conditions is more satisfactory than in cattle. Even then in humans, the basal body temperature has been shown to be of very limited value in fertility studies and an unreliable indicator of the time of ovulation (Siegler and Siegler 1951).

The lack of definite diphasic pattern in the rectal temperature of heifers in the present study shows that the influence of ambient factors including management on body temperature overshadows whatever effect circulating progesterone might have. Since in practical dairy

management exercise of cattle in the field can not be avoided, it is not likely that the diphasic change in rectal temperature during estrous cycle would be of any diagnostic value for reproductive biologists working with heifers in the field.

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II. Estrous cycles in heifers

Result:

(A) Pubertal age

The average age at which heifers of the Brown, Holstein and Fulani breeds reached puberty in months were 17.8 (range 16 - 20), 16.7 (range 14 - 19) and 20.3 (range 20 - 25) respectively. The breed difference was significant between the Holstein and Fulani (table IV).

(B) Estrual signs

The sign most often noticed was tail raising as described by Hammond (1927) and Hafez et al (1969). Rapid switching of the tail also described by the same authors as masturbatory rubbing of the perineum was noticed on very few occasions (Figs. 27 and 28).

Vaginal discharge occurred in all cases but was not commonly hanging from the vulva. Some times it was smeared on the buttocks and root of the tail. Intra-rectal manipulations as in ovarian examination at suspected or expressed estrus caused copious discharge of thin clear mucus through the vulva. This was used to detect some heifers showing subestrus especially when the bull's interest was not consistent. The discharge which was clear mucus sometimes also contained white flocculus, commonly in the Holsteins but less frequently in the Browns, on the day of estrus, and was blood tinged on the day after estrus. No growth of pathogenic microbes was observed

when the discharge containing white flocculus was cultured. The phenomenon was therefore not pathological. The flocculus was not noticed in the vaginal discharge of the Fulani heifers. The appearance of dry vaginal mucus was not examined.

The congestion of the vulva during estrus was noticed in only one heifer (B84) and not consistently. There was no obvious gross difference between the appearance of the vulva at estrus and diestrus, unlike previous reports (Hafez et al 1969).

From marks awarded for intensity of estrus (Table A 20) the Brown and Holstein scored higher than the Fulani heifers. Estrous intensity ranged from low to high in all the heifers and showed individual variations. The intensity was generally high in the morning and sometimes late in the evenings but low in the afternoon. It was also highest at the commencement of estrus. Intensity of estrus did not seem to have any relationship with the duration of estrus: a heifer showing low estrous intensity might remain on heat for as long as 20 hours or more. The intensity generally was more related to the weather of the day rather than the season. Estrus of low or high intensity could, therefore, occur in the wet and in the dry seasons.

Mounting behaviour, one of the attributes of estrus intensity, was exhibited more by the Brown and the Holstein and least by the Fulani. Mounting was influenced by the hierarchical order established:

the Browns were highest in the order, and they mounted more heifers in the group. Heifers in the low rank of the order mounted few or no animals since they were repulsed. Estrus however emboldened the heifers in the lower rank to approach and attempt mounting the heifer in the higher rank which they would have normally avoided. The mounting activity in the Fulani was in many cases nothing more than briefly raising of the fore legs from the ground. One Fulani (F23) however exhibited as much mounting as the temperate breeds. Placing of the jaw on the back of another heifer was most common with the Fulani.

A heifer at estrus exhibited general excitement and restlessness as described by Hammond (1927), Hafez et al (1969), Williamson et al (1972). She paced up and down in the pen and disturbed other females. Bellowing was not a common behaviour at estrus. Increased restlessness was noticeable in the Fulani which might also become unusually playful or troublesome in the pen, using her head to push weaker females or any female attempting to mount her.

The heifer on heat, when released into the paddock in the morning, did not graze but concentrated on mounting. Later she only interrupted grazing with mounting. She also accepted the bull frequently. As solar heat become more intense, the animal showed less interest in mounting and might go to the shade. Estrual behaviour might, therefore, stop temporarily.

A group of heifers often stayed around the heifer at estrus to mount her; and during this time she also mounted them. The bull attempted to disperse such other heifers. Only rarely was the Fulani in such a group except when another Fulani was on heat. Usually too, there was also one more dominant (B 84) or more "sexy" heifer (H 137, B 84) in the group that was always around to mount the heifer at estrus pushing other heifers out of the way.

Subestrus was noticed in a total of three heifers and on five occasions: H 13 and B 87 in February, H 13 in June, and B 84 in November of the same year. In subestrus, estrual behaviour was slight, or absent and estrus was only detected by the bull, although the bull showed only little intermittent interest in such a heifer. Estrus was confirmed by vaginal discharge on rectal manipulations.

The BN bull was more active than the WF bull. The BN bull was also more docile and easily led and amenable to training than the more sluggish WF bull. Most of the teasing of heifers was done by the BN Bull which dominated over the WF bull. Although the latter had low libido at the start of the experiment, he improved with time.

The presence of the teaser bulls seemed to aid the intensity of estrus exhibited since the heifer on estrus suddenly became more active when the teaser bull was introduced to her. The bull's interest in the heifer at estrus was diminished when hungry, after many services, or on hot afternoons. The bulls also showed preference

for one heifer or the other when two heifers were on heat simultaneously. A cow on heat was preferred by the BN bull to a heifer on heat at the same time.

The use of a halter with marker was sometimes misleading as the bull rubbed the paint on the body of many heifers, resented wearing it for a long time and some times forcibly removed it. The BN bull mounted more frequently and teased less at the commencement of estrus in a heifer than later during the estrous period. The WF bull did not tease when the BN bull was around but went straight to mount, in fear of being prevented by the latter. The BN bull was also more preferred by the heifers.

Time of onset of estrus

A twenty-four hour distribution of the time of onset of estrus was found in the three breeds as has been represented graphically (Figure II). The chi square test showed that there was no breed difference in the time of onset of estrus, chi square factor being 3.045 at 6 degrees of freedom. The highest percentage 41-48% (tables V & A21) occurred in the morning between 07.00 and 12.00 hours, followed by early morning period 01-07 h. (30.6-38.57%). Few estrous periods commenced in the afternoon till midnight. The times most favoured were between 0.5.0 and 10.00 hours in the morning. The release of the heifers into the paddock in the morning particularly seemed to trigger the commencement of mounting activity and standing estrus.

The analysis of variance of scores through the months of the year (Table A 22) showed that the effect of month on the time of commencement of estrus was not significant ($P > 0.05$). Breed effect was also not significant but the time of the day was highly significant ($P < 0.01$).

Duration of estrus

The percentage distribution of the duration of estrus is represented graphically (Fig. 12). The histogram for each breed is flat. For the Browns, the graph shows that the largest number of estrous periods (54.6%) lasted 15 - 16.00 hours. A few very long estrous periods lasting 27 - 31 hours occurred in some heifers occasionally but most often in B 84. Estrus as long as 30 - 31 hours constitute outliers of the graph. 57% of estrus in the Holsteins lasted from 11 - 16 hours, the 13 - 14-hour duration being more favoured. Outliers also occurred on the graph due to a few estrous periods lasting 29 - 30 hours. As for the Fulani, most estrus (47.5%) lasted 9 - 14 hours. The graph shows a lower flat level after the 14-hour duration due to some estrous periods lasting between 15 - 24 hours. Some outliers also occurred which are 8 estrous periods lasting 27 - 30 hours. The mean duration of estrus were 16.6 (± 5.95 S.D.), 15.9 (± 5.6 S.D.) and 14.5 (± 6.3 S.D.) for the Browns, Holstein and Fulani heifers respectively (Table VI). The difference between

the Browns and the Holsteins was not statistically significant ($P > 0.05$); but the difference between the Fulani and the Brown (table VII) was statistically significant ($P < 0.05$). Longer estrous periods were recorded in the wet season (March through October) than in the dry season (November through February); the difference was not statistically significant ($P > 0.05$) (tables VIII and A 23).

Most estrus ended between afternoon and night. Relatively shorter estrous periods recorded during the dry months, in some animals were probably due to the more intense environmental heat since mounting was less during very hot afternoons when the bull or the heifer might be rather lethargic and less interested. Short estrous periods also occurred in other months varying from one individual to another probably due to the same reason. Sometimes estrus temporarily ceased in the afternoon, and mounting resumed again in the late evening, night or the following morning. Heifers showing estrus in the paddock in the morning usually stopped mounting when the solar heat became more intense especially between 11 and 12.00 hours. When they had just returned into the pen, their time was spent feeding and drinking. Both the bull and the heifer on heat did not perform any mounting at that time. The heifer might allow mounting by the bull in about one or two hours after feeding or after a much longer time had lapsed depending on how hot the day was or how long the animals had been delayed in the sun before returning to the pen.

Ovulation

The mean intervals of ovulation after estrus ceased, with the standard errors, were 14.2 ± 0.6 , $14.7 \pm .6$ and 15.1 ± 0.9 hours in 44, 37 and 49 determinations in the Brown, Holstein and Fulani heifers respectively. The mean approximate time interval from the beginning of estrus to ovulation in these number of cases were 30.9; 29.9 and 30.7 hours in the Brown, Holstein and Fulani heifers respectively. There was no significant difference between the breeds in ovulation time ($P > 0.05$) Tables IX, X and XI).

Generally after a long estrus ovulation occurred within a short interval of 6 to 10 hours (table A 24). The total number of ovulations observed was 137. On eight occasions ovulation was not confirmed until developing corpora lutea were identified on days 4 and 5 of the estrous cycle. Out of the 129 ovulation that were timed, 105 (81.4%) occurred before or on the day following estrus, 22 (17%) a day after and 2 (1.5%) on the subsequent day. There was no seasonal difference in ovulation time (table XI).

Rhythmicity of estrous cycles

Heifers utilised for this study cycles all the year round. A total number of 108, 92 and 105 estrous cycles were considered during a year period in the Brown, Holstein and Fulani heifers respectively. Mean cycle lengths in days, with the standard errors, were 21.0 ± 0.3 , 20.1 ± 0.2 and 21.4 ± 0.2 in the order of the breeds (tables XII, XIII, XIV and XV).

The differences between the Holsteins and the Fulanis, and between the Browns and the Holsteins were statistically significant ($P < 0.05$). 95, 93.4 and 94.5 per cent of all the cycle lengths fell within the 18 - 24-day range in Brown, Holstein and Fulani heifers respectively. More estrous cycles shorter than 18 - 24-day range were recorded in the Holstein than in the Brown and the Fulani heifers, the scores being 6.5, 0 and 2.2% respectively. Estrous cycles longer than 18 - 24-day range are regarded as long and the Fulani scored higher than the Brown and the Holstein, the frequencies being 7.6, 3.5 and 1.3% respectively. The longest cycles recorded in the Brown, Holstein and Fulani heifers were 30, 28 and 29 days respectively.

The percentage frequency distribution of estrous cycle lengths are presented graphically (fig. 13). From the graph, estrous cycles in the Browns ranging between 20 - 22 days long showed the highest frequency. There was a gradual fall from 23-through 26-day frequencies and an outlier of 30-day length. As for the Holsteins, a peak occurred at 21 day frequency; there was a gradual increase in frequencies from 17 to 21 days after which a sharp fall occurred till the 24-day estrous cycle length. The graph for the Fulani heifers shows that estrous cycle lengths were mostly of the 20-day to 22-day durations. A smaller number lasted 23 - 25 days. A few cycle lengths, lasting 27 and 29 on the long side, or 16 to 18 days on the shorter side, tend to broaden the graph. In the Fulani, the mean estrous cycle length in days, with the standard deviation, in the wet season (21.8 ± 2.2) was

greater than in the dry season ($21.2 \pm .9$), but the statistical significance is quite low ($P = 0.05$). The values in the Browns, 21.3 ± 2.2 and 21.1 ± 1.4 days and in the Holsteins, 20.1 ± 1.5 and 20.6 ± 2.2 days, in the same order of the seasons, showed no significant seasonal difference ($P > 0.05$).

One Brown heifer (B1) had a peculiarly different pattern among the exotic breeds. The long cycles recorded in the Browns were actually all recorded in that particular animal. In that animal occurrence of 22-day cycles in December - January in the dry season was followed by 25 - 26-day cycles in February to March before the rains. She showed shorter cycles (21 - 23 days) from April till September; during October to November she showed long cycles of 26 - 30 days after which shorter cycles were shown again in December - January. One Holstein heifer showed a long cycle of 28 days in June in the wet season, but this was not repeated.

In the Fulani, most of the longer estrous cycles (25 - 27 days) occurred towards the end of the rains and at the beginning of the dry season, followed subsequently by shorter cycle lengths (19 - 23 days) through the dry season. There was a peculiar case in which one heifer (F 23) showed many estrus cycles ranging between 25 - 29 days from time to time through both seasons. Her longest cycle (29 days) occurred in the wet season.

Mid-cycle heats were recorded on five occasions in a total of four animals: H 148 on day 8 of estrous cycle (in December), B 91 on day 14 (April), F 23 on day 20

of 27-day cycle (January) and on day 9 (in June). They were standing heat in two cases (B 89 and B 91). A mature C.L. was palpable on one ovary in all cases.

Discussion

The *Bos taurus* heifers observed commenced cycling at a late age compared to a range of 5 - 15 months reported by previous workers under temperate conditions (Hammond 1927; Reynolds et al 1963; Wiltbank et al 1962). The delayed puberty may be caused by environmental heat stress as suggested by Vincent (1972). Inadequate nutritional level may also be a contributing factor.

The pubertal age recorded in the White Fulani heifers confirms the belief that the zebu cattle attain puberty much later than the *Bos taurus* cattle (Reynolds et al 1963; Plasse et al 1968). The pubertal age in the zebu cattle studied is, however, lower than what obtains under the range system of management reported by Johari and Talapatra (1957), Amble et al (1958) and Jochle (1972). The nutritional level is lower under the range system and is prone to mineral deficiencies compared to the semi-intensive system to which the experimental animals were subjected for the purposes of the present investigation; and this might have contributed to a relatively early onset of puberty in the Fulani heifers.

The high percentage of estrus detected in the heifers (100%) agrees with the reports by Donaldson (1968) and Wagnon et al 1972. Estrus not well expressed and termed subestrus might have been missed

but for the presence of the bulls and the rectal manipulations of heifers suspected or expected to be at estrus. The combination of different methods of detecting estrus employed in this study was responsible for the high percentage scored. Donaldson (1968) was able to detect 90% of heat with observations only at 07:00 and 16:00 hours only; the percentage increased to 100% when observations were increased to thrice on cows run with bulls carrying markers. It could have been possible to miss estrus in one Fulani (F20) but for the presence of the bulls and the continuous observation since she showed low estrous intensity. The combination of methods employed in the present study may however be too rigorous to be recommended for dairy farms.

Generally estrus was quite recognizable in the Fulani cattle. This was unlike the report by Anderson (1936) that estrus was scarcely recognizable in the East African zebu. It seems that the present results agree with the observations by Rakha et al (1970) with respect to estrual behaviour in African zebu cattle. The intensity of estrus observed in the zebu in the present study however, is higher than what the quoted authors observed. There was, in fact, no difference in the expression of estrus by two Fulani (F21 and F23) and the *Bos taurus* heifers.

It does not seem that the environment has altered the estrual behaviour in the *Bos taurus* heifers. The low estrual expression in the afternoon on hot days may just be an avoidance of exercise which might increase body heat load. A depressant effect of hot weather on estrus has previously been observed by Wolff & Monty Jr. 1974.

Mounting behaviour by the heifer at estrus and standing to be mounted by other heifers or by the bull were the most definitive estrual signs in the heifers examined. Mounting activity among^α group of heifers was quite indicative of an estrual animal among the group, as has been previously reported (Hammond 1927; Hafez et al 1969; Williamson et al 1972).

Most of the other signs of estrus described by previous workers were not exhibited consistently and were less reliable than mounting behaviour. A vaginal mucus flow can be quite useful in detecting heifers at estrus. It could particularly be useful in animals showing only slight estrual behaviour or when two heifers are on heat simultaneously and are both in the same group of mounting heifers. Since the heifer showing estrus also fed freely, like others while in the pen, anorexia was not particularly associated with estrus. During estrous period the interruption of grazing with mounting while in the paddock, however, meant that heifers might consume less grass at that time (fig. 28). In subestrual cases, a low level of circulating estrogen may be responsible for the low expression of estrus. The incidence was low involving only 17% of the heifers. The frequency (1.5%) of occurrence

of subestrus was also low and causal factors can not be identified. It was however not season dependent.

The economic importance of subestrus lies in the fact that in a dairy herd which is not run with a teaser bull, the subestrual animals can easily be unnoticed and so escape being bred. This will result in unduly prolonged calving intervals.

The distribution of the time of onset of estrus through the 24-hour day in the present study is similar to some previous reports. The greater concentration of onset of estrus between 05.0 a.m. and 12 noon is however slightly different from the reports on *Bos indicus* by Plasse et al (1970) with concentration at 4 a.m. to 6 a.m., 10 a.m. - 12 noon and 6 p.m. to 8 p.m. and the concentrations at sunrise and sunset observed by Rakha et al (1970).

Although no definite cause can be ascribed to the greater concentration of onset of estrus in the morning hours in the present study, the lower ambient temperature at that time of the day may be a predisposing factor. The release of the heifers into the field in the morning also seemed to trigger off mounting. The lack of significant effect of the month of year on the time of onset of estrus suggests that day length and season do not influence the time of onset of estrus.

The concentration of onsets of estrus at a time from very early to late morning hours means that the majority of estrus that occurred in a herd in this environment can be detected when farm

workers are on morning duty. The belief that zebu cattle are mostly on heat and copulate in the night (Anderson 1936; Roberts 1971; Rollinson 1962) is not borne out by the present result.

Since a proportion of the estrus also commenced in the evening, a twice daily check on the animals would be recommend for dairy herds in this environment. These times should be between 05.00 and 10.00 and between 17.00 and 20.00 hours in the morning and evening respectively in order that a very large percentage of animals that come on heat may be detected.

The range of the duration of estrus (8 - 31 hours) observed in this study agrees with the range (6 - 30 hours) reported by Hammond 1927 in European cattle under temperate climate. Generally, estrous periods as long as 20 hours were, however, not very many in the present study, constituting 44% of the total timed estrous periods.

The results here show that the zebu (Fulani) cattle show much longer estrus than 4.7 hours reported by Anderson (1944) for east African zebu, and 6.7 hours reported in Brahman heifers (Plasse et al 1970). The present result agrees with the reports in which estrous periods ranging between 12 - 24 hours were recorded in *Bos indicus* (Rollinson 1955; Villacorta 1960). Rakha, et al (1970) also reported that zebu and some other African breeds showed long estrus in Central Africa with a mean of about 16 hours, thus agreeing with the present observations. The observed long estrus in the Fulani in the present

study is particularly remarkable in that it negates the previous assumption that zebu cattle in this environment might be showing very short estrous periods.

Previous climatic laboratory reports have shown that the estrous period is shortened by exposing temperate types of cattle to hot environment (Branton et al 1957; Gangwar et al 1965). Normal duration of estrus was not regained after heifers had reestablished cycling in a hot environment that had caused temporary anestrus (Bond & MacDowell 1972). The present result shows that the shortened duration of estrus in cattle in the field due to environmental heat is not a permanent defect as the report by the latter authors tends to suggest. In the field, the effect on estrual expression of the environmental heat and particularly solar radiation may not even last much longer than the actual period of exposure.

The present result also does not agree with an earlier observation that estrus was hardly detectable in temperate breeds of cattle in hot weather (Poston, et al 1962). It shows some similarity to that of Wolff and Monty Jr. (1974) in which there was no significant difference between the duration of estrus in cool and hot seasons in non-lactating *Bos taurus* cows. An occurrence of estrus as short as 8 to 12 hours or as long as 20 hours or more, depending on the animal and the ambient condition of the day, and in both dry and wet seasons, suggests that the influence of the day is more important than that of the season in determining estrous period length in the field.

Direct solar radiation may be an important cause of the depression of estrual behaviour in some afternoons. The fact that the animals had to spend most of the afternoons in the shade of the pen has aided the prolonged estrous periods. It is most likely that exposure of a heifer at estrus to direct solar radiation continuously throughout the day may considerably shorten the estrous period. Keeping cows in the shade in a hot natural environment has been shown to improve fertility (Wiersma & Stott, 1969). By cutting off direct solar radiation which may impose severe heat load on the animals and which may therefore cause a depression of estrus, shade structures allow the prolonged expression of estrus which may increase the chances of the heifers being detected and bred at estrus artificially or by the bull.

Generally only an estimate of the time of ovulation was possible in view of the time intervals of ovarian examination which might have been responsible for the ovulation interval being longer than what was obtained (10.5 hours) in a much earlier report (Trimberger 1948). Since the first ovarian examination just a few hours after the end of long estrous periods revealed ovulation, it is possible that ovulation occurred before the end of some long estrous periods.

The present result, by showing no seasonal difference in ovulation, supports the reports by some previous workers that hot season did not adversely affect ovulation (Hall et al 1959; Plasse et al 1970; Wolff and Monty 1974). Spring and summer conditions have

been associated with reduced occurrence of abnormal ovulations in Friesians, Afrikaner crosses, and Afrikaners in South Africa (Van Rensburg and De Vos 1962). The relatively high ambient temperatures prevalent throughout the year in the sub-equatorial environment where the present study was conducted have been propitious for normal ovulations rather than being inhibitory.

Spermatozoa can survive for 15 to 24 hours in the reproductive tract of the cow after insemination (Krillov 1937; Andrew 1937). In view of the time of ovulation obtained in the present study insemination of heifers towards the end of estrus would ensure that ovulation coincides with the time when spermatozoa are present in the reproductive tract of the female, and hence a high conception rate may be achieved.

Hot conditions have been associated with reduced release of L.H. in cattle (Madan and Johnson 1972; Miller and Alliston 1974). This condition may be expected to affect ovulation. Since a large percentage of ovulation occurred before or on the day following estrus in the heifers examined, it is probable that pituitary L.H. release may not be adversely affected by the climate of the region where the present investigation was conducted.

The lack of breed differences in ovulation suggests that the ovulation process in *Bos taurus* cattle is not differently influenced by tropical climate from that in zebu cattle (Fulani). Van Rensburg and De Vos (1962) similarly found no breed differences in ovulation between the Afrikaners, Afrikaner crosses and Friesians in South Africa.

The result shows that variations may occur in the length of successive estrous cycles within and between breeds of cattle. The number of estrous cycles shorter than 18 days (2.8%) obtained in the heifers fall within the range (2.2 - 6.8%) in reports reviewed by Rollinson 1962; but cycles longer than 24 days (4.0%) were less than 13% given in that same report. The average lengths of estrous cycles in the *Bos taurus* heifers observed are similar to those previously reported (Trimberger and Hansel 1955; Hansel and Trimberger 1952; Asdel 1949). Results obtained in the Fulani heifers are also similar to some reports on zebu cattle (Villacorta 1960; Rakha et al 1970; Plasse et al 1970); the mean interval of estrus was, however, a little longer in those reports than in the present result.

Previous studies in the laboratory by Bond et al (1960), Gangwar et al (1965), and Bond and McDowell (1972), show that heat stress causes only temporary anestrus in *Bos taurus* cattle. This is prognostic for the continuous cycling of that type of cattle acclimatised to a tropical environment. The heifers observed could adjust as the seasons changed especially in an environment with relatively high ambient temperatures throughout the year.

The present result showing no seasonality in the sexual activity of zebu cattle does not corroborate previous reports that zebu show greater preference for the dry season for breeding (Wilson 1946). There is also no indication that the animals are influenced by

photoperiod in their sexual activity, unlike the postulate by Anderson (1948). It has been suggested that high ambient temperature is stimulatory to fertility in zebu or other African types of cattle (Thompson 1973; Jochle 1972; Steinbach & Balogun 1971; Wilson 1946). Probably, this meteorological factor has contributed to the regular recurrence of estrus in the zebu heifers throughout the year, thus agreeing with the report of Anderson (1948) that high temperature is stimulatory to sexual rhythms.

It is possible that the semi-intensive management to which the animals had been subjected for the present experiment, which is different from the range system by which zebu cattle are naturally maintained, had also contributed to the maintenance of regular estrous cycles during the dry season. There are indications that low nutritional levels can depress sexual rhythm and conception rate in *Bos indicus* (Rakha, et al 1970; Carneiro 1950). The seasonal peaks in breeding and conception rate in zebu herds under range management reported by the various authors quoted above, may be an ecological adjustment to increased breeding late in the dry season or early rainy season so that parturition will occur in the dry season or close to the rainy season but not so much in the rainy season. This avoids exposing young calves to high parasitic challenge. In spite of such adjustments under range management zebu cattle are shown by the present study not to show a preference for any particular period of

the year for increased sexual activity. The same is also true for the temperature breeds of cattle.

However, because of the problem of feeding during the dry season, it is advisable to regulate the breeding of cattle in the environment of the present study. Calving can be made to occur during dry the season when the calves would depend solely on milk and concentrate feeding. By the time the rains start and the grasses grow, the digestive system of the calves would be mature enough to cope with roughage. Calves can then utilize the pasture together with the concentrate supplement and maintain rapid growth through the rainy season. Thus, a steady rapid growth can be achieved in calves during the first year of life.

The cause of mid-cycle heat observed in the present study is not known. Wagnon et al (1972) found that the difference in the occurrence of mid-cycle heats or "short estrous cycles" between the stressed and unstressed cattle was not statistically significant. The stresses applied were managerial, including transportation, branding with hot iron and introduction to new environment.

From previous reports, it has been shown that a wave of follicular growth occurs in cattle ovaries from days 3 to 4 of the estrous cycle (Coles 1930; Hansel 1959; Rajakoski 1960). The largest of these follicles persists till mid-cycle before undergoing atresia. Large follicles (14 - 15 millimeters in diameter) have been found from the 8th to 13th day of estrous cycle in the ovaries of cows. Probably, the

estrogenic secretion from such follicles at their mature size at mid-cycle is sometimes high enough to cause behavioural estrus, and, even, standing heat observed in heifers in the present study. Wagnon et al (1972) found that two consecutive short estrous cycles were equal to one normal cycle and this agrees with the present observations. Although the frequency of occurrence of mid-cycle heat is very low, the implications in a breeding programme can be important. Cattle bred at mid-cycle estrus can not conceive since estrus is non-ovulatory and this can result in labour loss and waste of material in an artificial insemination programme.

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III. Plasma cortisol concentrations in heifers during estrous cycles and different quarters of the year

Results

The composite curves of plasma cortisol concentration during estrous cycles (Figs 14, 15, 16, 17) were broken before proestrus because of the differences in the number of estrous cycle days within breeds. For convenience of numbering, the day of estrus is referred to as day "0".

Plasma cortisol concentration in the heifers in the majority of cases ranged between 1.0 and 10 ng/ml. Marked occasional elevations in individuals are masked in the composite graphs. The graphs show that plasma cortisol levels fluctuated throughout the estrous cycle. Elevated levels or more pronounced fluctuation occurred around the day of estrus in individual heifers. In a few cases, however, the level on the day of estrus was not different from those of the other days of the cycle. Elevations might occur as early as three days before estrus. Plasma cortisol levels on the day after estrus, about ovulation time, was often lower than that on the day of estrus, though it was also high in a few cases. Lower levels and fluctuations in plasma cortisol occurred during diestrus especially about the middle of the estrous cycle. Such lower levels were however interrupted by occasional elevations. The fluctuations in the plasma cortisol

concentration were less marked and levels were generally lower during estrous cycles in November through the middle of January than in other periods of the year.

Analysis of variance (Table A 27) shows that the value on the day of estrus (mean of 8.9 ng/ml) for all the heifers through the year is significantly different ($P < 0.01$) from the mean for the cycles (5.8 ng/ml). The mid-cycle value (5.5 ng/ml) was not significantly different from the mean ($P > 0.05$).

The mean plasma cortisol levels in individual animals during the different periods of the year, and the mean for each breed are in tables A 25, A 26 and XVI. Analysis of variance (tables A 28a and b) shows that the effect of season on plasma cortisol level is significant ($P < 0.01$). Significantly higher levels ($P < 0.01$) of plasma cortisol were obtained in July/August (6.5 ng/ml) and March/April (6.1 ng/ml) periods. Analysis of variance also shows that there was no significant ($P > 0.05$) breed effect on plasma cortisol values. The grand means of plasma cortisol for the Brown, Holstein and Fulani heifers in the year were 5.9, 5.8 and 5.7 ng/ml respectively. Breed x season interaction was also not significant ($P > 0.05$).

The pattern of change in the plasma cortisol levels through the seasons was slightly different among the breeds. The relative rise in the mean levels during the March - April period in the Brown and Holstein did not occur in the Fulani in which the rise occurred only in the July - August period (fig. 18).

Discussion

Generally the values of 'basal' plasma cortisol concentration agree with the values obtained in cattle by competitive protein binding method in previous studies: for example, a mean of 2 - 5 ng/ml with elevations up to 25 ng/ml at estrus in heifers, a range of 1 - 5.4 ng/ml basal levels in heifers, and a mean of 8.04 ng/ml in steers reported by Gimenez et al (1974), Satterlee et al (1977), and Abilay, Mitra and Johnson (1975) respectively. A previous study using radioimmunoassay also obtained a similar range of basal plasma cortisol levels (3.6 - 10 ng/ml) in heifers (Dobson & Kancher 1977).

In the present study, the levels of plasma cortisol in the heifers are, however, quite low compared to those in a previous study in non-lactating cows using similar method of blood collection (Venkataseshu and Estergreen 1970). The cows in that study were not accustomed to handling and venipuncture. Previous studies have shown that low plasma cortisol levels can be obtained in sheep that are trained or adapted to the procedure of handling and venipuncture (Linder et al 1964; Basset and Hinks 1969, McNarthy and Thurley 1973). The adaptation is believed to be controlled by the central nervous system. In the present study the value of training animals was reflected in the low levels of plasma cortisol. The present study shows that such an endocrine study requiring repeated venipuncture should involve a period of training for the animals, and procedures should be uniformly continued throughout the duration of the study. The handler must

as much as possible not be changed and must use the same technique every time. The restraint period should be as short as possible as has also been indicated in other studies (Heitzman et al 1970; Gimenez et al 1974; Lee et al 1976).

The present results show that 'basal' plasma cortisol levels in the heifers were slightly but significantly higher during the wet months than during the dry hot months. In a few previous field studies, it has similarly been observed that plasma cortisol levels in cattle were depressed by seasonal heat (Stott and Wiersma 1971; Lee et al 1976). Excretion of 17-ketogenic steroids had similarly been found to be reduced in sheep in summer (Robinson and Morris 1960).

Seasonal differences in environmental temperature were less marked in the geographical area of the present study than in those of the previous studies quoted. The relatively low ambient temperature in July and August is however distinct when compared to other months of the year. It is possible that homeostatic mechanisms in tropical animals are sensitive to the relative seasonal changes in ambient conditions including environmental temperature just as animals in temperate and cold regions can be sensitive to the alternating cold and hot seasons. The lower adrenocortical function in the heifers during the dry hot months in the present study may, to some extent, be due to the depressant effect of heat.

In a previous study, it was found that adrenocortical function in sheep was increased under cold wet conditions (Panaretto and Vickey

1970). The wet and cooler conditions might also have been responsible for the slight increase in adrenocortical function during the rainy season in the present result. Future studies may explain the mechanism of such a phenomenon. However, the slight lowering of adrenocortical function in the dry hot months in the present study may be a form of thermoregulatory adjustment to reduce metabolic heat load due to circulating cortisol, since this hormone has been shown to be thermogenic (Yousef & Johnson 1967). This phenomenon has been described as protective (Yousef & Johnson 1967; Abilay, Mitra and Johnson 1975).

Since haematocrit values were not decreased in the hot dry season (table A 1D) the reduced levels of plasma cortisol during the dry season can not be attributed to haemodilution. Since Christison & Johnson (1972) have shown that in cattle, the release of cortisol is depressed by heat and that the metabolic clearance is constant, the seasonal changes in plasma cortisol in heifers in the present study may not be attributable to changes in metabolic clearance but to variation in the rate of functioning of the adrenal cortex. As part of the present investigation, high adrenocortical responses to exogenous ACTH both in magnitude and duration were found in the heifers, suggesting that the magnitude of response by adrenal cortex may be proportional to the level of circulating ACTH. Seasonal alteration in the level of circulating ACTH rather than changes in

adrenocortical responsiveness, or actual depression of adrenal cortex by heat per se, may therefore be responsible for the seasonal difference in adrenocortical function in the heifers. Further experimentation demonstrating seasonal levels of ACTH in heifers is suggested.

The fluctuations in the level of plasma cortisol from day to day in the heifers agree with previous reports (Sprague et al 1971; Arije et al 1971; Gimenez et al 1974; Abilay et al 1975). The more exaggerated responses occurring on the day of estrus or around estrus have also been similarly observed in some studies (Arije et al 1971; Gimenez et al 1974; Shaw, Dutta and Nichols 1960).

The fact that a sharp increase in plasma cortisol was not observed on every morning of the days of estrus may suggest that increased cortisol release did not occur continuously through the day of estrus. Blood samples might have been taken when the plasma cortisol levels were still low, preceding or after the rise. Perhaps more blood samples taken at other times of the day of estrus would have indicated the increase in plasma cortisol level as was observed in a previous study (Gimenez et al 1974). The elevation on the day of estrus was however found to occur both in the morning and afternoon samples in studies in a climatic laboratory (Miller and Alliston 1974). The last three quoted reports and the present study do not agree with the result of Abilay et al (1975) in the psychrometric chamber with

respect to the level of plasma cortisol on the day of estrus which was found to be lower than other days of the cycle in the latter report.

The elevated plasma cortisol level around estrus might in part be due to increased activity and excitement in the heifers about that time. If this is true, elevation of plasma cortisol concentration around estrus would hardly be avoidable in heifers in the field. Some other factors influencing the hypothalamic-pituitary-adrenocortical function may also be involved, and thus make the elevation more pronounced since similar reports had been obtained in restricted cattle in the reports quoted above.

If the finding by Sandberg and Slaunwhite (1959) that estrogen increases the amount of cortisol bound in the blood of man is true for cattle, a reduced feedback effect of cortisol at the pituitary level would be expected to occur during the estrogenic phase of the estrous cycle since less free cortisol would be in circulation. This may result in a general increase in plasma cortisol concentration around estrus. Increased estrogen just before or at estrus might also have stimulated increased ACTH since such effect of estrogen had been reported in rats (Gemzell, 1952).

It is not known if elevated plasma cortisol before and at estrus has any effect on corpus luteum similar to the suppressing effect on corpus luteal development observed when ACTH was administered from days 2 through 8 of the estrous cycle (Brunner et al 1969). The role of

plasma cortisol at estrus may be luteolytic as suggested for plasma cortisol in cows near term (Adams and Wagner 1970).

Since ovulation and expression of estrus occurred normally, increased circulating cortisol around estrus probably did not disturb the release of pituitary and gonadal hormones in the heifers. This is contrary to the observation in rats and cows injected with exogenous glucocorticoid in other studies (Baldwin and Sawyer 1974; Liptrap 1970).

A relatively low level of plasma cortisol during many days in the luteal phase of the estrous cycle may suggest a lowered adrenocortical secretion during the luteal phase. This is not similar to the results obtained by Abilay, Johnson and Madan (1975). It is however similar to the results obtained by Gimenez et al (1974) except that the present result shows relatively more elevations during the luteal phase. In the latter previous report, plasma cortisol levels were generally low during the luteal phase perhaps because the animals were restricted. Low adrenocortical secretion also occurs in cows during pregnancy, a luteal period (Heitzman et al 1970; Adams and Wagner 1970; Arije et al 1971). Low adrenocortical function during an active luteal stage may be desirable because of the suppressive effect of cortisol on corpus luteal function (Brunner et al 1969; Adams & Wagner 1970).

The generally lower levels of plasma cortisol over the estrous cycles during the drier hot months than during the wet cooler months may be an expression in the field of a similar phenomenon observed in heifers kept under constant high or circadian high and low temperatures of psychrometric chambers in two separate studies by Abilay, Johnson & Madan (1975) and Miller and Allison (1974) respectively. The role of such lowered adrenocortical functions on fertility has not been defined. Monty Jr. and Wolff (1974) suggested that it may not be the cause of reduced fertility in cows in the hot Arizona summer since injections of dexamethasone at the beginning of the breeding season did not improve fertility.

Higher circulating plasma cortisol concentrations during the wet and cooler season may imply an increased catabolic effect of cortisol on tissue protein and increased mobilisation of amino acids for gluconeogenesis (Ray 1968; Burkey 1973). Tissue protein catabolism during that time of the year may, therefore, effect a lowering of net weight gain. However, the terms 'low' and 'high' used in this discussion for the values of plasma cortisol obtained during the different quarters are only relative. Really high values that could cause marked metabolic problems did not occur. In fact the greatest weight gains in the heifers during the year were obtained in the wet season and tended to coincide with the rainfall peaks.

Low plasma cortisol level is also believed to occur in ruminants during drought as a result of low nutritional level and thus indicating low metabolic rate (Macfarlane 1968). The lower plasma cortisol concentration in the heifers during the dry hot months may therefore be suggestive of a lower metabolic rate during that period. A reduced performance associated with the lower metabolic rate was probably shown in the reduced body weight or weight gains in the heifers during November through January in the present study.

The onset of the rains in March through the rainy season was accompanied by growth of lush grass. This also resulted in increased grazing and growth rates, which could have been associated with increased metabolic rate and a general increase in adrenocortical function.

The mechanism by which cortisol increases the body resistance to disease is not fully known (Burke, 1973). It is however possible that increased adrenocortical function might increase the resistance of the animals to the stress of parasitic infestation and high numbers of biting insects prevalent during the wet season.

These results show that temperate-evolved cattle, once acclimatized to a tropical environment, may not necessarily have to maintain adrenocortical functions conspicuously different from local cattle, particularly when not under any stress of production. However, more investigations are still needed to explain the

mechanism involved in the role of adrenocortical hormones in homeostasis. More definite explanation for the seasonal variations in adrenocortical function in heifers under tropical condition can only emanate from such further work.

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IV. Diurnal and circadian changes in physiological responses and plasma cortisol concentration

Result

Respiratory rates and rectal temperatures

During the period when the physiological responses were recorded, air temperature varied from low morning values to peak values in the afternoon at about 16.00 hours. Declines in air temperature occurred from about 17.00 through 07.00 hours when the lowest values were recorded. The lowest relative humidities were also recorded at about 16.00 hour. Sunshine occurred before 09.00 or 10.00 hours and was very high before mid-day till about 16.00 hours.

Responses in heifers in the sun

Mean readings of rectal temperatures and respiratory rates are in tables XVII and A 29. The results of analysis of variance of the readings are in tables A 30 and A 31. See also fig. 19. Mean rectal temperatures ranged from low morning values of 100.0, 101.0 and 100.8^oF to high afternoon values of 103.2, 106, 102.8^oF, in the Brown, Holstein and Fulani respectively. Analysis of variance (table A 31) shows that time and breed effects on the rectal temperatures were each significant ($P < 0.01$). The difference between readings at 07.00 hours and other times of the day was significant

($P < 0.01$) but there was no significant difference between the readings at 11.00 hour and subsequent times till 18.00 hours. Thus, rectal temperature rose sharply to a maximum reading at 11.00 hours or at a later time. The significant ($P < 0.01$) breed effect was due to the abnormally high reading in two Holstein heifers (104.4°F (40.7°C) in H 145, and 106°F (41.1°C) in H 154) compared to the highest readings in the Brown (103.2°F (39.5°C) and the Fulani (102.8°F (39.3°). There was no significant ($P > 0.05$) difference between the Brown and Fulani in the rectal temperature change, but the Holstein was significantly different ($P < 0.01$) from these other two breeds in this respect. However, analysis of variance shows that the time of the day contributed more (60%) to the total (72.5%) variations that occurred than did breed effect (10%).

Mean respiratory rates ranged from low morning values of 26.8, 38.6 and 16.7 to high afternoon values of 50.1, 92.2 and 28.8 breaths per minute in the Brown, Holstein and Fulani heifers respectively. Analysis of variance (table A 30) shows that time ($P < 0.01$) and breed ($P < 0.01$) effects on respiratory rate were significant. Time and breed interaction was also significant ($P < 0.01$). By 11.00 hours, the Holsteins had started panting and this continued right into the afternoon when breathing rates as high as 110 per minute were recorded. Breathing rates in the Brown and Fulani were also elevated but not as much as in the Holsteins; and afternoon values ranged

from 58 to 68, and from 26 to 44 in the Brown and Fulani respectively. By 18.00 hours a little decline in respiratory rates occurred.

Correlation analysis (table A 32) shows that rectal temperatures ($P < 0.01$) and respiratory ($P < 0.01$) rates were significantly correlated with the environmental temperature, the correlation coefficients being 0.7 and 0.5 respectively. Rectal temperatures were also positively correlated with the respiratory rates in the different breeds, the correlation coefficient being 0.8, 0.9 and 0.7 for Holstein, Brown and Fulani respectively, and these were significant ($P < 0.01$). Using the Iberia Heat Tolerance test (IHT) on the mean rectal temperature at about 16.00 hour (ambient temperature of about 90°F) the heat tolerance coefficient for the Brown, Holstein and Fulani heifers were 85.2, 70.5 and 87.6. The lowest individual scores were 66 and 50 in Holsteins H 145 and H 154 respectively.

Physiological responses of heifers under shade

The mean rectal temperatures and respiratory rates, with the standard errors, in heifers kept in the shade are in table XVIII. The result is also graphically represented in fig. 20. Analysis of variance shows that the time effect on rectal temperature was significant ($P < 0.01$), but breed effect was not ($P > 0.05$). Time of the day effect contributed more (39.9%) than breed effect (3.6%) to the total variation (43.3%). The afternoon rectal temperatures were not abnormally elevated.

Analysis of variance shows that time ($P < 0.05$) and breed ($P < 0.01$) effects on respiratory rate were significant. There was, however, a significant ($P < 0.01$) breed x time interaction. Breed contributed more effectively (53%) than time of the day (31%) to the total variations that occurred (83.3%).

Correlation analysis shows that respiratory rate in the shade was positively correlated with the rectal temperature, the correlation coefficient being 0.38, 0.47, 0.48 in Brown, Holstein and Fulani heifers respectively and these were also significant ($P < 0.05$, $P < 0.01$, $P < 0.01$ respectively).

The results of analysis of variance comparing the readings of respiratory rate and rectal temperature in the shaded with unshaded heifers are shown in Table A 34, A 35 respectively. They show that the type of treatment (shade or sun) has significant ($P < 0.01$) effect on the rectal temperature and respiratory rate. The readings in the heifers when not shaded were significantly ($P < 0.01$) higher than the reading when shaded. As regards respiratory rate, significant ($P < 0.01$) two-way interactions were breed x time of the day, breed x type of treatment and time x type of treatment.

With regard to rectal temperature, time of the day was more effective (37%) than type of treatment (10%) or breed (7%) in contributing to the total variations (54%). On the other hand, breed effect contributed most (52%), compared with time of the day (27%) or type of treatment (5%), to the total variations (83.5%) that occurred in the breathing rates.

Circadian rectal temperature and respiratory rate in the shade

The mean values of the rectal temperature and respiratory rate are in table XIX. From the analysis of variance, time and breed had separate significant effects on the rectal temperature (Table A 33). Time factor was, however, more effective (54.7%) than breed effect (6%) in the contributions to the total variation (61.5%). Time and breed effects on respiratory rate were also separately significant ($P < 0.01$). Time x breed interaction was again significant ($P < 0.01$).

Rectal temperatures and respiratory rates between 11.00 and 23.00 hours were significantly ($P < 0.01$) higher than between 07.00 and 11.00 hours. The physiological responses were low and changed in a similar pattern as the ambient temperature but with some time lag (fig. 21).

Correlation analysis also shows that respiratory rate and rectal temperatures were positively correlated, $r = 0.6$, 0.9 and 0.6 in the Brown, Holstein and Fulani heifers respectively, and these were all significant ($P < 0.01$).

Plasma cortisol

1. Results from experiment 1 (tables XX and A 36) are presented graphically (Fig 22) and show little variation in the levels of plasma cortisol at the three times of blood collection. Analysis of variance of the values (table A 38) shows that breed and time had no significant effects ($P > 0.05$) on the values obtained. Breed x

time interaction was not significant ($P > 0.05$). Plasma cortisol level ranged between 2.2 and 8.8 ng/ml during the 3 sampling times.

Rectal temperature did not vary consistently with plasma cortisol level, correlation coefficients being -3, 0.5 and 0.5 in the Fulani, Holstein and Brown respectively (table A 39). The significance of the correlations also varied from low order ($P = 0.05$) in the Holstein and Brown to nil ($P > 0.05$) in the Fulani.

2. The three animals involved in experiment 2 showed reluctance when they were being separated from the group and throughout the morning they showed some desire to join the other heifers. The values of plasma cortisol obtained are shown in table XX. Morning high values of plasma cortisol 17.3, 14.1 and 9.7 ng/ml in the Brown, Holstein and Fulani respectively, were progressively decreased to lower values of 9.2, 5.1, and 3.5 ng/ml in the same order of breeds by 19.00 hours.

3. The diurnal plasma cortisol concentrations (tables XX and A 37) are shown in graphical form (fig. 23). There were individual variations in the level of plasma cortisol throughout the periods of blood sampling and there was no consistent pattern. Generally the values tended to be similar to morning values. In the Brown, the values tended to be generally higher in the morning than in the evening, while the values in the Holstein and Fulani as shown by the curves from the mean levels were fairly stable with a small relatively

higher level at 11.30 hours in the Holstein. Analysis of variance (Table A 40) shows that time and breed had separate significant effects ($P < 0.01$). Breed x time interaction was not significant ($P > 0.05$). One heifer (H 154) showed very high plasma cortisol level in the morning (19 ng/ml) (table A 37) but much lower level at subsequent times. Values from this animal were not used when the breeds were compared and in constructing the graph of the mean values.

4. The circadian levels of plasma cortisol (tables A 38 and A 41) are presented graphically (fig. 23). Analysis of variance shows that breed and time effects were not significant ($P > 0.05$). Breed x time interaction was not significant ($P > 0.05$) (table A 42). Fluctuations occurred in the plasma cortisol levels in individuals through the circadian period. Both the pattern and levels of plasma cortisol through the circadian period were not similar among the individual heifers. Values obtained in the coccygeal venous blood were less than 10 ng/ml of plasma and similar to those of jugular venous blood (Table A 41).

Plasma cortisol levels in heifers kept outdoors in experiment 3 were similar to values in experiment 4 in which animals were under shade throughout the day. Generally, values obtained in experiment 1 heifers outdoors in the afternoon were lower than the values obtained in experiments 3 and 4 in and out of shade respectively at about the same time of the day.

As typical of the weather conditions in this environment, daily dry bulb temperatures rose from a minimum value at 07.00 hours to a peak at 16.00 hours during the period of the investigation. A precipitous fall in air temperature occurred between 18.00 and 19.00 hours followed by a more gradual decrease through 07.00 hours.

The levels in plasma cortisol did not show a similar trend to the ambient temperature. Relatively elevated or low levels might occur in different individuals when the ambient temperature was quite high.

Discussion

The present study shows that circadian changes occur in the physiological responses of the heifers of *Bos taurus* and *Bos indicus* under natural hot climatic conditions; and this agrees with the previous observations in *Bos taurus* under subtropical hot climate (Berman 1967, 1968; Berman and Morag 1970). The similarity of the trend in diurnal and circadian physiological responses to that of ambient temperature indicates that the latter influences the former.

The high responses in the sun show the effectiveness of solar radiation as a strong heat stress factor, thus supporting similar prior observations (William et al. 1960). The lack of breed differences in the heifers continuously under shade is due to the fact that heat stress had been minimised by the shade structure.

The response of individual heifers also shows that when hyperthermia occurs in the hot weather, it lasts for only a short period during the day time and not continuously. This is not in agreement with a laboratory study in which responses were proportional to the applied heat stress (Worstell and Brody 1953).

The higher responses in the Holstein heifers particularly in the sun indicate a lower heat tolerance than in the Brown and Fulani, as confirmed by the Iberia heat tolerance test. The low heat tolerance in the sun also shows that the solar radiation must have imposed heat load more rapidly than the body could dissipate heat. A poorer cutaneous evaporative heat loss mechanism as well as a lower protection by the coat against heat load in the Holsteins than in the Browns and Fulanis is indicated.

The modifying effect of shade structures on the physiological responses observed in the present investigation agrees with the findings of Rainey, et al (1967) and Romance-Pounce et al (1977) in dairy cows. The result of a supplementary observation comparing the non-lactating cows with the heifers (table A 43), showing lower responses in the shade than in the sun, also indicates that both groups of animals would benefit from shade structures in the field.

The non-significant effect of the time of the day on plasma cortisol values obtained in experiment 1 indicates a more or less

uniform concentration through the day time, resembling the results from a similar investigation by Shaw et. al. (1960). In experiment 3, the higher mean values by 11.00 hours and lower mean values by 15.00 or 18.00 hours which were in many heifers similar to the morning (07.30 hours) values were responsible for the significant effect of time of the day in the analysis of variance.

The fact that relatively elevated levels of plasma cortisol occurred in some heifers at the time when the levels were relatively low in others signifies the lack of uniformity in the pattern of adrenocortical secretions among the heifers through the diurnal or circadian period. The significant effect of time shown by the analysis of variance on some values did not therefore indicate the existence of a definite rhythm in plasma cortisol. The factors that can be examined for their contribution to the result obtained include the experimental procedure, ambient conditions and hypothalamic-pituitary-adrenocortical rhythm. The heifers utilized for the study had been accustomed to handling including blood collection for 5 to 12 months before these investigations were carried out. The additional preconditioning before the blood samples were collected further helped in training the animals.

The intervals of sampling were over one hour so as to reduce the effect of handling since the heifers were not catheterised. As much as possible the procedure of blood collection was quite fast so that prolonged handling which could have resulted in struggling

was avoided. The advantage of speedy blood collection in obtaining physiological plasma cortisol levels had been noticed by other earlier workers (Heitzman et al. 1970; Gimenez et al. 1974; Lee et al. 1976). Collection of large volumes of blood, which was avoided, could have caused a prolongation of the time of handling and restraining the animal which could show up in elevated plasma cortisol levels as in some previous reports (Venkateseshu and Estergreen 1970; Patterson 1957).

With regard to the intervals of sampling, early studies on plasma corticosteroid periodicity in man had employed intervals of 4 to 6 hours which are longer than those used in the present study, and were able to describe a smooth rise and fall in corticosteroid levels (Migeon et al. 1956). The use of shorter intervals of sampling (20 - 30 minutes) only enabled more detailed description of the periodicity, with respect to the episodic secretions of plasma corticosteroid (Krieger et al. 1971; Hellman et al. 1970). The interval of sampling employed in the present study might not have precluded the observation of a plasma cortisol rhythm if it existed in the heifers. The low levels of plasma cortisol also indicate minimal influence of the method of blood collection and this could be a measure of adaptation. The use of the tail vein did not significantly alter the level or pattern of plasma cortisol concentration obtained despite the relatively lower excitement during tail blood sampling. This further justifies the view that the heifers were adapted.

The striking aspect of the results is the generally low levels of plasma cortisol concentration in the different investigations. Acute exposure to heat has been shown in laboratory studies to cause sharp elevations in plasma cortisol in cattle (Stott and Robinson 1970; Christison and Johnson 1972; Alvarez and Johnson 1973; Abilay, Mitra and Johnson 1975). The heat of the tropical afternoon did not evoke such marked elevations in adrenocortical functions in the heifers. Since the circadian plasma cortisol levels did not show a similar pattern to that of the ambient temperature, the latter might be influencing the former to a very limited extent.

It is possible that some of the low peaks at about 11 or 12 hours were due to the effect of ambient temperature and/or solar radiation. High ambient temperature has been shown in a previous study to be capable of causing increased cortisol release by setting up impulses at the skin thermoreceptors which then stimulate the hypothalamic CRF release (Chowers, et al 1966). The time point when the environmental heat or solar radiation was effective enough to excite the thermoreceptors might vary among the heifers depending on how quickly the skin temperature increased.

Under thermal exposure, the skin temperature of zebu cattle increases more slowly than that of *Bos taurus* (Ojo 1973; Allen 1962). The thermoreceptor of the *Bos taurus* might be more quickly excited under the present experimental conditions and this might be responsible

for some breed differences in the diurnal plasma cortisol among the heifers. The lack of uniformity in the values of plasma cortisol among the heifers in the afternoons does not, however, suggest that the adrenocortical function was responding to the ambient heat. From the results obtained, there was also no evidence to show that the adrenocortical response in the shaded and unshaded heifers differed.

The species differences in the binding capacity of cortisol binding globulin (CBG) has been reported to be responsible for species differences in plasma cortisol concentration (Linder 1964). CBG concentration was not determined in the heifers used for the present investigation and the contribution of this factor to the slight species difference in diurnal variation of plasma cortisol could not be assessed.

The lack of uniformity among the heifers in the circadian levels of plasma cortisol suggests that routine management practice might not have imposed any permanent influence on their adrenocortical function unlike the suggestion in a previous report on dairy cattle (MacAdam and Eberhart 1972). In species showing uniform variations in circadian plasma cortisol, the rhythm has been associated with circadian CRF and/or ACTH release. In the present observations, repeatability of cortisol periodicity was not demonstrable as to level and pattern in the different animals used unlike what was demonstrated in man (Krieger et al. 1971). This therefore does not

suggest the existence of a rhythm in ACTH release in the heifers, although it was said to be probably responsible for adrenocortical rhythm in cattle in another previous report (Wagner and Oxenreider 1972).

The present observation in a way, therefore, agrees with some other previous works in which definite diurnal adrenocortical rhythms could not be demonstrated in cattle (Paape et al 1973; Paape et al. 1974; Hudson et al. 1975; Shaw et al. 1960). Hudson et al. (1975) explained that the rhythm is lacking in cattle probably because cattle, like other ruminants, stay awake for considerable time in order to ruminate (Balch 1972; Morag 1967).

Some mechanism must be operating to keep plasma cortisol levels in cattle low in a hot environment (Bergman and Johnson 1963; Rhynes Ewing 1973; Christison and Johnson 1972). The purpose of such a mechanism may be to avoid increased metabolic heat production due to the thermogenic action of cortisol (Yousef & Johnson 1967). This mechanism may be preventing marked diurnal variations and a definite rhythm in plasma cortisol levels, such as have been described in other species. The results of experiment 2 further support this suggestion, and show that the mechanism operating to keep plasma cortisol levels low or to correct any elevations due to management or environmental stress may be of greater priority than that to maintain a diurnal or circadian rhythm in cattle, especially in a hot environment.

The reluctance shown by the animals in experiment 2 while they were being separated from their usual group was responsible for the elevated plasma cortisol values particularly in the morning. Separation of an animal from the usual group in the herd can be stressful. In normal dairy practice, this type of managerial stress is hardly avoidable since animals may be separated from their usual group for various reasons including breeding at estrus.

The inconsistent trend in the association between rectal temperature and plasma cortisol in experiment 1 (table A 36) is due to the more stable levels of the latter at the times of blood sampling than the values of the former. The more highly positive correlation between the physiological responses and the ambient temperature (table A 32), and the lack of a definite pattern of plasma cortisol through the day further indicate an absence of definite association between the physiological responses particularly the rectal temperature and plasma cortisol. In a laboratory study, similar trends in the elevations of plasma cortisol and rectal temperatures were found in cattle under thermal stress (Abilay, Mitra and Johnson 1975). It might be expected therefore that in the field, the diurnal plasma cortisol levels should show a trend similar to that of the rectal temperature of cattle. This was not found to be true in the present study, as the pattern of change in the levels of plasma cortisol did not resemble that of the rectal temperature in view of the more persistent rise in the latter in the afternoon till sometimes late in the evening.

In another study carried out in the climatic chamber, it has, however, been found that elevated plasma cortisol levels lasted for only a short period while elevated rectal temperatures persisted during thermal stress (Alvarez and Johnson 1973). This is prognostic for responses under natural hot climates where adrenocortical function in acclimatized cattle may, therefore, be less sensitive to ambient heat than is rectal temperature and may not show a similar trend as rectal temperature, diurnally. This suggestion is borne out by the present results.

The level and pattern of plasma cortisol during the circadian period in individual animals may have some relationship to adaptability. Further studies are still needed to examine this relationship in both adapted cattle and those newly introduced to hot natural climate.

v. Effect of exogenous corticotrophin on adrenocortical functions and estrous cycle

Result

The mean weight of heifers treated with ACTH was 317.5 (+17.9SE) kg. The minimum and maximum environmental temperatures on the day ACTH was injected were 72°F (22.2°C) and 89°F (31.7°C), and the relative humidities were 87% and 54% respectively. The plasma cortisol concentrations in the heifers are in table A 44 and are also presented graphically (figs. 25 and 26).

Mean pre-injection value of plasma cortisol concentration varied between 3.2 and 5.2 ng/ml. A sharp rise in the level was obtained at 30 minutes after ACTH injection. Peak values ranging between 41 and 62.4 ng/ml occurred between 30 - 140 minutes post-injection of ACTH and were 10 to 15 times higher than the initial levels. The percentage change in plasma cortisol concentration from the initial values ranged between 872 and 1304 among the heifers.

There was a tendency for a plateau to form before the decline in plasma cortisol concentrations occurred. The declines started between 140 - 300 minutes post-injection of ACTH and were either gradual or rapid. The initial pre-injection levels were closely approached but not reached in 5 of the six heifers. High levels occurred till after 6 hours post-injection. Plasma concentration

of cortisol 24 hours after ACTH injection were low. The response levels, that is, post-injection plasma cortisol concentration higher than initial levels, had a mean of 33.0 (+16.0 SD) and a range of 5.5 - 61 ng/ml in the heifers.

In the control experiment, the elevation in plasma cortisol was low compared to that after ACTH treatment, and ranged between 1.6 - 2.9 times the pre-injection level between 30 - 120 minutes post-injection of saline. The levels of plasma cortisol on the day following the ACTH injection were also low.

The analysis of variance of the results of the two treatments is in table A 45. It shows that the time interval, type of treatment and **breed** effects on the level of plasma cortisol were separately significant ($P < 0.01$), **F-ratio** being 103.79, 40.54 and 971.17 at degrees of freedom 8, 2, and 1 respectively. There were, however, significant ($P < 0.01$) two-way interactions between time interval and breed, time interval and type of treatment, and breed and type of treatment. There was a significant ($P < 0.01$) 3-way interaction between time interval, breed, and type of treatment. The total variations that occurred (70%) were due to the effect of the time interval (30%) and the type of treatment (36%), while the contribution from breed effect was very little (2%).

Analysis of variance on values of plasma cortisol obtained from ACTH treatment alone (table A 46) shows again the significant ($P < 0.01$)

breed, time interval and two-way interaction between the time interval and breed effects. With saline treatment alone (table A 47), breed effect was not significant ($P > 0.5$) as well as breed x time interval interaction ($P > 0.05$); but time interval effect was significant ($P < 0.01$).

Estrous cycles at the time of ACTH treatment lasted 18 - 23 days in the heifers. Estrous cycle rhythmicity was therefore not altered by the treatment.

Discussion

Because of the method of blood collection, it was not possible to collect blood samples at very short intervals of 10 minutes or less as was done in some previous studies which employed venous catheterisation (Paape et al 1973; Paape et al 1974; Shayanfar et al 1975; Satterlee et al 1977). The method employed here did not, however, prevent the observation of distinct adrenocortical response to ACTH, and this has similarly been observed in some earlier studies which employed venipuncture for blood collection (Venkataseshu and Estergreen 1970; Shaw and Nichols 1963; Dobson and Kanchev, 1977).

Pre-injection levels of plasma cortisol obtained in the present study were similar to values normally obtained in the same heifers during the year, and to values in some earlier reports by Swanson et al (1972) and other groups of workers quoted above.

The significant effect of the type of treatment on the level of plasma cortisol obtained was due to the stimulatory effect of ACTH on adrenal cortex as has been reported in earlier studies quoted. The elevation found in control experiment might have been due to the stimulation of the adrenal cortex by ACTH released from the pituitary in response to non-specific stimulus including the method of blood collection (Venkataseshu and Estergreen 1970; Shaw and Nichols 1963). The influence of the method of blood collection might have been responsible for the slightly higher levels of plasma cortisol in the control experiment (range 3.05 - 10.22 ng/ml) in the present investigation, compared to values obtained in an earlier report (0.7 - 7.7 ng/ml) under hot and thermoneutral conditions (Satterlee et al 1977). Compared to previous studies quoted above in which jugular venipuncture was employed, the values obtained **h e r e** in the control experiment were not high; and this can be attributed to adaptation in the heifers which had experienced protracted training and sampling through the year.

The significant effect of the time interval after the injection of ACTH on plasma cortisol concentration is mainly due to increasing secretion of cortisol into circulation as time progressed until a peak production was attained, thus agreeing with reports quoted above.

The significant two-way and 3-way interactions are due to the lack of uniformity in the pattern of change in the levels of plasma cortisol with time among the individual animals. Although the study

was not to compare the breeds, a breed factor was introduced to facilitate the use of analysis of variance to test the values. Incidentally, the values in the Brown heifers were higher than in the Holsteins and the Fulanis. Kajela (1973), quoted by Shayanfar (1975), did not find any breed difference between the Holstein and Jerseys in the adrenocortical response to ACTH. What is striking in the present result is the marked individual differences between the heifers in the responsiveness of the adrenal cortex to ACTH.

The weight differences between the heifers were small and could not have been responsible for the differences in the adrenocortical response among the individual heifers. The significance of this finding, however, is that although the basal levels of adrenocortical secretion, hence the plasma cortisol level, may be similar, the potential of the adrenal cortex for maximal secretion of corticoids varies among the heifers. This may also suggest individual differences in adrenal weight or the amount of cortical tissue in the adrenal gland. Rollinson (1962) stated that there had been indications of species variation in adrenal weight, with Brahman and Brahman crosses having heavier pituitary glands and lighter adrenal gland than British breeds. A definite work on this was, however, not quoted.

The adrenocortical response to ACTH in the present study are similar to those reported earlier in heifers (Satterlee et al 1977; Dobson & Kanchev 1977). This result is also comparable to that

obtained in dairy cows in another study (Shayanfar et al 1975). It therefore, suggests that the metabolic state may not alter the potentials of the adrenal cortex of cattle, inspite of the reported elevated normal basal glucocorticoid secretions in lactating cows (Vanjonak and Johnson 1975).

Under natural weather, adrenal responsiveness to ACTH was found to be less in dairy cows injected on days with environmental temperatures above 21.1°C than those injected below this temperature; it was suggested that adrenocortical responsiveness to ACTH could be depressed by environmental heat (Shayanfar et al 1975). In cattle raised in a hot environment, the responsiveness of their adrenal cortices to ACTH might, therefore, be expected to be low. The present result shows that the responsiveness of adrenal cortices of acclimatised heifers is not depressed by the tropical condition. This, therefore, substantiates the result of a study conducted in the climatic laboratory showing that adrenocortical responsiveness to ACTH in heifers acclimatised to hot environment (35°C 80% RH) was similar to those in a cold (5°C 30% RH) and thermoneutral (18°C 50% RH) conditions (Satterlee et al 1977).

The depressant effect of chronic environmental heat on adrenocortical function in cattle has been well documented as shown in various reports cited in previous chapters. The present result shows that the low adrenocortical function in cattle exposed for

prolonged periods to heat can be altered by ACTH. The normally low plasma glucocorticoid concentration in cattle exposed chronically to thermal stress may, therefore, be due in part to a depressed pituitary ACTH release rather than a depressed secretory ability of the adrenal cortex alone.

The result also shows that the normal functioning of the adrenal cortices of the heifers under the subequatorial and the experimental conditions described was much lower than the potentials of the glands. The heifers, therefore, had high adrenal reserves.

The lack of an effect of ACTH administration on the estrous cycle length suggests that induced elevation of endogenous glucocorticoid at and after mid-cycle period did not induce a luteolytic process. This corroborates previous report which showed that exogenous glucocorticoid did not alter estrous cycle when administered at mid-cycle period (Gimenez et al 1974); it is, however, contrary to the report that adrenal suppression with betamethasone caused prolongation of estrous cycles (Kanchev et al 1976). The fact that ACTH administration, hence increased circulating glucocorticoid, can suppress the development of corpus luteum in cycling heifers, as demonstrated by Brunner et al (1969), or that glucocorticoids can trigger luteolysis during late pregnancy may not necessarily mean that high levels of circulating endogenous cortisol at the middle of estrous cycle, and thereafter, can induce luteolysis.

SUMMARY AND CONCLUSION

It has been found that the basal level of plasma cortisol in the German Brown, Holstein-Friesian and White Fulani heifers under the sub-equatorial climate ranged between 1 - 10 ng/ml and showed no breed differences in the grand mean (5.8 ng/ml) through the year. This suggests that acclimatised temperate-evolved cattle do not necessarily have to maintain adrenocortical functions differently from local cattle in a tropical environment.

Seasonal difference in the level of plasma cortisol in the heifers was slight but significant, being lower in the dry hot season than in the wet relatively cooler season. This may result from a generally lowered metabolic rate during the former period. Frequently, higher adrenocortical activity occurred a few days before or on the day of estrus when plasma cortisol concentration might be as high as 22 ng/ml. This phenomenon was also more marked in the wet season than in the dry season; the fluctuations in the levels were less marked in the latter period.

Rectal temperatures were normal with a mean of 101.3°F (38.5°C) and 102.2°F (39.0°C) in the morning and afternoon respectively for all heifers together through the year. Although seasonal variation was little, it was significant, with lowest afternoon rectal temperatures occurring in the wet relatively cool months of July and August. There was some indication of slight seasonal shift in the basal rectal

temperature in some heifers showing higher values in the wet season than in the dry season. Differences between the morning and afternoon readings of rectal temperatures were lowest in the wet cool period. Breed differences were mainly due to the slightly lower body temperatures in the Fulani, and the greater occasional recurrence of hyperthermia in the Holstein heifers thus agreeing with previous reports that rectal temperatures in *Bos indicus* are less elevated than in *Bos taurus* under high-temperature environments (Findlay 1954; Worstell and Brody 1953).

Respiratory rate varied diurnally and with greater amplitudes between mean morning values of 14 - 44 and mean afternoon values of 17 - 75 breaths per minute in the shade. The low and high readings were recorded in the Fulani and Holstein heifers respectively. Breed differences were therefore highly significant. Seasonal variations were well marked as in subtropical observations in other earlier reports.

Although the mean afternoon rectal temperatures were generally not abnormal, the higher values in the relatively hotter and dry season was associated with high respiratory rates, suggesting accelerated stimulation of adaptive functions during that period of the year. These effects were more exaggerated in the Holsteins and suggest intolerance of the afternoon heat and of elevations in the body temperature. Generally, therefore, the Holsteins apparently attempted to maintain normal body temperatures by increased respiratory activity.

The results show that the lowest afternoon and diurnal variations in rectal temperatures and respiratory rates in the heifers occurred

during the period of the year when basal plasma cortisol levels were highest. If the increased adrenal function suggests higher metabolic rate, the low physiological response indicate that such increased metabolic rate was associated with greater comfort for the animal during that period of the year, that is, the wet relatively cooler season. It is not known if the seasonal variation in the quantity of available pasture, hence nutrition, influenced the suggested alterations in metabolic rate. The slight seasonal changes in both the basal (morning) and afternoon rectal temperatures, however, suggest that some shift in the set-point for temperature control may occur between the different periods of the year.

In spite of the relatively low basal secretions of cortisol, adrenocortical reserve was high since adrenal response to ACTH (200 I.U.) was high in magnitude and duration. Adaptation to a hot humid tropical climate did not alter the potentials of the adrenal cortex. Low normal basal secretions may therefore be part of a general adaptative thermoregulatory control in a tropical environment to avoid increased metabolic heat production which might result from the thermogenic action of cortisol. The adrenocortical responsiveness to exogenous ACTH may also suggest that the seasonal variations in adrenocortical secretions is due to seasonality in the levels of the circulating ACTH. This will require verification.

The changes in rectal temperatures and respiratory rates over the circadian period paralleled the changes in ambient temperature with high positive significant correlations and with peaks occurring in the

afternoons. These responses did not resemble that of the plasma cortisol concentrations which showed no consistent patterns. This suggests that adrenocortical function was less responsive to diurnal or circadian ambient conditions than were the physiological responses, in heifers in the shade and in the sun.

On one occasion in the sun, however, there was a tendency for higher adrenocortical secretions at about mid-day (11.00 - 13.00 hours) than in the evening (19.00 hours). Some species differences also occurred as the White Fulani showed lower responses than the Brown and Holstein heifers.

The physiological responses were more exaggerated in all heifers when exposed to the sun. Direct solar radiation was generally not tolerable to the *Bos taurus* breeds but the degree of tolerance varied between and within the breeds. The Holstein heifers were more hyperthermic in the sun; they panted, sought shade and therefore spent less time grazing than the other breeds when the heat was intense. The lower heat tolerance of the Holsteins, therefore, became more clearly evident on exposure to the sun. The Brown and the Fulani heifers did not pant in the sun. The Fulani heifers were generally able to tolerate the sun. The high breathing rate, and the avoidance of the sun plus the hyperthermia exhibited by the Holsteins in the sun, may suggest a lower efficiency of the increased population of sweat glands previously reported in animals of this breed when acclimatized to tropical conditions.

Estrus recurred in the heifers throughout the year. The majority of estrous periods commenced during daylight with the greatest concentration in the morning. It should be possible to detect almost all heifers on heat by close observation at between 07.00 and 10.00, and 18.00 and 20.00 hours, although with observations in the morning alone a large percentage of estrus should be detected. Mean estrous cycle lengths (with standard errors) were 21.0 ± 0.3 , 20.1 ± 0.2 and 21.4 ± 0.2 days in the Brown, Holstein and Fulani heifers respectively and showed no significant seasonal variations. The mean durations of estrus, with the standard errors, were 16.2 ± 0.7 , 15.6 ± 0.8 and 14.6 ± 0.8 hours in the Brown, Holstein and Fulani heifers respectively. Seasonal differences were not significant and the duration in the two species compared well, although the breed differences were slightly significant. Estrual behaviour was depressed by intense afternoon heat and direct solar radiation particularly in the *Bos taurus*. Estrus was well expressed in the *Bos taurus* and the *Bos indicus* heifers although the intensity was occasionally low in a few individuals among the latter.

Ovulations occurred well within a day after estrus with no significant breed and seasonal differences. Silent ovulations were not observed, but subestrous conditions occurred. Subestrous might pass undetected under systems utilising less vigorous estrous detection methods. Results of observations for estrous cycles, therefore, generally suggest that heifers of both *Bos taurus* and *Bos indicus* cattle can breed at all periods of the year under Ibadan conditions. However, because of

the seasonal nature of the available pasture, it is suggested that heifers and cows should be made to calve early in the dry season, when the calves would depend only on milk and concentrate feeding. By the time the rainy season starts and the grasses grow, the calves should be mature enough to digest roughage and thus utilize the pasture. A steady fast growth rate can thus be achieved through the first year of life.

Rectal temperatures did not show any cyclic pattern during the estrous cycles. Contrary to some previous reports on the effect of exogenous glucocorticoid in pigs and rats, normal elevation of endogenous plasma cortisol levels either at mid-cycle period or around estrus in the heifers probably did not disturb LH release since ovulations and the expression of estrus were generally normal.

Estrous cycle rhythmicity was also not altered by exogenous ACTH (200 I.U.) administered between days 10 to 15 of estrous cycle. Elevated endogenous glucocorticoids, therefore, did not elicit luteolytic effects in the cycling heifers, in spite of the reported depressing effect of ACTH administration on CL development (Brunner et al 1969).

The fact that the heifers were protected from solar radiation for most of the day throughout the year might have greatly contributed to the generally low seasonal differences in some of the parameters. The ability of the heifers to maintain homeostasis with relatively uniform effort throughout the year can be said to be reflected in the low seasonal shifts in rectal temperatures and plasma cortisol levels as well as the lack of seasonal differences in the estrous cycles. The

respiratory rates, however, showed that adaptive functions were brought to play to different magnitudes in the different breeds.

Because of their consistent avoidance of direct solar radiation and the ease with which hyperthermia developed in the Holsteins in the sun, *Bos taurus* cattle should be provided with shade structures under the conditions of Southern Nigeria. Grazing in the night throughout the early morning hours should also be mandatorily adopted.

The ease with which the Browns dominate over the Holsteins suggest that a mixed herd of these two breeds should not be encouraged. Access of the Holsteins to shade in the field and to feed in the pen is precluded in the mixed herd; and this can have adverse effects on production.

The German Brown cattle should be recommended over the Holsteins for the hot humid Southern Nigeria environment since the former are more adapted as reflected in their physiological responses. The Southern Nigeria conditions can definitely support large cattle establishments stocked with the German Brown cattle if the problem of seasonal availability of pasture can be overcome. There is need to utilize irrigation to make pastures grow all year round. Sufficiently large quantities of silage should be prepared against the dry season. Management should ensure that heifers show steady weight gains through the years until mature weight is attained.

These results show that the Fulani cattle are more adapted to the hot/humid sub-equatorial climate than the *Bos taurus* cattle. Their

qualities with respect to estrous cycles were also elucidated. Artificial breeding is as applicable for the Fulani as for temperate-evolved cattle.

Better management practices are needed for the Fulani cattle. Maintained under identical intensive or semi-intensive management, the temperate-evolved cattle in the tropical environment may prove to provide no advantage over the Fulani cattle in terms of reproductivity. The high cost of importing and managing the *Bos taurus* cattle calls for further investigations on the relative economics of production when this type of cattle and the *Bos indicus* are maintained under identical management in the tropical environment of southern Nigeria. Thus, the need to import temperate-evolved cattle may be re-evaluated.

Further areas of study in this particular environment that can be suggested include:

- (1) Investigations of adrenal reserves through the seasons in *Bos indicus* and *Bos taurus* cattle.
- (2) Investigation of possible progesterone/cortisol interaction (Adrenal secretion of progesterone is believed to contribute to plasma levels of that hormone; this has been suggested in previous works as a factor in the aetiology of infertility in cattle in a hot environment).
- (3) A study of gonadal functions with respect to hormone secretion in *Bos indicus* and *Bos taurus* cattle.

- (4) A study of the influence of seasons on fertility in *Bos taurus* and *Bos indicus* cattle under the same management with particular regard to effects on conception.
- (5) Investigations of adrenocortical functions and physiological responses in lactating cows of *Bos taurus* and *Bos indicus* breeds.

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Table 1

Mean Respiratory rates in heifers during different quarters of the year

	Brown 6a		Holstein 5		Fulani 6	
	AM	PM	AM	PM	AM	PM
Dec(4th) - Jan (14th)	20.8 ±0.5*	31.0 ±0.5*	27.1 ±0.6	55.6 ±1.6	19.3 ±0.4	24.6 ±0.5
n	252	252	210	210	252	252
March (9th) - April (19th)	30.6 ±0.6	30.0 ±0.9	45.1 ±1.1	65.1 ±1.2	21.7 ±0.4	27.4 ±0.5
n	252	252	210	210	252	252
July (5th) - August (17th)	16.0 ±0.6	19.5 ±0.7	22.5 ±0.6	27.4 ±1.2	15 ±0.4	18 ±0.4
n	264	264	220	220	264	264
October (23rd)- Dec. (2nd)	27.7 ±0.7	50.7 ±0.7	30.8 ±0.6	69.6 ±1.6	16.8 ±0.5	29.2 ±0.7
n	246	246	205	205	246	246

a - number of animals
* - standard error
n - number of observations

Table II

Mean percent change in respiratory rate at 15.00
from values at 07.00 hour.

PERIOD	Brown	Holstein	Fulani
DEC- JAN	71.8% (+12.1 SD)	104.4% (+17.2 SD)	27.5% (+ 3.0 SD)
MARCH TO APRIL	46.2 (+26.5 SD)	44.8 (+11.4)	27.4 (+ 4.8 SD)
JULY TO AUGUST	23.1 (+10.8 SD)	21.9 (+ 5.3 SD)	17.3 (+ 7.4 SD)
OCT- NOV	84.5 (+22.6 SD)	134.6 (+35.2 SD)	75.3 (+ 5.2 SD)

SD = Standard deviation.

Table III

Mean morning (07.00h) and afternoon (15.00h) rectal temperatures along with the standard deviations, in heifers during four quarters of the year

	Brown 6b		Holstein 5b		Fulani 6b	
	07.00h	15.00h	07.00h	15.00h	07.00h	15.00h
Dec. 4 - Jan. 14	101.2 \pm 0.4 $^{\circ}$ F (38.5 $^{\circ}$ C)	102.3 \pm 0.6 $^{\circ}$ F (39.0 $^{\circ}$ C)	101.2 \pm 0.6 $^{\circ}$ F (38.4 $^{\circ}$ C)	102.4 \pm 0.5 $^{\circ}$ F (39.4 $^{\circ}$ C)	101.2 \pm 0.3 $^{\circ}$ F (38.1 $^{\circ}$ C)	102.1 \pm 0.6 $^{\circ}$ F (38.9 $^{\circ}$ C)
n	252	252	210	210	252	252
March 9 - April 19	101.3 \pm 0.6 $^{\circ}$ F (38.5 $^{\circ}$ C)	102.2 \pm 0.6 $^{\circ}$ F (39.1 $^{\circ}$ C)	101.3 \pm 0.4 $^{\circ}$ F (38.5 $^{\circ}$ C)	102.4 \pm 0.5 $^{\circ}$ F (39.1 $^{\circ}$ C)	101.2 \pm 0.5 $^{\circ}$ F (38.4 $^{\circ}$ C)	102.1 \pm 0.3 $^{\circ}$ F (38.9 $^{\circ}$ C)
n	252	252	210	210	252	252
July 5 - August 17	101.3 \pm 0.5 $^{\circ}$ F 38.5	102.1 \pm 0.3 $^{\circ}$ F (39.0 $^{\circ}$ C)	101.3 \pm 0.4 $^{\circ}$ F 38.5 $^{\circ}$ C	102.2 \pm 0.6 $^{\circ}$ F (39.0 $^{\circ}$ C)	101.2 \pm 0.2 $^{\circ}$ F (38.4 $^{\circ}$ C)	102.1 \pm 0.4 $^{\circ}$ F (38.9 $^{\circ}$ C)
n	264	264	220	220	264	264
October 23 - Dec. 2	101.2 \pm 0.6 $^{\circ}$ F (38.5 $^{\circ}$ C)	102.3 \pm 0.5 $^{\circ}$ F (39.1 $^{\circ}$ C)	101.3 \pm 0.5 $^{\circ}$ F (38.5 $^{\circ}$ C)	102.5 \pm 0.7 $^{\circ}$ F (39.2 $^{\circ}$ C)	101.2 \pm 0.2 $^{\circ}$ F (38.4 $^{\circ}$ C)	102.2 \pm 0.5 $^{\circ}$ F (39 $^{\circ}$ C)
n	246	246	205	205	246	246

b = number of animals

n = number of observations.

Table IV

Mean age of commencement* of estrous cycles in Brown,
Holstein and Fulani heifers.

Breed	Brown	Holstein	Fulani
Mean	17.8	16.7	23.7
Standard deviation	<u>+1.6</u>	<u>+1.8</u>	<u>+1.9</u>
Range (months)	16-20	14-19	20-25
Number of animals	6	5	6

* = Pubertal age

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Table V

Percentage distribution of time of onset of estrus
in heifers

<u>Time of the day (hours)</u>	Fulani n=75	Brown n=70	Holstein n=74
20:00-01:00	8	4.3	4.0
01-07:00*	30.6	38.6	32.4
07-12:00	41.3	44.2	48.6
12-20:00	20.7	12.8	14.9

*Estrus commencing between 01:00 and 07:00 hours were more concentrated between 05:00 and 07:00 hours.

Generally most estrus commenced during the day time especially in the morning hours. n= number of observations

Table VI

Duration of estrus in heifers through one calendar year

Breed	Wet Season			Dry Season			All Year Round		
	Mean (h)	S E	n*	Mean (h)	S E	n*	Mean (h)	S E	n*
Brown n = 6	17.5	<u>+1.0</u>	36	15.6	<u>+0.9</u>	31	16.2	<u>+0.7</u>	67
Holstein n = 5	16.8	<u>+1.1</u>	31	15.2	<u>+0.9</u>	30	15.8	<u>+0.7</u>	61
Fulani n = 6	15.4	<u>+1.2</u>	37	13.3	<u>+0.7</u>	30	14.6	<u>+0.8</u>	67

S E = standard error

n* = number of observations

n = number of animals.

Table VII

Breed comparisons of the duration of estrus in heifers

	Brown versus Holstein	Brown versus Fulani	Holstein versus Fulani
DF	126	132	126
t	0.7	2.0	1.3
p	> 0.05	< 0.05	> 0.05
	NS	S	NS

t = student t-value; p = level of significance;

DF = degree of freedom; s = significant,

NS = not-significant.

Table VIII

Comparison of the duration of estrus in heifers during the
wet and dry seasons

	Brown	Holstein	Fulani
DF	65	59	65
t	1.3	1.2	1.3
p	> 0.05	> 0.05	> 0.05
	NS	NS	NS

DF = Degree of freedom; t = student t-value,

p = level of significance; NS = not significant.

Table IX

Ovulation interval (in hours)

	<u>Breeds</u>								
	Brown			Holstein			Fulani		
	\bar{x}	SE	n	\bar{x}	SE	n	\bar{x}	SE	n
After end of estrus	14.2	+0.6	44	14.7	+0.6	38	15.0	+0.9	49
From onset of estrus	30.9	+0.8	44	30.0	+0.7	38	30.7	+1.0	49

 \bar{x} = mean

SE = standard error

n = number of observations

Table X

Comparison of ovulation interval after the end of estrus in heifers

Breed	Brown Versus Holstein	Brown Versus Fulani	Holstein Versus Fulani
Degree of freedom	80	91	85
t	0.6	0.8	0.3
p	>0.05	>0.05	>0.05

t = student t -value; p = level of significance.

Table XI

Ovulation interval after the end of estrus in Brown, Holstein and Fulani heifers: observations in the wet and dry seasons compared

Wet Season (March - October)			Dry Season (November - February)		
$\bar{x}(h)$	SE	n	\bar{x}	SE	n
15.4	0.6	75	13.7	0.6	56

DF = 129

t = 1.9

p = 0.05

\bar{x} = mean; SE = standard error

n = number of observations during each season

DF = degree of freedom

t = student t - value

p = level of significance.

Table XII

The character of the frequency of estrous cycle lengths in heifers

	Brown	Holstein	Fulani
<u>Estrous cycle lengths</u> (in days)			
Mean	21.0	20.1	21.4
S.E.	0.3	0.2	0.2
Longest (days)	30	29	29
Shortest (days)	18	17	16
Between 18-24 days (%)	95	93.4	94.5
Above 18-24 days (%)	3.5	1.3	7.6
Below 18 days (%)	0	6.5	2.2
Number of observations	108	92	105
Number of animals	6	5	6

Table XIII

Estrous cycle lengths in heifers through the wet and dry seasons

		Dry season			Wet season			Total observations		
		Mean length (days)	SD	n	Mean length (days)	SD	n	Mean length (days)	SD	n
	n*									
Brown	6	21.3	2.2	51	21.1	1.4	57	21.0	3.0	108
Holstein	5	20.1	1.5	41	20.6	2.2	51	20.1	+1.6	92
Fulani	6	21.1	1.9	48	21.8	2.2	57	21.4	+2.1	105

SD = standard deviation

n = number of observations

n* = number of animals

Table XIV

Comparison of estrous cycle lengths in the
wet and dry seasons

	Brown	Holstein	... Fulani
Degrees of freedom	106	90	103
t	0.5	1.1	1.6
P	> 0.05	> 0.05	= 0.05
	NS	NS	NS*

t = student t-value

p = significance level

NS = not significant

NS* = not significant (but slightly longer values in the wet season).

Table XV

Comparison of the estrous cycle lengths in the Brown (B)
Holstein (H) and Fulani (F) heifers

	B versus H	B versus F	H versus F
Degrees of freedom	198	211	195
t	2.3	1.1	4.5
p	< 0.05	> 0.05	< 0.05
	S	NS	S

t = student t-value

p = level of significance

S = significant

NS = not significant.

Table XVI

Mean plasma cortisol concentration* in heifers during different quarters of the year

Season	Brown 6**	Holstein 5**	Fulani 6**
Dec. 4 - Jan. 14	5.1 +0.5a	4.6 +0.4a	5.7 +0.6a
n	87	87	96
March 9 - April 19	6.1 +0.5	6.3 +0.4	5.7 +0.4
n	96	96	110
July 5 - August 17	6.5 +0.4	6.6 +0.4	6.3 +0.4
n	98	96	98
October 23 - Dec. 2	5.6 +0.4	5.3 +0.4	5.2 +0.4
n	105	102	109

* = plasma cortisol concentration (ng/ml)

a = standard error

** = Number of heifers

n = number of observations.

Table XVII

Mean rectal temperatures and respiratory rates in heifers kept outdoors in the sun throughout the day time

Time of the day(h)	T _a (°F)	Brown n = 6		Holstein n = 5		Fulani n = 6	
		T _{re}	RR	T _{re}	RR	T _{re}	RR
0.700	75	100.9 +0.5 ^b	28 +2.3	101 +0.5 ^b	36.8 +0.5 ^b	100.8 +0.5 ^b	18.00 +3.2 ^b
11.00	84	102.0 +0.3	47.6 +6.2	102.9 +1.1	49.6 +1.3	101.7 +0.3	27.9 +6.3
15.00	88	102.0 +0.2	58.4 +0.5	103.9 +0.9	94 +5.7	102.5 +0.2	36.4 +6.1
18.00	82	102.1 +0.1	57.2 +5.0	103.2 +0.6	85.6 +3.3	101.7 +0.5	31.2 +4.1

n = number of animals

b = standard error

T_a = Ambient temperature (°F)

RR = respiratory rate

T_{re} = rectal temperature (°F)

Observations were made on three occasions.

Table XVIII

Diurnal rectal temperatures and respiratory rates of heifers
in the shade

Time of the day(h)	Mean T_a and RH		Brown n = 5		Holstein n = 5		Fulani n = 5	
	T_a	RH	T_{re}	RR	T_{re}	RR	T_{re}	RR
07.00	72.5	92	100.8 +0.4 _b	52.4 +3.8 _b	100.8 +0.4	34.8 +4.1	101.6 +0.4	16.7 +2.6
11.00	78	77	101.2 +0.4	35.4 +4.2	101 +0.2	70.3 +4.2	101.2 +0.4	21.8 +4.2
13.00	85	68	101.2 +0.2	37.5 +0.9	101.8 +0.3	74.4 +4.5	101.2 +0.1	28.9 +6.7
16.00	88	57	101.4 +0.1	38.0 +1.5	102.0 +0.3	74.4 +4.8	101.5 +0.2	28.0 +6.7
19.00	78	65	101.3 +0.2	35.8 +0.9	101.7 +0.4	68.6 +1.8	101.1 +0.3	21.4 +3.4
23.00	77	88	101.0 +2.6	28.0 +2.6	101.2 +0.4	47.6 +7.9	101 +0.3	16.0 +1.7

n = number of animals

T_{re} = Rectal temperature ($^{\circ}$ F)

RR = Respiratory rate (Breaths/minute)

T_a = Ambient temperature ($^{\circ}$ F)

RH = Relative humidity (%)

b = standard deviation

Observation were made on two occasions.

Table XIX

Circadian rectal temperatures and respiratory rates of heifers in the shade

TIME OF THE DAY (h)	BROWN*		HOLSTEIN*		FULANI*	
	T _{re}	RR	T _{re}	RR	T _{re}	RR
07 - 08	100.9 + .4 †	30.0 +2. †	100.9 +2.6 †	38 +3.2 †	100.9 +3.1 †	17.6 +2.6 †
10 - 11	101. + .1	34 +4	101.2 + .1	71.6 +3.8	101.2 + .1	25 +3.0
13.-14	101.3 +2	40 +6.4	101.8 + .1	74.8 +10.1	101.2 + .1	33.2 +5.2
16 - 17.00	101.5 + .2	42. 6.1	101.9 + .2	70 +6.6	101.3 + .1	33.6 +2.6
20 - 21	101.1 + .2	37.6 +3.8	101.7 + .1	60.8 +8.4	101.2 + .2	34.8 +4.1
01 - 02	101.1 + .2	27.6 +3.5	101.2 + .2	35.6 +3.8	101.0 + .3	16.8 +2.3
04 - 05	102.0 + .1	26.8 +2.7	101.6 + .2	33.6 +3.8	100.9 +1.0	16.4 +1.7

* number of animals = 5

† standard deviation.

RR = respiratory rates

T_{re} = rectal temperature (°F)

Table XX

Diurnal plasma cortisol concentration in heifers (ng/ml)
(Experiment 1)

TIME OF THE DAY (HOUR)	BROWN*	HOLSTEIN*	FULANI*	AMBIENT CONDITION
07.30	4.3 ± 0.5	4.2 ± 0.6	4.9 ± 0.7	73°F 92%RH
12.00	5.2 ± 0.4	6.4 ± 1.4	4.9 ± 1.9	89°F 70%RH
17.00	4.3 ± 0.2	5.1 ± 0.6	4.4 ± 0.3	90°F 68%RH

* = number of animals = 4

\pm = standard error

Blood samples were collected from heifers kept in the sun throughout the day as described in the text.

Table XXI

Plasma cortisol concentration in heifers separated from
the usual group

(Experiment 2)

		Time of the Day (h)			
		08.00 h	13.00 h	16.00 h	19.00 h
Heifer (identification no)		Plasma cortisol concentration (ng/ml)			
Brown	84	17.3	11.6	12.8	9.2
Holstein	145	14.1	7.9	10.9	5.1
Fulani	21	9.7	8.8	7.3	3.5

Heifers were kept outdoors all day away from the other mates, and bled at the time intervals shown in the table.

Table XXII

Mean, with standard error, diurnal plasma cortisol concentration in heifers

(Experiment 3)

Time of the Day (Hour)	Breed			T_a
	BROWN n = 4	HOLSTEIN* n = 4	FULANI n = 4	
Plasma Cortisol (ng/ml)				
7 - 8	6.8 ± 1.1	4.7 ± 0.9	3.9 ± 0.6	74.2°F (23.4°C)
11.30	7.4 ± 0.8	6.8 ± 0.2	4.3 ± 0.6	84°F (28.8°C)
15.30	5.0 ± 1.1	4.7 ± 0.7	4.6 ± 0.9	91°F (32.8°C)
18.30	4.4 ± 0.7	4.2 ± 0.3	4.0 ± 0.6	86°F (30°C)

Heifers were kept outdoors throughout the day and blood samples were collected at the times indicated in the table.

- n = Number of heifers
 T_a = Ambient temperature
 * = Values in Holstein 154 were excluded (see Table A 37).

Table XXIII

Circadian plasma cortisol, with the standard error, in
heifers of the Brown, Holstein and Fulani breeds
of cattle

(Experiment 4)

<u>Breed</u>	<u>Time of the Day (Hour)</u>						
	<u>07.30-08.00</u>	<u>12-13.00</u>	<u>16-17.00</u>	<u>19-20.00</u>	<u>22-23.00</u>	<u>01-02.00</u>	<u>04-05.00</u>
	<u>MEAN PLASMA CORTISOL (ng/ml) WITH THE STANDARD ERROR</u>						
<u>Brown</u>	4.6	7.2	6.8	4.5	5.0	6.2	4.6
<u>n = 3</u>	<u>+0.5</u>	<u>+0.7</u>	<u>+0.7</u>	<u>+0.3</u>	<u>+1.1</u>	<u>+1.4</u>	<u>+0.4</u>
<u>Holstein</u>	5.8	5.4	6.7	5.3	5.6	4.6	5.4
<u>n = 3</u>	<u>+0.1</u>	<u>+0.8</u>	<u>+0.4</u>	<u>+ .1</u>	<u>+ .4</u>	<u>+1.0</u>	<u>+0.6</u>
<u>Fulani</u>	5.1	6.4	5.8	5.3	4.2	5.0	4.5
<u>n = 3</u>	<u>+0.4</u>	<u>+0.6</u>	<u>+1.3</u>	<u>+0.4</u>	<u>+0.6</u>	<u>+0.5</u>	<u>+0.6</u>

Plasma cortisol levels were generally low and showed no rhythms.

n = number of heifers.

Table XXIII

Circadian plasma cortisol, with the standard error, in heifers of the Brown, Holstein and Fulani breeds of cattle

(Experiment 4)

Breed	Time of the Day (Hour)						
	07.30-08.00	12-13.00	16-17.00	19-20.00	22-23.00	01-02.00	04-05.00
	MEAN PLASMA CORTISOL (ng/ml) WITH THE STANDARD ERROR						
Brown n = 3	4.6 ±0.5	7.2 ±0.7	6.8 ±0.7	4.5 ±0.3	5.0 ±1.1	6.2 ±1.4	4.6 ±0.4
Holstein n = 3	5.8 ±0.1	5.4 ±0.8	6.7 ±0.4	5.3 ±.1	5.6 ±.4	4.6 ±1.0	5.4 ±0.6
Fulani n = 3	5.1 ±0.4	6.4 ±0.6	5.8 ±1.3	5.3 ±0.4	4.2 ±0.6	5.0 ±0.5	4.5 ±0.6

Plasma cortisol levels were generally low and showed no rhythms.

n = number of heifers.



Figure 1: A German-Brown heifer



Figure 2: A Holstein-Friesian heifer



Figure 3: A white Fulani heifer

Percentage monthly weight changes: composite of four heifers/breed.

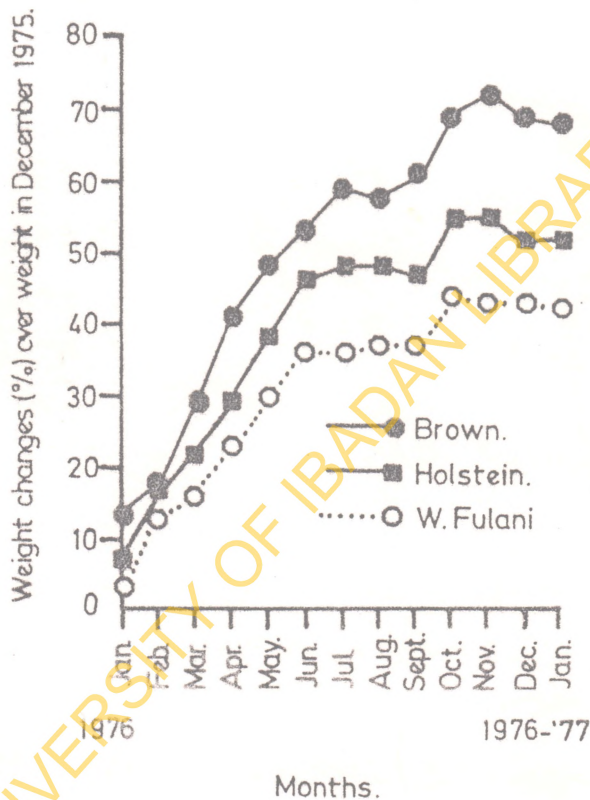


Figure 4: The period considered was from January 1976 through January 1977. Weight gain was reduced after the first rainfall peak of April - June. A second rainfall peak in September to November was associated with sharp increases in body weight gain, which declined with the onset of the dry season in November.

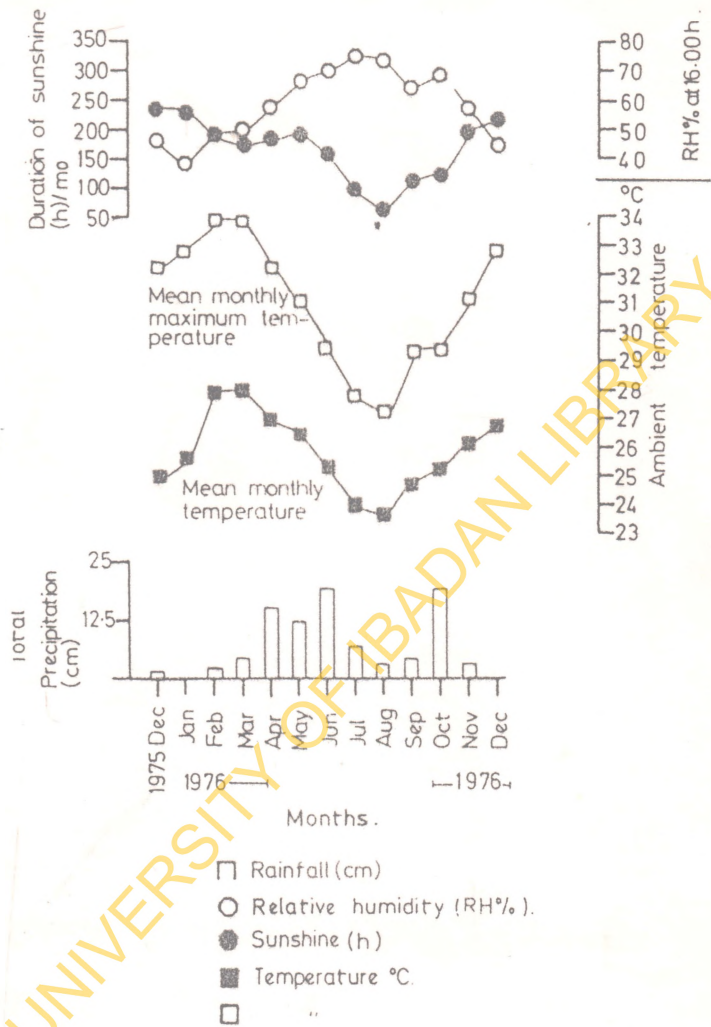


Figure 5

Mean environmental temperature and humidity during the periods when physiological studies on heifers were conducted.

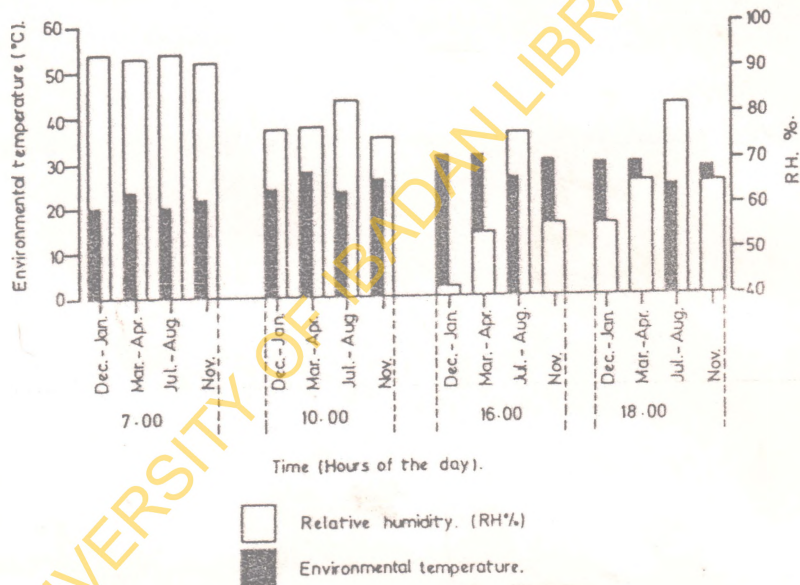


Figure 8. Minimum and maximum environmental temperatures occurred at 07.00 and 16.00 hours respectively- the reverse is true for the relative humidity. Lowest environmental temperatures and highest relative humidities occurred in July - August period. Morning environmental temperatures were highest and increased most rapidly during the March - April period.

MEAN RESPIRATORY RATES AND RECTAL TEMPERATURES IN HEIFERS.

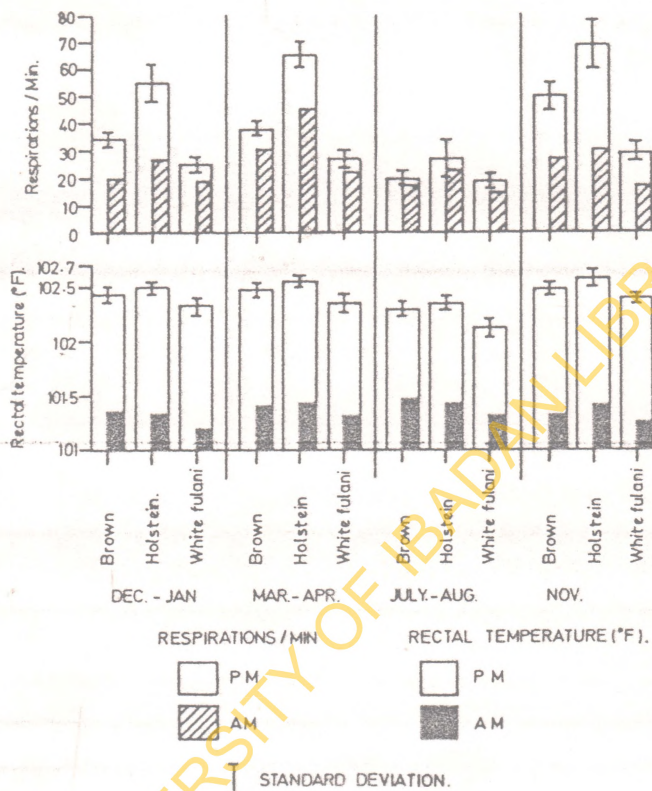


Figure 7: Breed, diurnal and quarterly differences in the respiratory rate were highly significant. Species and quarterly differences in mean rectal temperatures were slight but significant. Diurnal differences in rectal temperatures were also significant.

RECTAL TEMPERATURE : COMPOSITE OF
5-7 CYCLES / BREED.

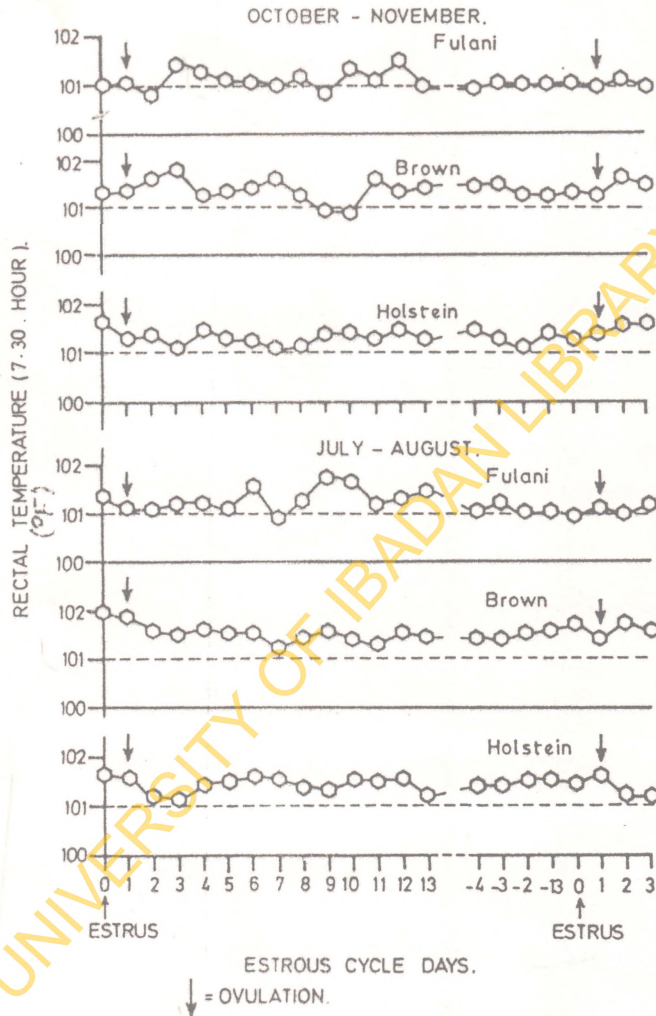


Figure 8: Examples of mean morning (07 - 07.30 hours) rectal temperatures through the estrous cycles. No definite rhythm was evident.



Scale = 1:99 cm

Figure 9: Shade seeking habit of heifers. Some Holstein and Brown heifers can be seen either lying or standing in the shade of a tree while the White Fulani and some Brown heifers remain grazing in the sun.



Figure 10: Panting Holstein heifer. Characteristically, panting is associated with opened mouth and loss of saliva.

PERCENTAGE DISTRIBUTION OF
TIME OF ONSET OF ESTRUS.

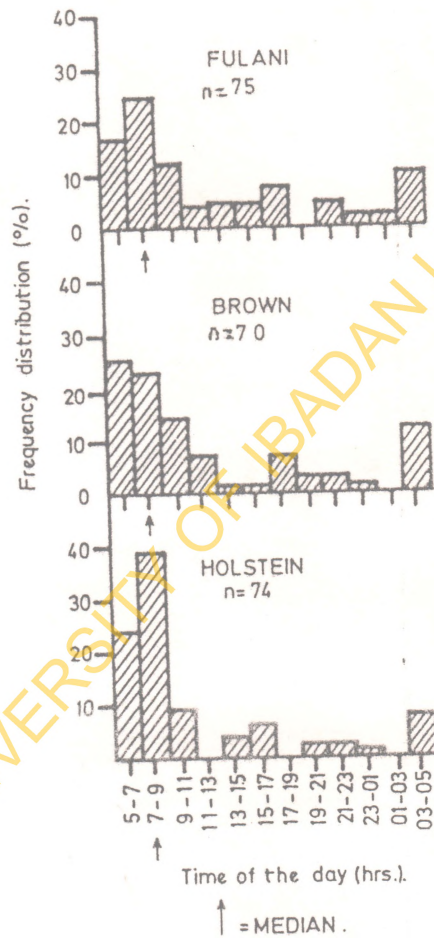


Figure 11: Most estrus commenced during the day time with a greater concentration in the morning hours and no significant breed difference. n = number of observations.

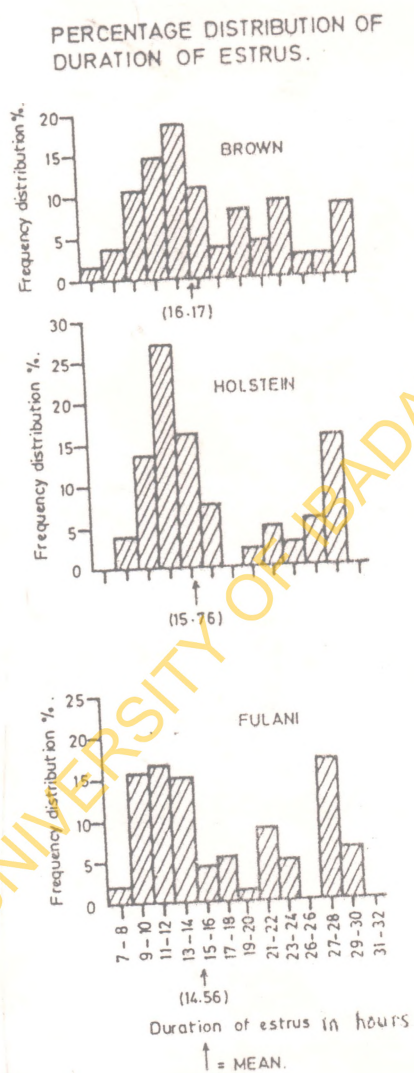


Figure 12: The range of the duration of estrus was smaller in the three breeds but mean values differed significantly between the Browns and the Fulanis.

PERCENTAGE DISTRIBUTION OF
ESTROUS CYCLE LENGTH.

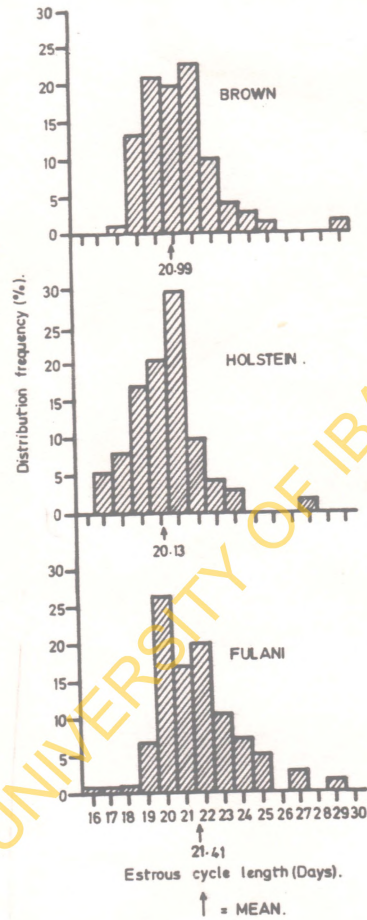


Figure 13: Mean estrous cycle length was significantly longer in the Brown and Fulani than in the Holstein heifers.

Plasma cortisol concentration during
estrous cycles: composite of 4 heifers/
breed (December - January).

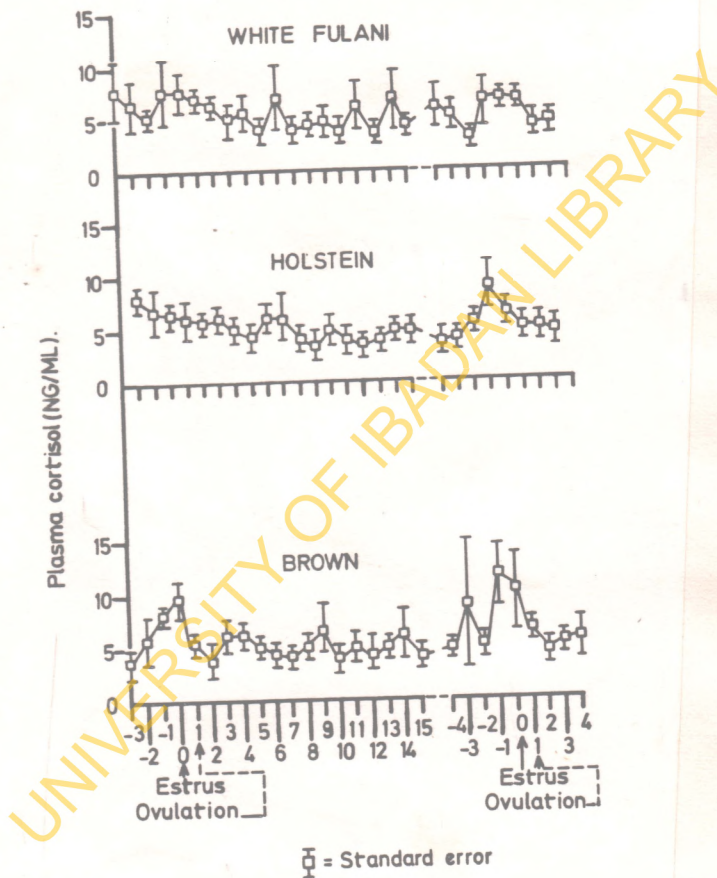


Figure 14: The period covered was from December 4, 1975 through January 15, 1976. Plasma samples were collected between 07:00 and 08:00 hours; and this applies to figures 15, 16, and 17.

Plasma cortisol concentration during
estrous cycles: composite of 4 heifers/
breed (March - April).

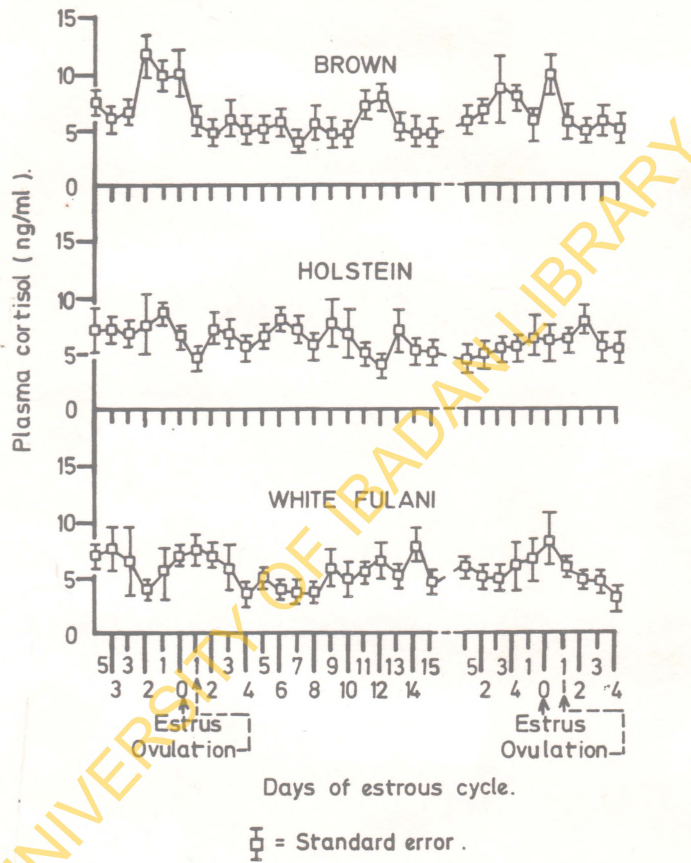


Figure 15: The period covered was between March 9 and April 19, 1978.

Plasma cortisol concentration during
estrous cycles: composite of
4 heifers / breed (July - August).

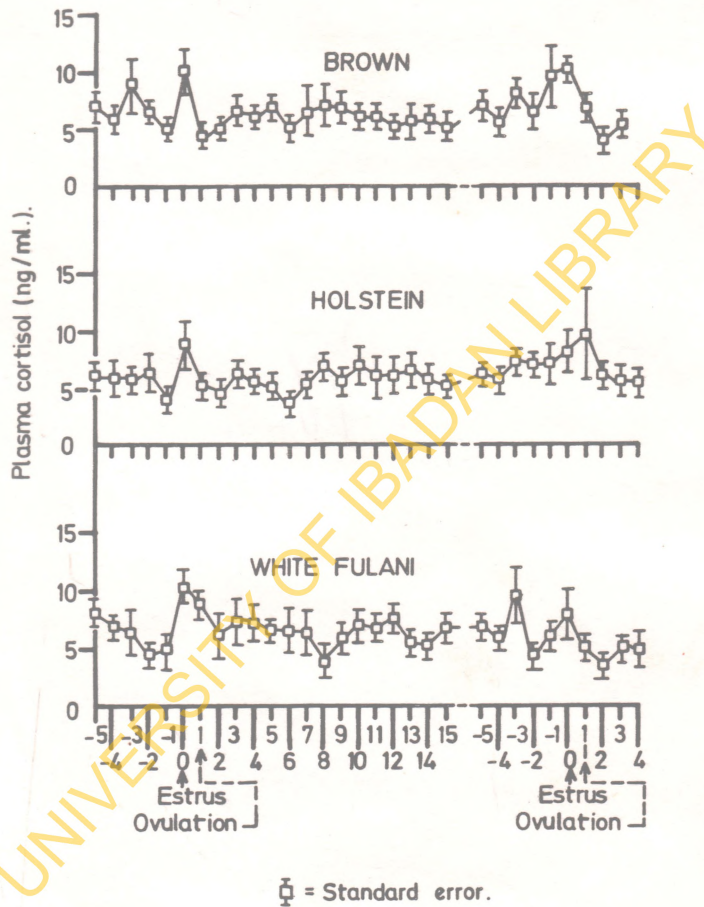


Figure 16: The period covered was between July 5 and August 14, 1976.

Plasma cortisol concentration during
estrous cycle: Composite of 4 heifers/
breed (November 1976).

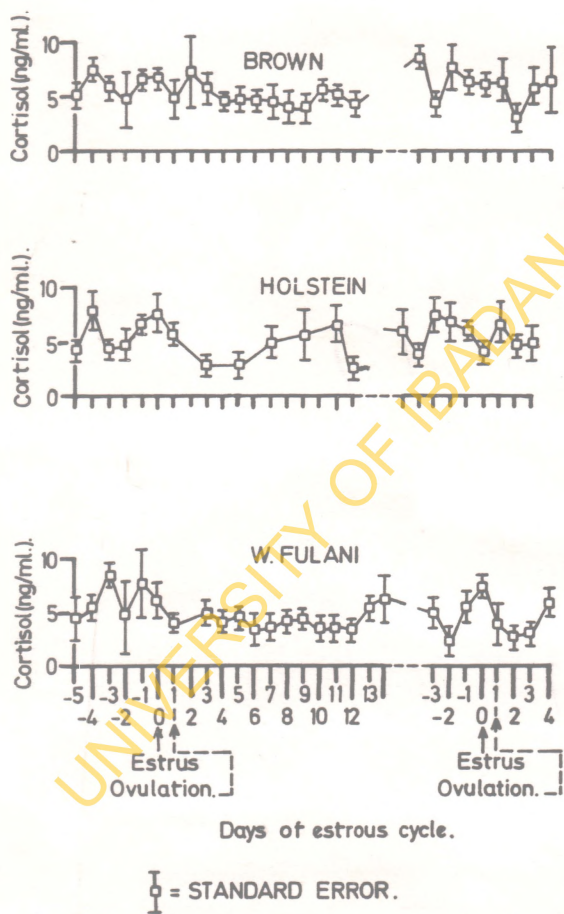


Figure 17: The period covered was late October through November 1976.

Mean plasma cortisol concentration in heifers during four periods of the year.

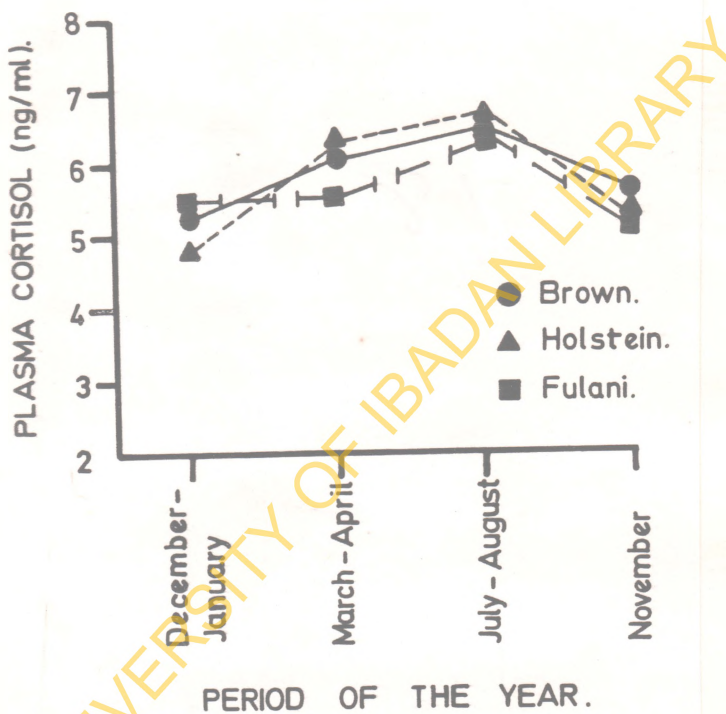


Figure 18

Determinations made on alternate days during periods indicated were plotted. See Table XVI (page 178) for the number of observation/breed/quartor.

Diurnal changes in rectal temperatures and respiratory rates in heifers (Unshaded).

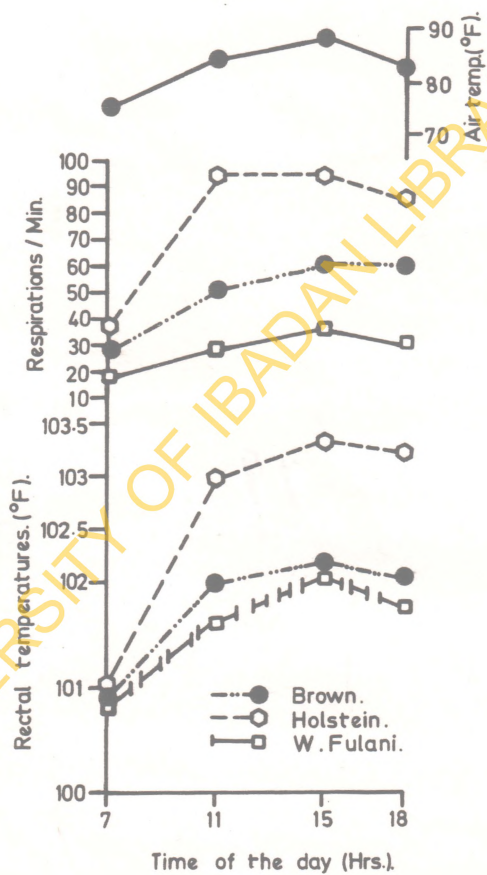


Figure 19

DIURNAL CHANGES IN RECTAL
TEMPERATURES AND RESPIR-
ATORY RATES IN HEIFERS
(Under shade).

92	77	68	57	65	88RH%
72.5	78	85	88	78	77Air Temp. (°F)

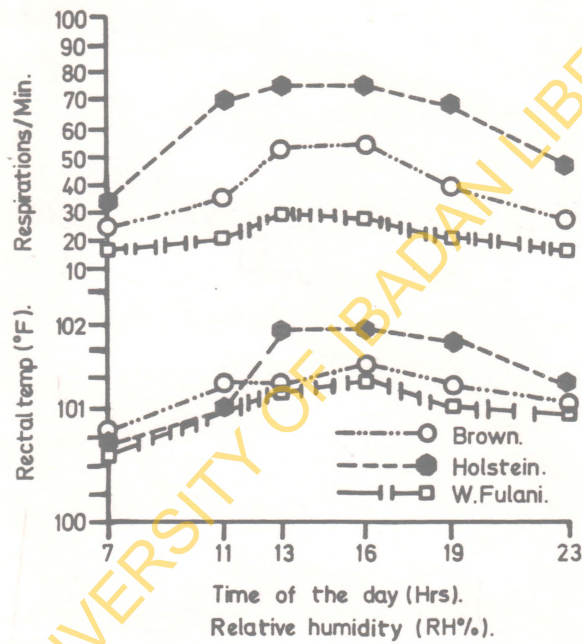


Figure 20

CIRCADIAN CHANGES IN RECTAL TEMPERATURES
AND RESPIRATORY RATES IN HEIFERS (UNDER
SHADE).

95%	74	60	57	65	80	90 RH%
72°F	82.2	88	90	80	75	73 Air temp.(°F).

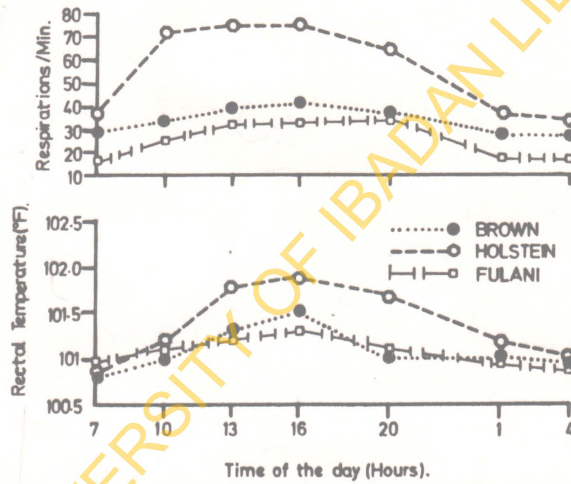
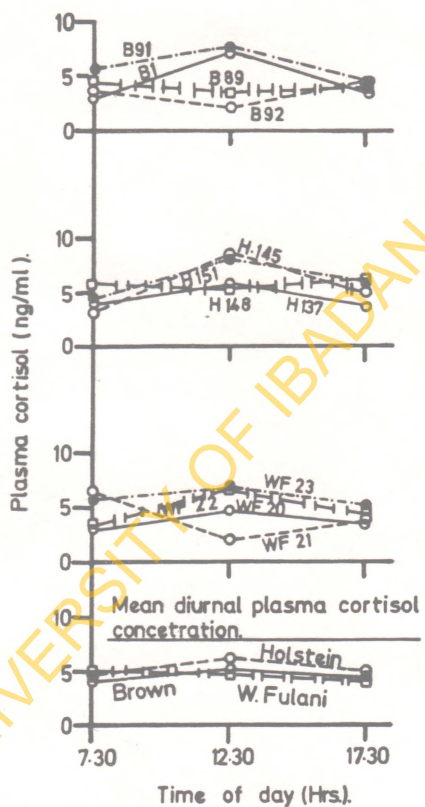


Figure 21

Diurnal plasma cortisol concentration in heifers.

73	89	91	90	Air temp. (°F).
92	70	62	68	RH %.



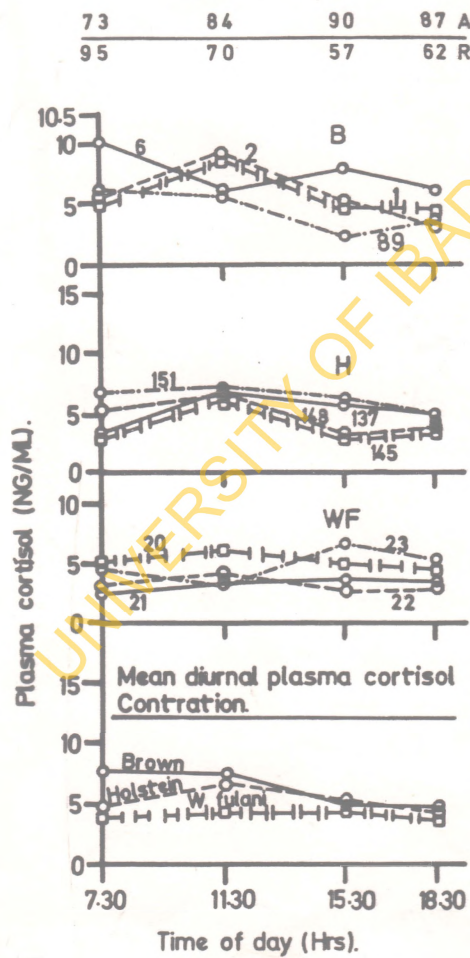
Mean diurnal plasma cortisol concentration.

Brown W. Fulani Holstein

Time of day (Hrs.)
 Relative humidity (RH%)
 Air Temperature = Air Temp (°F).
 Identification numbers of animals are indicated.

Figure 22

Diurnal plasma cortisol concentration in heifers.



CIRCADIAN PLASMA CORTISOL CONCENTRATION COMPOSITE OF THREE HEIFERS/BREED.

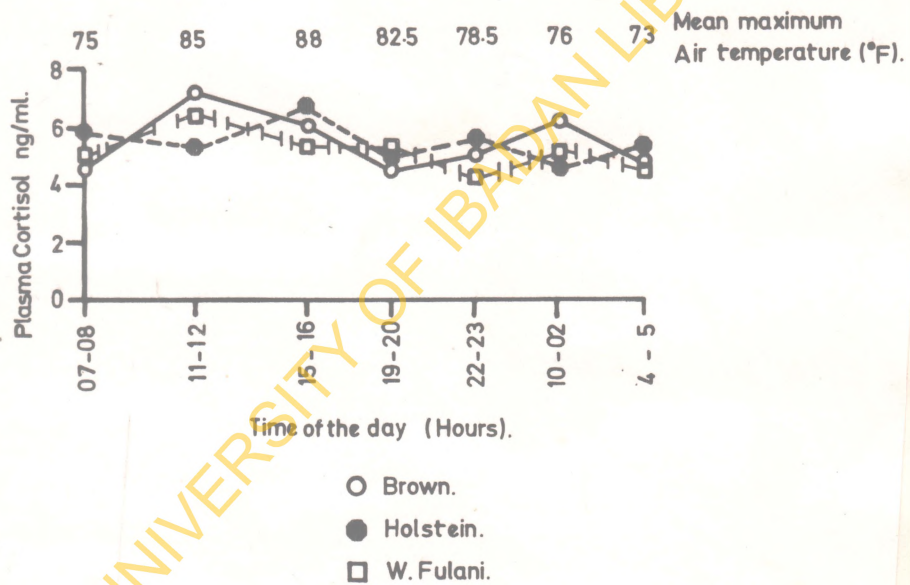
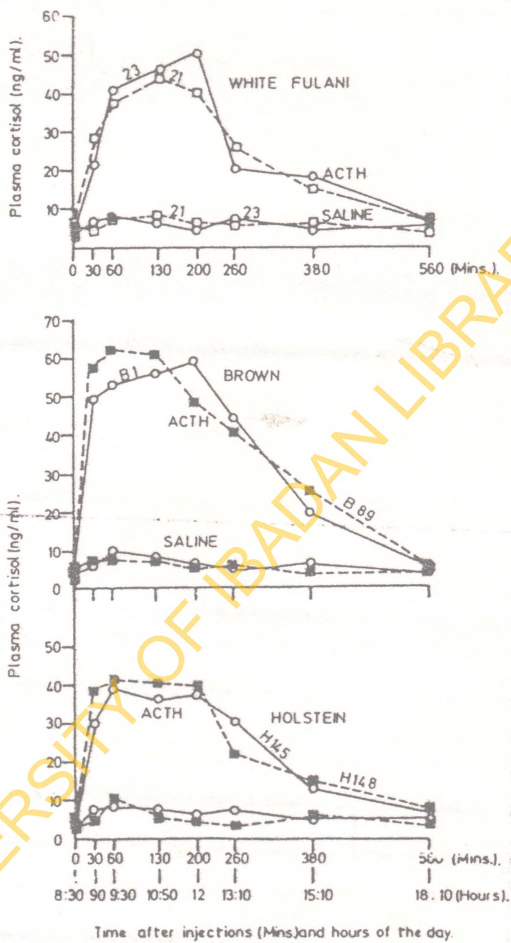


Figure 24

PLASMA CORTISOL CONCENTRATION AFTER SALINE AND ACTH INJECTIONS.



Identification numbers of heifers are indicated

Figure 25

PLASMA CORTISOL LEVELS AFTER SALINE AND ACTH INJECTIONS: COMPOSITE OF SIX HEIFERS.

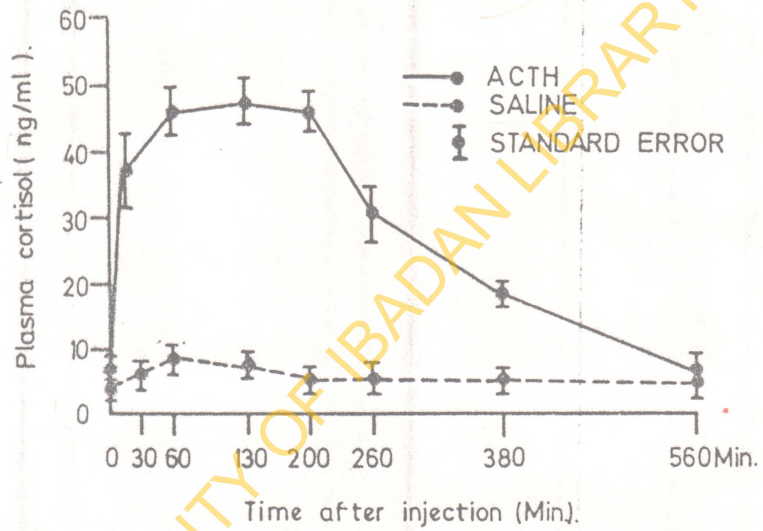


Figure 26



Figure 27: Standing-for-Mounting estrual behaviour

A White Fulani heifer being mounted by the BN teaser bull.



Figure 28: Estrual Sign (tail raising)

A holstein heifer on heat interrupts mounting with grazing despite continuous pressure from the bull.

B = Teaser bull

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APPENDIXTest of reliability of radioimmunoassay of cortisol

Fairly proportional increases in cortisol level were found when increasing volumes of a plasma sample were extracted and assayed (table A1); but there was a tendency for large volumes (0.6ml) to give lower values per milliliter of plasma than expected. The efficiency of extraction was the same when twenty volumes of solvent was used for the extraction of the larger plasma volumes (0.4 - 0.6 ml). Most of the assays were run using 0.2ml of plasma because better duplicate values were obtained than when 0.1ml of plasma were used, and because some values fell below the detection level of the chosen standard curve when the latter volume was used. The water blank gave zero values, that is, the values were far below the detection level of the standard curve most of the time.

The recovery of tritiated cortisol added to plasma was determined. Tritiated cortisol (10,000 d.p.m.) was dried in test tube; 0.2ml plasma was added and warmed for 10 minutes at 45°C, in the water bath and then mixed for 30 seconds on the vortex mixer. After allowing to stand for 20 minutes, the samples were extracted. The extracts were dried at 37°C in counting vials under nitrogen and then taken up in 0.5ml of assay buffer. The same amount of tritiated cortisol added directly to counting vials was dried and taken up in 0.5ml of assay buffer. After adding scintillant, the radioactivity was counted in every vial.

The recovery varied between 74.5 and 86%; the mean with standard error being 80.1 ± 1.0 per cent in a total of 18 determinations carried out in six different investigations.

The recovery was checked with each bottle of extraction solvent opened and from time to time with each assay. The mean recovery gave a correction factor of 1.25. Because of the fairly high precision of recovery, it has not been necessary to include tritiated cortisol as internal standard in the assays.

Increasing quantities of non-radioactive cortisol were dried in test tubes and taken up in 0.2ml of water. Cortisol was extracted and assayed; mean percentage of cortisol recovered ranged from 84 - 99.8 (Table A 2). In 20 different assays, the mean level of cortisol recovered in aqueous solutions of cortisol, 5 ng/ml, and 10ng/ml, were 5.69 (± 0.19 S.E.) and 9.97 (± 0.17) ng/ml with coefficients of variation of 15 and 8 respectively (table A 3).

Precision of assay was determined by finding the intra- and inter-assay variance by using the method of Abraham et al (1971). Measurements of the same samples in the same assay and in two different assays were done in duplicates. The coefficient of variation (CV) of the results of duplicate determination from their means was estimated by the following formula

$$CV = \sqrt{\frac{\sum d^2}{2n}}$$

where $d = \frac{\text{highest value of each duplicate} - \text{lowest value of same duplicate}}{2} \times 100$

n = number of duplicate determinations

For intra-assay variation, 12 duplicate determinations performed in the same assay with values ranging from 1.6 - 18.5 ng/ml had a coefficient of variation of 12.3% (Table A 4). For the inter-assay variation each of the groups of 10 and 9 different samples were assayed twice and the coefficients of variation were 14.9 and 18.5 respectively (Table A 5). The plasma pool run with the samples yielded a mean with standard error of 7.9 ± 0.2 ng/ml in 22 assays with a coefficient of variation of 10.4%. The reproducibility of the assay was fair as judged by the inter-assay variation. The recovery of cortisol added to plasma known to contain low levels of cortisol was also used to evaluate accuracy. 1, 2, and 4 ng of standard cortisol were dried in sets of tubes and were taken up in 0.2 ml of plasma. After extraction, assay and correction of assay value with recovery factor, the mean recoveries were 104, 110, and 98 per cent in the tubes containing 1, 2 and 4 nanograms of cortisol respectively (Table A 6).

The present method was used in participation with an RIA quality control programme arranged by the World Health Organisation (Geneva). There was close agreement in the value obtained with the present method and the mean of results from all the laboratories involved (table A 7).

The binding of tritiated cortisol at each level of the standard curve expressed as a percentage of the bound tritiated cortisol at zero concentration of the non-radioactive cortisol was fairly consistent through the assays (Table A 8). The mean (with the standard error) value of the slope of the standard curves drawn from the logit - log

transformation of the readings was 2.27 ± 0.03 in 20 assays, and the coefficient of variation was 6.3%. The consistency in the value of the slope can be a measure of accuracy as proposed by Cekan (1976)

Table A1

Assay of cortisol in increasing volumes of plasma

Plasma Volume (ml/tube)	Cortisol Value (ng)
0.6 n = 3	3.8 ± 0.1
0.4 n = 3	2.4 ± 0.2
0.2 n = 3	1.4 ± 0.1
0.1 n = 4	0.7 ± 0.1

The amount of cortisol recovered decreased at high plasma volumes.

Table A2

Recovery of cortisol in water in a single assay*

ng. of cortisol added	ng. found mean \pm standard error	% recovered \pm S. D.
1	0.9 \pm 0.1 n = 3	95 \pm 9.8
4	3.4 \pm 0.1 n = 3	84.6 \pm 2.2
8	7.98 \pm 0.9 n = 3	99.9 \pm 11.2

* The upper limit of the standard curve was 10 ng.

n = number of replicates

S D = standard deviation.

Table A3

Recovery of cortisol in aqueous solutions in 20
duplicate assays

Cortisol solution treated	Cortisol concentration determined (mean \pm standard error)	Coefficient of variation
5ng/ml	5.7 \pm 0.2 n = 20	15
10ng/ml	10.0 \pm 0.2 n = 20	8

n = number of duplicate assays.

Solutions containing cortisol, 5ng/ml and 10ng/ml of water were run in duplicates along with the samples during the assays of cortisol. The recoveries obtained were a measure of accuracy of the assay method.

254
Table A4

Duplication of samples in the same assay.
Within-assay variation is calculated from duplicate
values of plasma cortisol in 12 different samples
determined in the same assay

	Duplicate values of cortisol ng/ml	d	d ²
Samples 1	4.2 3.7	13.5	182.2
2	7.2 7.3	1.3	1.9
3	9.7 8.8	10.2	104
4	18.5 18.5	0	0
5	8 10	25	625
6	11 13	18.2	331.2
7	11.7 11.1	5.4	29.2
8	3.8 3.7	2.7	7.3
9	3.6 3.1	16.1	259.2
10	1.8 1.3	38.4	1474.6
11	2.9 2.4	20.8	432.6
12	3.9 4.4	12.8	163.8

$$d^2 = 3611.0$$

$$CV = 12.3$$

for d and CV, see footnote on page 255.

Table A5

Duplication in two different assays**
Inter-assay variation: plasma cortisol was
determined in the same plasma samples in two
different assays

	A*	B*	d	d ²
1	6.8	6.1	11.2	125
2	3.9	4.8	23.1	522.6
3	4.0	3.6	11.1	123.2
4	4.5	5.5	22.2	492.8
5	4.5	4.8	6.6	43.5
6	4.5	6.4	42.2	1780
7	13.8	14.3	3.6	13
8	12.5	14.2	13.6	185
9	7.6	5.8	31	961
10	13.5	12.0	12.5	156.2

$$\sum d^2 = 4418.7$$

$$CV = 14.9$$

d = deviation, calculated as in the text

CV = inter-assay coefficient of variation
 calculated as in the text.

* = set of plasma samples.

** = test of repeatability of assay.

Table A6

Test of Accuracy of Assay

A	B			C			D		
	(1) Amount of cor- tisol added (pg)	(2) Amount reco- vered (pg)	(3) % reco- vered	(1) Amount of cor- tisol added (pg)	(2) Amount reco- vered (pg)	(3) % reco- vered	(1) Amount of cor- tisol added (pg)	(2) Amount reco- vered (pg)	(3) % reco- vered
406	1000	1003	100.3	2000	2209	110.4	4000	3778	94.4
320	1000	1093	109.3	2000	2216	110.8	4000	4017	100.4
385	1000	1079	107.9	2000	2149	107.4	4000	3932	98.3
324	1000	1048	104.8	2000	2431	121.5	4000	3855	96.3
391	1000	999	99.9	2000	2012	100.1	4000	3803	95.0
		<u>Mean</u> 1044	<u>Mean</u> 104		<u>Mean</u> 2203	<u>Mean</u> 110.0		<u>Mean</u> 3877	<u>Mean</u> 96.9

To 0.2ml plasma containing low cortisol ^{level} in four sets of tubes (A-D) were added increasing amounts of cortisol 0 ng, 1 ng, 2 ng and 4 ng per tube per set; these were extracted and assayed; assay values were corrected for recovery and mean value of set A subtracted from sets B to D to obtain recovered value (column(2)). Within assay coefficient of variation was 11.1%.

Table A7

World Health Organisation (WHO) quality control of
radioimmunoassay

Date	Mean of results of all labora- tories (ug/100ml)	Value obtained by the present method (ug/100ml)
13/6/77	9.0	8.6 ± 0.5 SE
27/6/77	17.2	18.6 ± 0.4
11/7/77	14.6	10.6 ± 0.4
25/7/77	10.4	7.7 ± 0.5
8/8/77	16.8	16.30 ± 0.7
22/8/77	13.6	13.1 ± 0

Cortisol was assayed in plasma samples distributed by WHO to different laboratories including the laboratory where the present investigation has taken place.

Table A8

Standard curve of cortisol radioimmunoassay

Amount of steroid ng	% bound* radioactive cortisol	S E	n
5	20.7	0.6	20
2.5	30.7	0.8	20
1	48.7	1.2	20
0.5	64.3	1.6	20
0.25	80.3	1.3	20

SE = standard error.

n = number of observations considered.

* = the 100% binding of radioactive cortisol is that which occurs at zero concentration of the non-radioactive hormone.

(Antiserum was used at a total binding between 25 and 37%).

Table A9

SUMMARY OF MONTHLY WEATHER OBSERVATIONS FOR THE UNIVERSITY OF IBADAN CAMPUS
FROM DECEMBER 1975 THROUGH DECEMBER 1976

	DEC. 1975	JAN	FEB	MAR	APR	MAY	JUNE	JULY	AUG	SEPT	OCT	NOV	DEC	YEAR 1976
Mean Daily Minimum Temp.	°C 17.8	18.3	21.7	22.2	21.7	21.1	21.1	20.0	20.0	20.0	21.1	21.1	20.5	20.7
	°F 64	65	71	72	71	70	70	68	68	68	70	70	69	69.3
Mean Daily Minimum Temp.	°C 32.2	32.8	33.9	33.9	32.2	31.1	29.4	27.8	27.2	29.4	29.4	31.1	32.8	30.9
	°F 90	91	93	93	90	88	85	82	81	85	88	91	87.7	
Average Daily Mean Temp.	°C 25	25.5	27.8	28.0	26.9	26.1	25.3	23.9	23.6	24.7	25.2	26.1	26.7	
	°F 77	78	82	82	80.5	79	77.5	66.0	74	76.5	77	79	80	
Extreme Max.Temp.	°C	35.0	36.1	36.4	36.1	33.3	32.2	29.4	30.0	32.2	31.1	33.3	34.4	33.3
	°F 93	95	97	98	97	92	90	85	86	90	88	92	94	92.0
Extreme Minimum Temperature	°C	10	17.8	17.8	18.9	18.9	18.3	17.2	17.8	18.3	19.4	16.7	13.9	17.1
	°F 53	50	64	64	66	66	65	63	64	65	67	62	57	62.7
Total Precipitation	mm	10.7		19.3	43.4	129.8	129.8	196.9	67.6	25.7	39.4	195.3	29.5	0.5 877.2
	inches	0.4	11	0.8	1.7	5.1	4.8	7.7	2.7	1.0	1.5	7.7	1.7	0.0
Mean Relative Humidity (%) at 7.00 h		94	92	92	93	94	95	95	94	94	94	95	94	90 93.5
Mean Relative Humidity (%) at 16.00 h		46	37	48	50	58	66	70	75	74	64	69	57	44 59
Lowest Relative Humidity (%) for Month		25	15	35	30	30	55	59	63	62	50	57	33	22 42.6
Total Sunshine (hours)		237.1	235.6	186.9	178.0	186.7	195.0	160.4	95.0	59.0	113.3	127.9	197.6	218.9

Table A10

Mean haemoglobin concentration and packed cell volume during cool wet and dry hot seasons

Item	Wet Season				Dry Season			
	Breeds				Breeds			
	Brown	Holstein	Fulani	All	Brown	Holstein	Fulani	All
Hb g/100 ml								
Mean	8.8	8.8	10.9	9.5	8.8	8.8	11.6	<u>9.6</u>
S D	1.0	0.7	0.8	1.3	1.0	0.6	1.0	<u>1.6</u>
n	20	21	20	61	20	22	17	<u>59</u>
PCV (%)								
Mean	26.3	25.2	31	27.2	26.8	26.8	33.3	<u>28.7</u>
S D	2.5	2.5	2.3	5.1	3.8	2.0	4.0	<u>4.4</u>
n	20	221	20	61	20	22	17	<u>59</u>

Haemoglobin concentration (Hb g/100ml) and packed cell volume (PCV %) were determined in the Brown, Holstein and Fulani heifers with the conventional methods.

S D = standard deviation
 n = number of observations
 Wet Season = April - October
 Dry Season = November to mid-March.

Table A11

PCV (%) and Hb (g/100ml): cool wet and dry hot seasons compared

Hb (g/100ml)	Breeds			
	Brown	Holstein	Fulani	ALL heifers
Degree of Freedom	38	41	35	118
t	0.05	1.5	2.6	0.5
P	P > 0.05 NS	P > 0.05 NS	P < 0.01 S	P > 0.05 NS
Packed Cell Volume				
DF	37	41	35	117
t	0.5	2.2	1.2	1.7
P	P > 0.05 NS	P < 0.05 S	P > 0.05 NS	P > 0.05 NS

NS = Not significant
 S = Significant
 DF = Degree of freedom
 t = Student t value
 P = Level of significance
 Hb = Haemoglobin.
 PCV = Packed cell volume.

Table A12

Mean respiratory rates (breaths/minute) of individual Brown, Holstein and Fulani heifers at 07.00 and 15.00 hours during 42 consecutive days at different quarters of the year as indicated in the table

Heifer No.	DECEMBER- JANUARY		MARCH - APRIL		JULY - AUGUST		OCTOBER -NOVEMBER	
Brown 84	21.1	34.3	31.2	38.3	16.6	20.3	26.8	52.2
" 87	20.5	36.6	24.5	40.6	17.5	19.3	26.2	50.5
" 87	19.5	36.4	33.4	38.6	14.1	19.5	26.5	52.9
" 89	20.0	31.8	29.1	36.1	17.6	20.3	31.4	47.4
" 91	20.6	35.4	28.2	36.7	18.4	20.6	26.5	52.9
Holstein 137	25.8	56.2	35.7	60.5	23.5	28.6	34.1	70.3
" 145	28.3	54.2	44.3	67.1	22.0	25.5	27.7	65.3
" 148	26.6	58.2	28.6	63.5	21.2	27.3	24.0	68.4
" 151	28.6	53.4	38.8	69.6	23.3	28.3	37.2	74.7
" 154	27.5	69.7	42.6	72.4	26.3	30.2	34.5	75.5
Fulani 20	19.3	24.5	21.6	27.6	16.4	18.3	16.0	28.6
" 21	19.9	24.9	21.2	28.0	15.6	19.7	16.5	27.6
" 22	18.2	24.0	22	26.0	16.3	17.8	15.8	28.5
" 23	20.0	26.0	21.9	28.3	15.5	18.3	18.9	31.9
" 681	19.4	25.6	21.2	27.5	15.8	18.6	17.5	29.0

Table A13

Analysis of variance of respiratory rates in heifers
of three breeds of cattle during four periods of the year

Sources of Variation	Degree of Freedom	Mean Square	F ₁ Ratio	Significance level (P)
Season	3	4575.9	3.7	0.01*
Breed	2	4163.8	3.9	0.03*
Time of Day	1	9734.3	7.9	0.006*
Season x Breed	6	1201.2	0.98	0.99
Season x Time	3	1418.8	0.98	0.33
Breed x Time	2	1466.5	1.15	0.31
Season x Breed x Time	6	1076.2	1.19	0.99
Residual	72	1229.3	0.87	
Total	95	1485.8		

Table A 14

Contribution⁺⁺ to total variation that occurred in the respiratory rates

Season 9.6%

Breed 5.8%

Total 22.5%

Time of the Day 6.8%

⁺⁺ = as determined by the analysis of variance

* = significant.

Table A15

Mean rectal temperatures ($^{\circ}$ F) of individual Brown, Holstein and Fulani heifers at 07.00 and 15.00 hours during 42 consecutive days at different periods of the year as indicated in the table

HEIFER No.	DECEMBER-JANUARY		MARCH - APRIL		JULY - AUGUST		OCTOBER-NOVEMBER	
	TIME OF THE DAY		(HOURS)					
	07.00	15.00	07.00	15.00	07.00	15.00	07.00	15.00
Brown 84	101.4	102.3	101.3	102.3	101.4	102.1	101.3	102.3
" 86	101.1	102.3	101.3	102.4	101.3	102.4	101.3	102.3
" 87	101.2	102.2	101.2	102.3	101.4	102.3	101.4	102.4
" 89	101.3	102.2	101.3	102.3	101.3	102.2	101.3	102.3
" 91	101.3	102.3	101.3	102.3	101.3	102.4	101.2	102.4
Holstein 137	101.3	102.3	101.3	102.4	101.3	102.4	101.3	102.3
" 145	101.2	102.5	102.2	102.5	101.4	102.3	101.3	102.5
" 148	101.2	102.2	101.3	102.3	101.3	102.2	101.3	102.3
" 151	101.1	102.5	101.2	102.5	101.2	102.3	101.2	102.6
" 154	101.2	102.6	101.3	102.4	101.3	102.4	101.2	102.6
Fulani 20	101.1	101.9	101.2	102.2	101.2	102.0	101.1	101.8
" 21	101.2	107.1	101.1	101.9	101.2	101.8	101.2	102.3
" 22	101.2	102.3	101.1	102	101.1	101.9	101.2	102.1
" 23	101.2	102.1	101.2	102.3	101.3	102.2	101.2	102.3
" 681	101.2	102.3	101.2	102.0	101.2	102.2	101.2	102.2

Table A16

Analysis of variance of rectal temperatures in heifers of three breeds of cattle during four different periods of the year

S V	D.F.	Mean Square	F-ratio	Significance	L.S.D.
Season	3	0.05	6.77	0.001	0.12
Breed	2	0.54	78.60	0.001	0.13
Time	1	29.11	43.04	0.001	0.17
Season x Breed	6	0.03	4.32	0.001	
Season x Time	3	0.05	7.82	0.001	
Breed x Time	2	0.01	1.15	0.32	
Season x Breed x Time	6	0.01	1.05	0.40	
Residual	72	0.01			
Total	95	0.33			

Table A17

Contribution* to total variations that occurred in rectal temperatures

Season	0.004%
Breed	3.61%
Time of the day (morning and afternoon)	94.09%
Total	97+%

SV = source of variation.

DF = degree of freedom; L.S.D. = Least significant difference.

* = determined by the analysis of variance.

Table A18

Analysis of variance of shade seeking scores in heifers of Brown and Holstein cattle grazed in the sun

Sources of Variation	Degree of Variation	Mean Square	F-ratio	Significance
Breed	1	1.48	110.11	S
Season	3	0.19	14.88	S
Breed x Season	3	0.01	1	NS
Residual	24			
Total	31			

S = significant

NS = Not significant.

The Holstein and the Brown Heifers were considered: the Fulani did not seek shade.

Table A19

Mean percentage scores of shade seeking in the heifers during different periods of the year: observations were taken on 10 randomly chosen days of each quarter

	Brown	Holstein	Fulani
July - August 1976	19.0	43.65	0
November 1976	38.9	91.2	0
December 1976	37.15	89.12	0
March - April 1977	75.85	95.49	0

Table A20

Average scores of estrous intensity in heifers

Heifer No.	JAN	FEB	MR	AP	MY	JN	JL	AU	SEP	OCT	NOV	DEC
Brown 84	+++	+++	+++	+++	+++	+	+	+++	+++	++	+++	+++
" 86	+++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
" 87	+++	+	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
" 89	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
" 91	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Holstein 137	+++	+	+++	+++	++	++	+++	+++	++	+++	+++	+++
" 195	+++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
" 148	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
" 151	+++	+++	+++	++	+++	++	+++	+++	++	+++	+++	+++
" 154	++	++	++	++	+++	+++	+++	+++	+++	+++	+++	+++
Fulani 20	++	+	++	++	++	+++	+	++	++	++	+	+
" 21	++	++	++	+++	+++	++	+++	++	+++	++	++	+++
" 22	+++	++	+	++	++	++	+	++	++	++	+++	++
" 23	+++	+++	+++	++	+++	+++	++	+++	+++	+++	+++	+++
" 681	+++	++	++	++	+	++	+++	++	+	++	++	+

Average estimate of the intensity of estrus determined as in the text in heifers through the year; (+++) high (++) medium and, (+) low intensities.

Table A21

Distribution of the time of commencement of estrus
through the 24-hour day in heifers;
observations were made over one calendar year

	Brown	Holstein	Fulani
Time of the day (Hour)	n	n	n
5 - 7	18	18	13
7 - 9	16	29	19
9 - 11	10	7	9
11 - 13	5	-	3
13 - 15	1	3	4
15 - 17	1	5	4
17 - 19	5	1	6
19 - 21	2	2	1
21 - 23	2	2	4
23 - 01	1	1	2
01 - 03	-	-	2
03 - 05	9	6	8

\bar{n} = number of observations.

Table A22

Sources of Variation	Degree of Freedom	Mean Square	F-ratio	Significance
Month	11	1.5	1.45	NS
Breed	2	0.03	0.03	NS
Time	3	21.12	20.4	0.001**

Analysis of variance of time of onset of estrus in heifers through the year. Highly significant (**) effect of time of the day; and non-significant (NS) effects of month and breed.

Table A 23

Duration of estrous period in Brown (B),
Holstein (H) & Friesian (F) heifers through the year.

1973

	JAN	FEB	MAR	APR	MAY	JUNE	JULY	AUG	SEPT	OCT	NOV	DEC
Heifer No												
B 184	22	12	15	24	16	31	17	23	31	30	8	25
B 37	17	17	12	13	14		14	25	11	31	24	17
B 36	15	10	15	12	15	16	15	16	14	12	27	13
B 89	16	10	8	22	12	12	25	10	14	14		13
B 91			17	17	19	17	21	13	14	13	16	
				13		19	11				9	
B 92				13			15	25	13		13	
H 137	(16 (18	10 13	12	30	15	13	17	28	13	13	13	
H 145	16		16	26	12	15	14	13	12	24	29	21
H 148	(14 (9	10	11	30	16		12	30	12	14	
							23					
H 151	(11 (13	17	10	29	14 14		13	12 14	12 15	15
H 154	13		14	12			27		12	14 14	13 15	
F 20	(11 (12	10	13	12	23	22 23	30	27 29	27	12	11	12
F 21	15	8	12	11	10		17	27	9	14	13	13
F 22	15		8	21	9	9	27	10	9	14	14	24
F 23	(22	14 9	17	11 19	10 14	9 10	12 9		22 10	13 14	15	14 17
F 681							10	28	3	10	3	11
F 682								11	11	13		

Duration of estrus was determined as period during which the heifer stayed to be mounted by the teaser bull as described in the text.

Table A 24

Interval of ovulation from end (E) and onset (O) of estrus in
Brown (B), Holstein (H) and Fulani (F) heifers

Heifer No.	JAN		FEB		MAR		APR		MAY		JUN		JUL		AUG		SEPT		OCT		NOV		DEC	
	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O
B 84	8	30	13	25	10	25	13	42	14	30	6	37	14	31	14	37	15	28	10	40	45	59	13	30
			8	29																			17	27
B 87	16	34	18	33	13	28	19	31					10	27			28	42	16	35	7	27	9	24
	9	24			CL																			
B 86	16	31	10	24	14	24	13	26			16	36	23	48	CL						10	29	17	29
	16	22	15	32			15	25																
B 89										15	32	CL		19	29	18	34	19	36					
B 91							18	33	14	28	10	26		15	40									
B 92										18	28	13	27											
H 137	15	30	20	30	17	29	20	40	12	24	10	26	13	27		26	38	14	30	15	28	11	27	
H 145	16	32			13	32			16	28	13	26		19	36		CL							
H 151	8	24			18	36	17	32	8	18	18	32		10	29	17	30	14	34	10	29	14	30	
H 148	CL				16	35	23	36			15	34	17	30	18	32	10	23	14	28	CL	15	25	
H 154																15	27	16	29	15	29			
F 20	11	22	16	26	17	30	16	34	13	41	13	35	10	40	13	40	6	33			15	28		
	13	24	14	30																				
F 21	14	25	8	24	12	30	9	22	14	37			20	39					16	33	CL	12	27	
	18	32																						
F 22	16	31	CL		18	26	7	22	10	24	15	26		9	36	16	28	17	34					
	16	25																						
F 23	9	31	16	30	14	27	16	37	13	38	36	51	14	34			12	24	13	27	20	35	32	40
	9	26																						
F 681													13	26					19	29	16	24	10	31
F 682															20	31	9	20	10	23				

CL = Cases in which ovulation was doubted until corpus luteum growth was observed.

Table A 25

Mean plasma cortisol concentration in individual heifers
(Observations made on alternate days)

	Jan - Dec			March - April			July - Aug.			November			
	\bar{x}	SE	n	\bar{x}	SE	n	\bar{x}	SE	n	\bar{x}	SE	n	
Brown	84	5.3	+0.4	16	6.2	+1.0	20	6.4	+0.7	20	5.7	+0.3	23
"	86	5.6	+0.6	18	6.5	+0.6	19	7.2	+0.7	20	5.7	+0.6	19
"	87	4.3	+0.5	17	5.1	+0.6	20	6.4	+0.5	20	5.6	+0.5	20
"	89	5.0	+0.5	16	6.3	+0.5	18	7.5	+0.7		6.0	+0.5	22
"	91	4.4	+0.4	20	5.1	+0.4	19	7.4	+0.6	19	6.2	+0.8	21
Holstein	137	4.2	+0.4	15	5.7	+0.6	19	5.4	+0.4	20	5.0	+0.4	22
"	145	4.5	+0.3	19	6.9	+0.5	21	5.9	+0.4	19	5.1	+0.4	21
"	148	4.5	+0.3	16	7.0	+0.4	19	7.4	+0.1	19	5.7	+0.5	20
"	151	5.2	+0.4	19	6.0	+0.6	19	7.2	+0.6	18	5.7	+0.5	19
"	154	4.8	+0.6	18	6.1	+0.6	18	6.9	+0.8	21	7.6	+1.2	20
Fulani	20	4.8	+0.4	17	5.7	+0.5	20	5.2	+0.5	18	5.0	+0.4	23
"	21	5.5	+0.7	17	5.5	+0.5	21	5.8	+0.5	20	5.2	+0.5	11
"	22	6.8	+0.7	19	5.8	+0.6	18	6.9	+0.6	18	5.7	+0.5	23
"	23	5.6	+0.5	19	5.7	+0.6	20	6.1	+0.6	19	4.8	+0.4	20
"	681	4.5	+0.5	24	5.6	+0.4	21	6.8	+0.6	23	5.2	+0.6	22

Mean (\bar{x}) (with standard error (S E) plasma cortisol concentration in heifers on alternate days during different quarters of the year.

n = number of observations.

Table A 26

Mean plasma cortisol concentration in individual heifers

		January-December			March - April ¹¹			July - August			October-November		
		\bar{x}	SE	n	\bar{x}	SE	n	\bar{x}	SE	n	\bar{x}	SE	n
Brown	84	5.70	+0.45	35	5.93	+0.61	40	5.78	+0.39	40	5.70	+0.34	23
"	86	4.91	+0.36	36	5.97	+0.45	38	6.14	+0.37	38	5.55	+0.54	24
"	87	5.68	+0.42	36	6.45	+0.47	35	6.83	+0.46	40	5.30	+0.4	25
"	89	4.92	+0.35	36	6.11	+0.42	38	7.26	+0.60	37	6.0	+0.48	22
"	91	4.36	+0.38	20	5.12	+0.36	19	7.44	+0.57	32	6.19	+0.80	21
Holstein	137	4.62	+0.27	31	5.72	+0.41	37	5.45	+0.27	41	5.05	+0.41	22
"	145	4.86	+0.28	29	6.82	+0.43	40	6.46	+0.38	39	5.06	+0.45	24
"	148	4.93	+0.22	29	6.49	+0.2	38	7.4	+0.44	38	5.5	+0.4	27
"	151	5.08	+0.32	30	6.30	+0.36	38	6.96	+0.45	36	5.69	+0.53	24
"	154	4.81	+0.61	18	6.14	+0.63	18	6.9	+0.79	21	7.59	+1.18	20
Fulani	20	4.80	+0.42	32	5.58	+0.40	40	5.57	+0.41	36	4.96	+0.38	23
"	21	5.35	+0.54	33	5.22	+0.41	40	6.31	+0.42	39	5.09	+0.39	26
"	22	6.50	+0.53	35	6.07	+0.51	36	6.88	+0.49	33	5.72	+0.50	23
"	23	5.34	+0.29	37	5.84	+0.46	40	6.60	+0.48	34	5.06	+0.38	25
"	681	4.48	+0.48	24	5.61	+0.41	21	6.83	+0.56	23	5.22	+0.58	22

\bar{x} = Mean

SE = Standard error

n = Number of observations.

Table A 27

Analysis of variance of plasma cortisol levels in heifers; levels at diestrus, estrus and the mean of values during four different quarters of the year were compared

Source of variation	Degree of freedom	Mean square	F-ratio	Significance level	L.S.D.
Main effect	7	59.25	12.76	0.001**	
Cycle day	3	171.24	36.39	0.001**	3.45
Season	3	21.74	4.68	0.004**	2.99
Breed	2	3.53	0.76	0.99	
Day x season	6	5.88	1.2	0.23	
Day x Breed	4	3.92	0.8	0.99	
Season x Breed	6	5.49	1.13	0.32	
Day x Season x Breed	12	3.73	0.80	0.99	
Residual	108	4.64			
Total	143	7.37			

** = highly significant

L.S.D. = least significant difference.

Table A 28a

Analysis of variance of plasma cortisol concentrations in Brown, Holstein and Fulani heifers through four different quarters of the year

Sources of variation	Degrees of freedom	Mean squares	F-ratio	P level
Breed	2	0.25	1.01	6.37
Season	3	4.03	16.07	0.001**
Breed x Time	6	0.37	1.47	0.20
Residual	36	0.25		
Total	47	0.50		

P = Significance level

** = Highly significant

Table A 28b

Contribution* to total variation

Breed	2.2%
Season	50.4%
Total	52.8%

* = As determined by the analysis of variance.

Table A 29

Rectal temperature and respiratory responses of heifers in the sun

HEIFER NO.	07.00		11.00		15.00		18.00	
	T_{re}	RR	T_{re}	RR	T_{re}	RR	T_{re}	RR
Brown 84	100.2	30	101.8	40	102.4	52	102.2	50
" 87	101.5	28	102	52	102.2	64	102	56
" 92	101	32	102.4	54	102.2	60	101.9	64
" 86	100.8	28	101.1	50	102	56	102.2	58
" 91	101	26	102.4	42	102.4	60	102	58
Holstein 137	100.4	36	102	90	102.4	88	102.4	84
" 145	101.6	42	103.6	106	103.4	92	103.2	90
" 148	101.2	30	102	110	102.2	100	102	88
" 151	100.3	40	102.6	90	102.5	100	102.6	82
" 154	101.5	36	104.5	84	104.5	90	106	84
Fulani 20	100.6	22	101.2	28	102.2	44	101.4	32
" 21	101	14	101.6	40	102.4	28	101.8	26
" 22	101.2	16	101.7	36	102.8	40	102.3	28
" 23	100	20	101.8	37	102.6	36	102	34
" 681	101.2	18	102	26	102.4	34	101.5	36

T_{re} = Rectal Temperature ($^{\circ}$ F)

RR = Respiratory rate (breaths/minute)

Heifers were kept in the paddock throughout the day unshaded when the readings were taken.

Table A 30

Analysis of variance of respiratory rates of heifers
in the sun

SV	D F	Mean Square	F-ratio	Significance
Time	3	4069.75	71.57	0.001 S
Strain	2	9692.51	170.45	0.001 S
Time x Breed	6	359.85	6.33	0.001 S
Residual	48	56.86		
Total	59	618.35		

Contribution* to total variations that occurred

Time	33%
Breed	53%
Total	86.6%

S V = source of variation

DF = degree of freedom

S = significant effect

* = as determined by analysis of variance.

Table A 31

Analysis of variance of rectal temperature of heifers
in the sun

S V	DF	Mean Square	F-ratio	Significance level	L.S.D.
Time	3	8.81	41.35	0.001**	1.24
Breed	2	2.18	10.48	0.001**	1.43
Time x Breed	6	0.24	1.18	0.3	
Residual	48	0.21			
Total	59	0.71			

Contribution* to Total Variation that occurred

Time	62%
Breed	10%
Total	72.5%

Observations were made on three occasions in March 1977

S-V = Source of variation

DF = Degree of freedom

LSD = Least Significant Difference

** = Highly significant

* = As determined by analysis of variance.

Table A 32

Correlations between rectal temperature, respiratory rate and environmental temperature in the sun

	r	r ²	Significance	Slope	Intercept	S.E. of Estimation
(1) Rectal Temperature (T_{re}) Versus Environmental Temperature (T_a) (All breeds)	0.73	0.53	0.00001	0.129	91.67	0.579
(2) Respiratory Rate (RR) Versus (T_a) (All breeds)	0.54	0.29	0.00001	2.73	173.114	20.37
BROWN	0.85	0.73	0.00001	0.039	100.16	0.38
HOLSTEIN	0.86	0.73	0.00001	0.038	99.82	0.560
FULANI	0.73	0.53	0.00018	0.05	100.40	0.4035

r = Correlation coefficient

S E = Standard error.

Table A 33

Analysis of variance of readings of circadian rectal
temperatures of heifers in the shade

Sources of variation	Degree of Freedom	Mean square	F-ratio	Significance	Lowest significant difference
Main effect	8	3.21	18.69	0.001**	
Time of the day	6	3.86	22.47	0.001**	0.43
Breed	2	1.26	7.35	0.001**	0.22
Breed x Time	12	0.14	0.79	0.999 NS	
Residual	84	0.17			
Total	104	0.40			

Contribution* to total variations

Time	54.76%
Breed	6.25%
Total	61.5%

* = as determined by analysis of variance.

** = highly significant.

NS = Not Significant.

Observations were taken at 2-3 hourly intervals during the day and night on one occasion (March 1977).

Table A 34

Analysis of variance of respiratory rates of heifers kept
in the shade and in the sun

Sources of variation	Degree of freedom	Mean Square	F-ratio	Significance level
Main effects	6	8691.81	195.62	0.001**
Breed	2	16071.76	361.72	0.001**
Time of the day	3	5545.52	124.81	0.001**
Type of treatment	1	3370.8	75.87	0.001**
Breed x Time	6	579.21	13.04	0.001**
Time x Type	2	627.78	14.29	0.001**
Breed x Time x Type	6	70.72	1.59	0.157
Residual	96	44.43		
Total	119	525.09		

Contribution* to total variations that occurred

Breed	52%
Time of the day	27%
Type of treatment	5%
Total	83.5%

** = Highly significant effects

* = As determined by analysis of variance.

Table A 35

Analysis of variance of values of rectal temperature in heifers in the shade and in the sun

Sources of Variation	Degree of Freedom	Mean square	F-ratio	Significance level	
Breed	2	3.47	8.10	0.001	S
Time of the day	3	13.54	31.62	0.001	S
Type of Treatment	1	11.27	26.33	0.001	S
2-way interaction	11	0.62	1.45	0.16	N.S
Breed x Time	6	0.54	1.37	0.2	N.S
Breed x Type	2	0.15	0.35	0.9	N.S
Time x Type	3	1.0	2.35	0.07	N.S
BreedxTimexType	6	0.35	0.82	0.9	N.S
Residual	96	0.43			
Total	119	0.91			

Contribution* to total variations that occurred

Breed	7%
Time	37%
Type of treatment	10%
Total	54%

- * = As determined by analysis of variance
 S = Significant
 N-S = Not Significant

Observations in the shade were made in the pen.

Table A 36

Plasma cortisol concentration and rectal temperatures
of heifers kept in the sun throughout the day
 (Experiment 1)

TIME OF THE DAY (HOUR)						
		07.00	12.00		05.00	
PLASMA CORTISOL CONCENTRATION* AND RECTAL TEMPERATURE						
Heifer Identification Number	Cortisol (ng/ml)	T_{re} ($^{\circ}$ F)	Cortisol (ng/ml)	T_{re} ($^{\circ}$ F)	Cortisol (ng/ml)	T_{re} ($^{\circ}$ F)
Brown 84	3.4	101.4	7.4	102.6	3.6	102.8
" 92	3.7	101	2.0	102.4	4.6	102.5
" 89	4.3	101.5	3.4	103.0	4.4	103.3
" 91	5.7	100.7	7.8	102.0	4.6	102.0
Holstein 137	3.9	100.4	5.4	101.6	3.6	102
" 145	3.2	101.5	8.8	104.4	5.4	104.2
" 148	5.6	101	2.8	102.6	5.5	102.6
" 137	4.2	101.2	8.4	103.0	6.1	103.2
Fulani 20	3.7	101.6	3.6	102	3.9	102.4
" 21	6.4	101	2.2	101.8	3.8	101.6
" 682	3.5	101.2	6.8	102.5	4.6	102.8
" 23	5.8	100.3	7.0	101.6	5.3	101.6

T_{re} = Rectal temperature ($^{\circ}$ F)

* = Plasma cortisol concentration in ng/ml.

Observations were made thrice in the day in heifers kept in the paddock.

Table A 37

Diurnal plasma cortisol concentration in heifers (ng/ml) Experiment 3)

	Time (h)	7-8:00	11-12:00	15-16:00	18-19:00
	T _a (°C)	22.4	28	32.8	30
<hr/>					
Heifer identification					
Brown 84 (1)		5.0	4.8	4.8	4.7
87 (2)		5.2	10.0	5.1	3.2
86 (6)		10.3	6.0	7.8	5.9
89		6.2	6.0	2.4	3.5
Holstein 137		3.2	6.2	5.2	3.5
145		3.4	7.2	3.0	3.5
148		5.5	6.6	3.4	4.1
151		6.9	7.1	6.6	5.1
154		19.0	7.2	3.5	3.0
Fulani 20		5.2	6.1	5.1	4.5
22		3.0	3.9	2.9	3.0
23		4.5	3.6	6.8	5.3
21		2.9	3.4	3.6	3.0

Table A 38

Analysis of variance of diurnal plasma cortisol concentration in heifers (Experiment 1)

Sources of variation	Degree of freedom	Mean square	F-ratio	Significance	
Time of day	2	7.26	1.06	0.3	N.S
Breed	2	4.10	0.89	0.2	N.S
Time x Breed	2	3.30	0.71	0.9	N.S
Residual	27	4.48	0.97		
Total	35	4.5			

N.S = Not Significant

Table A 39

Correlation between plasma cortisol and rectal temperature
of heifers kept in the sun (Experiment 1)

Breed	r	r ²	Signi- ficance	Slope	Intercept	Standard error of determinati
Brown	0.5	0.26	0.055	1.90	-187.64	2.6
Holstein	0.49	0.25	0.05	0.80	- 76.73	1.82
Fulani	-0.27	0.07	0.194	-0.62	68.07	1.55

r = Correlation coefficient.

Table A 40

Analysis of variance of diurnal plasma cortisol concentrations
in heifers (Experiment 3)

Sources of variation	Degree of freedom	Mean square	F-ratio	Significance	
Time	3	15.10	2.81	0.05	S
Breed	2	17.14	3.19	0.05	S
Breed x Time	6	6.57	1.22	0.31	NS
Residual	36	5.37			
Total	47	6.64			

Contribution* to total variation that occurred

Time of the day	14%
Breed	11%
Total	25%

* = As determined by analysis of variance

S = Low significance

NS = Not significant

Table A 41

Circadian plasma cortisol concentration in heifers
(Experiment 4)

	Time of the day (hour)						
	07.30-08.00	12-13.00	16-17.00	19-20.00	22-23.00	01-02.00	04-05.00
	Plasma Cortisol (ng/ml)						
Heifer Number							
Brown 91*	5.12	6.07	6.59	3.74	7.21	8.92	4.56
" 81	5.29	8.61	4.62	4.5	4.25	4.28	5.54
" 6	3.53	6.98	7.02	5.15	3.28	5.48	3.86
Holstein 148*	8.68	5.4	10.32	5.35	5.20	6.84	4.63
" 13	5.48	6.42	3.28	5.08	6.10	3.92	6.25
" 145	3.02	4.26	6.5	5.47	5.5	3.04	5.26
Fulani 681*	6.86	7.40	8.07	5.10	3.41	5.74	6.00
" 21	5.21	6.22	3.51	4.53	3.57	5.26	3.62
" 23	3.15	5.68	5.80	6.36	5.68	4.00	4.02

Jugular (*) and tail vein blood samples were collected at the times shown on the table in restricted heifers of the Brown, Holstein and Fulani breeds; plasma cortisol levels showed no circadian rhythm.

Table A 42Analysis of variance of circadian plasma cortisol concentration (ng/ml) in heifers (Experiment 4)

Source of variation	Degree of freedom	Mean square	F-ratio	Significance	
Main effect	8	2.67	1.76	0.119	NS
Breed	2	0.69	0.45	0.999	NS
Time	6	3.33	2.19	0.062	NS
Breed x Time	12	1.52	1.00		
Residual	42	1.51			
Total	62	1.66			

Contribution* to total variation that occurred

Breed	1.4%
Time	19.36%
Total	20.7%

NS = Not significant

* = As determined by analysis of variance.

Table A 43

Mean rectal temperatures of nonlactating pregnant cows and nonlactating nonpregnant nulliparous heifers in the sun, unshaded and shaded

		T I M E of the day (Hour)		
		07-08.00 Unshaded	14-15.00 Unshaded	14-15.00 Shaded
COWS	BROWN	Rectal Temperatures ($^{\circ}$ F)		
		\bar{x} 101.38	102.68	101.50
		SD \pm .27	\pm .99	\pm .92
		n 5	5	5
	HOLSTEIN	\bar{x} 101.3	104.94	103.2
		SD \pm 0.28	\pm 1.17	\pm 1.69
n 5		5	5	
HEIFERS	BROWN	\bar{x} 100.82	102.08	101.22
		SD \pm .57	\pm .52	\pm .39
		n 5	5	5
	HOLSTEIN	\bar{x} 100.72	103.68	101.72
		SD \pm .51	\pm 1.47	\pm .54
		n 5	5	5
FULANI	\bar{x} 100.66	101.96	101.08	
	SD \pm .32	\pm .5	\pm 0.23	
	n 5	5	5	

n = number of animals

Table A 44

Plasma cortisol concentration at different time intervals
in heifers injected with saline and ACTH (200 IU)

Interval of Blood Collection (min)	Plasma cortisol in individual heifers (ng/ml)											
	B 80		B 84		H 145		H 148		F 21		F 23	
	Saline	ACTH	Saline	ACTH	Saline	ACTH	Saline	ACTH	Saline	ACTH	Saline	ACTH
0	5.0	4.2	3.2	5.1	3.1	3.5	4.1	3.4	5.2	3.4	3.7	3.9
+30	6.1	49.6	6.6	57.7	7.3	30.6	4.8	38.7	4.7	28.5	6.9	21.2
+50	7.9	53.2	9.4	62.4	8.9	37.7	10.2	41	7.3	38	7.5	41.3
+130	6.4	56.0	6.6	61	7.6	36.7	5.9	40.7	8.9	44	6.5	46.4
+195	6.4	50.4	5.1	48.8	6.0	37.7	4.6	40	5.9	40.5	4.8	50.3
+255	4.9	44.8	5.8	40.9	7.4	30.6	3.0	22.1	5.3	26.2	7.0	20.7
+375	5.9	19.6	3.8	25.5	4.9	16.3	5.4	15.2	5.2	15	4.5	18.4
+555	5.2	6.8	5.5	4.5	5.5	7.5	4.1	6.7	3.7	5.4	5.5	6.5
+24 HRS.	7.2	4.6	3.4	6.1	2.6	5.5	6.2	4.2	6.5	5.2	4.3	2.4

Identification numbers of heifers are indicated after
 B (Brown), H (Holstein) and F (Fulani).

Table A 45

Analysis of variance of plasma cortisol concentration
in heifers injected with saline and ACTH

Sources of variation	Degree of freedom	Mean square	F-ratio	Significance
Time interval	8	1173.17	103.79	0.001**
Breed	2	458.21	40.54	0.001**
Type of treatment	1	10976.61	971.17	0.001**
Time x Breed	16	45.29	4.00	0.001**
Time x Type	8	801.47	70.00	0.001**
Breed x Type	2	349.80	30.94	0.001**
TimexBreedxType	16	47.87	4.23	0.001**
Residual	54	11.30		
Total	107	285.35		

Contribution to total variations that occurred

Type	36%
Time	30%
Breed	2%
Total	70%

** = Highly significant.

Table A 46

Analysis of variance of plasma cortisol values in
heifers injected with ACTH

Source of variation	Degree of freedom	Mean square	F-ratio	Significance
Main effects	9	1686.25	110.89	0.001**
Breed	2	764.32	50.26	0.001**
Time	7	1949.66	128.21	0.001**
Breed x Time	14	64.61	4.25	0.001**
Residual	24	15.21		
Total	47	349.91		

** = Highly significant.

Table A 47

Analysis of variance of plasma cortisol values in
heifers injected with saline

Sources of variation	Degree of freedom	Mean square	F-ratio	Significance
Main effect	9	8.24	6.05	0.001**
Breed	2	0.15	0.11	0.9 N S
Time	7	10.56	7.75	0.001**
Breed x Time	14	0.46	0.33	0.9 N S
Residual	24	1.36		
Total	42	2.41		

** = Highly significant

N S = Not significant.