Environmental Quality and Safety

Supplement Volume V

Anabolic Agents in Animal Production

FAO/WHO Symposium Rome, March 1975
Guest Editors: Frank C. Lu and Jan Rendel

Georg Thieme Publishers Stuttgart 1976

IV/1 Quality of the Meat After the Application of Anabolic Agents in Young Calves

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Summary

Experiments were carried out to test the effect of implantation of Implix® (20 mg estradiol + 200 mg testosterone) or Revalor® (140 mg trienbolone acetate + 20 mg estradiol) on growth, feed conversion, slaughter quality, and residue levels in black Friesian bull calves at 4, 8, or 4 and 8 weeks before slaughtering. Weighings and calculations of feed conversion were carried out weekly. The control group of weight gain and feed conversion consisted of 22 calves; the chemical analyses of the control group was restricted to 8 animals.

Implantation of the hormones at 4 or 8 weeks before slaughtering resulted in a significant beneficial effect on weight gain, feed conversion and carcass index over the control. This effect was improved (up to 15%) by implanting the hormones at 8 and 4 weeks before slaughter. The effects obtained with Revalor seemed to be superior to those obtained with Implix. In 75% of the treated animals, some remainder of implants was found. No effect was found on water binding capacity and colour of raw meat. Carcass quality was not significantly improved as judged from water, fat, ash, and protein content of the *M. Longissimus Dorsi*. The collagen content of the animals treated with Revalor was slightly, though not significantly, increased. There is a slight decrease in the relative bone content of the treated animals over the control resulting in higher meat percentages. Residues were determined in meat obtained from rib and neck by biological and chemical methods. Oestrogenic activity was found in only 7 samples from the neck; all samples from the rib were negative. Chemical examination indicates the presence of oestradiol in these samples. No residues of trienbolone could be detected in the meat samples. The *Pars Dissiminata* of the prostate was examined histologically on frozen and paraffin sections. Although the latter permitted a sharper interpretation, both methods indicated an increased activity of the prostates induced by hormone treatment. Compared to Implix, Revalor treatment provoked a more pronounced mucous activity.

In recent years, the administration of estrogens in combination with testosterone or with trenbolone acetate (androst-4,9[10]-11-trien-3-one 17-acetate)[2,3,4,17] has been shown to improve the growth rate and feed conversion[5,14,17] in farm animals.

However, there is a lack of information on carcass quality, which is of interest to the producer as well as to the consumer. Moreover, there are sample data[14] about the residue levels remaining in the carcass following hormone implantation.

These experiments were carried out to test the effect of the implantation of Implix® (20 mg estradiol + 200 mg testosterone) or Revalor® (140 mg trienbolone acetate + 20 mg estradiol) on growth, feed conversion, slaughter quality, and residue levels in black Friesian bull calves.

1. Materials and Methods

The experiments were carried out on 40 black Friesian veal calves. The animals were purchased at the age of approximately 5 days, were housed in individual boxes in a ventilated

stable, and were fed a ration comparable to that used in practice. Implix or Revalor were implanted subcutaneously at the base of the left ear.

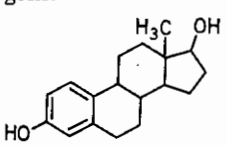
At the entrance of the stable, the animals were randomly sorted on body weight into 8 lots of 5 animals. Each lot contained its control and 4 animals treated with Revalor or Implix: 2 animals received implants at 4 **or** 8 weeks before slaughter, the other 2 animals were implanted at 4 **and** 8 weeks before slaughter. Weighings and calculation of feed conversion were carried out every 4 weeks. Feed conversion was calculated for each period from the ratio: weight gain/weight of feed consumed per animal. Fourteen animals housed in the same stable during the experiments were added to the control group for calculating weight gain, feed conversion, and carcass quality. The total rearing period was about 15 weeks.

After slaughter, the carcass was weighed, the left ear base was sampled for remainder of hormones and the prostate was removed for histological examination[12]. After being cooled for 24 h the carcass was weighed again and the slaughter yield calculated. The carcasses were graded by professionals ignoring the treatment into 3 classes (A: optimal, B: medium, C: low). This criteria is a measure of shape modification of the carcass with a high improvement of the posterior muscles. The carcass-index, calculated from the ratio: weight of carcass x 100/ carcass length (cm) from "atlas" to "pubis", was used as an objective parameter for meatiness. Since consumers prefer white meat over pink or red ones this parameter was also judged by professionals. Colour reflectance measurements (tristimuli: x, y, Z values) were made on diaphragma and cross-sections of *m. Long. Dorsi* of each cooled carcass.

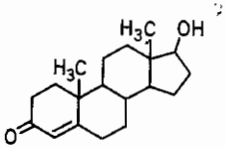
The *m. Long. Dorsi* was separated from the 3-rib cut and meat, tissue fat, and bone were weighed separately as an index of the meat/fat/bone content of the carcass. Water holding capacity[9], Warner-Bratzler shear values, weight loss after cooking samples (15 min at 80 °C) of meat slices or minced meat were all determined on samples from *m. Long. Dorsi.*

The chemical composition of defatted meat from the 3-rib cut was determined: moisture, protein, fat, and collagen[10] contents were estimated by standard methods.

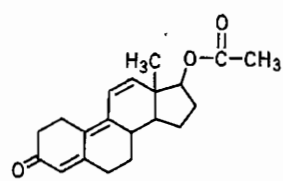
Residues in meat obtained from the neck between the 1st and 2nd cervical vertebra were assayed by two biological methods[1,16] and by chemical analysis[6,13,15,19]. The detection limit amounted to 1 ppm 17β-estradiol (or 0.6 ppm in the presence of testosterone) in the Tiecco-method. Subcutaneous injection of meat extracts to ovariectomized mice[7] and examination by the Allen-Doisy method[1] allowed detection of 40 ppb of 17β-estradiol in meat. Examination of meat extracts (50 g) by thin-layer chromatography after derivatization to fluorogenic compounds allowed detection of 50 ppb of either estrogens or androgens.



17β-Estradiol



Testosterone



Trienbolone acetate

2. Results

2.1. Body weight gain and feed conversion

The average growth of the animals over the experimental period is given in Table 1. Treatment of the calves with Implix or Revalor at 8 **or** 4 weeks before slaughter resulted in a

Table 1 Weight gain and feed conversion of bull calves during the experimental period (1x: single implantation; 2x: implantation at 8 and 4 weeks before slaughter)

Treatment	number of animals	Weight gain kg	Weight gain % of control	Feed conversion ratio	Feed conversion % of control
Control	22	124.4 ± 2.9	100	0.613 ± 0.008	100
Implix (1x)	8	135.5 ± 3.4[c]	108	0.661 ± 0.013[b]	107
Revalor (1x)	8	138.4 ± 5.5[b]	111	0.691 ± 0.007[a]	113
Implix (2x)	8	143.6 ± 3.8[a]	115	0.688 ± 0.015[b]	112
Revalor (2x)	8	146.3 ± 3.8[a]	118	0.707 ± 0.010[a]	115

Significant differences compared with control group:
[a] $p \leqslant 0.001$
[b] $p \leqslant 0.01$
[c] $p \leqslant 0.05$

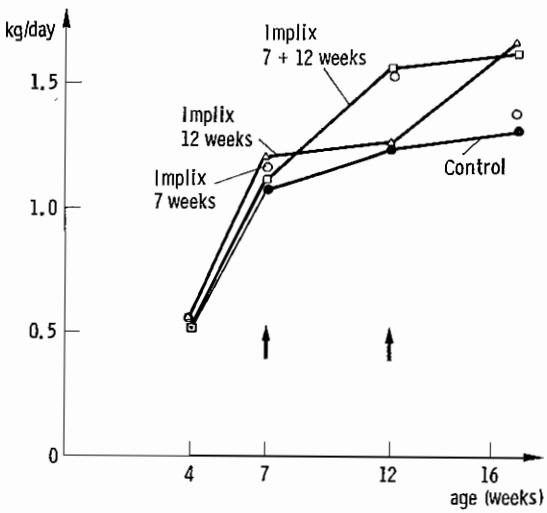


Fig. 1 Weight gain after Implantation of Implix®

significant weight gain over the controls. Implantation of the hormones at 8 **and** 4 weeks before slaughter had an additional growth promoting effect.

The increase in weight gain of the implanted and control animals during the trials are represented in Figs. 1 and 2. A relative decrease of the relative weight gain was observed in the period immediately before slaughter when calves were treated with Implix at 8 weeks before slaughter. This was not observed with Revalor implants. Implantation of the hormones at 4 weeks before slaughter caused a sharp increase in growth response bringing the calves to a higher weight (2–8 kg) than those injected at 8 weeks before slaughter. This effect is most pronounced with Revalor. The results (Figs. 1, 2, and Table 1) suggest that Revalor treatment improves weight gain over treatment with Implix.

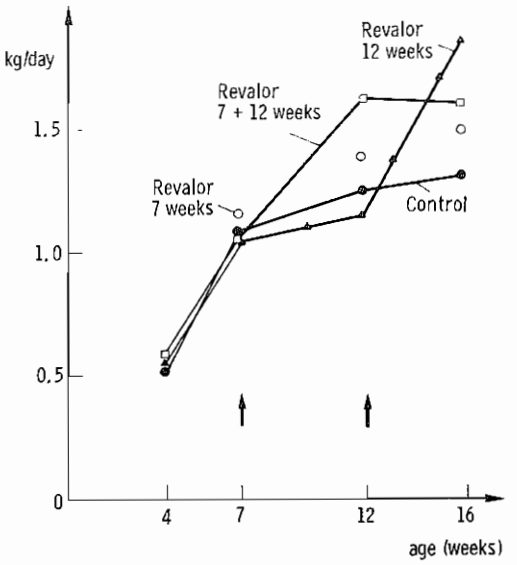


Fig. 2 Weight Gain after Implantation of Revalor®

Hormone treatment increased feed conversion significantly (Table 1) over the control group. This effect was pronounced in the groups receiving two implantations. Single or double implantation of Revalor resulted in a significant ($p \leqslant 0.05$) beneficial effect on feed conversion over the respective groups treated with Implix.

2.2. Carcass quality

Treated calves had always a better slaughter yield (1–2%) over the control animals. Treated carcasses were always significantly heavier than the control ones ($p \leqslant 0.01$).

Meatiness, as judged by professionals, was significantly improved after single ($p \leqslant 0.05$) or double ($p \leqslant 0.01$) implantation with Revalor (Fig. 3). Significant improvement was also observed after double treatment with Implix ($p \leqslant 0.01$) but not after single implantation. The carcass-index increased significantly in all treated groups (Table 2). Parallell with the

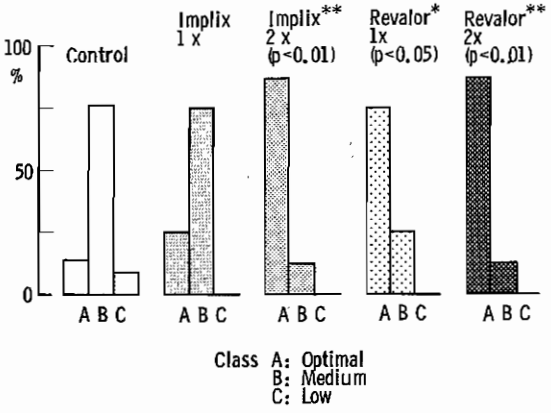


Fig. 3 Carcass Grade in Control, Singly Treated (1x), and Doubly Treated (2x) Groups

Table 2 Carcass-index and weight of the 3-rib cut (1x: single implantation; 2x: implantation at 8 and 4 weeks before slaughter)

Treatment	Number of animals	Carcass-index kg	Carcass-index % of control	3-rib cut Weight (kg)	3-rib cut % of control
Control	22	103.5 ± 1.8	100	3.48 ± 0.15	100
Implix (1x)	8	112.2 ± 2.4[c]	108	3.87 ± 0.08[c]	111
Revalor (1x)	8	114.5 ± 3.4[b]	111	3.85 ± 0.19	110
Implix (2x)	8	114.8 ± 2.2[b]	111	4.00 ± 0.11[c]	114
Revalor (2x)	8	118.2 ± 1.6[a]	114	4.09 ± 0.13[a]	117

Significant differences from control group:
[a] $p \leqslant 0.001$
[b] $p \leqslant 0.01$
[c] $p \leqslant 0.05$.

carcass-index, the weight of the 3-rib cut increased significantly in three of the treated groups. However, carcass colour was not significantly changed by treatment.

2.3. Carcass composition

The 3-rib cut is closely correlated with carcass composition. Treatment with Revalor increased the total meat percentage significantly over the control group (Table 3). Double treatment with Revalor reduced fat tissue ($p \leqslant 0.01$) and bone content ($p \leqslant 0.05$) of the 3-rib joint significantly. Both last effects are responsible for the increased meat yield observed with Revalor. Treatment with Implix increased meat yield and reduced bone content – though not significantly – of the 3-rib cut.

Table 3 Percentage composition of 3 rib cut (1x: single implantation; 2x: implantation at 8 and 4 weeks before slaughter)

Treatment	Number of animals	Musc. Long. Dorsi %	Total meat %	Fat %	Bone %
Control	8	11.06 ± 0.49	57.79 ± 0.86	23.85 ± 0.92	18.36 ± 0.49
Implix (1x)	8	10.22 ± 0.21	59.47 ± 1.00	23.24 ± 0.85	17.28 ± 0.32
Revalor (1x)	8	10.40 ± 0.53	61.29 ± 0.93[a]	21.73 ± 0.95	16.98 ± 0.49
Implix (2x)	8	10.20 ± 0.32	59.04 ± 0.92	24.02 ± 0.78	16.95 ± 0.88
Revalor (2x)	8	11.04 ± 0.18	63.11 ± 0.51[b]	20.49 ± 0.74[b]	16.49 ± 0.49[a]

Significant differences from control group:
[a] $p \leqslant 0.05$
[b] $p \leqslant 0.01$.

Chemical analysis of defatted meat obtained from the 3-rib cut (Table 4) showed not significant effect of treatment on moisture, protein, or ash content. The fat percentages were depressed and the collagen contents were increased ($p \leqslant 0.05$; paired samples) in the Revalor as compared to the control groups.

Table 4 Chemical analysis on defatted 3-rib cut (1x: single implantation; 2x: implantation at 8 and 4 weeks before slaughter)

Treatment	Number of animals	Moisture %	Protein %	Collagen %	Fat %
Control	8	76.12 ± 0.11	21.06 ± 0.20	0.58 ± 0.05	1.17 ± 0.09
Implix (1x)	8	76.43 ± 0.25	20.81 ± 0.22	0.74 ± 0.12	1.08 ± 0.11
Revalor (1x)	8	76.65 ± 0.39	20.77 ± 0.34	0.63 ± 0.07	1.02 ± 0.13
Implix (2x)	8	75.99 ± 0.23	20.68 ± 0.24	0.51 ± 0.02	1.19 ± 0.07
Revalor (2x)	8	76.36 ± 0.18	21.14 ± 0.31	0.76 ± 0.16	1.03 ± 0.09

2.4. Texture and water holding capacity of meat from m. Long. Dorsi

Increased shear values are observed after hormone treatment: this effect was significant ($p \leqslant 0.05$) in the Implix group treated at 8 and 4 weeks before slaughter (Table 5). Water holding capacity was not affected by hormone treatment. Increased cooking loss was observed in minced meat from the treated animals. This effect was significant ($p \leqslant 0.05$) in meat after treatment with Implix or single implantation of Revalor.

Table 5 Shear value, water holding capacity and cooking loss of meat from M. Long. Dorsi (1x: single implantation; 2x: implantation at 8 and 4 weeks before slaughter)

Treatment	Number of animals	Shear value	WHC Raw meat	Cooking loss mineed meat	Cooking loss meat slices
Control	8	8.8 ± 1.1	44.3 ± 1.7	21.1 ± 0.5	17.8 ± 1.6
Implix (1x)	8	9.3 ± 1.6	43.8 ± 1.4	23.1 ± 0.6	14.7 ± 1.1
Revalor (1x)	8	10.4 ± 0.7	44.7 ± 1.2	24.1 ± 1.1[a]	19.9 ± 1.6
Implix (2x)	8	12.2 ± 1.0[a]	44.8 ± 1.1	23.6 ± 0.8[a]	19.1 ± 1.2
Revalor (2x)	8	10.5 ± 1.2	45.4 ± 1.4	22.9 ± 1.2	17.9 ± 0.6

Significant differences as compared with control group:
[a] $p \leqslant 0.05$.

2.5. Residues of hormones in meat

No uterotropic effect was found in meat, sampled from the 3-rib joint. In meat sampled from the neck near the implantation site, 7 positive and 3 suspect samples were found by the Tiecco-method (Table 6) indicating a residue level of at least 0.5 ppm. By injection of these meat extracts to ovariectomized mice and examination by the Allen-Doisy method, 10 positive meat samples were found. The samples were evenly distributed between the Implix or Revalor groups implanted at 4 weeks before slaughter.

Examination by thin layer chromatography, after derivatization to fluorogenic compounds, indicated the presence of estradiol in at least 7 of these meat samples. One sample contained testosterone in addition to estradiol. No trienbolone was detected in any of the meat samples. The residues were detected in samples from the groups implanted at 4 or 4 and 8 weeks before slaughter. Three samples contained estrone in addition to estradiol.

In 13 of 32 of the treated animals, some remainder of the implants was found. Remainder of the implants was found after double implantation: 7 implants were recovered in the Revalor and 6 implants from the Implix group.

Table 6 Histological examination of prostate from veal calves after treatment with Implix or Revalor. Assay of residues in meat near the implanted site and remainder of implants (Number of animals: 8 controls, 32 treated animals)

Treatment		Histological examination of prostate		Meat sampled from neck (1–2 vertebra)			Remainder of implants
				Biological assays		Chemical analysis	
		Lab. 1	Lab. 2	Tiecco	Allen-Doisy		
Implix®	control	–	–	–	–	–	–
	8 wk before slaughter	4	3	–	1	–	–
	4 wk before slaughter	4	4	1	1	1 (oestradiol + testosterone)	–
	(8 + 4) wk before slaughter	8	8	2	4	a1 (oestrone + oestradiol) 1 (oestradiol)	6
Revalor®	8 wk before slaughter	4	4	–	–	–	–
	4 wk before slaughter	4	3	2	2	2 (oestradiol)	1
	(8 + 4) wk before slaughter	8	7	2	2	2 (oestradiol + oestrone)	6
	Total:	32	29	7	10	7a	13

a 2 samples lost.

2.6. Histological examination of the prostate

The *pars disseminata* was examined by two independent laboratories on HE-coloured frozen and paraffin sections. Both methods revealed, after hormone treatment, increased secretory activity, hyperplastic, and metaplastic changes. However, the hyperplasia and metaplasia were not generalized to the whole tubuli but were restricted to different foci. This necessitated a close examination of the whole section and frequently to substantiation of the diagnosis on frozen sections by the paraffin method. Compared to Implix, Revalor induced a more pronounced mucous activity. One laboratory failed to detect 3 treated animals on frozen sections but only failed to detect one after the examination of frozen and paraffin sections; the other indicated correctly all the treated animals.

3. Conclusions

Treatment of male veal calves with Implix or Revalor increased weight gain, feed conversion, and carcass weight significantly over the controls. The time of administration was of decisive importance for the effects obtained. Implix applied at 8 weeks before slaughter led to an improvement in growth rate and feed conversion which declined in the period immediatly before slaughter. This was not observed with Revalor. Treatment with Revalor resulted in a significant beneficial effect over the Implix groups treated similary. Meatiness, as measured from different parameters, was significantly increased by hormone treatment. Treatment with Revalor decreased bone and fat content of the carcass significantly over the controls. Increased shear values and cooking losses were observed in the treated calves. The chemical composition of the lean meat was not significantly modified by the treatments.

Biological tests indicated the presence of estrogenic residues in meat near the implanted site. No residues were found in the meat sampled from the 3-rib cut. The biological assays indicated the presence of estrogenic residue in 25 to 50% of the animals implanted at 4 or 4 and 8 weeks before slaughter. This was confirmed by chemical analysis of this meat: in the group treated twice, estrone was detected in addition

to estradiol. In the last groups some remainder of implants was detected in 75% of the treated calves.

Histological examination of the *pars dissimata* revealed increased secretory activity and hyperplasia restricted to different foci in the treated animals. Histological examination of paraffin and frozen sections enabled to detect practically all treated animals.

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