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4 1 **Evaluation of the illegal use of clenbuterol in feed, drinking water, urine,**
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6 2 **and hair samples collected in bovine farms in Portugal**
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22 **Abstract**

23 The recent discovery of clenbuterol contamination in Portuguese food led to a special
24 inspection of 16 cattle farms for β -agonists, which involved the analysis of a total of 486
25 samples (78 feed, 106 drinking water, 168 urine and 134 hair). The samples were screened for
26 the β -agonists bromobuterol, cimaterol, clenbuterol, clenpenterol, clenproperol,
27 hydroxymethylclenbuterol, mapenterol, salbutamol and terbutaline. Only clenbuterol was
28 found in all types of analyzed matrices and the most likely method of clenbuterol illegal
29 administration to animals was through drinking water. Of the samples analysed 14.15% of
30 drinking water were found positive in a range of 0.03 to 3.80 mg/l of clenbuterol. Inclusion of
31 hair samples in the Portuguese plan for clenbuterol residue control in live animals is
32 discussed.

34 **Key words:** Clenbuterol; drinking water; feeds; urine; hair

36 **Introduction**

37 Food with high fat content is considered one of the main causes of cardiovascular diseases.
38 For that reason, consumers in developed countries have been avoiding the consumption of
39 meat with higher fat content. So, animal producers have used some anabolic
40 substances which curtail the production of this kind of meat. β -agonists were utilized
41 because they result in animal carcasse meat with less fat tissue and, at the same
42 time, increased muscle protein, which provides a very attractive meat to consumers'
43 eyes (Asato et al., 1984; Mersmann, 1987; Inkster, Hovell, Kyle, Brown, & Lobley, G.,
44 1989). The search for food sources has increased, and consequently so has competition in the

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3 45 intensive meat production sector. The demand for higher productivity, preferably at lower
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5 46 cost and shorter time periods, has led to several changes in cattle production, such as the
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7 47 improvement of animal feeding and lodging, the use of genetic breeding and the control of
8
9 48 animal diseases. This pressure has also led to the illegal use of pharmacological agents,
10
11 49 specifically anabolic compounds, to promote the growth of animals (Directive n° 2003/74,
12
13 50 2003). Clenbuterol, a β -agonist, is an authorised drug in the veterinarian therapeutics of
14
15 51 bovines, equines and companion animals as bronchodilator or as tocolitic agent for cows. The
16
17 52 use of β -agonists as growth promoters is a public health risk; for example, animal-derived
18
19 53 foods containing clenbuterol have caused several outbreaks of food poisoning in Spain
20
21 54 (Martinez-Navarro, 1990; Garay et al., 1997), France (Pulce et al. 1991), Italy (Maistro,
22
23 55 Chiesa, Angeletti, & Brambilla, 1995; Brambilla, Loizzo, Fontana, Strozzi, Guarino, &
24
25 56 Soprano, 1997; Brambilla et al., 2000), China (Shiu & Chong, 2001) and Portugal (Barbosa et
26
27 57 al., 2005). In addition, the European Union (EU) has prohibited the use of growth-promoting
28
29 58 drugs in animals destined for human consumption, and the use of such prohibited substances
30
31 59 also constitutes an unfair practice in the market of animal production (Directive n° 96/22,
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33 60 1996).

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43 62 To implement the EU regulations, the EU Commission issues guidelines describing National
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45 63 Residue Control Plans for all Member States and these Plans involve sampling animals before
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47 64 and after slaughter (Directive n° 96/23, 1996). In the case of β -agonists in Portugal, the
48
49 65 Residue Control Plan stipulates the collection of liver and muscular tissue samples from
50
51 66 animals after slaughter, as well as samples from urine, drinking water, and feed from live
52
53 67 animals. GC-MS was used as the analytical method for determining the presence of β -
54
55 68 agonists, in agreement with the parameters established by Commission Decision n°
56
57 69 2002/657/CE (2002). The present paper is intended to contribute to a better understanding of

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2
3 70 the use (or misuse) of β -agonist substances in live animals in Portugal. The data obtained
4
5 71 from samples of urine, drinking water, feed, and hair taken from cattle farms is presented and
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7
8 72 the relevance of sampling drinking water and hair samples for Portuguese plan for clenbuterol
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10 73 residue control is discussed
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12 74

15 75 **Material and Methods**

17 76 *Reagents and equipments*

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19
20 77 Standards of mapenterol, cimaterol, clenproperol, clenpenterol, bromobuterol, and
21
22 78 hydroxymethylclenbuterol were kindly offered by Prof. François André (Laberca, Nantes,
23
24 79 France); salbutamol and terbutaline were acquired from Sigma (Madrid, Spain); and
25
26
27 80 clenbuterol and hexadeuterated clenbuterol (clenbuterol-d6) as an internal standard (IS) were
28
29 81 supplied, respectively, by Interchim (Montluçon, France) and by RIVM (Bilthoven, the
30
31 82 Netherlands). N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTA; Sigma, Madrid, Spain),
32
33 83 1,4-Dithioerythritol (DTE; Merck, Lisbon, Portugal), and trimethyliodosilane (TMIS; Sigma)
34
35 84 in the proportion of 5 ml:10 mg:10 μ l (MSTA:DTE:TMIS) were used as the derivatization
36
37
38 85 reagent. Purified water was obtained from a Milli-Q Plus system (Millipore, Bedford, MA,
39
40 86 USA) and all other reagents were from Merck, including *Helix pomatia* β -glucuronidase (30
41
42 87 U/ml) and arylsulfatase (60 U/ml).
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47
48 89 For storage and sample preparation, the following materials were used: an Electrolux freezer
49
50 90 (Coimbra, Portugal), a Mettler AG285 balance (Zurich, Switzerland), a Moulinex mixer
51
52 91 (Lisbon, Portugal), a 40-mesh Endecotts sieve (London, UK), vortex agitators from
53
54 92 UnimagZX (Reagente 5, Oporto, Portugal) and Edmund Bülher (KL2 model, Tübingen,
55
56 93 Germany), and a Memmert oven (Schwabach, Germany).
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3 95 Solid-phase extraction (SPE) was performed using a vacuum manifold system involving
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5 96 Clean Screen DAU mixed-phase (C8+Benzyl sulfonic acid) 500 and 1000 mg columns
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7
8 97 (Interchim, Montluçon, France). Centrifugation was done on a Sigma 3-16K centrifuge
9
10 98 (Reagente 5, Oporto, Portugal), and pH determination was done with a WPA CD7400
11
12 99 potentiometer (Cambridge, UK). Material for derivatization was supplied by Chromacol (Dias
13
14 100 de Sousa, Lisbon, Portugal). β -agonist determination was achieved by GC-MS using Agilent
15
16 101 Technologies (AT) equipment (Soquimica, Lisbon, Portugal), comprising an AT6890N gas
17
18 102 chromatograph equipped with a HP-5MS column (30 m x 0.25 mm I.D. x 0.25 μ m of film
19
20 103 thickness), an AT7683B automatic injector, an AT5975 MSD detector, an HP Compaq
21
22 104 computer, and an HP LaserJet 2420d printer. Nitrogen and helium gases were supplied by
23
24 105 Sogafer (Coimbra, Portugal).
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31 107 *Samples*

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34 108 Samples of feed, drinking water, urine, and hair were collected in 16 cattle farms during the
35
36 109 period between February 28 and August 24, 2007.

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38 110 Drinking waters were collected from troughs/tanks in minimum amounts of 100 ml per
39
40 111 sample. From farms that perform extensive growth enhancement (A, D, E, G, I and K),
41
42 112 samples were collected from the pasture tanks. For intensive farms (B, C, F, H, J, L, M, N, O
43
44 113 and P), samples were collected from individual bovine troughs from the cattle-shed water
45
46 114 system.
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51 115
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53 116 Feed samples were collected from the bovine mangers or the feed storage silos. When
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55 117 sampling was made in the storage silos, appropriate equipment was used in order to collect
56
57 118 sample aliquots at different depths. At least, 500 g of each feed were collected. Urine
58
59 119 sampling was always taken from male animals in disposable plastic bags adequately attached
60

120 to bovine's back, as well as by specialized technicians (Kurien, Everds & Scofield, 2004). 50
121 ml of urine was the minimum quantity of each sample.

122 Hair samples were collected using scissors and hair was cut as close as possible to the skin of
123 the animal. A quantity of 20 g, at least, was considered appropriate. Of the 37 hair samples
124 initially collected, only 5, one per farm B, C, D, E and F, were from animals that urine
125 samples had also been collected. After six months, on farm B, hair and urine samples were
126 simultaneously collected from 56 animals.

127
128 Table 1 shows the number of each sample matrices collected on each farm.

129 Urine and water samples were frozen at $-20\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ and hair and feed samples were stored
130 at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ until further analysis.

131

132 *Sample preparation*

133 Drinking water- Samples were thawed at room temperature and, after homogenization, 15 ml
134 were transferred to a centrifuge tube. After 10 min centrifugation at 4000g, an aliquot of 10
135 ml of clarified sample was transferred to another container and 100 μl of 1 $\mu\text{g}/\text{ml}$ IS and 5 ml
136 of 0.1 M phosphate buffer ($\text{pH } 6.0 \pm 0.1$) were added. Finally, pH was adjusted to 6.0 ± 0.3
137 with 1 M potassium hydroxide (Boyd, O'Keeffe, & Smyth, 1996; Ramos, 2000).

138

139 Feed- 5.0 g of ground and sieved sample were weighed into a centrifuge tube and 25 ml of
140 chloroform and 100 μl of 1 $\mu\text{g}/\text{ml}$ IS were added. After a 30 min agitation period at 420 rpm,
141 everything was filtered into a decantation funnel already containing 10 ml of 1 M
142 hydrochloric acid. The decantation funnel was mixed manually for 1 min and allowed to rest
143 until complete phase separation was achieved. The organic phase was discarded. Another 10
144 ml of chloroform were added to the decantation funnel and the previous step repeated. The

1
2
3 145 aqueous phase was recovered and 5 ml of 0.1 M phosphate buffer (pH 6.0 ± 0.1) were added.

4
5 146 After homogenization, pH was adjusted to 6.0 ± 0.3 with 2 M potassium hydroxide (Ramos,

6
7 147 Ribeiro, Saltão, Costa, & Silveira, 1999)

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9
10 148 Urine- Urine samples, collected each one for a different animal, were thawed at room

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12 149 temperature and, after homogenization, 15 ml were transferred into a centrifuge tube. After 10

13
14 150 min centrifugation at 4000g, pH was adjusted to 5.2 ± 0.3 with glacial acetic acid. Then, 10

15
16 151 ml of clarified sample were taken and the following were added: 100 μ l of 1 μ g/ml IS, 4 ml of

17
18 152 0.2 M acetate buffer (pH 5.2 ± 0.1) and 50 μ l of *Helix pomatia*. The mixture was vortexed for

19
20 153 20 sec, and then incubated overnight in an oven at $48 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$. Samples were left to cool at

21
22 154 room temperature. Then 5 ml of 0.1 M phosphate buffer (pH 6.0 ± 0.1) were added and the

23
24 155 pH was adjusted to 6.0 ± 0.3 with 1 M potassium hydroxide (Boyd et al., 1996).

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26 156

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29 157 Hair- Hair was washed with an aqueous solution of 5% Tween 80. The sample was then

30
31 158 rinsed with purified water, as often as necessary, until the washing water was clear. Hair

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33 159 samples were dried overnight in an oven at $80 \text{ }^\circ\text{C}$, and subsequently cut into small pieces 1-2

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35 160 mm in length. Pieces with a total mass of 1.0 g were transferred to a centrifuge tube to which

36
37 161 3 ml of 2 M sodium hydroxide and 100 μ l of 1 μ g/ml IS were added. Following

38
39 162 homogenization, the sample was digested at $80 \text{ }^\circ\text{C}$ for 1 h, with agitation every 15 min (Fente,

40
41 163 Vázquez, Franco, Cepeda, & Gigosos, 1999; Ramos et al., 2001). After cooling to room

42
43 164 temperature, 4 ml of 0.2 M acetate buffer (pH 5.2 ± 0.1) were added and the pH was adjusted

44
45 165 to 5.2 ± 0.3 with 2 M hydrochloric acid. The mixture was centrifuged for 5 min and the

46
47 166 supernatant was filtered. The sediment was resuspended in 5 ml of acetate buffer and

48
49 167 centrifuged again for 5 min. The second supernatant was also filtered and mixed with the

50
51 168 previous one (Cristino, Ramos, & Silveira, 2003). Then, 50 μ l of *Helix pomatia* were added,

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53 169 the mixture was vortexed for 20 sec, and incubated overnight at $48 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$. Samples were

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2
3 170 left to cool to room temperature. Then 5 ml of 0.1 M phosphate buffer (pH 6.0 ± 0.1) were
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5 171 added and pH was adjusted to 6.0 ± 0.3 with 1 M potassium hydroxide (Boyd et al., 1996;
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8 172 Ramos, 2000).

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10 17311
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13 174 *Solid-phase extraction*

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15 175 The sample extracts prepared as described above were purified by SPE using mixed-phase
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17 176 columns. A 500 mg column was used for urine and water samples, while a 1000 mg column
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19 177 was used for feed and hair; the volumes used for the former were one-half the volumes used
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21 178 for the latter. Sorbent was activated with methanol, water, and phosphate buffer (2 or 4 ml of
22
23 179 each solvent), after which the extract was loaded. Acetic acid (1 ml or 2 ml) was added to the
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25 180 columns and left for 10 min before being eluted. The washing step was performed using 6 or
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27 181 12 ml of methanol. After each of the previous two steps, the sorbent was dried using
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29 182 centrifugation at 4000g. Analyte elution was carried out using 5 or 10 ml of ethyl acetate:
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31 183 32% ammonia (97:3, v/v) (Montrade, le Bizec, Monteau, Siliart, & André, 1993; Ramos,
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33 184 Bañobre, Castilho, & Silveira, 1999)

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36 18537
38
39 186 *Derivatization*

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41 187 Eluates were evaporated to dryness under a nitrogen stream at 65 °C. The residue was
42
43 188 dissolved in methanol and transferred to a derivatization vial. Methanol was also evaporated
44
45 189 to dryness under the conditions mentioned above for the eluates, and the resulting dry residue
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47 190 was redissolved with 50 μ l of derivatization reagent. After vial encapsulation, derivatization
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49 191 was performed at 65 °C for 20 min (Ramos et al., 2003).

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3 193 *Chromatography*
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5 194 An aliquot of 2 μ l was injected into the GC-MS system in splitless mode (1 min), using
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8 195 helium as carrier gas with a constant flow of 1.4 ml/min through the column. The column
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10 196 was submitted to the following temperature gradient: 100 °C (0.4 min) -----> 10 °C/min ---->
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12 197 200 °C (3 min) ----> 10 °C/min ----> 300 °C (5 min). Injector and detector temperatures were
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14
15 198 270 and 280 °C, respectively, and detection was done in electron impact (EI) mode with
16
17 199 selective ion monitoring (SIM).
18

19
20 200 Table 2 shows m/z ions detected for each β -agonist, as well as for the IS (clenbuterol-d6).
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22 201 Figures 1 – 4 show chromatograms of a standard solution and of positives drinking water,
23
24 202 urine and hair samples.
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27 203

28
29 204 **Results and Discussion**
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31 205 *Method validation*
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34 206 Validation data was acquired for clenbuterol determination in urine, feed and hair samples.
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36 207 Urine data was used for drinking water samples. Specificity, linearity, decision limit ($CC\alpha$),
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38 208 detection capability ($CC\beta$), precision and accuracy were determined according to Commission
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40
41 209 Decision 2002/657/EC (2002). Specificity was demonstrated by analysing 20 blank of each
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43 210 samples before and after spiking at a concentration level of 1.0 μ g/l or 5.0 μ g/kg, respectively
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45
46 211 for urine and water samples and for hair and feed samples. No interfering peaks were
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48 212 observed at the retention time window for clenbuterol in the four analysed matrices. Linearity
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50 213 of the chromatographic response was tested for clenbuterol with five calibration points in the
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52 214 concentration range of 0.5–5.0 μ g/l and of 5.0–50.0 μ g/kg, respectively for urine and for hair
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54
55 215 and feed samples. The regression coefficients (r^2) for the calibration curves used in the
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57 216 method validation study were always above 0.993.
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218 $CC\alpha$ and $CC\beta$ were calculated as stated by Commission Decision 2002/657/EC (2002) and by

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3 219 ISO 11843 (2000). For $CC\alpha$, with $\alpha = 1\%$, the calculation was performed with the application
4 of the following formula:-
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9
10 223 $CC\alpha = b + 2.33S.D._b$
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12 224

13
14 225 where b is the concentration of the intercept of the regression line and $S.D._b$ is the standard
15 deviation from regression line.
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17 227

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19 228 $CC\beta$ at $\beta = 5\%$, was then calculated as:-
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21 229

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23 230 $CC\beta = CC\alpha + 1.64S.D._{CC\alpha}$
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26
27 232 wherein $S.D._{CC\alpha}$ is the standard deviation at $CC\alpha$ concentration.
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29 233

30
31 234 Clenbuterol accuracy, in terms of recovery, was measured using blank samples spiked at 0.5,
32 1.0, and 2.0 $\mu\text{g/l}$ and at 5.0, 10.0 and 20.0 $\mu\text{g/kg}$ respectively for urine and for hair and feed
33 samples. Precision, in terms of repeatability and intra-laboratorial reproducibility, was
34 evaluated in particular the within- and between days variation, by calculating the coefficient
35 of variation (CV) of clenbuterol mean concentrations at previous referred concentration levels
36 and matrices. For each level, six samples of each matrix were prepared and analysed every
37 day for 3 days. Obtained data at 1.0 $\mu\text{g/l}$ and at 10.0 $\mu\text{g/kg}$ levels, respectively for urine and
38 for hair and feed samples, were shown in table 3 and lead to the conclusion that the used
39 methodology could satisfy required reference parameters for the purpose.
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55 244 *Samples investigated*
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57 245 No β -agonists were found in any matrix from 12 of the 16 farms under inspection for using
58 growth-enhancing drugs. No β -agonists were found in any analyzed feed samples. In the other
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60 246

1
2
3 247 matrices analyzed, only one β -agonist was found, clenbuterol, in concentrations between 0.03
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5 248 – 3.80 mg/l, 0.3 – 11.6 μ g/l and 32.9 – 57.0 μ g/kg, respectively for drinking water, urine and
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7
8 249 hair samples. The results for the positive samples are shown in Table 1.

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10 250 Comparing the incidence of positive urine samples and positive hair samples, a discrepant
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12 251 value could be observed. However, one of the two hair samples where clenbuterol was
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14
15 252 detected was black and was the only hair sample collected from farm C. The urine sample
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17 253 collected from the same animal was also declared positive with a clenbuterol concentration of
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19 254 3.9 μ g/l. The other positive hair sample was brown and part of a group of 13 collected at farm
20
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22 255 B where 1 hair was white, 3 were blond, and 9 were brown. The urine sample collected from
23
24
25 256 the same animal was also considered positive for clenbuterol with a concentration of 4.8 μ g/l.
26
27 257 Clenbuterol was detected in the two positive samples at the levels of 57.0 μ g/kg (black) and
28
29 258 32.9 μ g/kg (brown), which is according to previously related melanin relevance in hair
30
31 259 clenbuterol detection (Cristino et al., 2003; Gallo, Brambilla, Neri, Fiori, Testa & Serpe,
32
33 260 2007, Salquèbre, Bresson, Villain, Cirimele & Kintz, 2006; Sauer & Anderson, 1994).
34
35 261 Effectively, melanin, the main hair pigmented component was responsible for clenbuterol
36
37 262 accumulation, due to chemical binding between their –SH radicals and the substituted
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39 263 aromatic ring of the β -agonist. (Howells, Godfrey & Sauer, 1994).

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45 265 Of the remaining hair samples, the great majority (97) were collected in two cattle farms that
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47 266 had been ordered to cease clenbuterol administration six months before, when clenbuterol had
48
49 267 been detected in 50% (Farm A) and 63% (Farm B) of the analyzed urine samples. In the 97
50
51 268 samples (1 white, 7 black, 40 brown, and 49 blonde), no clenbuterol was detected, which
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53 269 indicate, as previously related by Fente and co-workers (1999), that after six months,
54
55 270 clenbuterol is no longer detectable in hair samples.

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3 272 A total of 168 urine samples were tested. All of the 24 samples found to be positive had been
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5 273 collected in 3 farms, and they constituted 58.5% of such samples.
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8 274 A high number (15 of 22) of drinking water samples were positive in the farms where the β -
9
10 275 agonist was detected. All positive samples were collected from individual troughs of intensive
11
12 276 bovine production, where the animals were normally maintained in cattle-sheds during the
13
14 277 greater part of the day. It was also observed that clenbuterol was only put in the watering
15
16 278 system inside each cattle-shed. Thus, clenbuterol was pumped from a 50-liter water barrel into
17
18 279 the pipe that supplies bovine individual troughs. Indeed, clenbuterol water solubility allows an
19
20 280 easy and homogeneous mixture in the water that was supplied by the farms' wells and then
21
22 281 adequately managed and stored. The pump system was automatically activated by animals
23
24 282 when they drank water and the system could control clenbuterol concentration in drinking
25
26 283 water, according to animal size, age and feed and forage intake that were more or less
27
28 284 uniformly allocated in each cattle-shed. It was also confirmed that this pump system was easy
29
30 285 to make inoperative in order to difficult authority inspections.
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38 287 Our data suggests that a new methodology for illegal clenbuterol administration to the
39
40 288 animals is possible to use and should be inspected by authorities. However, clenbuterol was
41
42 289 also illegally used in extensive bovine growth promotion. Farm A that performs extensive
43
44 290 bovine growth promotion presented clenbuterol-negative drinking water samples. Samples
45
46 291 from farm A were collected from two different paddocks and the four animals that were
47
48 292 declared positive for urine analysis were from the same paddock. Clenbuterol administration
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50 293 to these animals could not be found, because drinking water and feed samples collected in the
51
52 294 same paddock were negatives.
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3 296 Our data for urine and drinking water samples reinforces the usefulness of these matrices for
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5 297 screening β -agonists in live animals, which is confirmed by the Canadian Food Inspection
6
7 298 Agency (Dickson, MacNeil, Lee & Fesser, 2005) or by the National Plans for Residue
8
9 299 Control in EU countries (Directive n° 96/23, 1996). Nevertheless, and with respect to live
10
11 300 animals and to the Portuguese Residue Control Plan, the hypothesis of analyzing hair samples
12
13 301 should also be considered. As it was observed in the present study, it would be relevant to
14
15 302 include hair samples. So, the present data will be shared by all the Portuguese authorities
16
17 303 involved in the official residue control plan in order to discuss the eventual inclusion of hair
18
19 304 samples for controlling β -agonists in bovines suspected of illegal fattening. Finally, this study
20
21 305 shows that clenbuterol still remains one of the most illegally used β -agonist substances on
22
23 306 farms that practice bovine growth promotion.
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307

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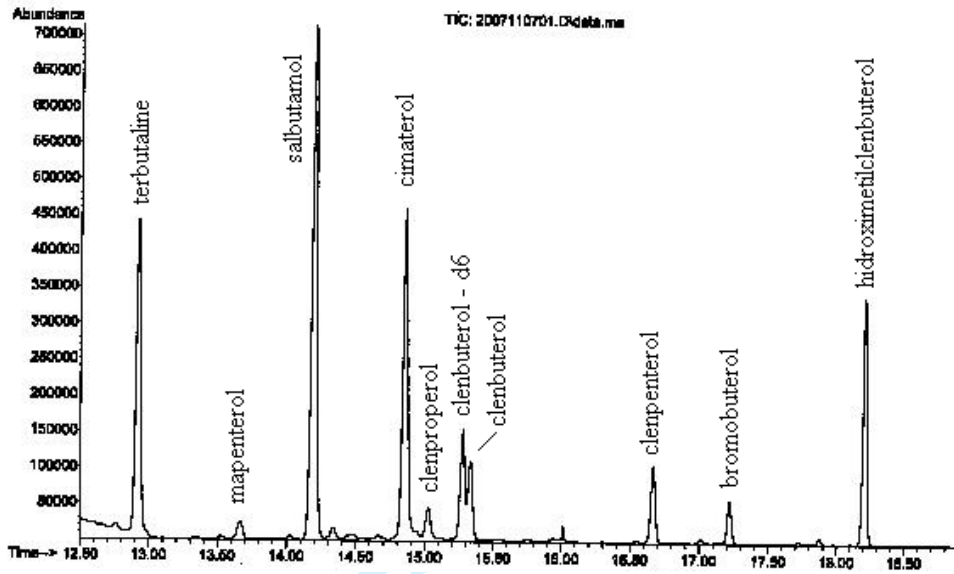
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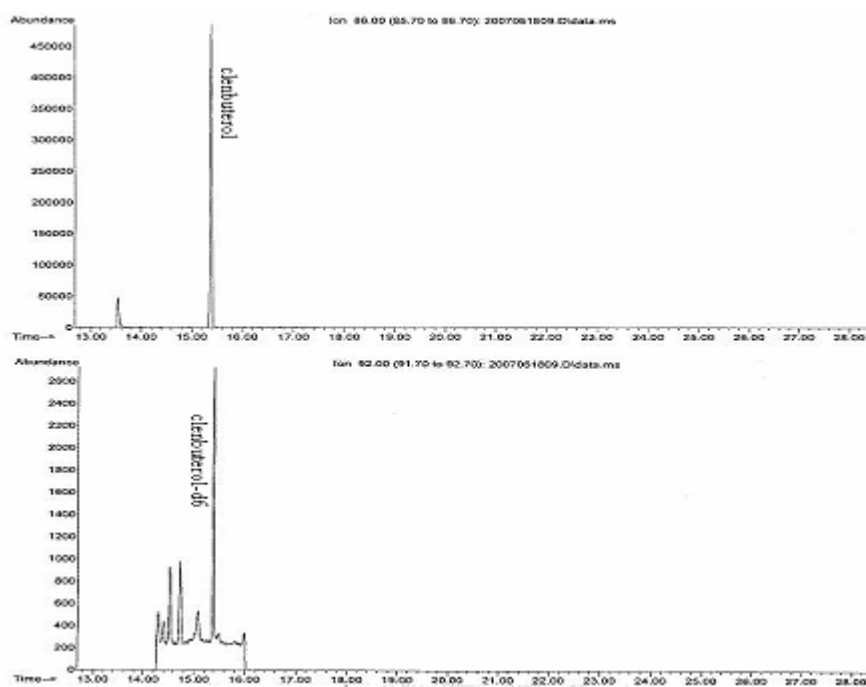
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Figure 1 – TIC chromatogram of β -agonist standards



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Figure 2 – Chromatogram of a drinking water sample with 0.28 mg/l of clenbuterol



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Figure 3 – Chromatogram of a urine sample with 1.1 µg/l of clenbuterol

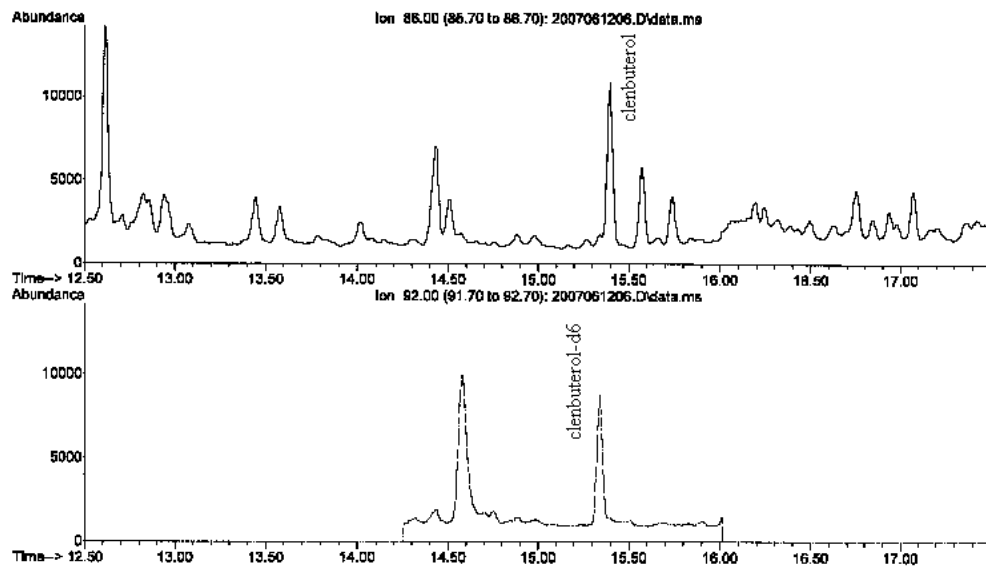
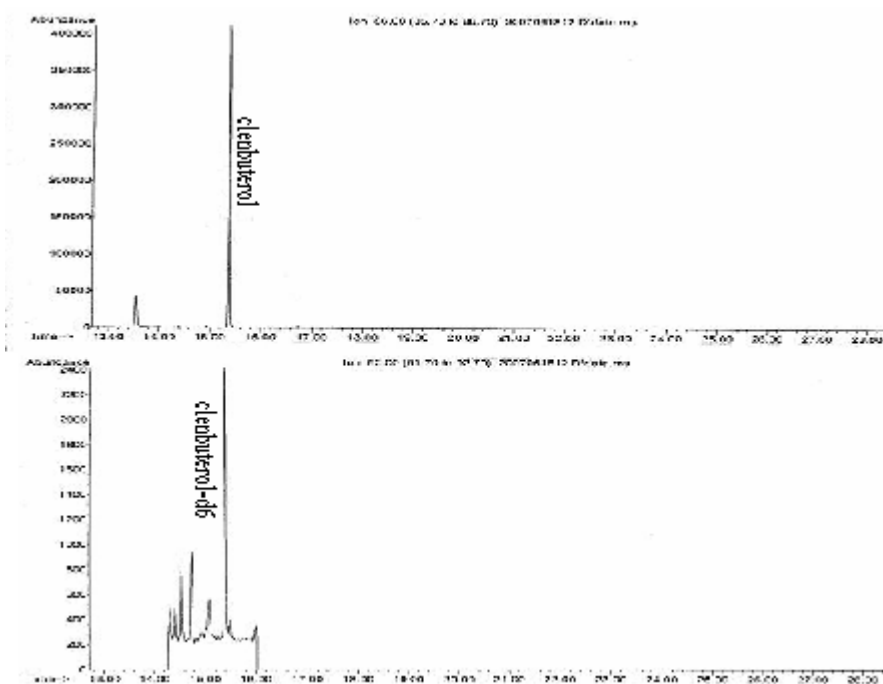


Figure 4 – Chromatogram of a hair sample with 57.0 µg/Kg of clenbuterol



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Table 1 – Number of samples collected on each farm

Farm	Water	Feed	Urine	Hair
A	2	3	8 (4)	2 + 41
B	7 (6)	2	22 (14) + 56	13 (1) + 56
C	4 (2)	2	11 (6)	1 (1)
D	1	2	1	5
E	3	2	6	1
F	11	11	25	6
G	2	4	1	9
H	8	8	14	-
I	1	1	6	-
J	14	-	5	-
K	2	3	13	-
L	11 (7)	-	-	-
M	8	-	-	-
N	6	-	-	-
O	18	-	-	-
P	8	-	-	-
TOTAL	106 (15)	78 (0)	168 (24)	134 (2)

Note 1 – Data in brackets are the number of clenbuterol positive samples

Note 2 – Data separated by a + mark indicate that samples were collected with a interval of six months

Table 2 – Detected m/z ions for each β -agonist

Time window	β - agonists	m/z ions
12.50 – 13.30	Terbutaline	86; 336; <u>356</u> ; 426
13.30 – 14.50	Mapenterol	<u>100</u> ; 313; 369; 453
	Salbutamol	86; 350; <u>369</u> ; 440
14.50 – 16.00	Cimaterol	72; 219; <u>291</u> ; 418
	Clenproperol	<u>72</u> ; 300, 335; 391
	Clenbuterol-d6 (IS)	92; 411
	Clenbuterol	<u>86</u> ; 300; 335; 405
16.00 – 17.70	Clenpenterol	<u>100</u> ; 300; 335; 421
	Bromobuterol	<u>86</u> ; 344; 425; 495
17.70 – 19.00	Hydroxymethylclenbuterol	145; <u>174</u> ; 315; 493

Table 3 – Performance characteristics of the method for clenbuterol

Matrices	CCα	CCβ	Accuracy (n=6)	Repeatability (n=6)	Reproducibility (n=18)
	[$\mu\text{g/l}$] or [$\mu\text{g/kg}$]	[$\mu\text{g/l}$] or [$\mu\text{g/kg}$]	[%]	[% (CV)]	[% (CV)]
Urine	0.12	0.25	98.45	2.31	9.93
Feed	0.73	1.92	92.10	6.97	12.23
Hair	1.05	2.53	93.31	5.82	13.55