Effects of illegal treatments on meat quality of Charolaise bulls

Barbera S.[1], Cannizzo F.T.[2] and Biolatti B.[2]

[1] Dipartimento di Scienze Zootecniche – Università degli studi di Torino – Italy.
[2] Dipartimento di Patologia Animale - Università degli studi di Torino – Italy.

Abstract — **This study investigates the effects of illegal administration of growth promoters on meat quality of Charolaise bulls. Thirty finishing Charolaise bulls (initial LW=596±21.0kg; age=479±47.7days), were randomly divided into four groups: D, E, P (n=6 animals each group) and C (n=12). The D, E and P groups were respectively administered: 0.7mg head/day/os of dexamethasone for 40 days, 20mg head/week im of 17-beta-estradiol for 5 times, 15mg head/day/os of Prednisolone for 30 days. The C group served as control. Samples of *m. longissimus thoracis* were taken to evaluate: pH1h, drip loss, meat color, total WHC and ring area and WHC trend, meat cooking shrinkage (MCS), MCS cooking loss, peak in Stress resistance and Relaxation (SRR) and SRR elastic coefficient. The treated groups showed a different behavior. P group had the lowest pH1h, CIE a* and b* and total WHC area values and the highest Drip Loss and MCS values. The Canonical Discriminant Analysis shows a significant separation between control and treated groups. The first canonical variable explains the 75% of between-class separation distinguishing the P group from the others, due to the total WHC area, the color parameters a* and b* and pH1. The second canonical variable explains the 16% and divides the two treated D and E groups from group C, due to the color parameters a* and b*. Meat quality is affected by growth promoter administration. The observed parameters clearly separate group D from the other groups and less clearly group E and P from control group.**

Keywords— **Beef, Growth Promoters, Meat Quality.**

I. INTRODUCTION

Whilst the administration of anabolic agents in farm animals is known to increase the efficiency of meat production, their use has been prohibited within the European Union, due to the potential risks to human health, since the late 1980s [1, 2].

Glucocorticosteroids are widely used in buiatrics to limit inflammatory processes that otherwise would significantly contribute to pathology, prolong recovery time, and compromise animal welfare [3]. Dexamethasone is a potent synthetic analogue of hydrocortisone, illegally used in association with anabolic steroids as growth promoter in veal calves and beef production, in order to improve quality and quantity of meat [4, 5, 6].

In the European Union, some corticosteroids are permitted for therapeutic use in livestock. However, the use of corticosteroids is regulated by the Commission Regulation (EEC) N° 37/2010, which sets maximum residue limits (MRLs) for betamethasone, dexamethasone, methylprednisolone and prednisolone. The member states are required to monitor the use of pharmacologically active substances in animals used for the production of food for human consumption. They must follow the indications of Council Directive 96/22/EC, which was amended by Directive 2003/74/EC and 96/23/EC. Because of their steroidal structure, the corticosteroids are included in group A3 (substances with an anabolic effect and unauthorised substances/steroids) of National Residue Control Plan (NRCP) of some member states (Italy, the Netherlands and Denmark), whereas other states allocate them to the B2f group (other pharmacologically active substances). The 2009 Italian NRCP reports stated that corticosteroid residues in cattle were an emerging problem.

Due to the economic benefits that can be gained from the use of illegal growth promoters in beef cattle, producers continue in their illegal administration. Recent surveys revealed that dexamethasone is often present at detectable concentrations in the liver of slaughtered animals [7, 8, 9].

In the last 10 years, farmers have progressively reduced the dosages of illegal administration of these drugs to avoid penalties committed by the public authority. Such changes in hormone abuse have highlighted the need for developing new techniques to improve the detection of growth promoter use during meat production. A number of innovative methods to achieve this goal have been examined previously, including the receptor concentration determination

[10] and gene expression profiling [11, 12, 13, 14]. Illegal treatments are carried out in different categories of fattening animals, particularly to improve performances of male bulls.

Meat tenderness, colour, marbling, flavour and juiciness are qualitative parameters that influence consumer's decisions to purchase meat and this study was conducted to determine the effects of administration of illegal substances on some meat quality traits of Charolaise male cattle.

II. MATERIALS AND METHODS

Thirty finishing Charolaise bulls (initial LW=596±21.0kg; age=479±47.7days) were randomly divided into four groups, kept in separate boxes (10 × 15 m): D, E, P (n=6 for each group) and C (n=12). The D, E and P groups were respectively administered: 0.7mg head/day/os of Dexamethasone-21-sodium-posphate for 40 days, 20mg head/week im of 17-beta-Estradiol for 5 times, 15mg head/day/os of Prednisolone for 30 days. The C group served as control. Dosages were chosen according to literature [14, 15]. The animals were fed with a diet consisting of corn silage, corn, hay and a commercial protein supplement; water was supplied *ad libitum*. Animals were slaughtered after a 6-days drug withdrawal. Italian Ministry of Health and the Ethic Committee of the University of Turin authorized the experiment. Carcasses of treated animals were destroyed (2003/74/CE - DL 16-3-2006, 2 n.158).

Samples of m. *longissimus thoracis* were taken to evaluate: pH 1h and 24h, Drip Loss, meat color (CIE L*, a*, b*, Chrome, Hue), Meat Cooking Shrinkage (MCS), MCS Cooking Loss, peak in Stress resistance and Relaxation (SRR) and SRR elastic coefficient, WHC total and halo area and WHC trend. Analyses were done according to the protocol developed at the Dipartimento of Scienze Zootecniche [16, 17, 18].

Data analysis was performed by SAS/ STAT in SAS 9.2 [19] using one-way analysis of variance (GLM) and treatment as independent variable. Results are expressed as LSmeans and MSE. A Stepwise Discriminant Analysis was applied to the full set of variables (17 parameters) to select the best discriminating ones among treatments. Only variables with significance level, to enter or to stay, at 0.15 were retained at the end of the stepwise procedure. The selected variables were then submitted to a Canonical Discriminant Analysis, a dimensional reduction technique that performs both univariate and multivariate one-way analysis to derive canonical functions. Finally a Discriminant Analysis was applied to evaluate the model.

III. RESULTS AND DISCUSSIONS

The treated groups D, E and P (Table 1), showed, in a different way, a significant influence on meat quality. The estimate difference between Control minus treated groups resulted in a lower temperature at 1h (-1.1°C) and higher a* (2.6), b* (1.7) and Chroma (3.1) indicating a general stress and a less reddish, yellowish and saturated meat in treated bulls. Among treated groups there were substantial difference. The P group showed the lowest pH and temperature 1h, a*, b*, Chroma, Hue, SRR Peak, WHC Total Area and highest Meat Cooking Shrinkage. The meat from P treated group tends to be more interesting for Italian consumer [20, 21] with the exception of the MCS regarded by consumers as an indicator of unhealthy meat. The E group was less tender and more similar to C group but with high temperature at 1 h to indicate stressed animals.

From the 17 qualitative parameters measured in meat, 6 significant parameters were retained at the end of the Stepwise Discriminant Analysis: Carcass Temperature at 1h, Drip Loss, L*, Chroma, WHC Halo Area, MCS. The L* and WHC Halo Area did not differ among groups in the GLM model. This is a possible result, as the Stepwise Discriminant Analysis is a multivariate technique that evaluates all involved variables to determine which one contributes most to the discrimination among groups. The univariate R^2 are variable and range between 0.12 for L* and 0.57 for the Carcass Temperature at 1h. The multivariate test for differences between the classes is significant at the <0.0001 level.

The Canonical Discriminant Analysis (Figure 1) shows a significant separation between control and treated groups. The first canonical variable accounts for the 79% of the total variability and divides P group from the others and is due to the Drip Loss and the Carcass Temperature at 1h. The second canonical variable accounts for the 20% and divides the two D and E groups from the Control group due to the Chroma more saturated in the last one.

Finally a Discriminant Analysis was applied to evaluate the model. In classification the accuracy was interesting with a 4% of total misclassification error. In cross-validation the accuracy was very variable. The P group had 0% of misclassification error, Control group 10%, E group 33% and the D group was confused between E and C groups. The multivariate approach to the qualitative analysis could be ideally used to classify meat treated by illegal substances. The effectiveness is variable depending on the used substances.

IV. CONCLUSIONS

The illegal use of growth promoters positively influenced some meat quality traits, making the meat more attractive to consumers. The three treatments gave variable results. The Prednisolone improved the meat tenderness and the six selected parameters clearly identified this treatment. The 17-beta-Estradiol gave the tougher meat, lower Meat Cooking Shrinkage and higher carcass temperature but it could be confused with the meat treated with Dexamethasone, which are very similar. The Control group is quite well separated from the treated groups to confirm the interest in a protocol, based on qualitative parameters, to identify meat treated by illegal substances.

These results confirm farmers' interest for both livestock and qualitative results but there are side effects for animals and possible health risks for consumers.

Table 1. LSMeans and MSE of the qualitative meat parameters (N=30)

LSMeans by parameter in the same row with different letters are significantly different (a, b, c: P<=.05; A, B, C: P<=.01)

		GROUPS				
Parameters		C	D	E	P	MSE
pH 1h		6.57^{a}	6.60^{a}	6.55^{ab}	6.40^{b}	0.026
Temperature 1h	°C	38.5^{a}	40.0^{b}	40.0^{b}	38.7^{a}	0.58
pH 24h		5.36	5.49	5.42	5.38	0.022
Drip Loss	%	2.8^{ab}	2.0^{ab}	1.5^{a}	3.5^{b}	2.30
L*		43.3	43.3	42.5	41.2	5.35
a*		27.0^{aA}	24.4^{B}	25.5^{bAB}	23.2^{aB}	2.16
b*		10.2^{aA}	8.5^{bc}	9.5^{abA}	7.4^{cB}	1.52
Chroma		28.9^{aA}	25.9^{B}	27.2^{aAB}	24.4^{bB}	3.10
Hue		0.361^{a}	0.332^{ab}	0.355^{ab}	0.316^{b}	$1.6E^{-3}$
MCS	%	16.3^{a}	15.5^{ab}	12.9^{b}	16.8^{a}	6.82
Cooking Loss mcs	%	24.0	23.4	21.3	22.0	9.56
SRR Peak	N	176.9^{ab}	175.0^{ab}	184.4^{a}	155.5^{b}	654.73
SRR k2 before	N	0.069	0.066	0.067	0.070	$4.2E^{-5}$
SRR k2 after	N	1.344	1.320	1.316	1.327	$2.38E^{-3}$
WHC Total Area	mm^2	1380^{A}	1453^{B}	1407^{aAB}	1349^{bA}	2387.7
WHC Halo Area	%	42.9	45.3	45.1	41.6	11.54
WHC trend k2	mm^2	-0.0018	-0.0019	-0.0019	-0.0018	$1.54E^{-8}$

ACKNOWLEDGMENT

The authors are grateful to the Centro di Referenza di Patologia Comparata 'Bruno Maria Zaini'.

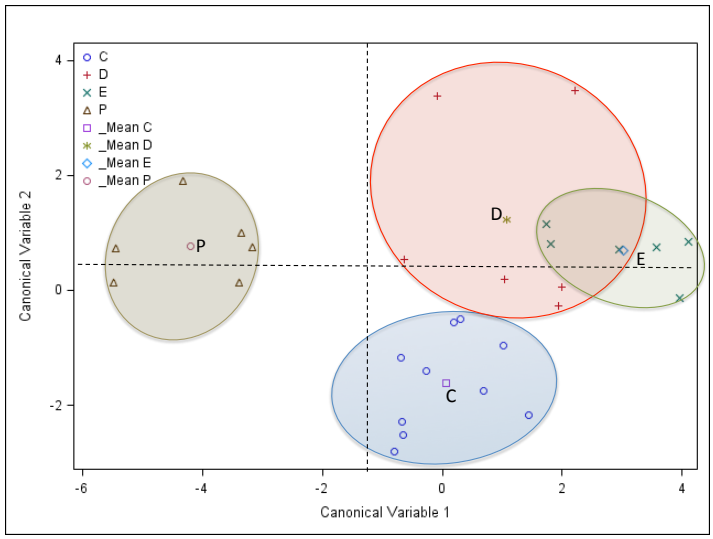

Figure 1. Canonical discriminant analysis of selected qualitative meat parameters (Carcass Temperature at 1h, Drip Loss, L*, Chroma, WHC Halo Area, MCS)

This work was partially supported by Regione Piemonte "Prevenzione dell'uso di anabolizzanti in zootecnia: le bioteconologie nello sviluppo di disciplinari per la qualità e salubrità della carne e derivati", by Ministero delle Politiche Agricole Alimentari e Forestali, project SAFORISK.

REFERENCES

1. Stephany RW (2001) Hormonal growth promoting agents in food producing animals. Handbook of Experimental Pharmacology, 195:355-367, DOI 10.1007/978354079088416
2. EU (2002) Scientific Committee On Veterinary Measures Related to Public Health: Review of Previous SCVPH Opinions of 30 April 1999 and 3 May 2000 on the Potential Risks to Human Health from Hormone Residues in Bovine Meat and Meat Products. <http://www.ec.europa.eu/food/fs/sc/scv/out50_en.pdf>
3. Moiré N, Roy O, Gardey L (2002) Effects of dexamethasone on distribution and function of peripheral mononuclear blood cells in pneumonic calves. Vet Immunology and Immunopathology 87(3-4):459-466
4. Istasse L, Evrard P, et al. (1988) Trenbolone acetate in combination with 17 beta-estradiol: influence of implant supports and dose levels on animal performance and plasma metabolites. J. Anim. Physiol. A: Anim. Nutr. 62:150-158
5. Courtheyn D, Le Bizecb B, et al. (2002) Recent developments in the use and abuse of growth promoters. Analytica Chimica Acta 473(1-2):71-82
6. Biolatti B, Bollo E, et al. (2005) Effects of low-dose dexamethasone on thymus morphology and immunological parameters in veal calves. J Vet Med Series A: Physiology Pathology Clinical Medicine 52(4):202-208
7. Biolatti B, Rosmini R, et al. (1999) Genital and accessory reproductive organs, mammary gland and thymus changes following illegal anabolic treatment in veal calves. Proc. 17th Meeting of the European Society of Veterinary Pathology, Nantes, 175:14-17
8. Biolatti B, Valpreda M, et al. (2002) Anabolic target organs pathology in cattle slaughtered in Piemonte region (Italy). Proc. 20th Meeting ESVP, Grugliasco (TO) Italy 18-21 Sept. p58
9. Carraro L, Ferraresso S, et al. (2009) Expression profiling of skeletal muscle in young bulls treated with steroidal growth promoters. Physiol. Genomics 38:138-148
10. Odore R, Badino P, et al. (2006) Changes in lymphocyte glucocorticoid and β-adrenergic receptors in veal calves treated with clenbuterol and steroid hormones for growth-promoting purposes. J Vet Pharmacology and Therapeutics 29(2):91-97
11. Nebbia C, Urbani A, et al. (2011) Novel strategies for tracing the exposure of meat cattle to illegal growth-promoters. The Veterinary J 189:34-42
12. Toffolatti L, Gastaldo LR, et al. (2006) Expression analysis of androgen-responsive genes in the prostate of veal calves treated with anabolic hormones. Domestic Anim Endocrinology 30(1):38-55
13. Reiter M, Walf VM, et al. (2007) Modification of mRNA expression after treatment with anabolic agents and the usefulness for gene expression-biomarkers. Analytica Chimica Acta 586:73-81
14. De Maria R, Divari S, et al. (2009) 17beta-oestradiol-induced gene expression in cattle prostate: biomarkers to detect illegal use of growth promoters. Vet Record 164:459-464
15. Meyer HH (2001) Biochemistry and physiology of anabolic hormones used for improvement of meat production. APMIS: Acta Pathologica, Microbiologica, et Immunologica Scandinavica 109:1-8
16. Barbera S, Tassone S (2006) Meat cooking shrinkage: Measurement of a new meat quality parameter. Meat Science 73(3):467-474
17. Prandi M, Barbera S (2009) Stress Resistance and Relaxation: an Instrumental Method for the Texture Analysis and Sensory Evaluation of Meat. ICoMST 55th International Congress of Meat Science and Technology. Copenhagen, Denmark, 2009, ICoMST_PS4_Part2_ver2_Final.pdf. PE4.47 pp 621-626
18. Isoppo S, Sala G, Barbera S (2009) A Protocol for the assessment of physical and sensorial characteristics of meat. ICoMST 55th International Congress of Meat Science and Technology. Copenhagen, Denmark, 2009, ICOMST_PS4_Part2_ver2_Final.pdf. PE4.75 pp 736-739
19. SAS (2011) The SAS System for Windows, Release 9.02. SAS Institute Inc., Cary, NC, USA. At http://support.sas.com/documentation
20. Zamora F, Aubry L, et al. (2005) Serine peptidase inhibitors, the best predictor of beef ageing amongst a large set of quantitative variables. Meat Science 71(4):730-742
21. Verbeke W, Federico JA, et al. (2010) European citizen and consumer attitudes and preferences regarding beef and pork Review Article. Meat Science 84(2):284-292