FULL PAPER *Public Health*

A Histologic Study on Growth Promoter Target Organs of Slaughtered Beef in Molise Region (Italy)

Pierluigi IMBIMBO[1)], Lorenzo CASTIGLIEGO[1)]*, Andrea ARMANI[1)], Bartolomeo BIOLATTI[2)], Francesca Tiziana CANNIZZO[2)], Daniela GIANFALDONI[1)] and Alessandra GUIDI[1)]

[1)]*Department of Animal Pathology, Prophylaxis and Food Hygiene, University of Pisa, Via delle Piagge 2a–56124 Pisa, Italy*
[2)]*Department of Animal Pathology, University of Turin, Via Leonardo da Vinci 44–10095 Grugliasco (TO), Italy*

(Received 28 October 2011/Accepted 2 May 2012/Published online in J-STAGE 22 May 2012)

ABSTRACT. A gross pathology and histological investigation was carried out on bovine target organs of anabolic substances in the Molise Region (Italy). One hundred forty-four bovines (12–24 months old, 123 males and 21 females) were included in the survey. An ante-mortem assessment of their behavior and clinical examination were performed. After slaughter, samples of prostate, Cowper's glands, Bartholin's glands, mammary gland, ovaries, thymus and thyroid were collected, inspected and processed for histopathology, as suggested in the guidelines of the Italian national program for residue surveillance (PNR). Overall, 15.3% of the examined animals were classified as "suspect," 44.4% were classified as "uncertain," and the remaining 40.3% were classified as "negative." The most frequent lesion was a severe thymus atrophy with fat infiltration (15.4% of males and 14.3% of females), strongly suggesting the illegal use of corticosteroids.
KEY WORDS: bovines, growth promoters, histological investigation, illegal drug treatments.

doi: 10.1292/jvms.11-0489; *J. Vet. Med. Sci.* 74(10): 1253–1259, 2012

Since the 1960s, the administration of growth promoters has been prohibited in Italy for auxinic purposes in animals intended for human consumption. After the first non-comprehensive laws, legislative updates were produced, in parallel with the European regulations, which led to Decree 148/2009 [14], which implemented European Directive 2008/97/EC [16]. In particular, at present it is prohibited to administer "to farm or aquaculture animals, by any means whatsoever, thyrostatic substances, stilbenes, stilbene derivatives, their salts and esters, estradiol 17β and its ester-like derivatives, beta-agonists and substances having estrogenic (other than estradiol 17β and its ester-like derivatives), androgenic or gestagenic action," except in the case of regulated therapeutic or zootechnical treatments.

Among growth promoter molecules, sex steroids, beta agonists and corticosteroids are the most frequently used. Nevertheless, controversial growth promoter effects of dexamethasone have been discussed in several studies [21]. Corticosteroids showed the ability to increase the external fat thickness, carcass muscularity and dressing percentage. Courtheyn *et al.* [12] reported that providing glucocorticoids in low dosages increases feed intake and average daily gain (ADG) and improves the feed conversion ratio. Tarantola *et al.* [38] observed the lowest daily gain and the worst feed conversion ratio in veal calves receiving a prolonged oral low dose of dexamethasone. Corah *et al.* [11] reported a greater thickness of external fat in treated steers.

*CORRESPONDENCE TO: CASTIGLIEGO, L., Department of Animal Pathology, Prophylaxis and Food Hygiene, University of Pisa, Via delle Piagge 2a–56124 Pisa, Italy.
e-mail: lcastigl@vet.unipi.it

However, excessive exposure to sex hormones or residues of other growth promoters may lead to harmful effects on the human endocrine glands, altering growth and puberty, and the immune system and may exert genotoxic and carcinogenic effects [22]. In spite of strict legislation and a national program for residue surveillance (PNR), which specifies rules about the number of samples to be taken and the sampling procedures, the problem of illegal treatments with growth promoters is still extremely topical. The use of these substances is facilitated by the fact that they cannot be easily detected, either because of their low concentration in biological samples, the very large number of molecules potentially used, often modified from those indicated in the official pharmacological protocols, or the use of so-called "cocktails." These are a mixture of molecules that act synergistically, permitting improved zootechnical results, while lowering the relative concentration of each compound used and thus the concentration of any residue, leading to further difficulties in terms of analysis. However, illegal substances used in livestock cause the occurrence of alterations, to different extents, in organs that respond to their stimulating or inhibiting effects [1]. Indeed, over the years, studies on this topic have suggested that the use of growth promoters can be highlighted by both macroscopic and histological changes of the genital tract organs and sex accessory glands, in both males and females [6, 23, 24, 32], or other organs such as the thymus [7, 28] or thyroid [37]. These lesions persist long enough to be detected at slaughter. For this reason, in addition to the traditional chemical analysis, which can give unsatisfactory results [25], other auxiliary diagnostic approaches to detect illegally treated animals are based on anatomo-histopathological examination of target organs [1]. Recently, biotechnological approaches [4, 33]

Table 1. List of organs examined and type of lesion considered

Organ	Type of lesion
Prostate (urethra)	Hyperplasia, light or severe metaplasia
Prostate (glandular tissue)	Hyperplasia, light or severe metaplasia; hypersecretion or cysts
Cowper's glands (ducti)	Hyperplasia, light or severe metaplasia
Cowper's glands (glandular tissue)	Hyperplasia, light or severe metaplasia; hypersecretion or cysts
Bartholin's glands (ducti)	Hyperplasia, light or severe metaplasia
Bartholin's glands (glandular tissue)	Hyperplasia, light or severe metaplasia, hypersecretion or cysts
Ovaries	Cysts and corpora lutea
Mammary gland	Secreting alveoli
Thymus	Atrophy of the parenchyma with fat tissue infiltration
Thyroid	Follicular epithelium hyperplasia

to the research of biomarkers, mainly with biomolecular methods, have been suggested [8, 13, 17, 39].

At present, a histopathological test is already routinely employed in the Netherlands to evaluate the illegal use of hormones, and it was officially introduced in the Italian PNR for screening purposes in 2008.

In this work, we adopted the criteria described in the Italian PNR to examine a sample of regularly slaughtered beef cattle in the Molise Region and to verify a possible use of growth promoters in that Region.

MATERIALS AND METHODS

Experimental design and sample collection: A group of 144 beef cattle (12 to 24 months old, excluding 11 older animals from 25 to 28 months old), 123 males and 21 females, was examined. The animals were slaughtered in five slaughterhouses located in the Molise Region (Italy) during 2009. About 61.8% (n=89) of them were raised in Molise; 35.4% (n=51) of them were raised in Campania Region, and the other 2.8% (n=4) were raised in other Italian Regions. Based on the local production data, the number of animals to be examined was calculated using the "Campionamenti" software (free software of the Experimental Zooprophylactic Institute G. Caporale, Teramo, Italy) in order to have a statistically representative number of batches and of total subjects. For calculation of the sample size, an expected prevalence of 2% was considered, instead of the 5% imposed by the Italian PNR. Out of a population of about 11,000 bovines slaughtered per year, grouped into about 8,000 lots, and assuming a confidence level of 95%, 147 animals should have been examined. Lots consisted of 1–2 animals on average. For lots composed of three or less subjects, all the subjects were examined.

Data acquisition and assessment of gross lesions: Documents for each animal were checked particularly to exclude that the animals had undergone drug treatments in the last 90 days. Each animal was submitted to regular antemortem clinical examination to assess the possible presence of morpho-functional alterations in specific organs. In particular, for the evaluation of treatment with estrogens, testicular size and anomalous development of the mammary gland were checked in males, while in females, size and development of the mammary gland and nipples and presence of secretions were investigated. For the evaluation of treatment with androgens, males were checked for testicular size, and females were checked for clitoris size. Finally, as a hint concerning treatment with beta-agonists or corticosteroids, we proceeded to assess the eventual absence of the tracheal ridge or the presence of thymus atrophy, respectively.

Histological investigation: Samples for histological analysis were collected from the thymus, thyroid, prostate, Cowper's glands, Bartholin's glands, mammary gland and ovaries. The biological material was immediately fixed in 4% formalin solution and stored at room temperature until inclusion in paraffin blocks for sectioning on a microtome. Slices 5 μm thick were produced, fixed on slides and then stained with hematoxylin-eosin according to standard protocols.

Lesions were classified as described in Table 1.

An evaluation sheet was then filled as indicated in the 2009 Italian PNR [35]. The individual organs were classified as "negative" when lesions were absent, "uncertain" if the sample presented minor or new onset lesions and "suspect" when even one of the tissues examined presented severe lesions.

RESULTS

No macroscopic alterations were detected in the genital tracts and mammary glands of the examined subjects. Seven thymi (4.9%) showed severe macroscopic atrophy.

Overall, 15.3% (n=22) of the subjects were classified as "suspect," 44.4% (n=64) were classified as "uncertain," and the remaining 40.3% (n=58) were classified as "negative." Percentages for each class were similar for males and females: 15.4% (n=19) and 14.3% (n=3), respectively, for the suspect group, 45.5% (n=56) and 38.1% (n=8) for the uncertain group and 39% (n=48) and 47.6% (n=10) for the negative group, although the total number of males was markedly higher. With regard to the individual organs, results of histological findings are summarized in Table 2 and indicate the number of subjects classified according to criteria previously described. Tables 3 and 4 report outcomes in more detail.

In 23.1% (n=28) of cases, prostate hypersecretion was detected, mostly associated with other changes, such as hy-

Table 2. Histological changes of the examined target organs

	Negative	Uncertain	Suspect	na	Total
Prostate	82 (67.8%)	38 (31.4%)	1 (0.8%)	2	123
Cowper's glands	112 (91.8%)	10 (8.2%)	0 (0%)	1	123
Bartholin's glands	8 (47.1%)	9 (52.9%)	0 (0%)	4	21
Ovaries	8 (100%)	0 (0%)	0 (0%)	13	21
Mammary gland	13 (100%)	0 (0%)	0 (0%)	8	21
Thymus	86 (62.3%)	31 (22.5%)	21 (15.2%)	6	144
Thyroid	140 (97.9%)	3 (2.1%)	0 (0%)	1	144

The total number of animals (percentage) are reported for each group. na: not analyzed.

perplasia or light metaplasia, which were observed in 63.1% (n=77) of the examined males (gland or urethra); however, only one case of severe metaplasia was detected in the urethra (Fig. 1), and this was associated with hyperplasia and hypersecretion of the gland tissue. This was the only case classified as suspect for sex steroids treatment.Hypersecretion of Cowper's glands was observed in 9% (n=11) of cases and was not associated with metaplasia or hyperplasia; the latter was detected in 4.1% (n=5) of glands (secreting portion or ducts). With regard to females, 35.3% (n=6 out of 17 examined samples) showed hypersecretion in the Bartholin's gland associated with hyperplasia or light metaplasia, with the exception of one case. Overall, slight epithelial hyperplasia was observed in glands or ducti of 16 samples.

Mammary glands did not show lesions.Three thyroids (2.1%) showed hyperplasia. Histological atrophy of the thymus parenchyma, lymphocyte depletion and fat tissue infiltration were detected in 15.4% of examined males (n=18) and 14.3% of females (n=3), for a total of 21 subjects (15.2%) (Fig. 2).

DISCUSSION

In 2010, the official national monitoring plans of European Countries showed a 0.24% positivity in cattle for residues of illegal growth promoters [10]. There is evidence, however, that these figures may underestimate the real incidence of growth promoter abuse in meat cattle breeding. In fact, using alternative screening tests like the histological test, the number of animals suspected of receiving illegal treatments with growth promoter dramatically increases. A pilot study conducted in Italy involving 1330 animals reported a high incidence of histological lesions, possibly connected with the illegal administration of growth promoters; 144 (10.8%) animals were classified as "suspect," and 477 (35.9%) were classified as "uncertain" (Master Plan of the Ministry of Health—Phase II, 2006—data not published). Similar results were recorded in a recent official monitoring plan of Piemonte Region [36]. The results of the present work confirm the frequency of target organ lesions in beef slaughtered in Molise Region. The high number of Bartholin's glands classified as uncertain is probably due to the ages of the animals, which had already reached puberty; therefore some physiological changes represented by slight epithelial hyperplasia or sometime metaplasia may appear even in untreated subjects. Only one case of suspected steroid administration was found, which was confirmed by metaplastic lesions in the urethra and hypersecretion and hyperplasia in the prostate. The most severe and frequently observed lesion was thymus atrophy represented by lymphocyte depletion and fat tissue infiltration of the parenchyma. This finding in young and healthy bovines, without a declaration of drug administration for therapeutic purposes, becomes a strong indicator of illegal treatment with corticosteroids. Thymus atrophy following corticosteroid administration has been reported, either with long-term and low-dosage administration [2, 3, 5, 7, 27]. Thymus atrophy could also be the consequence of infectious diseases, intoxication and stress [26]. However, in these cases, the affected animals should show typical clinical signs and lesions [18–20, 29–31, 34, 40].

To avoid penalties due to positivities for drug residues detected by the public veterinary controls, farmers reduced the dosages of administered molecules. This change in strategy reduced the severity of the thymus atrophy in treated animals, making detection of alterations by veterinary inspectors a difficult task [3], although the histological test is still able to show microscopic changes. Whenever evident lesions in target organs are found after histological investigation, the current legislation provides clear guidance on procedures to be applied on farms from which the subjects suspected of illegal pharmacological treatments come from. In particular, in Italy, the PNR specifies that in cases of suspect diagnostic outcomes (from 2010, also individual cases), the official institution that performed the sampling must activate, directly or indirectly, the investigations specified in the Article 18 of Decree 158/2006 [15]. In brief, the farm where the suspected animals come from should be checked for the presence of possible implants in bred animals, and random sampling must also be planned for a chemical analysis, designed to detect the presence of prohibited or unauthorized substances on farms where animals are bred, kept or finished, as well as in companies linked to them. To this end, an official sampling of drinking water and feed should also be prescribed. As specified in the PNR, it is worth remembering that monitoring plans based on histological analysis represent an additional and non-substitutive approach to controls based on official chemical procedures validated in compliance with Decision 2002/657/EC [9] and the subsequent amendments, which currently are the only procedures to have legal validity. This results in the obligation to unambiguously identify the

Table 3. Histological changes in target organs of males. Only animals with at least one lesion classified as "uncertain" are reported

N°	Age	Prostate			Cowper's glands			Thymus		Thyroid	
		Urethra	Gland	Resp	Ducti	Gland	Resp	Atrophy	Resp		Resp
2	22	LM		**U**			N		N		N
4	16	M	H+I	**S**			N	Mo	N		N
5	21	H	I	**U**			N	P	**S**		N
6	22	H	I	**U**			N	P	**S**		N
10	16			N			N	Mo	**U**		N
11	20			N			N	Mo	**U**		N
14	18		I	**U**		I	**U**		N		N
15	22	H	I	**U**			N		na		N
16	22	H	H	N			N	P	**S**		N
17	22	H		N			N	P	**S**		N
18	21	H		N			N	Mo	**U**		N
19	23		H	N			N	Mo	**U**		N
20	20	H		N			N	Mo	**U**		N
21	23	H		N			N	Mo	**U**		N
22	22	LM	H	**U**		I	**U**	Mo	**U**		N
23	22	H	I	**U**			N	Mo	**U**		N
24	22	H		N		I	**U**		N		N
25	21	LM		**U**			N		N		N
31	22			N			N	P	**S**		N
32	16	H	I	**U**			N		N		N
33	14		F	N			N		N		N
34	14		I	**U**		I	**U**		N		N
35	13		I	**U**		C	**U**		N		N
40	17	H	I+F	**U**			N		N		N
41	24	H	I	**U**			N	Mo	**U**		N
43	21	LM	H+I	**U**			N		N		N
44	17			na			N	Mo	**U**		N
47	19		I+F	**U**			N	P	**S**		N
48	21	H	I+F	**U**			N	P	**S**		N
49	19		H+F	N			N	Mo	**U**		N
50	22	LM	I	**U**			N	Mo	**U**		N
51	21	H	H+F	N			N	Mo	**U**		N
52	19	LM	I	**U**			N	Mo	**U**		N
54	19			N			N	P	**S**		N
57	16			N		H	N	Mo	**U**		N
59	23	H	I	**U**			N	L	N	H	**U**
60	20			N			N	Mo	**U**		N
63	15	H	H+I	**U**			N		N		N
65	21	LM	I	**U**		F	N		N	H	**U**
66	16	LM		**U**			N	na	na		N
70	22			N		I	**U**		N	H	**U**
72	18		I	**U**			N		N		N
73	13		I	**U**			N		N		N
74	15		H+F	N			N	Mo	**U**		N
76	22			N			N	P	**S**		N
77	28	H		N			N	Mo	**U**		N
78	25	LM	F	N		I+F	N	P	**S**		N
79	25	LM	F	N			N	Mo	**U**		N
82	25	H		N			N	Mo	**U**		N
85	17	H	I	**U**			N		N		N
86	19		F	N		I	**U**	L	N		N
87	20	H	I	**U**			N		N		N
88	14	H	I+F	**U**		I+F	**U**		N		N
89	19	H	H+I	**U**		H+I	**U**	P	**S**		N
93	18	LM		**U**			N	L	N		N
96	19	H	F	N			N	Mo	**U**		N
97	22	LM		**U**		I	**U**		N		N
98	22	LM	F	**U**			N		N		N
104	19			N			N	Mo	**U**		N
106	18			N			N	P	**S**		N
108	18		I	**U**			N	Mo	**U**		N
110	22	LM	H	**U**			N	Mo	**U**		N
111	12	LM		**U**			N		N		N
112	15	H	H	N			N	Mo	**U**		N
113	24	H		N		I	N	P	**S**		N
115	14		I	**U**			N	Mo	**U**		N
117	24	H		N			N	P	**S**		N
119	21	LM	H+I	**U**			N		N		N
121	13			N			N	P	**S**		N
124	17			N			N	Mo	**U**		N
131	20	H		N			N	P	**S**		N
135	17	LM		**U**			N		N		N
136	19	LM		**U**			N		N		N
137	17			N			N	P	**S**		N
143	19			N			N	P	**S**		N

LM=light metaplasia; M=metaplasia; H=hyperplasia; I=hypersecretion; F=flogosis; L=light; Mo=moderate; P=prominent; N=negative; U=uncertain; S=suspect; na=not analyzed.

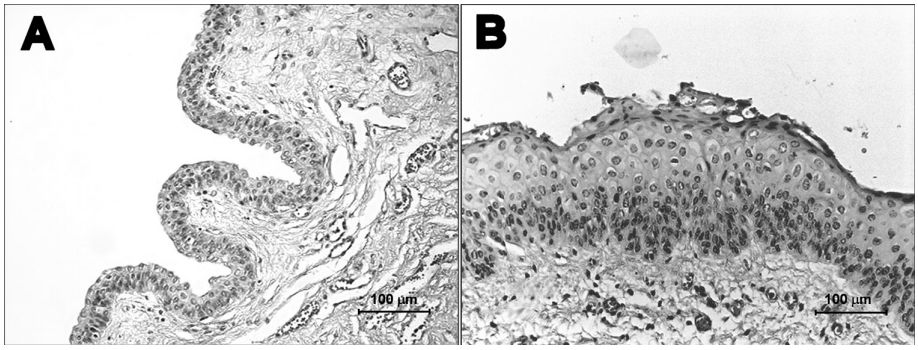

Fig. 1. Histological findings of a negative urethra (A) compared with epithelial metaplasia in the urethra of the only animal classified as "suspect" for steroids (B) (200× magnification). Staining: HE.

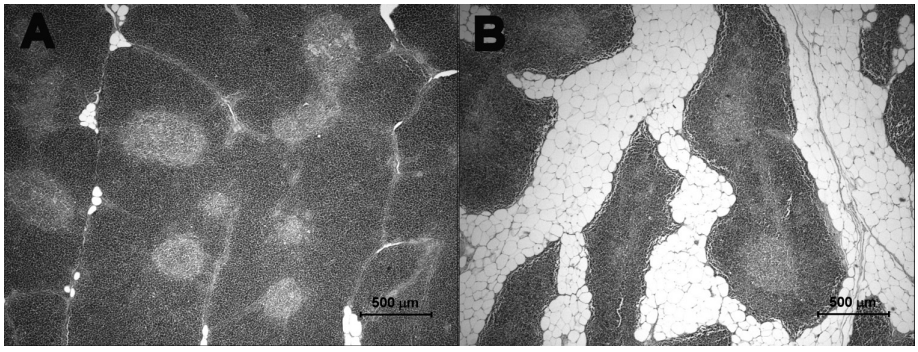

Fig. 2. Histological findings of a negative thymus (A) compared with an atrophic thymus classified as "suspect" (B) (40× magnification). The latter shows severe atrophy of the parenchyma, lymphocyte depletion and diffuse fat tissue infiltration. Both samples come from 17-months-old males. Staining: HE.

Table 4. Histological changes in target organs of females. Only animals with at least one lesion classified as "uncertain" are reported

N°	Age	Bartholin's glands			Ovaries		Mammary gland		Thymus		Thyroid
		Ducti	Gland	Resp		Resp		Resp	Atrophy	Resp	Resp
1	12	M	LM+I	**U**	na	na	A	N		N	N
8	21	M	LM+I	**U**	na	na	Sec	N		N	N
12	25	LM		N	na	na	Sec	N	P	**S**	N
38	14	M		**U**			A+Sec	N	Mo	**U**	N
102	38	H+F	H	N	na	na	A	N	P	**S**	N
103	19	M	LM	**U**	CL	N	A	N	Mo	**U**	N
114	24			na	C	N		na	P	**S**	N
120	27	M	I	**U**	CL	N		na		N	N
124	17	M	LM+I	**U**		N		na	Mo	**U**	N
125	21	M	LM+I	**U**		N		na		N	N
129	17	M	H+I	**U**		N		na	Mo	**U**	na

LM=light metaplasia; M=metaplasia; H=hyperplasia; I=hypersecretion; F=flogosis; C=cysts; CL=corpus luteum; A=alveoli; Sec=secretion; Mo=moderate; P=prominent; N=negative; U=uncertain; S=suspect; na=not analyzed.

molecule responsible for the observed histological changes in biological samples belonging to the suspected animals before setting up any official accusation of a farm manager or veterinarians, who are considered co-responsible for control of the ban. However, if histological changes and organ lesions persist up until slaughter, the analytical methods used to date may not be sufficiently sensitive to confirm positivity.

In conclusion, the results of this survey suggest that the use of growth promoters prohibited by law is a practice still widespread in some areas of Italy. In fact, the percentage of suspected animals was anything but small, revealing that the extent of this occurrence, if confirmed, would represent a major problem and that new counteraction strategies should be planned. Furthermore, our data are strongly in disagree-

ment with the data obtained annually by the Italian PNR, in which positivities that are found for residues from growth promoters represent only a fraction of a percent. Although histological screening has no value for legal purposes, it represents a useful supplementary method to support the control of illegal treatment with growth promoters and enables the veterinary inspectors to apply specific procedures at the farm.

REFERENCES

1. Biolatti, B., Cabassi, E., Rosmini, R., Groot, M., Castagnaro, M., Benevelli, R., Giglioli, G., Ghizzinardi, A., Giorgi, P., Mazzini, C., Comellini, F., Alberghini, L., Zancanaro, G., Cannizzo, T., Amedeo, S., Poppi, L. and Cantoni, A. 2003. Lo screening istologico nella prevenzione dell'uso di anabolizzanti nel bovino. *Large Anim. Rev.* **9**: 9–19.
2. Biolatti, B., Cannizzo, T., Zancanaro, G., Amedeo, S., Cesano, L. and Barbarino, G. 2003. Effects of low doses of dexamethasone on bovine thymus morphology. *Res. Vet. Sci.* **74**: 12. [CrossRef]
3. Biolatti, B., Bollo, E., Cannizzo, F. T., Zancanaro, G., Tarantola, M., Dacasto, M., Cantiello, M., Carletti, M., Biolatti, P. G. and Barbarino, G. 2005. Effects of low-dose dexamethasone on thymus morphology and immunological parameters in veal calves. *J. Vet. Med. A Physiol. Pathol. Clin. Med.* **52**: 202–208. [Medline] [CrossRef]
4. Bovee, T. F. H., Heskamp, H. H., Hamers, A. R. M., Hoogenboom, R. L. A. P. and Nielen, M. W. F. 2005. Validation of a rapid yeast estrogen bioassay, based on the expression of green fluorescent protein, for the screening of estrogenic activity in calf urine. *Anal. Chim. Acta* **529**: 57–64. [CrossRef]
5. Canese, M. G., Guarda, F., Pancani, I., Leonardo, E. and Bianco, S. 1985. Involuzione timica nel vitello indotta da desametazone. *Summa* **2**: 275–280.
6. Cannizzo, F. T., Zancanaro, G., Spada, F., Mulasso, C. and Biolatti, B. 2007. Pathology of the testicle and sex accessory glands following the administration of boldenone and boldione as growth promoters in veal calves. *J. Vet. Med. Sci.* **69**: 1109–1116. [Medline] [CrossRef]
7. Cannizzo, F. T., Miniscalco, B., Riondato, F., Bollo, E., Barbarino, G., Giorgi, P., Mazzini, C. and Biolatti, B. 2008. Effects of anabolic and therapeutic doses of dexamethasone on thymus morphology and apoptosis in veal calves. *Vet. Rec.* **163**: 448–452. [Medline] [CrossRef]
8. Carraro, L., Ferraresso, S., Cardazzo, B., Romualdi, C., Montesissa, C., Gottardo, F., Patarnello, T., Castagnaro, M. and Bargelloni, L. 2009. Expression profiling of skeletal muscle in young bulls treated with steroidal growth promoters. *Physiol. Genomics* **38**: 138–148. [Medline] [CrossRef]
9. Commission Decision 2002/657/EC of 12 August 2002. 2002. Official Journal of the European Union 17.8.2002 L221 8–36.
10. Commission of the European Communities. 2011. On the implementation of national residue monitoring plans in the Member States in 2010. Part III. Commission staff working document. Brussels, 23.12.2008. [cited 2012 August 7] Available from http://ec.europa.eu/food/food/chemicalsafety/residues/workdoc_2010_en.pdf.
11. Corah, T. J., Tatum, J. D., Morgan, J. B., Mortimer, R. G. and Smith, G. C. 1995. Effects of a dexamethasone implant on deposition of intramuscular fat in geneticallyidentical cattle. *J. Anim. Sci.* **73**: 3310–3316. [Medline]
12. Courtheyn, D., Le Bizec, B., Brambilla, G., de Brabander, H. F., Cobbaert, E., Van de Wiele, M., Vercammen, J. and de Wasch, K. 2002. Recent developments in the use and abuse of growth promoters. *Anal. Chim. Acta* **473**: 71–82. [CrossRef]
13. De Maria, R., Divari, S., Goria, M., Bollo, E., Cannizzo, F. T., Olivero, M., Barbarino, G. and Biolatti, B. 2009. 17β-oestradiol-induced gene expression in cattle prostate: biomarkers to detect illegal use of growth promoters. *Vet. Rec.* **164**: 459–464. [Medline] [CrossRef]
14. Decree n.148 of 29 October 2009. 2009. Italian Official Journal n. 254 of 31 October 2009.
15. Decree n.158 of 6 March 2006. 2006. Italian Official Journal n.98 of 28 April 2006.
16. Directive 2008/97/EC of the European Parliament and of the Council of 19 November 2008. 2008. Official Journal of the European Union 28.11.2008, L318 9–11.
17. Divari, S., Cannizzo, F. T., Uslenghi, F., Pregel, P., Mulasso, C., Spada, F., De Maria, R. and Biolatti, B. 2011. Corticosteroid hormone receptors and prereceptors as new biomarkers of the illegal use of glucocorticoids in meat production. *J. Agric. Food Chem.* **59**: 2120–2125. [Medline] [CrossRef]
18. Durchfeld, B. 1988. Pathologisch-anatomische und pathologisch-histologische Untersuchung der akzidentellen Thymusatrophie bei Kälbern. Vet. Med. thesis. Justus- Liebig Universität. pp 61.
19. Durchfeld, B., Kaufer-Weiss, I. and Weiss, E. 1989. Accidental thymus involution in calves. *Berl. Munch. Tierarztl. Wochenschr.* **102**: 400–405. [Medline]
20. Durham, P. J., Lax, A. and Johnson, R. H. 1985. Pathological and virological studies of experimental parvoviral enteritis in calves. *Res. Vet. Sci.* **38**: 209–219. [Medline]
21. Gottardo, F., Brscic, M., Pozza, G., Ossensi, C., Contiero, B., Marin, A. and Cozzi, G. 2008. Administration of dexamethasone per os in finishing bulls. I. Effects on productive traits, meat quality and cattle behaviour as indicator of welfare. *Animal* **2**: 1073–1079. [Medline] [CrossRef]
22. European Food Safety Authority. 2007. Opinion of the scientific panel on contaminants in the food chain on a request from the European commission related to hormone residues in bovine meat and meat products. Question N° EFSA-Q-2005–048. *EFSA J.* **510**: 1–62.
23. Groot, M., den Hartog, J. M. and Gruys, E. 1989. Influence of androgens on the genital tract of cyclic heifers. *Vet. Q.* **11**: 198–209. [Medline] [CrossRef]
24. Groot, M. and Den Hartog, J. M. P. 1990. Histological changes in the genital tract of female veal calves implanted with naturally occurring anabolic steroids. *J. Vet. Med. A* **45**: 425–440.
25. Groot, M. J., Ossenkoppele, J. S., Bakker, R., Pfaffl, M. W., Meyer, H. H. and Nielen, M. W. 2007. Reference histology of veal calf genital and endocrine tissues—an update for screening on hormonal growth promoters. *J. Vet. Med. A Physiol. Pathol. Clin. Med.* **54**: 238–246. [Medline] [CrossRef]
26. Gruver, A. L. and Sempowski, G. D. 2008. Cytokines, leptin, and stress-induced thymic atrophy. *J. Leukoc. Biol.* **84**: 915–923. [Medline] [CrossRef]
27. Guarda, F., Valenza, F., Biolatti, B., Quaglia, F. and Emanuel, C. 1983. Sull'atrofia precoce del timo in seguito a somministrazione prolungata di glicocorticoidi nei vitelli sanati. *Nuovo Progr. Veterinario* **38**: 434–442.
28. Guarda, F., Biolatti, B., Valenza, F. and Miglietti, M. 1990. Pathologico-anatomic findings in thymus gland and female sex organs of slaughtered calves after treatment with anabolically effective substances. *Deut. Tierarztl Woch.* **97**: 313–315.
29. Heilmann, P., Steinbach, G. and Schulze, F. 1982. Pathoanatomical and pathohistological studies of cyclophosphamide-induced

organ changes in calves with special reference to lymphatic organs. *Arch. Exp. Veterinarmed.* **36**: 623–633. [Medline]
30. Hughes, B. J., Forsell, J. H., Sleight, S. D., Kuo, C. and Shull, L. R. 1985. Assessment of pentachlorophenol toxicity in newborn calves: clinicopathology and tissue residues. *J. Anim. Sci.* **61**: 1587–1603. [Medline]
31. Inui, S., Narita, M. and Kumagai, T. 1978. Bovine virus diarrhea-mucosal disease. I. Pathological changes in natural and experimental cases. *Natl. Inst. Anim. Health Q. (Tokyo)* **18**: 109–117. [Medline]
32. Jansen, E. H., Stepany, R. W., Vos, J. G., Ruitenberg, E. J., Benraad, T. J., De Boer, F., De Ruig, W. G., Weijman, J. and Schmidt, N. A. 1989. Application of diethylstilbestrol dipropionate in bulls. Excretion of residues in urines and faeces and histological and immunohistochemical changes in the prostate. *Vet. Q.* **11**: 1–11. [Medline] [CrossRef]
33. Mooney, M. H., Bergwerff, A. A., van Meeuwen, J. A., Luppa, P. B. and Elliott, C. T. 2009. Biosensor-based detection of reduced sex hormone-binding globulin binding capacities in response to growth-promoter administrations. *Anal. Chim. Acta* **637**: 235–240. [Medline] [CrossRef]
34. Peters, W., Liess, B., Frey, H. R. and Trautwein, G. 1987. Incidence and impact of persistent infections with BVD virus in the field. pp. 133–145. *In*: Agriculture. Pestivirus Infections of Ruminants (Harkness, J. W. ed.), Office for the Offical Publications of the European Communities, Brusselles.
35. PNR. 2009.Program of Surveillance by Histological Test. Department of Veterinary Public Health, General Direction of Food Safety and Nutrition, Ministry of Health of the Italian Republic, Rome.
36. Regione Piemonte. 2009. [cited 2011 October 15] Available from http://www.regione.piemonte.it/sanita/cms/pubblicazioni/category/97-2010.html?download=978%3Aarea-c-piano-regionale-di-controllo-dei-residui-indesiderati-nelle-carni-e-negli-animali-allevati-pdf-180.49-kb&start=60.
37. Serakides, R., Nunes, V. A., Santos, R. L., Cassali, G. D. and Costa Neto, P. P. 1999. Histomorphometry and quantification of nucleolar organizer regions in bovine thyroid containing methylthiouracil residues. *Vet. Pathol.* **36**: 574–582. [Medline] [CrossRef]
38. Tarantola, M., Schiavone, A., Preziuso, G., Russo, C., Biolatti, B. and Bergero, D. 2004. Effects of low doses of dexamethasone on productive traits and meat quality of veal calves. *Anim. Sci.* **79**: 93–98.
39. Toffolatti, L., Rosa Gastaldo, L., Patarnello, T., Romualdi, C., Merlanti, R., Montesissa, C., Poppi, L., Castagnaro, M. and Bargelloni, L. 2006. Expression analysis of androgen-responsive genes in the prostate of veal calves treated with anabolic hormones. *Domest. Anim. Endocrinol.* **30**: 38–55. [Medline] [CrossRef]
40. Uno, K., Takesue, K., Nakanisi, K., Nakagawa, K., Murakami, K. and Homma, S. 1990. Thymuses atrophy in calves associated with infectious diseases. *J. Jpn.Vet. Med. Assoc.* **43**: 655–660.