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## Introduction:

The effects of anabolic sex hormones play a significant role in the establishment of muscle tissue. Therefore anabolic steroids have been used in animal production management schemes for several years to increase growth rate and feed efficiency. In the EU the use of hormonal growth promoting substances has been banned since 1988, because used in cattle they might possess a potential health risk to consumers. In other countries, e.g. USA and Canada, six hormones are allowed as growth promoters: zeranol, trenbolone acetate (TBA), melengestrol acetate (MGA), 17 $\beta$ -oestradiol, progesterone and testosterone (Figure 1).

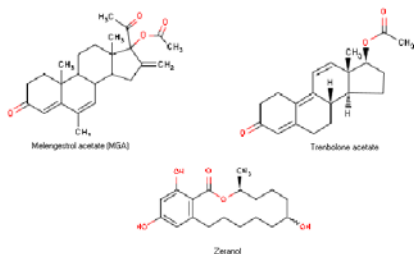


Fig. 1: Hormonal growth promoter

## Study design:

For this study 24 heifers were separated into three groups of eight heifers and were treated over eight weeks with multiple-dosages (0-, 1-, 3- and 10-fold) of either MGA (Melengestrol acetate, synthetic progestin), Ralgro (Zeranol, synthetic oestrogen) or Finaplex (Trenbolone, synthetic androgen). In each study two animals served as control, two received single, two 3-fold and two 10-fold dose of the recommended preparation. After selecting an assortment of anabolic sex hormone dependent genes from different biological functional groups (endocrine, adipolysis, oncogene, protein and amino acid metabolism, inflammatory, apoptosis or transcription) in the bovine liver, measurements of expression patterns were undertaken by quantitative real-time polymerase chain reaction (qRT-PCR).

**Aim of this study was to find treatment specific differentially regulated target genes, which can be used for further more detailed investigations**

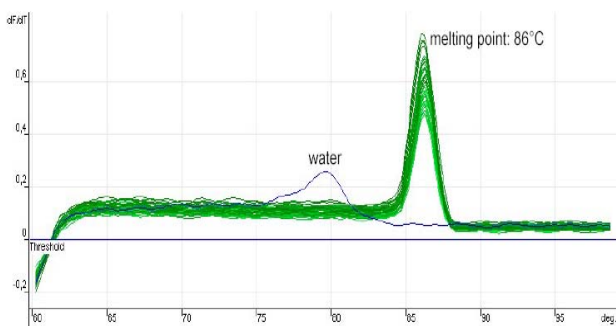


Fig. 2: Representative Melting curve for CTSL- PCR product

## Results:

All investigated genes showed specific products and single peaks in melting curve analysis (Figure 2). Out of 24 examined genes, 6 genes showed a significant positive or negative regulation ( $p < 0.05$ ) under the influence of MGA, 6 genes under the influence of Finaplex, 3 genes under the influence of Ralgro (Figure 3), 3 genes under the influence of two different treatments either MGA or Finaplex and 2 genes under the influence of two different treatments either Finaplex or Ralgro. Only 4 of the observed genes remained unaffected by any of these treatments. The number of examined regulations confirm the used approach in selecting specific genes from functional groups which are associated with an anabolic sex hormone treatment, as shown in Table 1.

**Table 1:** Linear regression coefficients of dosages regressed on  $\Delta\Delta CP$  with corresponding significance level based on a 10- fold treatment as shown in Figure 3. Significant up-regulated genes are highlighted in green, and down-regulated genes in red [ \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ ]

Group	Target gene	MGA				FINAPLEX				RALGRO			
		up	down	r	sig. level	up	down	r	sig. level	up	down	r	sig. level
endocrine	IGF-1												
	IGF1-R												
	IGF-BP3												
	IR-alpha					+0.86		0.09	*	-0.06		0.07	*
	IR-beta	+0.36		0.08	*								
	PRLR	+0.84		0.24	**								
adipolysis	GR-alpha									+0.86		0.34	***
	GHR	+0.87		0.08	*	+1.02		0.25	**				
	EnoylCoA					+0.52		0.18	**				
oncogene	ACADvl					+0.61		0.25	**				
	p53		-0.65	0.09	*								
prot&amino	v-myb	+1.75		0.21	**	+1.45		0.09	*				
	CAST	+0.53		0.07	*								
inflammatory	CTSB					+0.96		0.33	***				
	CTSL	+1.17		0.24	**								
apoptosis	IL-1-beta					+0.78		0.23	**				
	Cox2		-1.3	0.07	*								
transcription	TNF-alpha												
	bcl-xl					+0.66		0.46	***				
others	FasL					+0.43		0.08	*	-1.42		0.29	***
	c-fos	+1.16		0.1	*			-1.6	0.19	**			
others	c-jun												
	CALR					+0.53		0.06	*				
	Cyp11A1										-1.07	0.12	**

## Discussion:

The study gives a first impression about the role of possible candidate genes and its expression patterns in drug screening sorted by its functional groups, with the aim to be used for veterinary drug screening at the level of toxicological relevance. After these examinations it seems interesting for the future to follow this candidate gene approach and to add more well-characterized genes to the functional groups and even extend the group selection.

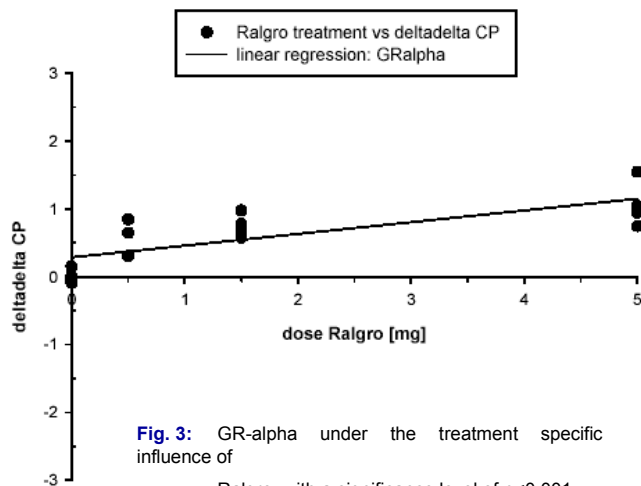


Fig. 3: GR-alpha under the treatment specific influence of Ralgro with a significance level of  $p < 0.001$