The effect of clenbuterol on the steroidogenesis

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Conclusion

- There was a clear separation based on steroid patterns in urine and serum between control group and clenbuterol treated group.
- Two (conjugated) estrogens are down regulated in urine after clenbuterol treatment.
- Underlying mechanism is still unknown.

Introduction

Clenbuterol is a well-known growth promotor in cattle. Although its use for this purpose is forbidden, clenbuterol is still used on a large scale in countries outside Europe. It has been found that people traveling to China were tested positive for clenbuterol in urine after consuming clenbuterol contaminated meat. In Mexico almost all tested soccer players competing in the FIFA U-17 World Cup 2011 were found to be positive for clenbuterol[1,2].

Objective

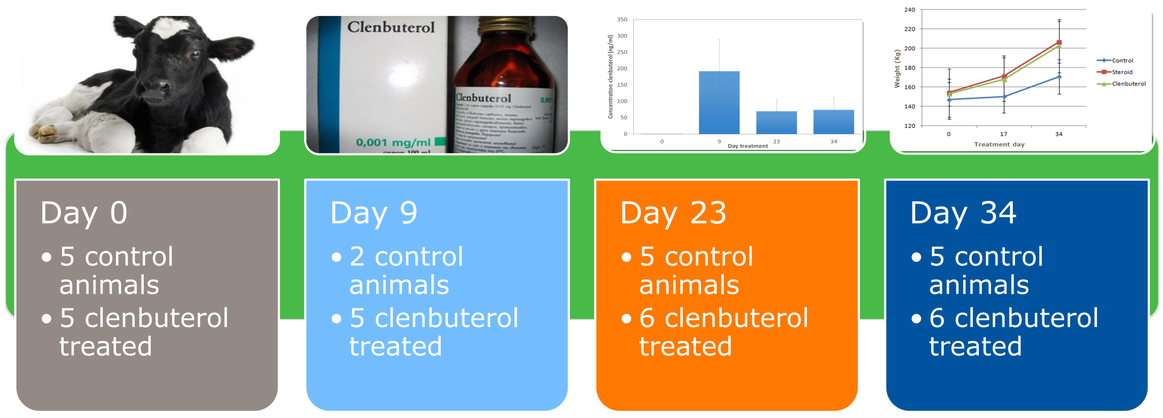
The underlying mechanism explaining why clenbuterol is a growth promotor is not well known[3]. The aim of the experiments described was to assess whether there is an effect of clenbuterol on the steroidogenesis in cattle.

Experimental

Bovine urine (500 µl) or plasma (150 µl) is processed using a combination of 96-well Oasis HLB and 96-well WAX sorbents plates. Conjugates (glucuronides and sulphates) are separated from the free compounds (aglycons). The aglycon fraction is derivatised with picolinic acid to increase the sensitivity of in particular estrogens. Concentrations of 60 compounds involved in the steroidogenesis are determined. Analysis is performed on an UPLC-MS/MS system within 6 minutes. Conjugates are analysed by CSH C_{18} UPLC-MS/MS. Aglycons are analysed by BEH C_{18} UPLC-MS/MS.

Animal trial

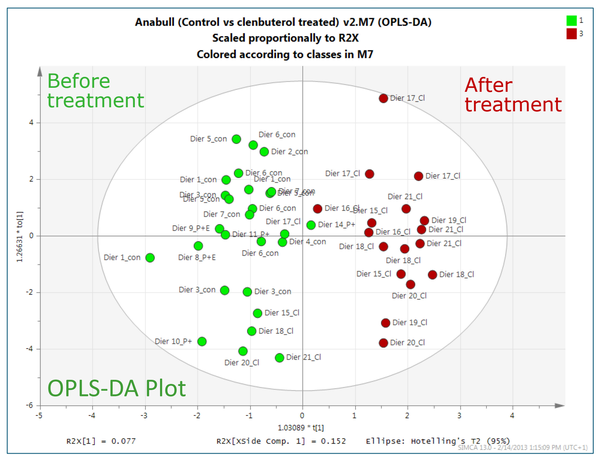
In an animal trial conducted by the Technical University of Munich[3]. The animal experiment consisted of several male control animals and animals treated with clenbuterol (10µg/kg BW/day). Samples of urine and plasma were taken before treatment and 9, 23 and 34 days after treatment start.

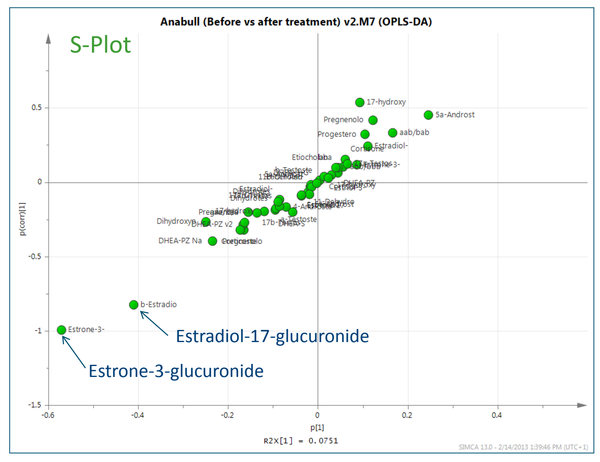


Results

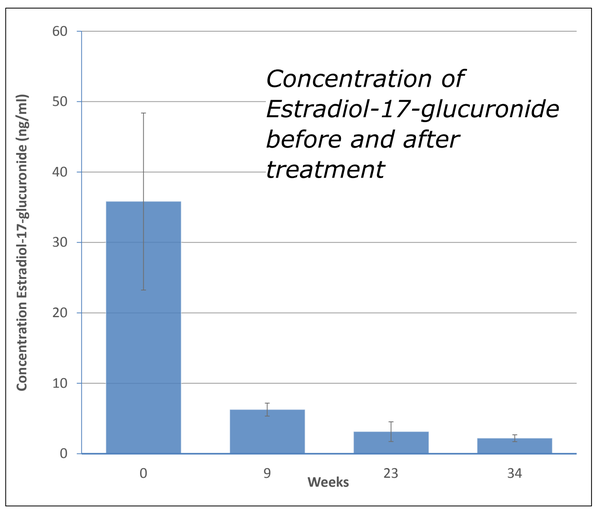
After quantitative analysis of the urine and plasma samples, multivariate statistics was performed to determine which compounds are up- or down-regulated after treatment. First, the treated and non-treated groups of animals were trimmed using PCA. Subsequently, the groups were successful separated using OPLS-DA. Validation of the model was performed using cross validation and a permutation test. The compounds contributing to the separation of the groups were identified by means of an S-Plot.

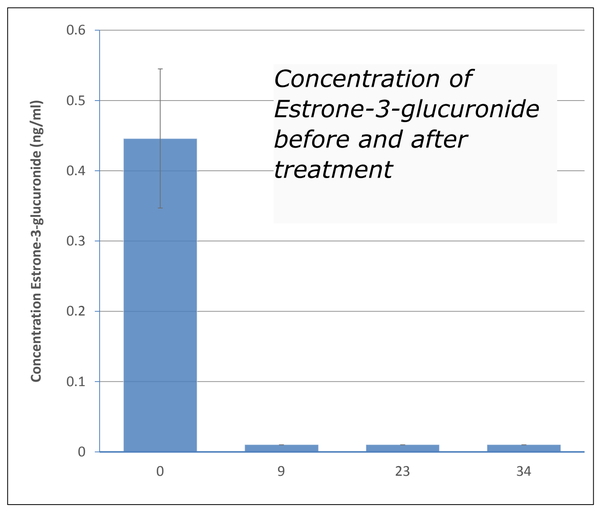
Results urine analysis



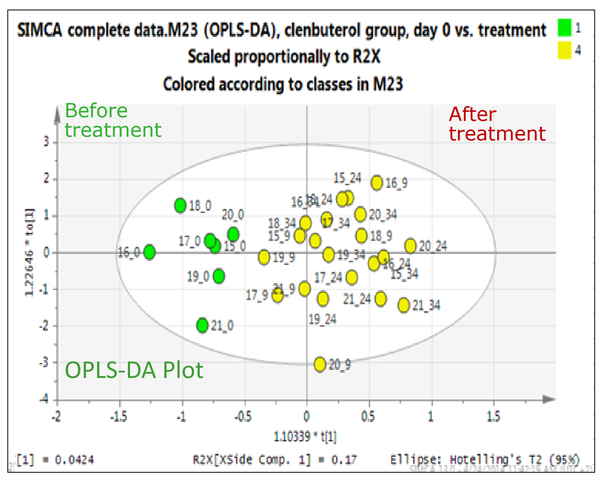


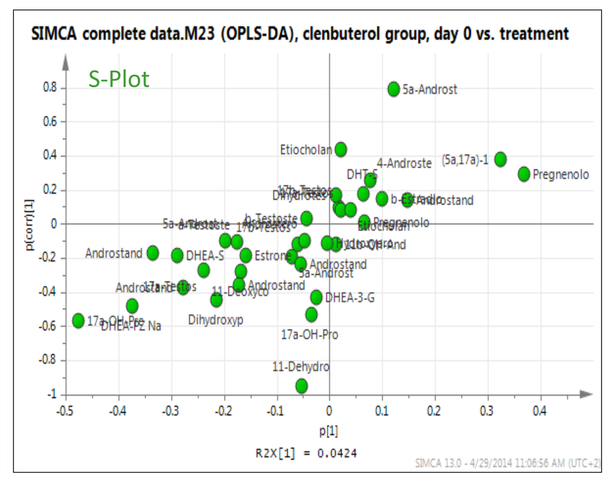
Using the S-Plot it was determined that Estradiol-17-glucuronide and Estrone-3-glucuronide were down regulated after treatment with clenbuterol. Quantitative details are shown in the figure below.





Results plasma analysis





The S-plot for the plasma samples leads to the conclusion that the treatment of clenbuterol negatively correlates to the steroid synthesis as can be observed by a general shift to the lower left corner of the S-plot. However, individual examination of compounds does not show a clear effect on concentrations.

Other related studies

- Studies show up-regulation of estrogen and progesterone receptors in the reproductive system of female veal calves induced by dietary clenbuterol[4].
- DHEA-S ($p < 0.002$), DHEA ($p < 0.03$), Estradiol ($p < 0.02$), and Estrone ($p < 0.02$) measured from 22 postmenopausal asthmatic and 22 age-matched, postmenopausal, nonasthmatic women were lower in asthmatic patients caused by use of beta-agonists[5].

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References
1. Drug Test Anal. 2013; 5(5):372-6
2. Presentation Saskia Sterk on Wednesday at 14.45
3. Presentation Irmgard Riedmaier on Wednesday at 10.10
4. Am J Vet Res. 1995; 56(11): 1493-7
5. J Allergy Clin Immunol. 1996; 97: 1-8