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Original Article

Canonical discriminant analysis and meat quality analysis as complementary tools to detect the illicit use of dexamethasone as a growth promoter in Friesian bulls

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ABSTRACT

A screening method based on meat quality parameters and production traits for detecting the effects of illegal administration of dexamethasone in Friesian bulls was assessed. Twenty finishing bulls were divided into an untreated control group ($n = 8$) and two treatment groups receiving dexamethasone orally at dosages of 1.4 ($n = 6$) or 0.7 ($n = 6$) mg per head per day for 60 days. The animals were slaughtered 26 days after cessation of treatment. Thirty-six parameters were measured on live animals, carcasses and samples of the longissimus thoracis muscle. The production traits were similar between groups, but there were significant differences in meat quality between treatment groups. The higher dosage of dexamethasone improved meat tenderness, while the lower dosage resulted in more saturated red meat, with increased meat cooking shrinkage and cooking loss. The use of a portable 'electronic nose' as a screening tool was not successful in discriminating between treated and untreated meat. These results indicate that a multivariable approach using canonical discriminant analysis may be a complementary tool to identify meat from animals illegally treated with dexamethasone, based on several parameters (meat flavour, cooking and thawing loss, tenderness, colour and live weight gain), which are part of the normal analysis of meat quality.

Introduction

According to European Union (EU) Council Directive 96/22/EC,[1] the use of certain compounds capable of manipulating the growth of food producing animals is banned in the EU (Stephany, 2010; Valladares-Carranza et al., 2015). Synthetic glucocorticoids, including dexamethasone, have a wide range of therapeutic applications in veterinary clinical practice (Corah et al., 1995; Ferguson and Hoenig, 1995), but are also used illegally as growth-promoters in veal calves and finishing bulls, either alone or in combination with other banned substances (Courtheyn et al., 2002; Tarantola et al., 2004; Gottardo et al., 2008; Cannizzo et al., 2008, 2010; Girolami et al., 2010). The low dosages of glucocorticoids used for promotion of growth often result in urine concentrations below the limits of detection of the current screening methods (Vincenti et al., 2009).

Italy has the highest detected frequency of dexamethasone misuse in cattle in the EU, with 12/24 non-compliant results in 2013.[2] A pilot study conducted in Italy and involving 295 veal calves and 1035 finishing bulls, reported typical thymic lesions (i.e. parenchymal atrophy) due to the misuse of glucocorticoids in 17.7% of cases examined.[3] Several biomarkers for exposure to dexamethasone or other glucocorticoids have been identified in cattle (Girolami et al., 2010; Rijk et al., 2010; Nebbia et al., 2011; Ludwig et al., 2013; Guglielmetti et al., 2014; Pegolo et al., 2014).

Changes in the physical traits of retail cuts of meat from cattle treated with glucocorticoids include improvement in warm carcass dressing percentage, meat colour and tenderness (Tarantola et al., 2004; Gottardo et al., 2008); producers and consumers consider

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[1] See: http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:31996L0022 (accessed 12 March 2016).

[2] See: http://www.efsa.europa.eu/it/supporting/pub/723e (accessed 5 March 2016).

[3] See: http://www.salute.gov.it/imgs/C_17_pubblicazioni_1146_allegato.pdf (accessed 13 May 2016).

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these traits to be indicators of high quality meat. Several variables assessed routinely in the meat industry, such as tenderness, colour and yield, may be useful for establishing a protocol for the detection of animals illegally treated with glucocorticoids.

Meat aroma measured by a portable 'electronic nose' (EN) has been used to evaluate the shelf life of livestock products (Tikk et al., 2008; Berna, 2010; Narsaiah and Jha, 2012) and to analyse meat quality (Cornale and Barbera, 2009; Isoppo et al., 2009); we hypothesised that this variable could be used to identify meat from animals treated with dexamethasone.

A number of studies have addressed the effects of low dosages of dexamethasone on carcass characteristics and meat quality in cattle (Corah et al., 1995; Tarantola et al., 2004; Gottardo et al., 2008). However, none of these studies have included Friesian finishing bulls, for which the illegal use of glucocorticoids could be attractive, in view of their potential high carcass dressing percentages and meat quality.

The main aims of this study were: (1) to examine the effects of dexamethasone on production traits and meat quality in Friesian finishing bulls after a long withdrawal time; (2) to ascertain whether meat quality parameters, including analysis of aroma using a portable EN, could be a useful tool for identifying illegal treatment with glucocorticoids; and (3) to test the practical applicability of the abattoir as a suitable location to collect data and samples for the proposed screening test using a multivariable approach (canonical discriminant analysis).

Materials and methods

The study was approved by the Ministry of Health and the local committee for animal welfare on 20 September 2006. The trial used 20 Italian Friesian finishing bulls, with an initial mean (±standard deviation) body weight of 439.7 ± 53.4 kg and an age range of 329–443 days. The number of experimental animals was selected according to the sample size test by the GLMPOWER procedure in SAS 9.4.[4]

The animals were housed in outside pens with an overhead shelter on a farm in Piemonte, Italy. They were fed a total mixed ration with ad libitum access to fresh water. After an acclimatisation period of 4 weeks, the bulls were randomly divided into three groups based on convenience sampling. Treated animals received either 1.4 (high dose, HD; $n = 6$) or 0.7 mg (low dose, LD; $n = 6$) dexamethasone (sodium phosphate salt, Dexadreson, Intervet Italia) per animal per day orally for 60 days, mimicking a growth-promoting protocol (Gottardo et al., 2008), while animals from the control group ($n = 8$) were untreated. The commercial preparation was diluted with tap water to a volume of 10 mL and orally administered using a plastic syringe without a needle.

Cattle were slaughtered 26 days after cessation of treatment. The animals were weighed immediately prior to slaughter and muscle sampling and data recording at the abattoir were performed on slaughtered animals. Temperature, pH and hot carcass weight were recorded at the level of the twelfth rib on the left side, after 1 h in a cool chamber (0–4 °C), using a pH meter (pH 211) provided with an FC200B electrode and an automatic temperature compensator (HANNA Instruments).

Production traits examined were initial live weight (measured on farm), final live weight, 1 h carcass weight and yield, live weight gain and live percent gain, average daily gain, and 1 h carcass pH and temperature. Meat analysis was performed on both raw and cooked meat samples, measuring commonly used parameters in meat science,[5] as well as additional parameters (i.e. total cooking loss, cooling and cooking loss, and meat cooking shrinkage). A sample of longissimus thoracis muscle (between the ninth and eleventh ribs) was collected from each carcass, vacuum packaged, stored for 7 days at 2–4 °C and then frozen at −20 °C for 2 months. The sample was thawed for 48 h at 2–4 °C and thawing loss was measured. The raw meat chemical composition was determined by the oven method for dry matter and ash, the Kjeldhal method for crude protein and the Soxhlet extraction technique for ether extract (Helrich, 1990). Collagen content was assessed as hydroxyproline multiplied by 7.4 (Vázquez-Ortíz et al., 2005). Results are expressed as percentage of fresh meat.

Meat colour of raw meat was evaluated after 60 min of exposure to room temperature using a colourimeter (CR300; Konica Minolta Sensing), by determining lightness (L*), red index (a*), yellow index (b*), saturation index (chroma) and hue using the CIELab standard illuminant D65.[5] Three readings were taken for each sample, which consisted of a 2.5 cm thick raw meat slice.

Water holding capacity (WHC) was measured as drip loss (on raw meat) and cooking loss (on cooked meat).[5] Meat cooking shrinkage (MCS) on cooked meat was measured with a video image analyser using the formula: (raw area – cooked area)/raw area. Fluid lost during cooking and after 20 min of cooling was designated 'total cooking loss', while fluid loss occurring during the 20 min of cooling was designated 'cooling loss'. The 'cooking loss' was the difference between total cooking loss and cooling loss (Barbera and Tassone, 2006). The tenderness of cooked meat was evaluated by the Warner-Bratzler (WB) shear force (Instron 1011) using cylinders of meat (2.54 cm diameter), based on the highest load (WB peak) and the break force (WB break).

Aroma was measured on warmed meat samples using a portable EN (PEN 2; Airsense Analeptics) using 2 g sample per vial, according to a modified vial method (Haugen et al., 2006). Statistical analysis was performed on the 5 s average around the maximum value. The EN had 10 metal oxide sensors and analysed an air flow of 150 mL/min, providing output in a data matrix for 10 classes of chemical compounds (Table 3).

Results are expressed as least square means (LSmeans) ± standard error of the mean (SEM). A univariable model was applied to assess the effects of the treatments in SAS 9.4 STAT[4] using a general linear model (GLM) and multiple comparisons for unbalanced data using Tukey's test. After the application of a stepwise discriminant analysis (SDA), selected parameters were subjected to canonical discriminant analysis (CDA), a dimensional reduction technique performing a multivariable one-way analysis to derive canonical functions, i.e. linear combinations of the quantitative variables, summarising the variation among groups. Discriminant analysis was applied to validate the model.[4]

Results

Animals in the HD group were significantly younger than those in the LD and CT groups (355 days vs. 406 and 396 days, respectively; $P = 0.03$) and also had a significantly lower initial live weight (389.5 kg vs. 453.8 and 466.8 kg for the HD, LD and CT groups, respectively; $P = 0.01$). To avoid a biased analysis,[4] the initial live weight was used as a covariate for the analysis of production traits. At growth-promoting dosages in both HD and LD groups, dexamethasone was not effective in altering production traits upon univariable analysis (Table 1). The multivariable approach may increase accuracy, power, and efficiency of data analysis. SDA was then applied and five parameters were selected among production traits. The CDA on the five selected parameters derived the first and second canonical variables (CAN1 and CAN2, respectively). CAN1 explained 99% of variability and separated the CT group from the treated groups (Fig. 1a). The largest contribution to the CAN1 was due to the live weight gain.[4]

The effects of dexamethasone treatment on chemical and meat quality parameters are summarised in Table 2. Eight out of 18 tested parameters showed statistically significant differences following data analysis in the univariable model. Overall, dexamethasone-treated bulls exhibited higher raw meat colour values, water holding capacity and tenderness, as illustrated by a lower WB peak and break of cooked meat, compared to untreated bulls. HD-treated bulls had significantly lower WB peak and WB break values compared with controls. However, for other parameters, dose-related effects were not observed. The meat in

Table 1
Productive traits (least square means ± standard errors of the means) in untreated Friesian finishing bulls (control) vs. bulls treated with two dosages of dexamethasone (low dose: 0.7 mg dexamethasone/day; high dose: 1.4 mg dexamethasone/day).

Parameters	Control	Low dose	High dose
Final live weight (kg)	520.1 ± 6.62	504.2 ± 7.16	518.6 ± 8.52
Carcass weight 1 h (kg)	298.9 ± 5.66	285.8 ± 6.12	304.1 ± 7.29
Carcass yield 1 h (%)	57.2 ± 1.13	56.8 ± 1.22	58.8 ± 1.46
Live weight gain (kg)	80.4 ± 6.62	64.5 ± 7.16	78.9 ± 8.52
Live weight gain (%)	18.8 ± 1.56	14.8 ± 1.69	18.2 ± 2.01
Average daily gain (g)	874 ± 72.6	709 ± 78.5	867 ± 93.5
Carcass pH 1 h	6.8 ± 0.07	6.6 ± 0.07	6.8 ± 0.09
Carcass temp. 1 h (°C)	35.8 ± 0.63	35.5 ± 0.68	36.8 ± 0.81

[4] See: http://support.sas.com/documentation (accessed 13 May 2016).
[5] See: http://www.meatscience.org/publications-resources/printed-publications (accessed 13 May 2016).