

Do testosterone and cortisol assays correctly target metabolites of these hormones in carnivore faeces? Insights from spotted hyenas.

Marion L. East¹, Martin Wicke¹, Roberto Bonanni², Klaus Eulenberger³, Heribert Hofer¹ & Martin Dehnhard¹

¹Leibniz Institute for Zoo & Wildlife Research, Berlin, Germany; ²Dept of Evolutionary & Functional Biology, University of Parma, Italy; ³Zoo Leipzig, Pfaffendorferstr. 29, 04105 Leipzig, Germany

Faecal assays for the non-invasive measurement of hormone concentrations are normally based on an antibody directed against a specific hormone. However, an antibody designed to target a specific hormone may cross-react with metabolites derived from another hormone. Previously we have suggested that faecal "testosterone" assays probably cross-react with faecal glucocorticoid metabolites because they measured a large increase in the concentration of metabolites following an adrenocorticotrophic hormone (ACTH) challenge experiment (Bonanni et al., 2007; also Fig. 1).

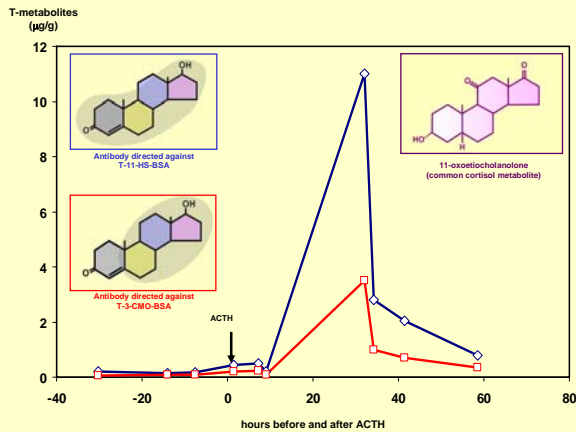


Fig.1: An increase in steroid metabolites detected by two different antibodies directed against testosterone (T) after an ACTH-challenge indicate high cross-reactivity with glucocorticoid metabolites (Bonanni et al., 2007)

Assessment of faecal metabolites of testosterone and cortisol in spotted hyena faeces

We conducted radio-labelled metabolism studies on spotted hyenas at Leipzig Zoo. One male received [3H]testosterone, another male received [3H] cortisol. We obtained faeces before and after application. Enzyme immunological determinations were assessed using antibodies raised against testosterone-3-CMO and cortisol-3-CMO-BSA. HPLC-immunograms were generated on a reversed-phase RP18 column separating faecal metabolites according to their polarity.

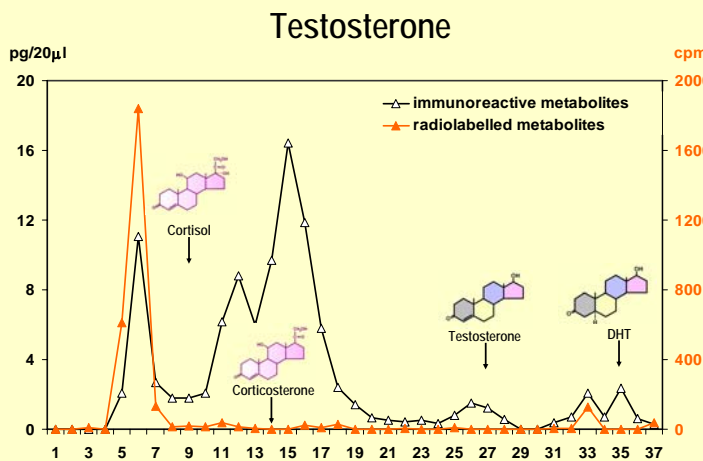


Fig.2: HPLC separation of radio-labelled (cpm) and immunoreactive (pg/20µl) faecal testosterone metabolites. The arrows indicate the positions of the steroid standards used in separate runs.

- Radio-labelled testosterone (T) was largely metabolised into polar, probably conjugated metabolites (fig. 2: fraction 6).
- The T antibody predominantly traced metabolites (fraction 9 – 19) that did not overlap with the radio-labelled T peak (fraction 4-7) and thus produced false results.

Table 1

Androgen	Antibody type
testosterone	4-androsten-17β-ol-3-one-11-HS-BSA
testosterone	4-androsten-17β-ol-3-one-CMO-BSA
dihydrotestosterone (DHT)	5α-androstan-17β-ol-3-one-CMO-BSA
epiandrosterone	5α-androstane-3,17-dione-3-CMO-BSA

Using the T HPLC immunograms (Fig. 2) we assessed the efficacy of 4 androgen antibodies (Table 1) and found that they all measured large amounts of non-T metabolites as shown in Fig. 2.

The specificity of antibodies used to detect faecal T-metabolites produced by spotted hyenas and probably other carnivore species should be assessed to avoid false interpretation of results.

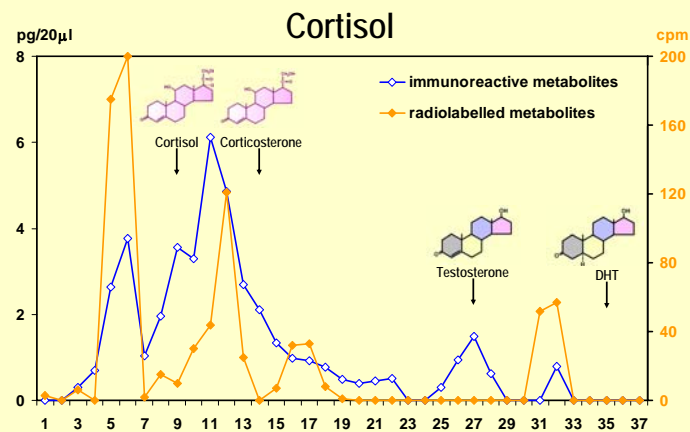


Fig.3: HPLC separation of radio-labelled (cpm) and immunoreactive (pg/20µl) faecal cortisol metabolites. The arrows indicate the positions of the steroid standards.

- Radio-labelled cortisol was metabolised to polar, probably conjugated metabolites (fraction 5,6), an unknown metabolite (fraction 12) and two minor metabolites in fractions 16,17 and 31,32.
- The cortisol antibody traced the polar metabolites (fraction 5,6), the metabolite in fraction 12 and fraction 31,32.
- The antibody also showed immunoreactivity with metabolites between fraction 9 and 27 which were not supported by distinct peaks of radio-labelled cortisol metabolites.
- The antibody showed immunoreactivity with a non-cortisol metabolites in fraction 22-27.

Although the cortisol antibody traced some it did not trace all major radio-labelled cortisol metabolites. This problem coupled with the considerable overlap of T (Fig 2) and C (Fig 3) metabolite fractions suggests that a more specific antibody targeting C specific metabolites is required.