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# Determination of serum biochemical reference intervals for the Iberian lynx (*Lynx pardinus*)

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ABSTRACT

Biochemical reference intervals were determined for 31 clinically healthy Iberian lynxes (*Lynx pardinus*) between 1 and 6 years of age. Thirteen of the lynxes were wild-caught and the other 18 were captive-reared animals. The samples were collected between November 2004 and December 2006. The influence of sex (males vs. females), age (juveniles vs. adults) and habitat condition (free-living vs. captive) on the biochemical analytes were evaluated. Serum albumin concentrations were significantly higher in females than in males, while creatine phosphokinase was higher in males. The levels of alkaline phosphatase and lactate dehydrogenase were higher in juvenile lynxes, while gamma glutamyl-transferase and creatinine values were higher in adults. Lynxes captured in the wild had higher concentrations of iron, calcium, alkaline phosphatase and creatinine, but lower aspartate aminotransferase and alanine aminotransferase than lynxes maintained in captivity. The results were generally comparable to commonly reported reference intervals for other lynx species, the domestic cat and other felid species.



## Introduction

The Lynx genus is one of the 14 living genera that comprise the family *Felidae* (Wilson and Reeder, 2005). There are 40 known species of felids in the world today, including the domestic cat, which have all descended from a common ancestor (Wilson and Reeder, 2005). The Iberian lynx (*Lynx pardinus*) is one of the four species of lynxes that currently exist, originating from the Iberian Peninsula (Beltrán et al., 1996; Johnson et al., 2004).

In the past few decades, the progressive disappearance of Iberian lynx populations has been mainly attributed to the drastic reduction in the numbers of its staple prey, the wild rabbit (*Oryctolagus cuniculus*), as well as habitat fragmentation, illegal trapping, roadkill, illegal hunting and diseases (Ferreras et al., 1992, 2001; Rodríguez and Delibes, 2004). All of these factors have led to the Iberian lynx becoming the most threatened felid species in the world (Nowell and Jackson, 1996); it is considered as 'critically endangered' with a distribution that has been reduced by 99% in the last 50 years, with fewer than 200 individuals currently inhabiting only two isolated metapopulations in Southern Spain (Sierra Morena and the Doñana Natural Park) (Palomares et al., 2002).

A number of conservation activities have been carried out in recent years (LIFE project, NGO programmes, regional-level laws), and several scientific studies and research projects have contributed to expanding the knowledge on the biology, threats and ecological interactions of the Iberian lynx (Ferreras et al., 2004; Johnson et al., 2004; Rodríguez and Delibes, 2004). Published information concerning the pathologies that may affect the Iberian lynx have increased (Torres et al., 1998; Briones et al., 2000; Pérez et al., 2001; Vicente et al., 2004; Roelke et al., 2008) but studies investigating the use of haematological and biochemical analytes as health indicators are limited (Beltrán et al., 1991). Knowledge of base-line biochemical values is especially important in threatened species for the interpretation of laboratory data, which is often the first indicator of disease.

The main objectives of the present study were, firstly, to provide reference intervals for serum biochemical analytes for free-living and captive Iberian lynxes; secondly, to compare these values with those of other lynx species, the domestic cat and other felid species, thirdly, to assess whether there are significant variations in these analytes depending on factors such as sex, age or habitat condition.

### Material and methods

From November 2004 to December 2006, 31 blood samples were collected from free-living ($n = 13$) and captive ($n = 18$) Iberian lynxes. Fifteen animals were from

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Sierra Morena (Jaén province, 38° 13′N, 4° 11′W) and 16 from Doñana National Park (Huelva province, 37° 9′N, 6° 26′W).

Age was estimated by weight, tooth wear and facial, body and pelt features. Lynxes were classified as either juveniles (2–17 months) or adults (⩾18 months). Lynxes >18 months were classified as adults (as this coincides with the age for dispersal in this species; Ferreras et al., 2004). Seventeen (55%) of the samples were taken from juvenile lynxes and 14 (45%) from adults. Sixteen blood samples (52%) were collected from males while 15 (48%) were taken from females.

All of the animals were captured using box-traps and anaesthetised with a combination of ketamine and medetomidine (Ferreras et al., 1994). A tiletamine and zolazepam combination was only used in males in which semen was obtained by electroejaculation. All drugs were administered IM (Ferreras et al., 1994).

A physical examination was performed on each animal and any clinical signs of disease (including anorexia, lethargy, depression, ataxia, fever, dehydration, anaemia, jaundice, pallor of mucous membranes, dyspnoea, swelling, eye or nasal discharge, vomiting or diarrhoea) recorded. Temperature, weight, pulse and respiration were also measured. Only samples from animals that did not show any signs of disease, and with normal temperature, weight, pulse and respiration were included in the study. Six of the animals (three with traumatic lesions, two with conjunctivitis and one with signs of respiratory disease) were excluded from the study. A total of 31/37 individuals were selected to establish the reference intervals for the serum biochemical analytes. An epidemiological questionnaire was also completed for each animal in order to obtain information on individual and environmental characteristics.

Using disposable syringes and 23-gauge needles, 2 mL of blood were obtained from each animal by venepuncture of either the cephalic or the jugular vein. Samples were collected into a lithium heparin Vacutainer (Becton-Dickinson), and then centrifuged at 400*g* for 15 min. The plasma was removed, kept at 4 °C and analysed within 24 h of extraction. Biochemical analyses were determined using an automated analyser (RA115000 – Climate MC 150). A total of 20 biochemical analytes were analysed (Table 1). Means, standard deviations (SD) and ranges were calculated for each analyte and the normality of data distribution and the variance homogeneity of each variable were assessed by a Kolmogorov–Smirnov test and Levene F-test, respectively.

Correlation among age (juveniles vs. adults), sex (male vs. females) and habitat conditions (captive-reared vs. free-living) was previously tested using a Pearson's correlation test. A one-way ANOVA was used to determine whether there were differences among groups after assumptions of normality and homocedasticity were checked. When a variable did not fit a normal distribution or variance homogeneity, a Kruskal–Wallis test was selected. Values with $P < 0.05$ were considered as statistically significant. Statistical analyses were performed using statistical software (SPSS-PC 13.0, SPSS).

## Results

The range values for the 20 tested serum biochemical analytes were reported as means ± SD (Table 2). The data represent 31 healthy Iberian lynxes of different sexes, ages and habitat conditions. The values obtained were usually comparable to the reference intervals reported for the other lynx species, other wild felid species and domestic cat. Correlation analysis showed that the different groups analysed (age, sex and habitat) were independent. Analytes with statistically significant differences between sexes, age classes and habitat conditions are shown in Table 3.

## Discussion

Some biochemical analytes were similar to the reported reference values for other lynx species (Fuller et al., 1985; Weaver and Johnson, 1995; Miller et al., 1999), the domestic cat (Bush, 1991; Jain, 1993; Kaneko et al., 1997; O'Brien et al., 1998) and other wild felid species (Currier and Russell, 1982; Hawkey and Hart, 1986; Marco et al., 2000).

The differences observed between the values previously obtained for the Iberian lynx (Beltrán et al., 1991) and our values are likely to be related to the sample size, the habitat condition or the capture methods used. The activities of enzymes such as lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT) or creatinine phosphokinase (CK) have been widely used as indicators of physical stress in felid species (Currier and Russell, 1982; Fuller et al., 1985; Marco et al., 2000). Higher values of these analytes obtained by Beltrán et al. (1991) may be due, at least partially, to the use of coil-spring foothold traps as one of the capture methods. In the present study all animals were captured using a trap-box to avoid variations in biochemical values due to the use of different capture procedures. However, the lower levels of analytes obtained in our study compared to those reported by Beltrán et al. (1991) were in accordance with those published for other wild felid species (Hawkey and Hart, 1986; Weaver and Johnson, 1995; Miller et al., 1999).

Several analytes showed significantly higher levels than those found in domestic cats (Bush, 1991; Jain, 1993; Kaneko et al., 1997; O'Brien et al., 1998). These results were generally in accordance with those previously reported for other lynx species (Kocan et al., 1985; Weaver and Johnson, 1995; Miller et al., 1999). Species variability, nutritional factors, muscle damage, stress, muscle mass or reproductive condition may explain these differences (Currier and Russell, 1982; Fuller et al., 1985; Meyer et al., 1992; Marco et al., 2000). Higher glucose levels in Iberian lynxes compared to

**Table 1**
Summary of analytical methods used to determine the biochemical analytes.

| Analyte | Analytical method[a] | Reference |
|---|---|---|
| Glucose | Colorimetric enzymatic method. Hexokinase method | Thomas (1998) |
| Cholesterol | Colorimetric enzymatic method. CHE/POD method | Thomas (1998) |
| Triglycerides | Colorimetric enzymatic method. GPO-PAP method | Thomas (1998) |
| Uric acid | Colorimetric enzymatic method. Uricase method | Thomas (1998) |
| Lipase | Colorimetric enzymatic method[b] | Thomas (1998) |
| Cholinesterase | Colorimetric enzymatic method[b] | Thomas (1998) |
| Total proteins | Colorimetric method. Biuret method | Thomas (1998) |
| Albumin | Colorimetric method. Bromocresol green method | Grant et al. (1999) |
| Iron | Colorimetric method. TPTZ method[b] | Tietz (1995) |
| Calcium | Colorimetric method. Arsenazo III method | Thomas (1998) |
| Phosphorus | Colorimetric method. Molybdate method | Thomas (1998) |
| Creatinine | Colorimetric kinetic method. Jaffé method | Thomas (1998) |
| Gamma glutamyl-transferase (GGT) | Colorimetric kinetic method[b] | Thomas (1998) |
| Alkaline phosphatase (ALP) | Colorimetric kinetic method[b] | Thomas (1998) |
| Amylase | Colorimetric kinetic method[b] | Thomas (1998) |
| Aspartate aminotransferase (AST) | Ultraviolet kinetic method[b] | Thomas (1998) |
| Alanine aminotransferase (ALT) | Ultraviolet kinetic method[b] | Thomas (1998) |
| Lactate dehydrogenase (LDH) | Ultraviolet kinetic method[b] | Thomas (1998) |
| Urea | Ultraviolet kinetic method. GLDH method | Thomas (1998) |
| Creatine phosphokinase (CK) | Ultraviolet enzymatic method[b] | Schumann and Klauke (2003) |

[a] CHE, Cholesterol esterase; POD, peroxidase; GPO, glycerophosphate oxidase; PAP, peroxidase anti-peroxidase; TPTZ, 2,4,6-tri-(2-piridil)-5-triazine; GLDH, glutamate dehydrogenase.
[b] Recommended method based on IFCC (International Federation of Clinical Chemistry).

**Table 2**
Serum biochemical analytes values from 31 Iberian lynxes (*Lynx pardinus*).

| Analyte | *n* | Mean (±SD) | Range | Normal range for domestic cat[a] |
|---|---|---|---|---|
| Glucose (mmol/L) | 10 | 7.3 (3.8) | 2.1–13.8 | 3.3–5.5 |
| Cholesterol (mmol/L) | 30 | 5.4 (2.7) | 2.1–12.7 | 1.9–6.3 |
| Triglycerides (mmol/L) | 31 | 0.3 (0.2) | 0.1–0.9 | 0.5–1.1 |
| Uric acid (mmol/L) | 31 | 24.0 (23.7) | 0.0–95.2 | <59.5 |
| Total proteins (g/L) | 31 | 82.2 (13.8) | 53.0–291.7 | 50–80 |
| Albumin (g/L) | 31 | 38.6 (8.0) | 23.0–62.0 | 25–40 |
| Phosphorus (mmol/L) | 31 | 2.4 (0.6) | 1.3–3.6 | 1.0–2.6 |
| Iron (μmol/L) | 28 | 13.1 (6.4) | 2.3–24 | 12.2–38.5 |
| Calcium (mmol/L) | 31 | 2.1 (0.5) | 1.3–3.2 | 2.1–2.6 |
| GGT (IU/L) | 30 | 6.6 (4.1) | 2–17 | 1.3–5.1 |
| ALP (IU/L) | 31 | 120.5 (68.7) | 28–286 | 10–93 |
| LDH (IU/L) | 16 | 415.6 (533) | 78–1843 | 10–273 |
| Urea (mmol/L) | 31 | 10.9 (4.7) | 1.7–25.4 | 7.3–11.0 |
| Creatinine (μmol/L) | 31 | 152.8 (60.4) | 53.0–291.7 | 44.2–132.6 |
| AST (IU/L) | 30 | 79.6 (90.6) | 19–385 | 10–60 |
| ALT (IU/L) | 30 | 62.8 (39.9) | 20–138 | 6–83 |
| Pancreatic amylase (IU/L) | 31 | 875.2 (253.9) | 404–1359 | 40–1800 |
| Lipase (IU/L) | 29 | 11.8 (2.8) | 8–21 | <250 |
| CK (IU/L) | 28 | 723.2 (578.3) | 4–2691 | 50–480 |
| Cholinesterase (IU/L) | 27 | 6461.8 (2461.6) | 2034–14209 | 640–1400 |

[a] From Kaneko et al. (1997), Bush (1991), Jain (1993).

**Table 3**
Biochemical analytes that differed significantly between sex, age and habitat.

| Category | Analyte | Mean (±SD) | | *P* |
|---|---|---|---|---|
| Sex | | Males | Females | |
| | Albumin (g/L) | 35.4 (6.9) | 42.1 (7.7) | 0.02 |
| | CK (IU/L) | 940.3 (677.3) | 472.7 (300.8) | 0.03 |
| Age | | Juveniles | Adults | |
| | GGT (IU/L) | 5.1 (3.4) | 8.4 (4.3) | 0.03 |
| | ALP (IU/L) | 156.5 (71.3) | 76.9 (30.0) | 0.00 |
| | LDH (IU/L) | 516.9 (585.1) | 111.8 (33.5) | 0.04 |
| | Creatinine (μmol/L) | 127.9 (36.6) | 183.1 (70.5) | 0.02 |
| Habitat | | Captive | Free-living | |
| | Iron (μmol/L) | 16.5 (4.5) | 9.3 (6.3) | 0.00 |
| | Calcium (mmol/L) | 2.3 (0.4) | 1.8 (0.6) | 0.03 |
| | ALP (IU/L) | 140.8 (80.9) | 92.5 (32.7) | 0.03 |
| | Creatinine (μmol/L) | 177.3 (65.1) | 119.0 (31.4) | 0.03 |
| | AST (IU/L) | 40.3 (22.3) | 131.0 (118.9) | 0.00 |
| | ALT (IU/L) | 49.1 (33.4) | 80.8 (41.7) | 0.02 |

domestic cats were found in our study and these findings are similar to those published for other wild felids (Currier and Russell, 1982; Fuller et al., 1985; Weaver and Johnson, 1995; Miller et al., 1999), which, compared to domestic cats, are more negatively affected by handling, capture and immobilization. The increased stress during capture and the use of immobilizing drugs decrease liver glycogen output, and therefore increase serum glucose concentrations (Miller et al., 1999).

Urea concentrations in Iberian lynxes were also higher than in domestic cats (Bush, 1991). Elevated urea has also been reported in other wild felids (Fuller et al., 1985; Marco et al., 2000). Weaver and Johnson (1995) found higher urea concentrations in old Canadian lynxes reflecting subclinical renal dysfunction, and autoimmune membranous glomerulonephritis has been reported in adult Iberian lynxes (Jiménez et al., 2008; Peña et al., 2006). Although we found higher urea concentrations in adults compared to juveniles, these differences were not statistically significant ($P = 0.09$).

Females showed higher concentrations of albumin ($P = 0.02$) and lower concentrations of CK ($P = 0.03$) than males. These results are different from those previously published by Beltrán et al. (1991), who did not observe significant differences in any of the biochemical analytes between sexes in the Iberian lynxes they studied. However, higher albumin values were reported in female European wildcats by Marco et al. (2000). The larger muscle mass and the increased physical activity as a consequence of territorial control may explain the higher CK values observed in male lynxes.

Higher ALP activity in young lynxes compared to adult animals was also expected because ALP activity decreases as bone growth slows towards adulthood (Kaneko et al., 1997). This finding was previously observed in the Iberian lynx (Beltrán et al., 1991), the Canadian lynx (Weaver and Johnson, 1995) and other wild felid species (Paul-Murphy et al., 1994).

Serum creatinine concentrations are directly related to the muscular mass of an animal, and the higher concentrations in the adult lynxes might therefore have been anticipated. Greater physical activity and dietary factors (i.e. a strictly meat diet) are also associated with higher creatinine levels (Miller et al., 1999). A positive correlation between age and creatinine values was also noted by Beltrán et al. (1991). Recently, Jiménez et al. (2008) found higher creatinine levels in lynxes affected by glomerulopathy, which is more prevalent in adult animals.

Free-living and captive-raised lynxes showed statistically significant differences in some biochemical analytes. The increased levels of iron, calcium, ALP and creatinine in captive lynxes may be attributed to their diet; captive-reared lynxes are subjected to a very controlled nutrition plan, with a diet composed mainly of rabbits and quails. Similarly, previously published studies have also reported higher AST and ALT levels in free-living bobcats and Iberian lynxes (Fuller et al., 1985; Beltrán et al., 1991) as compared to captive Canadian lynxes (Weaver and Johnson, 1995). Higher concentrations of enzymes in muscle obtained in free-living wild felid species was attributed to the physiological stress, strenuous exercise and damage of muscle tissue during capture handling (Seal and Hoskinson, 1978; Currier and Russell, 1982; Fuller et al., 1985; Marco et al., 2000).

Even though differences were observed between sexes, ages and habitat conditions, the results for both subgroups in each category fell within the ranges reported for the 31 lynxes. However, to evaluate the real clinical or biological relevance of the differences observed, a larger number of samples would be needed.

## Conclusions

The results obtained in this study serve to establish the biochemical reference ranges for the Iberian lynx and may contribute to the conservation of the species. Owing to the small number of individuals (approximately 200), the samples may be considered as representative of the whole population. The differences found in the ranges of some analytes between lynxes and other felid species may be due to intrinsic factors, related to the species, or to extrinsic factors, including the environment and the methodologies used by the authors. The lynxes were grouped according to factors that might have influenced their biochemical variables, such as age, sex and habitat. Although further studies are needed to assess the clinical or biological relevance of the differences observed between subgroups, we consider that these differences should also be taken into account when evaluating the health and physiological status of the Iberian lynx.

## Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper

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