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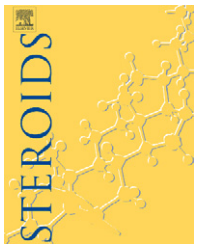

Criteria to distinguish between natural situations and illegal use of boldenone, boldenone esters and boldione in cattle 1. Metabolite profiles of boldenone, boldenone esters and boldione in cattle urine

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ABSTRACT

Boldenone is an androgenic steroid that improves the growth and food conversion in food producing animals. In most countries worldwide, this anabolic steroid is forbidden for meat production. Until recently, the control of its illegal use was based either on 17β-boldenone or 17α-boldenone (its main metabolite in cattle) identification in edible tissues, hair, faeces or urine. Recent observations and data tend to demonstrate the natural occurrence (but not ubiquitous) in cattle of these steroids, making the analytical strategy of the control more complicated. We investigated the metabolism of boldenone in cattle after intramuscular and oral treatment of boldenone, boldenone esters and boldione. The central objective was to elucidate the structures of the main metabolites (phase I and phase II) in urine, with main objective to be further in position to compare boldenone urinary profiles of treated and non-treated animals. Nine metabolites have been identified, only four were present whatever the treatment and the administered boldenone source. Nevertheless, all of them have been detected at least once in non-treated animals which did not permit us to use them as biomarkers of an illegal treatment. At last, but not at least, all metabolites were found mainly glucuro-conjugated, and rarely sulfo-conjugated, with the only exception of 17β-boldenone. Current investigations are showing the absence of 17β-boldenone sulfoconjugate in non-treated animals; that would permit to distinguish non-treated from treated animals with boldione, boldenone and boldenone esters.

1. Introduction

Boldenone (1-dehydrotestosterone or androsta-1,4-dien-17β-ol-3-one), is a steroid which differs from testosterone only by one double bond in-between carbons 1 and 2 (Fig. 1). Boldenone and its esters (mainly the undecylenate form) are commercially available as ready-to-use anabolic preparations, either for human, horse or cattle. The oxidised form

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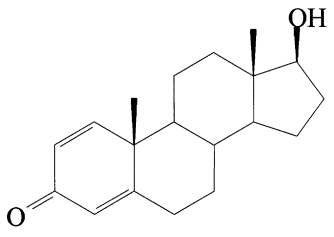
Table 1 – Sampling times relative to the five different treatments (A–E)

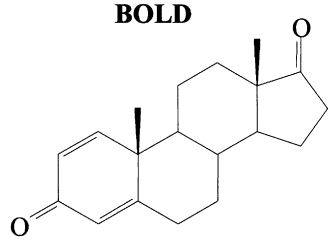
A	B	C	D	E
Sampling times				
Ante treatment	Ante treatment	Ante treatment	1 day before treatment	1 day before treatment
20 min after treatment	55 min after 1 day of treatment	3 h after 1 day of treatment	Ante treatment	Ante treatment
50 min after treatment	5 h after 1 day of treatment	24 h after 1 day of treatment	1 day after treatment	1 day after treatment
90 min after treatment	24 h after 1 day of treatment	24 h after 2 days of treatment	2 days after treatment	2 days after treatment
24 h after treatment	35 min after 2 days of treatment	24 h after 6 days of treatment	4 days after treatment	5 days after treatment
4 days after treatment	65 min after 2 days of treatment	12 days after suspension	7 days after treatment	6 days after treatment
5 days after treatment	50 min after 3 days of treatment	–	14 days after treatment	7 days after treatment
–	24 h after suspension	–	–	14 days after treatment
–	5 days after suspension	–	–	–

of boldenone, e.g. boldione or androsta-1,4-dien-3,17-dione (ADD), is a recent popular over-the-counter (OTC) steroid effortlessly obtainable via Internet. It is illegally use as a growth promoter in cattle husbandry. The recent increasing number of positive cases for boldenone in cattle was firstly attributed to the improvement of analytical methodologies, which permit nowadays to improve the limit of detection and quantification of main steroids in biological matrices, especially with the introduction of powerful MS/MS technologies. Then, because most of the positive cases were difficult to understand and sometimes impossible to explain, the possible endogenous status was envisaged. In 2003, several official working party involving government experts permitted to follow the various research projects held in Official European Laboratories (ISS/Roma, RIVM/Utrecht, LCA-FVM/Ghent, TNO/Zeist, LABERCA/Nantes). A report and a paper were published in 2003 and 2004, respectively [1,2] reviewing the different aspects and works on boldenone until nowadays.

Official criterions are currently missing for control laboratories to discriminate natural occurring boldenone metabolites and presence related to a misuse situation. The objective of this paper is to study exhaustively the phases I and II metabolites of boldenone including some of its precursors, e.g. boldione or different ester forms, either after oral or intramuscular administration.



BOLD



ADD

Fig. 1 – Structures of boldenone (BOLD) and androsta-1,4-diene-3,17-dione (ADD, boldione).

2. Experimental

2.1. Animals

Five different calves have been treated according to five different procedures (sampling times are described in Table 1). Treatments were performed in Italy and The Netherlands using Holstein-Friesian veal calves and set-up at a farm under controlled experimental conditions, according with the animal wellness protocols. The animals were housed in ventilated stables and appropriate measures were taken to avoid any kind of cross contamination between the different animals. The calves were fed with a diet usually employed in the zootechnical practice composed of products available on the market, *i.e.*, milk replacers, calf starters and forage. *Ad libitum* access to water and feeds was allowed to the animals, which were regularly monitored by the specialized staff. The access to the experimentation environments was restricted to the personnel involved in the study. The animals underwent a 7 days adaptation phase. Then, during a 30-day phase, the calves were managed under farm techniques comparable to those adopted in the current zootechnical practices.

- *Treatment A*. Oral administration of boldenone and ADD, single administration dose, 5 mg/calf.
- *Treatment B*. Oral administration of boldenone esters (acetate + benzoate + propionate, 2 + 2 + 1), daily treatment over 3 days, 100 mg/(calf day).
- *Treatment C*. Oral administration of ADD, daily treatment over 6 days, 100 mg/(calf day).
- *Treatment D*. Oral administration of boldenone undecylenate, daily treatment over 5 days, 100 mg/(calf day).
- *Treatment E*. Intramuscular injection of boldenone undecylenate, single administration in the neck, 250 mg/calf.

2.2. Reagents and chemicals

All solvents and reagents were of analytical and HPLC grade quality. All SPE (C18, SiOH, NH_2, SiOH, SCX cartridges) were single use cartridges. Purified *Helix pomatia* preparation was used for steroid glucuronide deconjugation; solvolysis was used for sulfoconjugates hydrolysis. Trimethyliodosilane (TMIS) and *N*-methyl-*N*-(trimethylsilyl)-trifluoro-acetamide (MSTFA) were purchased from Fluka (Buchs, Switzerland). Dithiothreitol (DTE) and ammonium iodine (NH_4I) were from