wild ruminant animals and steers. *Journal of Food Quality* **9,** 331-343
MOORE, J. H. & SIKES, S. K. (1967) The serum and adrenal lipids of the African elephant. *Comparative Biochemistry and Physiology* **20,** 779-792
NISSEN, H. P. & KREYSEL, H. W. (1983) Polyunsaturated fatty acids in relation to sperm motility. *Andrologia* **15,** 264-269
NISSEN, H. P., KREYSEL, H. W. & SCHIRREN, C. (1981) Composition of the lipid-bound fatty acids of human semen in relation to its fertility values. *Andrologia* **13,** 444-451
NOBLE, R. C., MCCARTNEY, R. & FERGUSON, M. W. (1993) Lipid and fatty acid compositional differences between eggs of wild and captive-breeding alligators: an association with reduced hatchability? *Journal of Zoology (London)* **230,** 639-649
NOBLE, R. C., SPEAKE, B. K., MCCARTNEY, R., FOGGIN, C. M. & DEEMING, D. C. (1996) Yolk lipids and their fatty acids in the wild and captive ostrich. *Comparative Biochemistry and Physiology* **113B,** 753-756
O'BRIAN, E., HOFFMAN, S., SCHAWANG, T., SUEDMEYER, W. K., EASTON, E., SCHMIDT, D. & LOSKUTOFF, N. (1998) An update to improve the short- and long-term storage of elephant semen. Proceedings of the Third International Elephant Research Symposium. Springfield, USA, June 6 to 7, 1998. p 7
O'KEEFE, J. H. Jr & HARRIS, W. S. (2000) From Inuit to implementation: omega-fatty acids come of age. *Mayo Clinic Proceedings* **75,** 607-614
OKUYAMA, H., KOBAYASHI, T. & WATANABE, S. (1997) Dietary fatty acids – the n-6/n-3 balance and chronic elderly diseases. Excess linolenic acid and relative n-3 deficiency syndrome seen in Japan. *Progress in Lipid Research* **35,** 409-457
PARKS, J. E. & LYNCH, D. V. (1992) Lipid composition and thermotropic phase behavior of boar, bull, stallion and rooster sperm membranes. *Cryobiology* **29,** 255-266
PAULENZ, H., TAUGBOL, O., HOFMO, P. O. & SAAREM, K. (1995) A preliminary study on the effect of dietary supplementation with cod liver oil on the polyunsaturated fatty acid composition of boar semen. *Veterinary Research Communications* **19,** 273-284
PETERS, J. M., MAIER, R., HAWTHORNE, B. E. & STORVIK, C. A. (1972) Composition and nutrient content of elephant milk. *Journal of Mammalogy* **53,** 717-724
RATCLIFFE, H. L. & CRONIN, M. T. (1958) Changing frequency of arteriosclerosis in mammals and birds at the Philadelphia zoological gardens. *Circulation* **18,** 41-52
SCOTT, J. W. (1973) Lipid metabolism of spermatozoa. *Journal of Reproduction and Fertility Supplement* **18,** 65-76
SEBASTIAN, S. M., SELVARAJ, S., ARULDHAS, M. M. & GOVINDARAJULU, P. (1987) Pattern of neutral and phospholipids in the semen of normospermic, oligospermic and azoospermic men. *Journal of Reproduction and Fertility* **79,** 373-378
SHELDRICK, D. (1990) Raising baby orphaned elephants: part II. *Swara* **13,** 23-31
SIKES, S. K. (1968a) Observations on the ecology of arterial disease in the African elephant in Kenya and Uganda. *Proceedings of the Symposium of the Zoological Society of London* **21,** 251-273
SIKES, S. K. (1968b) Habitat stress and arterial disease in elephants. *Oryx* **9,** 286-292
SIKES, S. K. (1969) Habitat and cardiovascular diseases. Observations made on elephants and other free-living animals in East Africa. *Transactions of the Zoological Society of London* **32B,** 1-104
SIMOPOULOS, A. P. (1991) Omega-3 fatty acids in health and disease and in growth and development. *American Journal of Clinical Nutrition* **54,** 438-463
SIMOPOULOS, A. P. & SALEM, N., Jr (1989) n-3 fatty acids in eggs from range-fed Greek chickens. *New England Journal of Medicine* **321,** 1412
SREEKUMAR, K. P. & NIRMALAN, G. (1990) The fatty acid composition of plasma lipids in the Indian elephant (*Elephas maximus*). *Veterinary Research Communications* **14,** 427-431
SWAIN, J. E. & MILLER, R. R. (2000) A post-cryogenic comparison of membrane fatty acids of elephant spermatozoa. *Zoo Biology* **19,** 461-473
VAN HOVEN, W., PRINS, R. A. & LANKHORST, A. (1981) Fermentative digestion in the African elephant. *South African Journal of Wildlife Research* **11,** 78-86
VAN VLIET, T. & KATAN, M. B. (1990) Lower ratio of n-3 to n-6 fatty acids in cultured than in wild fish. *American Journal of Clinical Nutrition* **51,** 1-2
VASTESAEGER, M. M. & DELCOURT, R. (1961) Spontaneous atherosclerosis and diet in captive animals. *Nutritio et Dieta* **3,** 174-188
WHORTON, A. R. & CONIGLIO, J. G. (1977) Fatty acid synthesis in testes of fat-deficient and fat-supplemented rats. *Journal of Nutrition* **107,** 79-86
ZALATA, A. A., CHRISTOPH, A. B., DEPUYDT, C. E., SCHOONJANS, F. & COMHAIRE, F. H. (1998) The fatty acid composition of phospholipids of spermatozoa from infertile patients. *Molecular Human Reproduction* **4,** 111-118

SHORT COMMUNICATIONS

Measurement of 25-hydroxycholecalciferol in captive grey parrots (*Psittacus e erithacus*)

M. STANFORD

HYPOCALCAEMIA is a common syndrome in grey parrots (*Psittacus e erithacus*) in captivity, although the aetiology is still unconfirmed. It is expressed clinically by hypocalcaemic seizures, poor breeding performance and osteodystrophy (Rosskopf and others 1985, Hochleithner 1989). Vitamin D deficiencies are common in poultry kept indoors in an ultraviolet (UV)-deficient environment with insufficient dietary vitamin D (Edwards 1994), and a similar situation might be expected to occur with captive psittaciforms. Seed-based diets contain low levels of calcium and vitamin D_3 and these are traditionally fed to grey parrots in captivity. It has been postulated that this contributes to a nutritional secondary hyperparathyroidism (Hochleithner and others 1997, Klasing 1998). A deficiency of UV light in the 285 to 315 nm (UVb) range may also be implicated in the aetiology of the disease, leading to a functional vitamin D_3 deficiency.

The vitamin D_3 metabolism of birds has been extensively reviewed (Taylor and Dacke 1984, Bentley 1998), and it has been established that domestic chickens secrete 7-dehydrocholesterol (provitamin D) onto the featherless skin of the skin and feet (Tian and others 1994). Conversion of the provitamin D to cholecalciferol (vitamin D_3) occurs by a UV light-dependent isomerisation reaction. Cholecalciferol is a sterol prohormone which is subsequently activated by a two-stage hydroxylation process. It is initially metabolised to 25-hydroxycholecalciferol in the liver, and the synthesis of 25-hydroxycholecalciferol is regulated by product inhibition. 25-hydroxycholecalciferol is transported to the kidney via carrier proteins and converted to either 1,25-dihydroxycholecalciferol or 24,25-dihydroxycholecalciferol, the active metabolites of cholecalciferol in the domestic fowl (Elaroussi and others 1994).

The concentration of 25-hydroxycholecalciferol in serum is considered to be the most reliable measure of the vitamin D status of an individual due to its long half-life compared with other vitamin D metabolites (Hollis and others 1999). Traditionally, radioimmunoassays (RIAs) have been used to assay 25-hydroxycholecalciferol but, more recently, enzyme

Veterinary Record (2003) **153,** 58-59

M. Stanford, BVSc, MRCVS, Birch Heath Veterinary Clinic, Birch Heath Road, Tarporley, Cheshire CW6 9UU

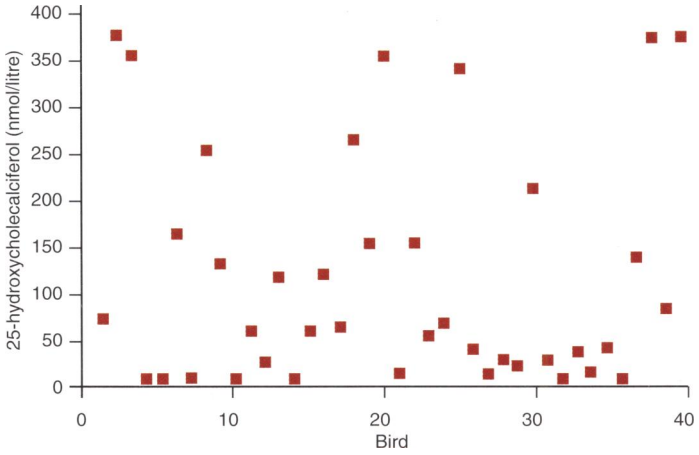

FIG 1: Concentrations of 25-hydroxycholecalciferol in 40 grey parrots

immunoassays (EIAs) have become available with the advantages of both convenience and economy. This short communication describes the measurement of 25-hydroxycholecalciferol in captive grey parrots using an EIA.

Forty birds were randomly selected from a group of 100 adult grey parrots of known sex, which had been fed an unsupplemented seed diet for the previous 12 months. As part of their annual health checks, faecal samples were taken from each bird for parasitology and Gram stain examination. Blood samples taken from each bird were subject to routine haematological and biochemical tests, including circovirus, polyoma and chlamydophilia PCR. Each bird was examined clinically by laparoscopy. On the basis of these tests, only birds with no evidence of clinical disease were included in the study.

The birds were blood sampled for 25-hydroxycholecalciferol under isoflurane anaesthesia with the informed consent of the owner. The blood testing was performed outside the breeding season to minimise the effects of both oestrogen and seasonality on both the levels of vitamin D_3 and calcium (Bentley 1998). All the blood samples were taken into heparin and immediately cooled to –70°C before analysis. Each sample was assayed in duplicate using the OCTEIA 25-hydroxycholecalciferol assay (IDS), with no significant difference between assay results for the same sample.

The results are shown in Fig 1. 25-hydroxycholecalciferol was consistently recovered from blood samples taken from the group of 40 healthy grey parrots using the EIA. The results show a wide variation in the level of 25-hydroxycholecalciferol, with a range of 7·2 to 380 nmol/litre. The mean (sd) level was 116·52 (126·70) nmol/litre. A study in captive green iguanas (*Iguana iguana*) revealed a similar wide variation in 25-hydroxycholecalciferol concentrations (Nevarez and others 2002). A simple explanation for this would be that different birds were subject to varying levels of unfiltered UVb light. A further study is under way to investigate the effects of UVb on 25-hydroxycholecalciferol on the same group of birds.

In the laying hen, 25-hydroxycholecalciferol would not be expected to fall below 26 nmol/litre and would normally be above 50 nmol/litre (Dacke 2000). Although no normal ranges are available for psittaciforms at the present time, 16 of the 40 birds had 25-hydroxycholecalciferol levels below 50 nmol/litre. Chronic deficiency of vitamin D would be expected to lead to hypocalcaemia and secondary hyperparathyroidism. This would potentially have significant consequences for the grey parrot, which is known to suffer from hypocalcaemia and related disorders of calcium metabolism. In addition, vitamin D has been found to have a profound effect on the immune system (Aslam and others 1998).

Further studies are ongoing using the 25-hydroxycholecalciferol assay to derive an adequate level of vitamin D in complete psittaciform diets under different UV light regimes. The 25-hydroxycholecalciferol EIA also has practical uses with other species known to suffer from disorders of calcium metabolism such as *I iguana*.

References

ASLAM, S. M., GARLICH, J. D. & QURESHI, M. A. (1998) Vitamin D deficiency alters the immune responses of broiler chicks. *Poultry Science* **77,** 842-849

BENTLEY, P. J. (1998) Hormones and calcium metabolism. In Comparative Vertebrate Endocrinology. Ed P. J. Bentley. Cambridge, Cambridge University Press. pp 269-301

DACKE, G. C. (2000) Parathyroids, calcitonin and vitamin D. In Sturkie's Avian Physiology. 5th edn. Ed G. C. Whittow. London, Academic Press. pp 472-485

EDWARDS, J. R., HARDY, M., ELLIOT, M. A., SOONCHARERNYING, S. & BRITTON, M. W. (1994) Quantitive requirement for cholecalciferol in the absence of ultraviolet light. *Poultry Science* **73,** 288-294

ELAROUSSI, M. A., FORTE, L. R., EBER, S. L. & BELLIER, H. V. (1994) Calcium homeostasis in the laying hen. Age and dietary calcium effects. *Poultry Science* **73,** 1581-1589

HOCHLEITHNER, M. (1989) Convulsions in African grey parrots in connection with hypocalcaemia: five selected cases. Proceedings of the 2nd European Symposium on Avian Medicine and Surgery. pp 44-52

HOCHLEITHNER, M., HOCHLEITHNER, C. & HARRISON, G. L. (1997) Evidence of hypoparathyroidism in hypocalcaemic African grey parrots. In Avian Examiner Special Supplement, Spring. Palm Beach, HBD International

HOLLIS, B. W., CLEMENS, T. L. & ADAMS, J. S. (1999) Vitamin D metabolites. In Primer on the Metabolic Bone Disease and Disorders of Bone Metabolism. Ed M. J. Favus. Philadelphia, Lippincott, Williams & Wilkins. pp 124-128

KLASING, K. C. (1998) Minerals. In Comparative Avian Nutrition. Ed K. C. Klasing. New York, CAB International. pp 290-295

NEVAREZ, J. G., MITCHELL, M. A., LE BLANC, C. & GRAHAM, P. (2002) Determination of plasma biochemistries, ionised calcium, vitamin D_3 and haematocrit values in captive green iguanas (*Iguana iguana*). Proceedings of the Association of Reptile and Amphibian Veterinarians 9th Annual Conference. pp 87-95

ROSSKOPF, W. J., WOERPEL, R. W. & LANE, R. A. (1985) The hypocalcaemic syndrome in African greys: an updated clinical viewpoint. Proceedings of the Association of Avian Veterinarians. pp 129-132

TAYLOR, T. G. & DACKE, C. G. (1984) Calcium metabolism and its regulation. In Physiology and Biochemistry of the Domestic Fowl. Vol 5. Ed B. M. Freeman. London, Academic Press. pp 125-170

TIAN, X. Q., CHEN, T. C., LU Z., SHAO, Q. & HOLICK, M. F. (1994) Characterisation of the translocation process of vitamin D_3 from the skin into the circulation. *Endocrinology* **135,** 655-661