Trop Anim Health Prod (2012) 44:1323–1328
DOI 10.1007/s11250-012-0089-1

BRIEF COMMUNICATION

Growth performance in heavy lambs experimentally treated with 17 ß-estradiol

Guido Ruggero Loria · Roberto Puleio · Maria Rita Schiavo · Paolo Giambruno · Elena Bozzetta · Massimo Todaro

Accepted: 24 January 2012 / Published online: 3 August 2012

Abstract European and Italian legislation have banned use of growth promoters in livestock since 1988, but epidemiological data show that anabolic drugs are still being used illegally. Recent surveys carried out on the cattle farms in Northern Italy have confirmed the presence of growth-promoting hormones. Authors report data on growth performances in 80 Valle del Belice×Comisana weaned lambs experimentally treated with 17 beta-estradiol with 0.5 ml solution of oil Depot Estradiol ® (containing 5 mg of 17β-estradiol valerate) by intramuscular injection into the thigh. The experiment was founded by the National Ministry of Health, to validate histological test for surveillance and control of growth-promoting hormones in sheep. This study confirmed the strong correlation between clinical and anatomopathological features and growth performances of treated animals. Otherwise, no significant differences were found on in vivo performance of the lambs. Estradiol treatment showed heavier shoulders and necks on treated lambs, while the loins were significantly lighter. Moreover, lamb-estradiol-treated groups showed lower separable and inseparable fat percentage than lamb-control groups.

Keywords Growth hormone · 17 Beta-estradiol · Growth performance

Introduction

EU and Italian legislation (Italian D.Lgs. n. 336/99 and UE Directive n. 96/22/CE) banned the use of growth hormones in livestock in 1988. Directive 97/EC (2008) specifically forbade 17 β-estradiol in food-producing animals due to its potential carcinogenicity (Laviolette et al. 2010; Yu 2002; Newbold and Liehr 2000).

Despite this regulation, EU data confirm that anabolic drugs are still being used illegally as shown by constant high sales of anabolic drugs in the livestock sector (Reig and Toldrá 2009).

The laboratory tests used for detection of these undesirable residues display low sensitivity (Ministry of Health 2009). Moreover, the metabolization of new anabolic cocktails is rapid, which means that positive results are rarely obtained (Courtheyn et al. 2002). Some surveys carried out, at slaughterhouse, have confirmed the presence of growth-promoting hormones by histological lesions, in target organs (sexual accessory glands, thymus, thyroid; Reig and Toldrá 2009).

Histology represents an effective screening test for slaughterhouse control, identifying those herds where there is illegal use of anabolic drugs (Pezzolato et al. 2011). In fact, a standardized histological test was added to the official

G. R. Loria · R. Puleio (✉) · M. R. Schiavo
Istituto Zooprofilattico Sperimentale of Sicily,
Laboratory of Histology and Immunohistochemistry,
Palermo, Italy
e-mail: roberto.puleio@izssicilia.it

P. Giambruno
Department of Veterinary Public Health,
ASP n. 6,
Palermo, Italy

E. Bozzetta
Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta,
Torino, Italy

M. Todaro
Dipartimento Demetra, Università degli Studi di Palermo,
Palermo, Italy

surveillance national plan in 2008; while this histological approach has been applied in cattle in some EU countries, no data are available for small ruminants.

In the EU countries, 159 millions of sheep and 18 millions of goats are reared, mainly in Mediterranean countries. In Italy, the current demand for small ruminant meat exceeds internal supply, so there is a significant import of sheep meat (live lambs and carcasses), which requires an efficient surveillance system in order to monitor health and quality of meat products (ERSAF 2008). In Sicily, dairy sheep production represents an important resource for the economy of hilly and mountainous areas, where other economical activities are difficult to be developed (Scintu and Piredda 2007). The major advantage of sheep, in our agricultural system, is its ability to utilize pasture to produce milk, meat, and wool.

In tropical and subtropical countries (including the Mediterranean basin), there is no reported evidence of treatment with anabolic substances in small ruminant meat farming. Actually, the traditional production, still classifiable as “pastoralism” in these countries, does not concern this kind of practice. Nevertheless, the new global market has stimulated meat trades among several countries; in Sicily where we record, for internal consumption, more than 25% of total lamb meat imported from Eastern Europe (ERSAF 2008). This massive flow of import live animals and carcasses needs to implement a specific surveillance to estimate the potential risk for consumers.

In this study, in order to investigate the effects of anabolic treatment in lambs and to strengthen surveillance tools for sheep meat production, authors collected data on growth performance to be related to histological changes, observed in heavy lambs, experimentally treated with 17 beta-estradiol.

Materials and methods

Sheep farming and feeding

The trial was conducted at an experimental station located in Western Sicily between December and April and lasted 105 days. A total number of 80 Valle del Belice × Comisana weaned lambs were shared in four homogeneous groups according to age (55±10 days), sex, and body weight. The mean body weight for each group were similar (16.51±0.77 kg for male lamb-control group, 15.57±1.12 kg for female lamb-control group, 17.03±1.12 kg for male lamb-estradiol-treated group, 15.82±1.07 kg for female lamb-estradiol-treated group). Lambs were slaughtered at 160 days of age, when sheep reach commercial weight, requested by our internal market (about 35 kg). All lambs were fed with vetch and oat hay (crude protein=10.31% on dry matter (DM), neutral detergent fiber=57.86% on DM) offered ad libitum and 520 g/head/day of commercial concentrate (CP=20.37% on DM, ether extract=2.32% on DM); freshwater was always available. For each group, daily fed intake (offered minus residual) was recorded weekly; hay and concentrate were analyzed for chemical composition monthly. All lambs were weighted at the beginning, at the end of treatment, and monthly.

All experimental procedures were designed in order to avoid any animal distress and according to the European (EC Directive 86/609/EEC for animal experiments) and Italian animal health and well-being regulation.

Experimental treatment with 17 β-estradiol

The animals were monitored daily for clinical signs and behavioral abnormalities occurring during the period of the experimental trial. Any deviation from physiology was noted in a clinical form with special reference to under development, expressed as a percentage relative to control groups (percent size improvement). After 54 days from the start of the study, two groups of 20 lambs (male lamb-estradiol-treated group and female lamb-estradiol-treated group) were both treated with 0.5 ml/head solution of oil Depot Estradiol ® (containing 5 mg of 17β-estradiol valerate) by intramuscular injection into the thigh. The estradiol administration was repeated weekly for four times. The control group lambs were injected with placebo (saline solution 0.5 ml per head). The treatment was stopped 20 days before slaughtering. Estradiol dose utilized (5 mg/head) was established according to project guidelines, in order to obtain significant histological changes in target organs.

Slaughter data

The animals were slaughtered at 160 days of age. They were fed until 12 h before slaughtering, and then stunned and bled out. The gastrointestinal content was removed to calculate empty body weight. At necropsy, macroscopic changes of target organs (prostate, bulbo-urethral glands, ovary, udder, Bartolin’s glands) and of thymus, hearth, lung, liver, spleen, adrenal glands, and striated muscle were examined. After anatomopathological evaluation, all samples were immediately fixed in 10% buffered formalin. The tissue samples were later processed routinely, sectioned at 5 μm, and stained with hematoxylin-eosin as standard procedures. Sections were examined by light microscopy in order to evaluate histological changes to be related to anabolic treatment. The carcasses were split and the right side was weighed and sectioned into commercial cuts according to Preziuso et al. (1999). The right proximal pelvic limb (sample cut) was removed from each carcass, and was dissected according to European Association for Animal Production

Table 1 Voluntary feed intake of lambs

	Female		Male	
	Control	Treated	Control	Treated
Hay intake (g/head/day of DM)	846	727	846	727
Concentrate intake (g/head/day of DM)	470	466	470	466
Dry matter intake (g/head/day)	1316	1.193	1316	1.193
Daily gain (g/day)	147	115	151	160
Food conversion ratio	8.95	10.38	8.72	7.46

Guidelines (Working Group on Carcass Evaluation; Fisher and De Boer 1994) to determine the lean meat percentage, fat, bone, and other tissues. After pelvic limb dissection, meat was immediately homogenized and lyophilized. Dry matter (at 105°C), fat extracted (with petroleum ether Soxhlet apparatus), ash (at 525°C) and protein (by the Kjeldhal procedure) were determined (AOAC 2003) in the lyophilized samples.

Statistical analysis

All data were analyzed with a statistical two-factor model of analysis of variance with interaction; the fixed factors were sex and group. Least square means were compared with Student's *t* test. Statistical analysis was carried out, using the general linear model of SAS software (SAS 2004).

Results

Clinical data, pathology, and histology

Clinical changes started to be visible after the third injection of estradiol valerate. In treated female group, mammary glands were all enlarged by 20% compared with udder of control female group and nipples looked similar to an adult lactating ewe. Sexual behavior was aroused and they behave like adults during the reproductive season. Nevertheless, there were no clinical changes in the control group.

The anatomopathological examination showed a general enlargement of target glands, in all treated lambs: bulbo-urethral glands and prostate for males, ovaries and genital tract for females. Identification and inspection of Bartolin's glands were often difficult or inconclusive because the Bartolin's glands in sheep are only present in one animal out of five (Barone 2003).

Histological changes of treated animals showed marked metaplasia of prostatic tissue surrounding urethra lumen and bulbourethral glands. In control animals, treated with placebo, there were no remarkable histological changes of the prostate, bulbo-urethral glands, ovary, udder, Bartolin's glands, although in some cases they showed a moderate hyperplasia of the urethra.

In vivo performance and carcass characteristics

The voluntary feed intake of dry matter is reported in Table 1. The hay/concentrate ratio is 1.7 without significant differences between groups. Daily gain, did not show any differences between sex and between groups. As regards the food conversion ratio, no significant differences were found too.

The in vivo lamb performance is reported in Table 2. At the start of trial, males were heavier than females but no significant differences were found between control and

Table 2 In vivo lambs performance (LSM±ES)

	Female		Male		Significance		
	Control	Treated	Control	Treated	S	G	S×G
BW start the trial (kg) (A)	15.57±1.12	15.82±1.07	16.51±0.77	17.03±1.12	NS	NS	NS
BW start treatment (kg) (B)	23.50±1.34	22.04±1.29	24.66±0.93	25.65±1.12	*	NS	NS
BW end trial (kg) (C)	30.05±1.52	28.15±1.45	33.55±1.05	32.91±1.12	**	NS	NS
Daily gain (kg/day) before treatment: (B−A)/54	0.147±0.01	0.115±0.01	0.151±0.01	0.160±1.12	*	NS	NS
Dailygain(kg/day)after treatment: (C−B)/51	0.128±0.01	0.120±0.01	0.174±0.01 A	0.142±1.12 B	**	*	NS

BW body weight, *S* sex, *G* group, *S*× *G* sex×group, *NS* not significant

On the row, different letters shown significant differences for $P<0.01$

*$P<0.05$, **$P<0.01$

Table 3 Slaughter and dissection data (LSM±ES)

	Female		Male		Significance		
	Control	Treated	Control	Treated	S	G	S×G
Slaughter weight (kg)	27.64±1.43	27.33±1.43	30.52±0.97	31.90±0.97	**	NS	NS
EBW[a] (kg)	25.00±1.31	23.80±1.26	26.54±0.88	2729±0.88	*	NS	NS
Hide+limbs (% EBW)	13.53±0.40	14.62±0.39	13.86±0.27	14.32±0.27	NS	*	NS
Head (%EBW)	6.50±0.19	6.79±0.18	7.07±0.13	6.98±0.13	*	NS	NS
Organs[b] (%EBW)	4.48±0.18	4.70±0.18	4.80±0.13	4.87±0.13	NS	NS	NS
Carcass (kg)	12.82±0.91	12.34±0.91	12.60±0.91	13.70±0.91	NS	NS	NS
Dressing (%)	49.76±1.04	51.19±1.04	49.75±1.04	51.63±1.04	NS	NS	NS
Right side RS (kg)	6.41±0.46	6.17±0.46	6.30±0.46	6.85±0.46	NS	NS	NS
Shoulder (% RS)	18.08±0.34A	19.42±0.34B	18.67±0.34AB	18.60±0.34AB	NS	*	*
Pelvic limb (% RS)	28.97±0.51	29.76±0.51	30.17±0.51	30.39±0.51	*	NS	NS
Bacon (% RS)	3.76±0.21	3.41±0.21	3.51±0.21	3.29±0.21	NS	NS	NS
Loin (% RS)	12.17±0.59	10.15±0.59	10.95±0.59	9.60±0.59	NS	**	NS
Neck (% RS)	9.04±0.43	10.51±0.43	8.67±0.43	11.19±0.43	NS	***	NS
Steak+brisket(% RS)	26.25±0.54	25.15±0.54	27.03±0.54	26.41±0.54	*	NS	NS

On the row, different letters shown significant differences for $P<0.01$

NS Not significant

*$P<0.05$, **$P<0.01$, ***$P<0.001$

[a] Empty body weight

[b] Lungs, trachea, heart, liver

treated groups. In the first period, before treatment with estradiol (lasting 54 days), daily gains ranged from 115 to 160 g/head/day but no significant differences were found between groups. Instead, during the experimental period of 51 days (estradiol treatment), male-treated group showed lower significant daily gain ($P<0.01$) than male control group; while no significant differences were found between the two female groups.

Results from slaughter house are reported in Table 3. The effect of treatment was showed only for some anatomic parts: hide plus limbs that resulted heavier than control groups. Moreover, the estradiol treatment determined shoulders and necks heavier in treated males and females, while the loins resulted lighter, expressed as percent of right side. The interaction between sex and group was significant only for the shoulders, where the estradiol treatment determined an increase of shoulders incidence only for the female lambs. Results showed as the estradiol produced a negative effect on meaty cuts. Tissue composition data and chemical composition of pelvic limb meat are reported in Table 4. Treated lambs resulted less fat than control lambs, showed lower separable fat percentage and a lower ether extract percentage of pelvic limb meat. Otherwise, protein and water percentages in pelvic limb of treated lambs (males and females) resulted higher than control lambs.

Table 4 Tissue composition and meat chemical composition of pelvic limb (LSM±ES)

	Female		Male		Significance		
	Control	Treated	Control	Treated	S	G	S×G
Muscle (%)	56.48±1.78	56.77±1.78	52.71±1.78	54.26±1.78	*	NS	NS
Fat (%)	11.08±0.92	8.58±0.92	10.59±0.92	8.87±0.92	NS	*	NS
Bone (%)	30.87±2.17	27.44±2.17	32.66±2.17	30.95±2.17	NS	NS	NS
Other tissues (%)	0.80±0.09	0.31±0.09	0.86±0.09	0.26±0.09	NS	***	NS
Water (%)	73.48±0.87	75.90±0.77	73.50±0.94	76.26±0.73	NS	**	NS
Protein (% DM)	85.40±0.68	85.45±0.68	85.81±0.68	87.93±0.68	NS	*	NS
Ether extract(%DM)	10.04±0.83	7.30±0.73	9.00±0.89	5.59±0.69	NS	***	NS
Ash (% DM)	4.98±0.16	4.91±0.14	4.74±0.17	5.18±0.13	NS	**	NS

NS not significant

*$P<0.05$, **$P<0.01$, ***$P<0.001$

Discussion

The in vivo performance of the lambs showed no significant differences between groups, but the feed conversion ratio was higher than that reported in previous studies (Bonanno et al. 2011) on the same breed. The body weight recorded was in accordance with other studies on the same breed (Pace et al. 2006).

The results obtained for slaughter weight and carcass are comparable with slaughtered lambs of the same age (Bonanno et al. 2011). As regards the lack of treatment effect observed on growth performance of lambs, Meyer (2001) reports that dose of estradiol can inhibit lambs growth; high doses inhibit livestock growth, whereas low doses enhance weight performance above 5%. Therefore, we can assume that our dose was high for lambs, in particular for male lambs that showed the worst growth performance. In fact, other authors (Pace et al. 2006), monitored the beneficial anabolic action of the estrogens contained in the subterranean clover (Mediterranean pasture), with a low content of phytoestrogen (0.8 mg/g of dry matter), that induced a significant improvement in animal weigh gain (13.47%). The effect of treatment at slaughter and dissection showed differences between groups only for some anatomical parts: hide plus limbs, shoulders, loins, and necks. The interaction between group and sex resulted statistically significant only for shoulder percentage that resulted higher for female treated lambs probably for different bone growth. As far as tissue composition, results showed that treated lambs were less fat than control lambs. These results are in accordance with the international literature (Carson et al. 2001; Scarth et al. 2009), that reports as internal body fat and dissectible carcass fat deposition are reduced by hormone administration.

Conclusions

This study confirms the strong correlation between clinical and anatomopathological features, which have also showed some interesting implications on growth performances of treated animals. In term of carcass characteristics, *intra vitam* and *post mortem* data showed that estradiol treatment at this concentration did not influence the body weight and weight gain of female lambs while induced a worsening of male lambs performance. The treatment significantly influenced some carcass cuts: as shoulder, loin, and neck. The major effect of anabolic treatment was observed in tissue composition and meat chemical composition, showing meat with lower fat contents (separable and not) and higher water and protein percentages.

In spite of scarce response in term of body weight, relate to estradiol treatment in heavy lambs, the experiment confirmed severe anatomopathological and histological changes in target organs, as observed in cattle (Groot 2006). Future work will investigate same parameters in groups of lambs treated with lower doses of hormone.

Acknowledgments This research was founded by the Italian Ministry of Health (Projects RF-IZP-2006-364645).

References

Association of Official Analytical Chemists (AOAC). 2003. Official Methods of Analysis, 17th edition, revision 2, Gaithersburg, MD, USA.

Barone R., 2003. Anatomia comparata dei mammiferi domestici. (Edagricole, Bologna).

Bonanno A., Di Miceli G., Di Grigoli A., Frenda A. S., Tornambè G., Giambalvo D., Amato G., 2011. Effects of feeding green forage of sulla (*Hedysarium coronarium* L.) on lamb growth and carcass and meat quality. Animal, 5 (1),148–154

Carson A.F., Elliott C.T., Mackie D.P., McCaughey W.J., 2001. Active immunization of lambs with a monoclonal antibody against clenbuterol. Livestock Production Science, 68, 87–91.

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. Analytica Chimica Acta, 473, 71–82.

ERSAF, 2008. Il mercato delle carni, produzione e consumo. www.ersaf.lombardia.it

Fisher A.V., De Boer H., 1994. The EAAP standard method of sheep carcass assessment. Carcass measurements and dissection procedures. Report of the EAAP Working Group on Carcass Evaluation, in cooperation with the CIHEAM Instituto Agronomico Mediterraneo of Zaragoza and the CEC Directorate General for Agriculture in Brussels. Livestock Production Science. 38,149–159

Groot MJ., 2006. Effects of phyto-oestrogens on veal calf prostate histology. Veterinary Research Communication;30(6),587–98

Laviolette L.A., Garson K., Macdonald E.A, Senterman M.K., Courville K., Crane C.A. and Vanderhyden B.C., 2010. 17ß-estradiol accelerates tumor onset and decreases survival in a transgenic mouse model of ovarian cancer. Endocrinology, 151, 3, 929–938.

Meyer HH, 2001. Biochemistry and physiology of anabolic hormones used for improvement of meat production. APMIS.109(1),1–8.

Ministry of Health, 2009. D.G. della Sicurezza degli Alimenti e della Nutrizione, Ufficio III. Relazione finale Piano Nazionale Residui: 16–18.www.salute.gov.it/imgs/C_17_pubblicazioni_1296_allegato.pdf

Newbold R.R., Liehr J.G., 2000. Induction of uterine adenocarcinoma in CD-1 mice by catechol estrogens. Cancer Research. 15;60(2): 235–237.

Pace V., Carbone K., Spirito F., Iacurto M., Terzano M.G., Verna M., Vincenti F., Settineri D., 2006. The effects of subterranean clover phytoestrogens on sheep growth, reproduction and carcass characteristics. Meat Science. 74, 616–622

Pezzolato M., Maurella C., Varello K., Meloni D., Bellino C., Borlatto L., Di Corcia D., Capra P., Caramelli M., Bozzetta E., 2011. High sensitivity of a histological method in the detection of low-dosage illicit treatment with 17b-estradiol in male calves. Food Control. 22,1668–1673.

Preziuso, G., Russo, C., Casarosa, L., Campodoni, G., Piloni, S., Cianci, D., 1999. Effect of diet energy source on weight gain and carcass characteristics of lambs. Small Ruminant Research. 33, 9–15.

Reig, Milagro; Toldrá, Fidel, 2009. Safety of meat and processed meat, food microbiology and food Safety. Springer, New York.

SAS, 2004. Statistical Analysis System. SAS Institute Inc. Version 9.1.2, Cary, NC, USA.

Scarth J, Akre C, van Ginkel L, Le Bizec B, De Brabander H, Korth W, Points J, Teale P, Kay J., 2009. Presence and metabolism of endogenous androgenic–anabolic steroid hormones in meat-producing animals: a review. Food Additive Contaminants: Part A. Vol.26(5),640–671.

Scintu M.F., Piredda G., 2007. Typicity and biodiversity of goat and sheep milk products. Small Ruminant Research, 68,221–231.

The European Parliament and the Council of the European Union, 2008. Directive 2008/97/EC of the European Parliament and the Council of 19 November 2008 amending Council Directive 96/22/EC concerning the prohibition on the use in stock farming of certain substances having a hormonal or thyrostatic action and of beta-agonists. Official Journal of the European Union, L 318, 9–1.

Yu FL., 2002. 17 Beta-estradiol epoxidation as the molecular basis for breast cancer initiation and prevention. Asia Pacific Journal of Clinical Nutrition;11 Suppl 7, 460–466.