



APPENDIX

Table 1. Total prolactin immunoreactive images (PII) counted, Mean prolactin immunoreactive image surface area(MPIIA), Mean prolactin immunoreactive image surface area after transformation (MPIAT), Standard Deviation (SD) and Proportion of total area stained (PTAS) for the non-lactating (NL) and lactating (L) groups

Animal/Group	Total PII	MPIIA (μm^2)	MPIAT	S D	PTAS
NL 1	183	161,1424	3,0500	0,3513	0,01966
NL2	108	142,7271	2,9896	0,3469	0,01028
NL3	127	167,7681	3,0370	0,3814	0,01420
NL4	134	159,4177	3,0264	0,3667	0,01424
NL5	165	158,7227	3,0308	0,3565	0,01746
NL6	117	206,1310	3,1286	0,3905	0,01608
NL7	82	132,6842	2,9716	0,3317	0,00725
NL8	56	162,7280	3,0584	0,3536	0,00608
NL9	54	252,5954	3,2038	0,4230	0,00909
NL10	64	213,0313	3,1474	0,4048	0,00909
Non-Lactating	1090	175,6948	3,0467	0,3680	0,01277
L1	265	158,5346	3,0304	0,3639	0,02801
L2	138	182,9749	3,0930	0,3743	0,01683
L3	44	160,4264	3,0567	0,3440	0,00471
L4	155	144,4156	3,0042	0,3367	0,01492
L5	80	146,9649	3,0211	0,3396	0,00784
L6	118	204,8796	3,1398	0,3760	0,01588
L7	73	169,1121	3,0486	0,3783	0,00823
L8	106	204,8505	3,1377	0,3856	0,01448
L9	113	211,7866	3,1649	0,3728	0,01595
L10	110	185,8706	3,1024	0,3716	0,01363
Lactating	1202	176,9816	3,0751	0,3680	0,01418

Table 2. Total somatotropin immunoreactive images (SMTII) counted, Mean somatotropin immunoreactive image surface area (MSMTIA), Standard Deviation (SD) and Proportion of total area stained (PTAS) for the juvenile male (J) and adult male (A) groups

Animal/Group	Total SMTII	MSMTIA (μm^2)	S D	PTAS
J1	320	764,3250	1077,0379	0,1631
J2	380	632,0500	883,3562	0,1601
J3	317	487,9590	478,4198	0,1031
J4	331	849,1964	1138,8335	0,1874
J5	328	793,5427	955,3352	0,1735
J6	379	1121,9868	1526,7062	0,2835
J7	263	1048,4563	1318,5661	0,1838
J8	397	965,2317	1353,0254	0,2555
J9	212	1132,1274	1555,8380	0,1600
Juvenile	2927	855,8241	1192,4024	0,1856
A1	214	554,7804	583,9511	0,0791
A2	236	466,2415	335,5042	0,0734
A3	255	472,8824	412,3376	0,0804
A4	194	488,7062	687,4381	0,0632
A5	257	642,8405	593,2428	0,1101
A6	244	674,8770	947,9141	0,1098
A7	227	630,5595	666,4675	0,0954
Adult	1627	563,7167	633,9105	0,0874

Table 3. Total ACTH immunoreactive images(TACTHII) counted, Mean ACTH immunoreactive image surface area(MACTHIA), Mean ACTH immunoreactive image surface area after transformation (MACTHIAT), Standard Deviation (SD) and Proportion of total area stained (PTAS)

Animal/Group	TACTHII	MACTHIA (μm^2)	MACTHIAT	S D	PTAS
NL1	305	146,9474	2,1406	0,1496	0,02988
NL2	223	148,1042	2,1439	0,1505	0,02202
NL3	256	146,3174	2,1395	0,1474	0,02497
NL4	237	133,9093	2,1028	0,1411	0,02116
NL5	223	142,4180	2,1255	0,1524	0,02117
NL6	243	152,0364	2,1532	0,1571	0,02463
NL7	154	144,9896	2,1314	0,1596	0,01489
NL8	240	145,7887	2,1353	0,1550	0,02333
NL9	247	135,1972	2,1086	0,1364	0,02226
NL10	376	147,5416	2,1393	0,1582	0,03698
Non-lactating	2504	144,5411	2,1327	0,1514	0,02413
L1	250	143,5588	2,1321	0,1458	0,02393
L2	297	143,1917	2,1281	0,1525	0,02835
L3	231	137,2140	2,1094	0,1517	0,02113
L4	385	151,0205	2,1516	0,1531	0,03876
L5	313	145,9223	2,1364	0,1527	0,03045
L6	354	156,2297	2,1670	0,1522	0,03687
L7	301	156,1572	2,1672	0,1511	0,03134
L8	397	142,1178	2,1260	0,1498	0,03761
L9	415	148,9726	2,1445	0,1565	0,04122
L10	309	141,5818	2,1257	0,1460	0,02917
Lactating	3252	147,0579	2,1401	0,1522	0,03188

Animal/Group	TACTHII	MACTHIA (μm^2)	MACTHIAT	S D	PTAS
J1	219	160,0457	2,1773	0,1534	0,02337
J2	387	139,0992	2,1177	0,1463	0,03589
J3	396	146,3304	2,1390	0,1489	0,03863
J4	408	147,8847	2,1418	0,1544	0,04022
J5	356	140,2346	2,1195	0,1505	0,03328
J6	329	143,7588	2,1288	0,1554	0,03153
J7	319	161,7561	2,1794	0,1603	0,03440
J8	349	145,5935	2,1344	0,1555	0,03388
J9	350	137,1162	2,1086	0,1522	0,03199
Juvenile	3113	146,0933	2,1363	0,1542	0,03369
A1	201	132,0617	2,0959	0,1414	0,01770
A2	146	129,6295	2,0839	0,1509	0,01262
A3	266	139,6412	2,1153	0,1560	0,02476
A4	159	134,5107	2,1050	0,1418	0,01426
A5	230	154,0403	2,1566	0,1633	0,02362
A6	324	130,4483	2,0898	0,1441	0,02818
A7	333	151,3522	2,1516	0,1559	0,03360
Adult	1659	139,9017	2,1172	0,1535	0,02210



Animal/Group	TACTHII	MACTHIA (μm^2)	MACTHIAT	S D	PTAS
E1/1	336	156,0525	2,1627	0,1623	0,03496
E1/2	373	157,4315	2,1713	0,1495	0,03915
E1/3	144	149,0677	2,1440	0,1572	0,01431
E1/4	70	137,1347	2,1100	0,1514	0,00640
E1/5	185	146,1126	2,1355	0,1572	0,01802
E1/6	290	134,6537	2,1030	0,1467	0,02603
E1/7	277	141,9683	2,1258	0,1473	0,02622
Enclosure (D 1)	1675	147,8367	2,1414	0,1547	0,02358
E2/1	324	138,0198	2,1120	0,1514	0,02981
E2/2	264	141,0728	2,1233	0,1478	0,02483
E2/3	253	137,1311	2,1066	0,1587	0,02313
E2/4	268	138,5315	2,1159	0,1461	0,02475
E2/5	337	149,8059	2,1442	0,1626	0,03366
E2/6	386	145,2232	2,1332	0,1562	0,03737
E2/7	188	131,6023	2,0926	0,1464	0,01649
E2/8	291	142,5605	2,1250	0,1562	0,02766
Enclosure (D 7)	2311	141,3022	2,1215	0,1544	0,02721
E3/1	333	150,0471	2,1440	0,1644	0,03331
E3/2	223	134,5490	2,1004	0,1519	0,02000
E3/3	248	128,7412	2,0883	0,1339	0,02129
E3/4	214	132,3134	2,0971	0,1416	0,01888
E3/5	312	132,2790	2,0951	0,1457	0,02751
E3/6	213	128,2147	2,0840	0,1399	0,01821
E3/7	233	148,8463	2,1455	0,1523	0,02312
E3/8	567	153,4129	2,1566	0,1578	0,05799
Enclosure (D 21)	2343	141,0414	2,1209	0,1533	0,02754

REFERENCES

1. Anthony E L P, Bruhn T O, Weston P J 1991 Immunocytochemical localization of growth hormone and growth hormone-releasing hormone immunoreactivity in the brain and pituitary of the little brown bat. *The American Journal of Anatomy* 190: 1-9
2. Armario A, Restrepo C, Castellanos J M, Balasch J 1985 Dissociation between adrenocorticotropin and corticosterone responses to restraint after previous chronic exposure to stress. *Life Sciences* 36: 2085-2092
3. Banky Z, Nagy G M, Halasz B 1994 Analysis of pituitary prolactin and adrenocortical response to ether, formalin or restraint in lactating rats: Rise in corticosterone, but no increase in plasma prolactin levels after exposure to stress. *Neuroendocrinology* 59(1): 63-71
4. Berardinelli J G, Godfrey R W, Adair R, Lunstra D D, Byerley D J, Cardenas H, Randel R D 1992 Cortisol and prolactin concentrations during three different seasons in relocated Brahman and Hereford bulls. *Theriogenology* 37: 641-654
5. Borson S, Schatteman G, Claude P, Bothwell M 1994 Neurotropins in the developing and adult primate adenohypophysis: a new pituitary hormone system? *Neuroendocrinology* 59(5): 466-476

6. Bruno J F, Olchovsky D, White J D, Leidy J W, Song J, Berelowitz M 1990 Influence of food deprivation in the rat on hypothalamic expression of growth hormone-releasing factor and somatostatin. *Endocrinology* 127(5): 2111-2116
7. Burns J 1979 Immunohistochemical methods and their applications in the routine laboratory. *Recent Advances in Histopathology* 10: 337-349
8. Christian JJ, Ratcliffe HL. 1952 Shock disease in captive wild mammals. *American Journal of Pathology* 28(4): 725-37
9. Clarke I J, Fletcher T P, Pomares C C, Holmes J H G, Dunshea F, Thomas G B, Tilbrook A J, Walton P E, and Galloway D B 1993 Effect of high protein feed supplements on concentrations of growth hormone (GH), insulin-like growth factor-I (IGF-I) and IGF-binding protein-3 in plasma and on the amounts of GH and messenger RNA for GH in the pituitary glands of adult rams. *Journal of Endocrinology* 138(3): 421-427
10. Colborn D R, Thompson Jr. D L, Rahmanian M S, Roth T L 1991 Plasma concentrations of cortisol, prolactin, luteinizing hormone, and follicle-stimulating hormone in stallions after physical exercise and injection of secretagogue before and after sulpiride treatment in winter. *Journal of Animal Science* 69: 3724-3732
11. Coppinger T R, Minton J E, Reddy P G, Blecha F 1991 Repeated restraint and isolation stress in lambs increases pituitary-adrenal secretions and reduces cell-mediated immunity. *Journal of Animal Science* 69: 2808-2814

12. Day L R, Bolton A E 1982 Radiation safety in the radioimmunoassay laboratory. Irish Veterinary Journal 36: 44-48
13. Duvilanski B H, Zambruno C, Seilicovich A, Pisera D, Lasaga M, Del C, Diaz M 1995 Role of nitric oxide in control of prolactin release by the adenohypophysis. Proceedings of the National Academy of Sciences of the United States of America 92(1): 170-174
14. Franzmann A W, Flynn A, Arneson P D 1975 Serum corticoid levels relative to handling stress in Alaskan moose. Canadian Journal of Zoology 53: 1424-1426
15. Ganhao M F, Hattingh J, Pitts N, Raath C, de Klerk B, De Vos V 1988 Physiological responses of blesbok, eland, and red hartebeest to different capture methods. Suid Afrikaanse Tydskrif vir Natuurnavorsing 18(4): 134-136
16. Gericke MD, Hofmeyr JM, Louw GN. 1978 The effect of capture stress and haloperidol therapy on the physiology and blood chemistry of springbok, Antidorcas marsupialis. Madoqua 11(1): 5-18
17. Goluboff L G, Ezrin C 1969 Effect of pregnancy on the somatotroph and the prolactin cell of the human adenohypophysis. Journal of Clinical Endocrinology 29: 1533-1538
18. Gupta D 1980 Radioimmunoassay of Steroid Hormones (2nd ed) Verlag Chemie Weinheim . Deerfield Beach, Florida.

19. Halmi N S 1983 The Hypophysis. In: Weiss L (ed) *Histology Cell and Tissue Biology* (5th ed) The Macmillan Press: 1054-1078
20. Hattingh J, Pitts N I, Ganhao M F 1989 Immediate responses to repeated capture and handling of wild impala. *The Journal of Experimental Zoology* 248: 109-112
21. Hofmeyer J M, Louw G N, Du Preez J S 1973 Incipient capture myopathy as revealed by blood chemistry of chased zebras. *Madoqua* 7: 45-50
22. Hofmeyer J M, Luchtenstein H G, Mostert P K N 1976 Capture, handling and transport of springbok and the application of haloperidol as a long-acting neuroleptic. *Madoqua* 10: 123-130
23. Huang W M, Gibson S J, Facer P, Gu J, Polak J M 1983 Improved section adhesion for immunocytochemistry using high molecular weight polymers of lysine as a slide coating. *Histochemistry* 77: 275-279
24. Ingvarsten K L, Andersen H R 1993 Space allowance and type of housing for growing cattle. *Acta Agriculturae Scandinavica Section A, Animal Science* 43(2): 65-80
25. Janssens C J J G, Helmond F A, Wiegant V M 1994 Increased cortisol response to exogenous adrenocorticotrophic hormone in chronically stressed pigs: Influence of housing conditions. *Journal of Animal Science* 72(7): 1771-1777

26. Klemcke H G 1994 Responses of the porcine pituitary-adrenal axis to chronic intermittent stressor. *Domestic Animal Endocrinology* 11(1): 133-149
27. Knox C M, Hattingh J, Raath C 1990 The effect of tranquilizers on the immediate responses to repeated capture of boma-kept impala. *Comparative Biochemistry and Physiology* 95C: 247-251
28. Knox C M, Hattingh J, Raath C 1991 The effect of Zeranol on body mass and physiological responses to repeated capture of boma-confined impala. *South African Journal for Wildlife Research* 21: 38-42
29. Knox C M, Hattingh J, Raath C 1992 Physiological responses of boma-confined impala to repeated capture. *South African Journal for Wildlife Research* 22: 1-6
30. Knox C M, Zeller D A, Hattingh J 1993 Comparison of two methods for the capture of boma-confined impala. *South African Journal of Wildlife Research* 23(1): 1-5
31. Koelkebeck K W, Cain J R, Amoss M S J 1986 Use of adrenocorticotropin challenges to indicate chronic stress responses of laying hens in several housing alternatives. *Domestic Animal Endocrinology* 3(4): 301-305
32. Kotze S H, Van Aswegen G 1990 An Immunohistochemical study of various peptide-containing endocrine cells and neurones at the equine ileocaecal junction. *Onderstepoort Journal of Veterinary Research* 57: 13-17

33. Lopez M E, Hargis B M, Dean C E, Porter T E 1995 Uneven regional distributions of prolactin- and growth hormone-secreting cells and sexually dimorphic proportions of prolactin secretors in the adenohypophysis of adult chickens. *General & Comparative Endocrinology* 100(2): 246-254
34. Marple D N, Aberle E D, Forrest J C, Blake W H, Judge M D 1972 Effects of humidity and temperature on porcine plasma adrenal corticoids, ACTH and growth hormone levels. *Journal of Animal Science* 34(5): 809-812
35. Marple D N, Judge M D, Aberle E D 1972 Pituitary and adrenocortical function of stress susceptible swine. *Journal of Animal Science* 35(5): 995-1000
36. Martucci R W, Jessup D A, Gronert G A, Reitan J A, Clark W E 1992 Blood gas and catecholamine levels in capture stressed desert bighorn sheep. *Journal of Wildlife Diseases* 28(2): 250-254
37. Meyer B J 1979 *Die fisiologiese basis van Geneeskunde* (2nd ed) HAUM Pretoria
38. Munksgaard L, Lovendahl P 1993 Effects of social and physical stressors on growth hormone levels in dairy cows. *Canadian Journal of Animal Science* 73(4): 847-853
39. Narinder S, Chaudhary K C, Singh N 1992 Plasma hormonal and electrolyte alterations in cycling buffaloes (*Bubalus bubalis*) during hot summer months. *International Journal of Biometeorology* (36)3: 151-154

40. Opel H, Proudman J A 1984 Two methods for serial blood sampling from unrestrained, undisturbed turkeys with notes on the effects of acute stressors on plasma levels of prolactin. *Poultry Science* 63: 1644-1652
41. Parrott R F, Misson B H, De La Riva C F 1994 Differential stressor effects on the concentrations of cortisol, prolactin and catecholamines in the blood of sheep. *Research in Veterinary Science* 56: 234-239
42. Pliska V, Hari J, Heiniger J, Neuenschwander S, Stranzinger G 1992 Stress-like changes in the histological structure of pig adrenals and pituitaries: Effect of total body fat but not of predisposition to malignant hyperthermia. *Journal of Animal Breeding and Genetics* 109: 51-63
43. Polak J M, Van Noorden S 1987 *Immunocytochemistry, modern methods and applications* (2nd ed) Wright Bristol
44. Przekop F, Wolinska-Witort E, Mateusiak K, Sadowski B, Domanski E 1984 The effect of prolonged stress on the oestrus cycles and prolactin secretion in sheep. *Animal Reproduction Science* 7: 333-342
45. Ramesh R, Proudman J A, Kuenzel W J 1995 Changes in pituitary somatotrophs and lactotrophs associated with ovarian regression in the turkey hen (*Meleagris gallopova*). *Comparative Biochemistry and Physiology* 112C(3): 327-334

46. Rampacek G B, Kraeling R R, Fonda E S, Barb C R 1984 Comparison of physiological indicators of chronic stress in confined and unconfined gilts. *Journal of Animal Science* 58(2): 401-408
47. Recabarren M S, Ramirez R J, Manriquez A L, Orellana B P, Parilo V J 1991 Plasma levels of luteinizing hormone and of growth hormone in ewe lambs with food restriction. *Agro Ciencia* 7(2): 155-160
48. Rojkittikkhun T, Uvnas-Moberg K, Einarsson S, Lundeheim N 1991 Effects of weaning on plasma levels of prolactin, oxytocin, insulin, glucagon, glucose, gastrin and somatostatin in sows. *Acta Physiologica Scandinavia* 141: 295-303
49. Sagrillo C A, Voogt J L 1991 Endogenous opioids mediate the nocturnal prolactin surge in the pregnant rat. *Endocrinology-Philadelphia* 129(2): 925-930
50. Selye H 1973 *The Evolution of the Stress Concept*. *American Scientist* 61: 692-699
51. Skinner J D, Smithers R H N 1990 *The Mammals of the Southern African Subregion* (2nd ed) University of Pretoria Pretoria
52. Spraker T R 1978 Pathophysiology associated with capture of wild animals. *Proceedings of a Symposium: National Zoological Park: Smithsonian Institute* 403-414

53. Stefaneanu L, Kovacks K, Lloyd R V, Scheithauer B W, Young W F J, Sano T, Jin L 1992 Pituitary lactotrophs and somatotrophs in pregnancy: a correlative in situ hybridization and immunocytochemical study. *Virchows Archives B, Cell Pathology Including Molecular Pathology* 62(5): 291-296
54. Sternberger L A 1986 *Immunocytochemistry* (3rd ed) Churchill Livingstone New York
55. Steward M W 1976 *Immunochemistry*. William Clowes & Sons Ltd. London
56. Stupnicki R 1982 A Single-Parameter Quality Control in Radioimmunoassays. *Endokrinologie* 80: 48-51
57. Teng C S 1991 Correlation of plasma growth hormone level with wasting syndrome in feline AIDS. *AIDS* 5(12): 1542-1543
58. Van Nesselrooij J H J, Kuper C F, Bosland M C 1992 Correlations between presence of spontaneous lesions of the pituitary (adenohypophysis) and plasma prolactin concentration in aged wistar rats. *Veterinary Pathology* 29: 288-300
59. Vidal S, Sanchez P, Roman A, Moya L 1994 Immunocytochemical study of the growth hormone and prolactin pituitary cells in male and female suckling mink. *General and Comparative Endocrinology* 93(3): 337-344

60. Williams T D, Rebar A H, Teclaw R F, Yoos P E 1995 Influence of age, sex, capture technique, and restraint on hematologic measurements and serum chemistries of wild California sea otters. *Veterinary Clinical Pathology* 21(4): 106-110

61. Worthy K, Escreet R, Renton J P, Eckersall P D, Douglas T A, Flint D J 1986 Plasma prolactin concentrations and cyclic activity in pony mares during parturition and early lactation. *Journal of Reproduction and Fertility* 77: 569-574