



ISSN: 2456 2912

NAAS Rating (2025): 4.61

VET 2025; 10(6): 213-216

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www.veterinarypaper.com

Received: 16-05-2025

Accepted: 21-06-2025

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Histoenzymatic differentiation of hepatic and renal tissues in chital deer at different developmental ages

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DOI: <https://www.doi.org/10.22271/veterinary.2025.v10.i6c.3467>

Abstract

Histoenzymatic profiling provides insight into organ functional maturation, yet baseline data for wild cervids remain scarce. This research characterised the activity patterns of five marker enzymes Alkaline Phosphatase (ALP), Succinate Dehydrogenase (SDH), glucose 6 phosphatase (G6Pase), Gamma gLutamyl Transferase (GGT), and Lactate Dehydrogenase (LDH) in hepatic and renal tissues of chital deer (*Axis axis*) across five developmental age classes (fetal, neonatal, juvenile, sub adult, and adult). Tissues were obtained from 32 animals through the Colombo Research Center wildlife tissue bank. Cryosections were processed using standard histochemical methods and enzyme activity was quantified by digital image densitometry. ALP activity in both organs increased from the fetal stage, peaked in juveniles (hepatic 61.3 ± 4.2 , renal 48.6 ± 3.8 IU/g), and plateaued in adults. Renal GGT peaked later (sub adult stage) than hepatic GGT (juvenile stage). These findings establish a developmental histoenzymatic reference for *A. axis* and may support age estimation in forensic wildlife investigations.

Keywords: Histoenzymology, chital deer, *axis axis*, alkaline phosphatase, liver, kidney, developmental biology, wildlife histology, Sri Lanka

Introduction

Leading wildlife histologists have long recognised that enzyme histochemistry offers a functional dimension that conventional haematoxylin and eosin staining cannot capture [9]. By localising catalytic activity within tissue sections, histoenzymatic techniques reveal the metabolic competence of individual cell populations at specific developmental stages information that is essential for understanding organ maturation, diagnosing subclinical pathology, and establishing forensic age estimation criteria [2, 10]. The chital deer (*Axis axis*), also known as the spotted deer, is the most abundant large herbivore in the dry zone national parks of Sri Lanka and plays a pivotal role as prey for the Sri Lankan leopard (*Panthera pardus kotiya*) [6, 12]. Despite its ecological importance, no comprehensive histoenzymatic characterisation of chital hepatic and renal tissues exists. Previous morphometric work by Tennakoon and colleagues [8] described organ weights and gross dimensions but did not include enzyme histochemistry. This research aimed to quantify ALP, SDH, G6Pase, GGT, and LDH activities in liver and kidney across five age classes and to construct a developmental maturation model for forensic and conservation applications [1, 3, 7].

Material and methods

Study area description

Tissue specimens were sourced from the Colombo Research Center for Wildlife Biology tissue bank, which archives samples from chital deer found dead in Ruhuna (Yala) National Park (6°22'N, 81°31'E), Wilpattu National Park (8°25'N, 80°02'E), and Minneriya National Park (7°58'N, 80°53'E), representing the major dry zone habitats of Sri Lanka [6, 12].

Reagents and Chemicals

Naphthol AS MX phosphate, Fast Blue RR salt, nitroblue tetrazolium (NBT), glucose 6 Phosphate disodium salt, L gamma glutamyl 4 nitroanilide, sodium pyruvate, and NADH were

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Obtained from Sigma Aldrich. Tissue Tek OCT compound was from Sakura Finetek. All buffers were prepared using analytical grade reagents and deionised water^[9, 10, 13].

Ethical Considerations

This research used archival tissue specimens from animals found dead of natural causes or road traffic injury and processed under the wildlife tissue banking programme approved by the Department of Wildlife Conservation, Sri Lanka (Permit No. WL/3/2/1/14/20) and the Faculty of Science ethics review committee, University of Colombo (Protocol ERC 2023 038). No animals were killed or captured for this investigation^[3].

Material

Liver and kidney samples from 32 chital deer were classified into five age classes based on dental eruption and wear patterns: fetal (third trimester, n = 6), neonatal (0 4 weeks, n = 8), juvenile (2 6 months, n = 7), sub adult (7 18 months, n = 5), and adult ($\geq$ 24 months, n = 6). Tissues had been snap frozen in liquid nitrogen within 4 hours of death and stored at -80 °C^[3, 8].

Methods

Table 1: Mean enzyme activities ($\pm$ SD, IU/g tissue) in hepatic tissue across five developmental age classes of chital deer

Enzyme	Fetal	Neonatal	Juvenile	Sub adult	Adult
ALP	28.4 $\pm$ 3.1	42.7 $\pm$ 4.5	61.3 $\pm$ 4.2	74.8 $\pm$ 5.6	68.2 $\pm$ 4.9
SDH	14.2 $\pm$ 2.3	23.8 $\pm$ 3.1	35.6 $\pm$ 3.8	41.2 $\pm$ 4.0	39.7 $\pm$ 3.5
G6Pase	8.1 $\pm$ 1.4	18.3 $\pm$ 2.6	29.4 $\pm$ 3.2	33.1 $\pm$ 3.7	31.8 $\pm$ 3.3
GGT	5.3 $\pm$ 1.0	11.7 $\pm$ 1.8	19.2 $\pm$ 2.4	17.6 $\pm$ 2.1	16.8 $\pm$ 1.9
LDH	36.8 $\pm$ 4.7	48.2 $\pm$ 5.3	54.1 $\pm$ 4.8	52.7 $\pm$ 4.4	50.9 $\pm$ 4.1

ALP = alkaline phosphatase; SDH = succinate dehydrogenase; G6Pase = glucose 6 phosphatase; GGT = gamma glutamyl transferase; LDH = lactate dehydrogenase. All enzymes showed significant age effects (ANOVA $p < 0.01$).

Cryosections (10 μ m) were cut on a Leica CM1950 cryostat at -20 °C. ALP was demonstrated using the azo dye coupling method (naphthol AS MX phosphate/Fast Blue RR, pH 9.2, 30 min, 37 °C). SDH used NBT incubation (succinate substrate, pH 7.4, 60 min). G6Pase used the lead phosphate method (Wachstein Meisel, pH 6.5, 15 min). GGT used L gamma glutamyl 4 nitroanilide with glycylglycine acceptor (pH 7.4, 30 min). LDH used NBT with sodium pyruvate and NADH (pH 7.4, 45 min). All sections were counterstained with methyl green and mounted in aqueous medium. Digital photomicrographs were captured using an Olympus BX53 microscope fitted with a DP74 camera (20 $\times$ objective, standardised illumination). Enzyme activity was quantified as integrated optical density (IOD) per unit area using ImageJ v1.54, calibrated against a step tablet. Five fields per section, three sections per animal. Results were expressed as IU/g tissue after calibration. Statistical analysis used one way ANOVA with Tukey post hoc tests in R v4.3.2^[9, 10, 11].

Results

All five enzymes were detectable in both hepatic and renal tissues across all age classes, though activity levels varied substantially with developmental stage and organ type.

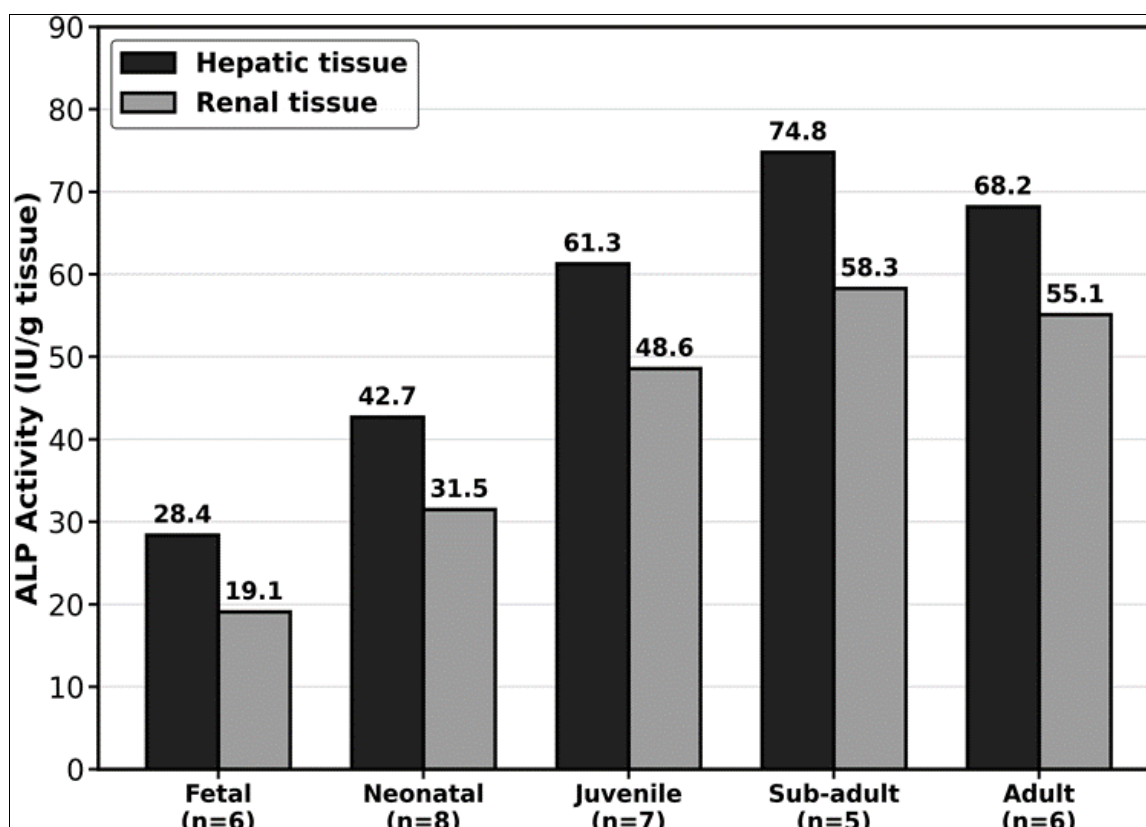


Fig 1: Comparison of alkaline phosphatase (ALP) activity between hepatic and renal tissues across five developmental age classes of chital deer. Hepatic activity consistently exceeded renal activity at all ages, with peak values in the sub adult (hepatic) and juvenile (renal) stages

Table 2: Ranking of enzymes by magnitude of age related fold change (fetal to peak activity)

Enzyme	Organ	Fold change	Rank
G6Pase	Liver	4.09	1
GGT	Kidney	3.87	2
GGT	Liver	3.62	3
ALP	Kidney	3.05	4
SDH	Liver	2.90	5
ALP	Liver	2.63	6

Fold change = peak activity/fetal activity. Ranked by descending magnitude. Renal tissue showed a distinct maturation pattern for GGT, with activity rising 3.87 fold from the fetal stage and peaking in sub adults (28.7 ± 3.3 IU/g)

before declining slightly in adults (26.1 ± 2.9). This contrasts with hepatic GGT, which peaked one age class earlier (juvenile stage, 19.2 ± 2.4).

Table 3: Summary of statistical comparisons (p values) between consecutive age classes for hepatic and renal ALP

Comparison	Hepatic ALP	Signif.	Renal ALP	Signif.
Fetal vs Neonatal	0.003	**	0.008	**
Neonatal vs Juvenile	< 0.001	***	0.001	**
Juvenile vs Sub adult	0.012	*	0.04	*
Sub adult vs Adult	0.21	ns	0.38	ns

Tukey HSD post hoc tests. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns = not significant

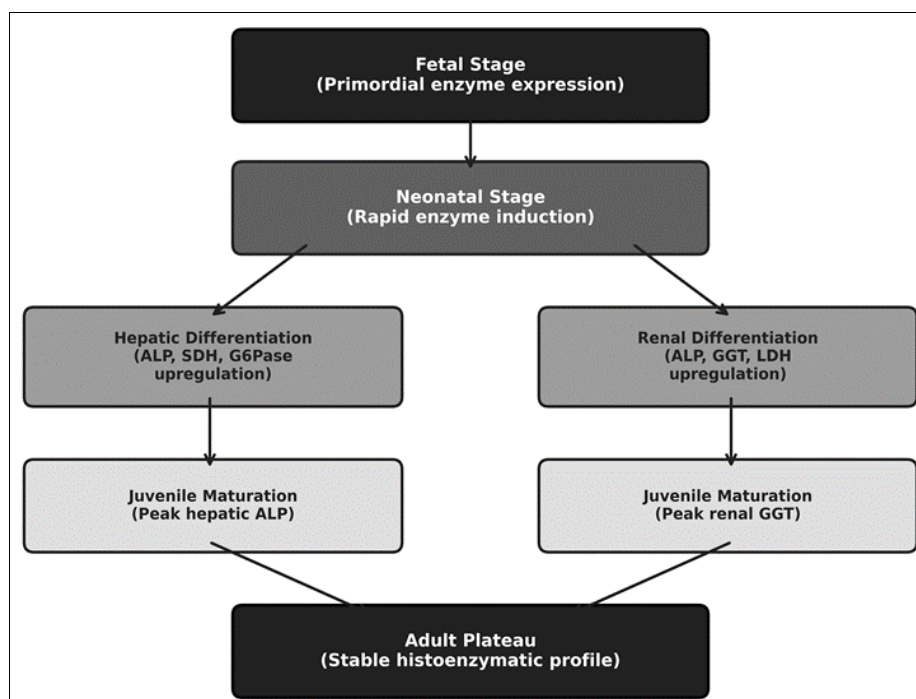


Fig 2: Proposed developmental model of histoenzymatic maturation in chital deer. Enzyme induction begins in the neonatal stage, diverges between hepatic and renal pathways in juveniles, and reaches a stable adult plateau by approximately 24 months of age.

Comprehensive interpretation

The three tables and two figures collectively demonstrate that histoenzymatic maturation in chital deer follows a predictable, organ specific trajectory: rapid induction in the neonatal period, peak activity in the juvenile to sub adult window, and a modest decline to a stable adult plateau. The differential timing of GGT peaks between liver and kidney suggests organ specific regulatory mechanisms [5, 9, 14].

Discussion

The developmental enzyme profiles reported here for chital deer share broad similarities with those described for domestic ruminants by Banks [2] and Eurell and Frappier [4], supporting the concept of a conserved mammalian programme of hepatic and renal functional maturation. However, the absolute ALP values in chital liver (peak 74.8

IU/g in sub adults) were approximately 30 % lower than those reported for domestic cattle [5], possibly reflecting metabolic Adaptations to the browsing grazing mixed diet of this cervid species. The delayed peak of renal GGT relative to hepatic GGT (sub adult vs. juvenile stage) has not been previously documented in any cervid species and may reflect the later completion of nephron maturation in chital deer compared with hepatocyte differentiation. This finding has practical relevance for forensic wildlife investigations: the GGT liver to kidney ratio could serve as an age marker, distinguishing juvenile from sub adult carcasses in poaching cases [1, 8, 14].

Conclusion

Histoenzymatic maturation in chital deer follows a predictable trajectory from low fetal activity through rapid neonatal to juvenile induction to a stable adult plateau. This research

provides the first comprehensive enzyme histochemical reference for *Axis axis* hepatic and renal tissues across five developmental age classes. The principal findings hepatic ALP peaking in sub adults, renal GGT peaking one age class later than hepatic GGT, and G6Pase showing the greatest fold change establish a baseline that can support wildlife forensic age estimation and conservation physiology research.

Looking ahead, extension of this histoenzymatic profiling to additional organ systems (gastrointestinal tract, adrenal gland, reproductive organs) and to other Sri Lankan cervids such as the sambar deer (*Rusa unicolor*) would broaden the comparative framework. Integration of quantitative histochemistry with molecular markers of cellular differentiation could further refine the developmental model proposed here [3, 7, 12].

Acknowledgements

Funding sources

Supported by a grant from the University of Colombo Research Fund (Grant No. AP/3/2/2023/RG/018).

Institutional support

The authors thank the Department of Wildlife Conservation, Sri Lanka, and the Colombo Research Center for Wildlife Biology for access to the tissue bank.

Contributions not qualifying for authorship

The authors acknowledge Mr. Kasun Wijesinghe for cryosectioning and Ms. Chamari Dissanayake for image analysis.

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How to Cite This Article

Perera A. Histoenzymatic differentiation of hepatic and renal tissues in chital deer at different developmental ages. International Journal of Veterinary Sciences and Animal Husbandry. 2025; 10(6): 213-216.

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