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# Differences in Natural Steroid Hormone Patterns of Beef from Bulls and Steers<sup>1</sup>

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**ABSTRACT:** This investigation gives an overview of the concentrations of naturally occurring androgens, progestogens, corticosteroids, and their precursors and metabolites in meat from bulls and steers. A recently developed gas chromatography-mass spectrometry (GC-MS) method with improved sensitivity for steroid analysis was used. Eighty-two beef samples were analyzed using the GC-MS method. Beef from bulls contained higher concentrations of testosterone, which is an anabolic androgen, and its metabolite epitestosterone ( $P < .01$ ) and the androgen precursor dehydroepiandrosterone ( $P < .05$ ) than beef from steers. Beef from steers contained higher

concentrations of the basic hormone precursor pregnenolone and cortisol, which is a catabolic corticosteroid, than beef from bulls. A classification of an unknown beef sample to one of the categories (bull or steer) was possible in most cases (>90%) using a masculinity index (MI) that was calculated using the concentrations of testosterone, epitestosterone, and pregnenolone. Because the hormonal status of beef cattle is related to meat quality characteristics, such as tenderness or fat and protein distribution, the MI may contribute to meat quality assessment and meat quality control.

Key Words: Beef, Steers, Bulls, Androgens, Progestogens, Corticoids

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## Introduction

In some countries (e.g., Germany), finishing bulls rather than steers is the predominant practice because of bulls' faster growth rate. This higher growth rate compared with that of castrated or female cattle seems to be due to the higher production of natural anabolic hormones by the testes (Lee et al., 1990). Because the use of hormones as growth promoters is not allowed in the European Union for finishing purposes (Directive 88/146/EEC), the finishing of steers and heifers is less economical. However, meat from steers and heifers is preferred because of its better sensory traits (Seideman et al., 1989). Some of the quality characteristics, particularly the proportions of protein and fat, are influenced by steroid hormones (Gariépy et al., 1990). Relationships between serum androgen levels and connective tissue and muscle fiber composition have also been reported (Young and Bass, 1984; Gerrard et al., 1987).

A distinction between beef from male and female cattle is made possible by determining the ratio of progesterone to its precursor, pregnenolone, in their tissues (Hartwig et al., 1997). A distinction between beef from bulls and steers has not been possible because the physiological concentrations of androgens and their metabolites often are below the determination limit of analytical methods. Recently, Hartmann and Steinhart (1997) reported a sensitive gas chromatography-mass spectrometry (GC-MS) method that can distinguish among structurally related hormone and metabolite compounds.

The purpose of the current research was to use the GC-MS method of Hartmann and Steinhart (1997) to compare the hormone patterns in meat samples from beef steers and bulls. The samples were taken from animals of different breeds, feeding conditions, and slaughter weights; the animals had not been treated with anabolics. The cuts contained inter- and intramuscular fat and were taken from different parts of the carcasses in an attempt to get universal patterns.

## Materials and Methods

*Meat Samples.* All samples analyzed were of known origin (i.e., taken from various animal trials that were performed for different purposes). The assignment of

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each animal to a classification (bull or steer) and the lack of treatment with steroid hormones were verified. Bulls ( $n = 40$ ) were of the German Simmental breed, and steers were German Simmental ( $n = 30$ ) and Hereford  $\times$  Angus ( $n = 9$ ). Additionally, one Holstein, one Red Pied, and one Holstein  $\times$  Red Pied steer were analyzed. Sixty-one samples were taken from mature animals (slaughter weights of 450 to 650 kg). Four samples were taken from bulls and four from Simmental steers slaughtered at 200 kg. Seven and six samples were taken from Simmental bulls and steers, respectively, slaughtered at 350 kg. The rearing and fattening of the Simmental cattle has been described by Schwarz et al. (1992). The meat samples were taken from the back (longissimus muscle) and the leg (semitendinosus and extensor carpi ulnaris muscles). Samples were stored at  $-20^{\circ}\text{C}$  before analysis. Subcutaneous fat and epimysium were removed before homogenization.

**Steroid Hormones.** The steroid hormones and their precursors and metabolites analyzed in this study are presented in Figure 1. The following reference standards were purchased 1) from Serva (Heidelberg, Germany): androstenedione (4-androstene-3,17-dione), androsterone (5 $\alpha$ -androstane-3 $\alpha$ -ol-17-one), testosterone (4-androstene-17 $\beta$ -ol-3-one), and progesterone (4-pregnene-3,20-dione); 2) from Sigma (Deisenhofen, Germany): cortisone (4-pregnene-17 $\alpha$ ,21-diol-3,11,20-trione), dehydroepiandrosterone (**DHEA**; 5 $\alpha$ -androstene-3 $\beta$ -ol-17-one), dihydrotestosterone (5 $\alpha$ -androstane-17 $\beta$ -ol-3-one), epitestosterone (4-androstene-17 $\alpha$ -ol-3-one), hydroxyprogesterone (4-pregnene-17 $\alpha$ -ol-3,20-dione), and pregnenolone (5-pregnene-3 $\beta$ -ol-20-one); and 3) from Merck (Darmstadt, Germany): cortisol (4-pregnene-11 $\beta$ ,17 $\alpha$ ,21-triol-3,20-dione).

**Hormone Analysis.** A 20-g portion of each meat sample was subjected to hormone analysis. Analysis was performed with GC-MS according to Hartmann and Steinhart (1997), excluding the determination of the estrogens. In brief, sample preparation consisted of the extraction of steroids with methanol/H<sub>2</sub>O and the removal of proteins, fat, carbohydrates, and salts by heating, liquid-liquid extraction, and solid-phase extraction. Acidic and phenolic compounds (including estrogens) were removed by extraction with KOH and discarded. Corticosteroids were separated from the less-polar steroids and purified separately. For GC-MS determination, the purified extracts were combined and derivatized to trimethylsilyl ethers and trimethylsilyl enoethers.

**Statistics.** Because some of the physiological hormone concentrations were below the determination limit, median values and the range (5th to 95th percentile) are given. Variances were analyzed by *F*-test (using a statistical program package; SPSS, 1995). To compare the hormone concentrations of bulls and steers, the nonparametric *U*-test of Mann and Whitney was used (Brosius and Brosius, 1995).

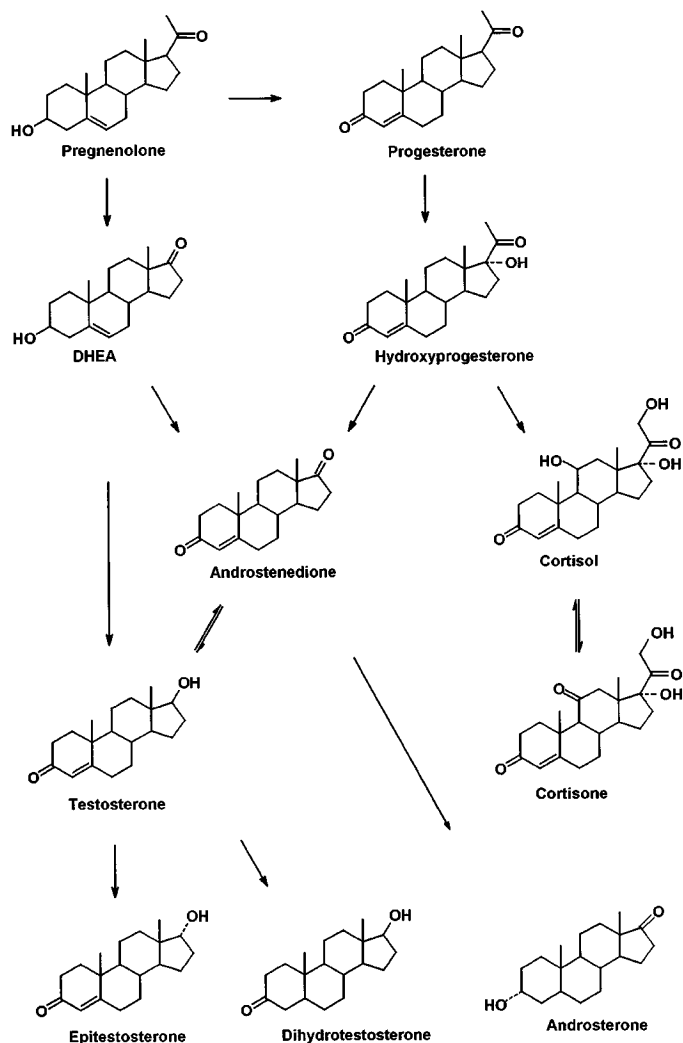


Figure 1. Biosynthetic relationships between the investigated steroids. DHEA = dehydroepiandrosterone.

## Results and Discussion

Steroid hormones were selected on the basis of their biosynthetic relationships and their known occurrence in beef. They included anabolic and catabolic steroid hormones as well as their precursors and metabolites to establish comprehensive hormone patterns. Estrogens were not considered because their physiological concentrations in muscle tissue of bulls and steers were at or below the determination limit of the GC-MS method chosen. There is little evidence that physiological concentrations of estradiol are important in determining relative carcass composition of bulls and steers because little difference occurs in concentrations in meat from bulls and steers (Gettys et al., 1988). Existing data concerning estrogens in beef are summarized in a review published recently (Hartmann et al., 1998).

Previous studies on the contents of steroid hormones in beef have primarily focused on testosterone, progesterone, and estrogens. Gaiani and Chiesa

Table 1. Concentrations of steroid hormones in beef from bulls and steers ( $\mu\text{g}/\text{kg}$ ); total number of samples; number of samples with determinable steroid concentrations, median, and range (5 to 95 percentile); and differences between the contents (*U*-test)<sup>a</sup>

Steroid	Bulls				Steers				U <sup>b</sup>
	n	Positive	Median	Range	n	Positive	Median	Range	
Pregnenolone	40	40	.67	.35–2.37	42	42	1.24	.31–3.07	S
Dehydroepiandrosterone	40	40	.24	.08–1.51	42	42	.19	.08–.53	S
Progesterone	40	39	.17	.06–.60	42	40	.23	.01–2.33	NS
Hydroxyprogesterone	36	23	.01	<.01–.12	42	27	.03	<.01–.15	NS
Androstenedione	40	40	.39	.04–.78	42	42	.24	.05–.92	NS
Testosterone	40	40	.34	.08–1.05	42	28	.01	<.01–.14	HS
Dihydrotestosterone	40	19	<.03	<.03–.56	42	16	<.03	<.03–.50	NS
Epitestosterone	38	38	.31	.08–.80	42	42	.12	.03–.28	HS
Androsterone	39	38	.07	.02–.19	42	42	.07	.02–.38	NS
Cortisone	34	34	1.25	.17–3.39	42	41	1.28	.06–3.17	NS
Cortisol	34	34	4.33	1.07–10.1	42	42	5.78	.42–14.5	S

<sup>a</sup>Brosius and Brosius, 1995.

<sup>b</sup>Resulting *U*-test, where S = significant ( $P < .05$ ), HS = highly significant ( $P < .01$ ), and NS = not significant ( $P > .05$ ).

(1986) also considered the hormone precursor androstenedione, and Gariépy et al. (1990) considered the catabolic corticosteroid cortisol. Just one study considered hormones as well as precursors and metabolites in muscle tissues of bulls, steers, and heifers (Hartwig et al., 1997). Only the basic precursor pregnenolone could be determined in 100% of the beef samples analyzed by these authors. Other steroids analyzed were detectable in about 10% (dihydrotestosterone, hydroxyprogesterone, and pregnene-20 $\alpha$ -ol-3-one), 30% (testosterone and androsterone), 40 to 50% (androstenedione, DHEA, and epitestosterone), and 70% (progesterone) of meat samples from bulls and steers.

Using the GC-MS method developed by Hartmann and Steinhart (1997), almost all physiological levels of the hormones investigated were measurable (Table 1), except for dihydrotestosterone (below the determination limit in 50% of the bull and 60% of the steer samples) and hydroxyprogesterone (below the determination limit in 40% of the bull and steer samples). Testosterone could be determined in 67% of the meat samples from steers.

**Concentrations of Hormone Precursors.** The concentrations of the basic precursor, pregnenolone, were higher in beef from steers than in beef from bulls ( $P < .05$ ); however, the ranges overlapped (Table 1). Hartwig et al. (1997) investigated the hormone patterns of 11 bulls and 30 steers and found no differences in pregnenolone concentrations, which ranged from .8 to 5.0  $\mu\text{g}/\text{kg}$  for bulls and .5 to 5.9  $\mu\text{g}/\text{kg}$  for steers, with median values of 1.7  $\mu\text{g}/\text{kg}$  for both bulls and steers. We could not detect differences between the tissue concentrations of the metabolic intermediates progesterone and hydroxyprogesterone in the bulls and steers. However, concentrations of these intermediates tended to be slightly higher for steers than for bulls. The values obtained were somewhat similar to the results of Hartwig et al.

(1997) of <.3 to .4 and <.3 to 1.7  $\mu\text{g}/\text{kg}$  for progesterone and of <.3 and <.3 to .3  $\mu\text{g}/\text{kg}$  for hydroxyprogesterone in bulls and steers, respectively. The higher concentrations of the lipophilic C<sub>21</sub> precursors in meat from steers may be due to the higher relative amounts of fat in their beef samples (e.g., roast beef samples from the Simmental cattle analyzed: steers 1.9 to 7.7% intramuscular fat vs bulls 1.7 to 4.2%; from Otto et al., 1993 and Reimann et al., 1993). Other researchers have reported progesterone concentrations in meat from steers of  $.27 \pm .33 \mu\text{g}/\text{kg}$  (Kushinsky, 1983) and  $3.89 \pm .77 \mu\text{g}/\text{kg}$  (Tsujioka et al., 1992). The latter seems to be comparatively high and might be due to differences in analytical procedures.

The concentrations of the weakly androgenic intermediates DHEA and androstenedione were higher in beef from bulls than from steers ( $P = .03$  and  $.05$ , respectively, Table 1) and are in accordance with results of Hartwig et al. (1997), who reported median values of .3 (a range of .2 to .5)  $\mu\text{g}/\text{kg}$  for DHEA and .6 (<.2 to 1.2)  $\mu\text{g}/\text{kg}$  for androstenedione in bulls and of <.2 (<.2 to .7)  $\mu\text{g}/\text{kg}$  for DHEA and <.2 (<.2 to 2.5)  $\mu\text{g}/\text{kg}$  for androstenedione in steers. Gaiani and Chiesa (1986) reported similar androstenedione concentrations of  $.37 \pm .05 \mu\text{g}/\text{kg}$  in muscle tissue of bulls.

**Concentrations of Androgens and Metabolites.** The concentrations of testosterone and its metabolite, epitestosterone, were higher in meat from bulls than in meat from steers ( $P < .001$ , Table 1). Testosterone concentrations in meat from bulls were similar to others reported:  $.73 \pm .10 \mu\text{g}/\text{kg}$  ( $n = 6$ ; Gaiani and Chiesa, 1986) and  $.78 \pm .73 \mu\text{g}/\text{kg}$  ( $n = 8$ ; Hoffmann and Rattenberger, 1977). Hartwig et al. (1997) were able to determine testosterone in 10 of 11 meat samples from bulls (median, .5; range, <.2 to 2.8  $\mu\text{g}/\text{kg}$ ) but only in 1 of 30 meat samples from steers (.4  $\mu\text{g}/\text{kg}$ ). Gariépy et al. (1990) could not determine significantly different testosterone concentrations in

beef from castrated and intact male cattle (steer:  $1.05 \pm .57 \mu\text{g/L}$  muscle fluid [ $n = 7$ ] vs bull:  $1.44 \pm .86 \mu\text{g/L}$  [ $n = 8$ ]). The only report of tissue concentrations of epitestosterone (Hartwig et al., 1997) indicated that a considerable percentage of samples was below the determination limit of the analytical method (steers:  $<.2$  to  $.4 \mu\text{g/kg}$ , 8 out of 10 samples  $<.2 \mu\text{g/kg}$  vs bulls:  $<.2$  to  $.6 \mu\text{g/kg}$ , 2 out of 11 samples  $<.2 \mu\text{g/kg}$ ). We could not find any difference between the concentrations of the androgen metabolites dihydrotestosterone and androsterone in meat samples from bulls and steers (Table 1). The values agree with Hartwig et al. (1997), who reported  $<.2$  to  $.5 \mu\text{g/kg}$  androsterone (21 out of 30 samples  $<.2 \mu\text{g/kg}$ ) and no determinable concentrations ( $<.2 \mu\text{g/kg}$ ) of dihydrotestosterone in beef from steers and  $<.2$  to  $.2 \mu\text{g/kg}$  androsterone (9 out of 11 samples  $<.2 \mu\text{g/kg}$ ) and dihydrotestosterone (7 out of 11 samples  $<.2 \mu\text{g/kg}$ ) in beef from bulls.

**Concentrations of Corticosteroids.** Cortisol concentrations are reported to be higher in serum of steers than in serum of bulls (Lee et al., 1990). Circulating cortisol concentrations are negatively correlated with growth rate and lean tissue accumulation, as a result of enhanced protein degradation (Gettys et al., 1988). However, only few data are available concerning corticosteroids in tissues. Sauerwein et al. (1991) measured  $.52$  to  $.57 \mu\text{g/L}$  cortisol in muscle cytosol of Simmental calves (live weight: 155 kg). They could not detect any difference between male and female calves or between muscles from the neck, shoulder, abdomen, and hind leg. Gariépy et al. (1990) reported  $10.62 \pm 8.33 \mu\text{g/L}$  and  $6.52 \pm 5.16 \mu\text{g/L}$  of cortisol in muscle of steers and bulls, respectively. The difference between the concentrations was not significant. In the present study, we observed no difference in cortisone concentrations but did observe a difference in cortisol concentrations ( $P < .05$ ) between bulls and steers. The cortisol concentrations were in good agreement with the data of Gariépy et al. (1990) but exceeded the values of Sauerwein et al. (1991) approximately 10-fold. This discrepancy might be due to differences in the types of samples (cytosol vs whole muscle), indicating the occurrence of matrix-bound cortisol, or to the differing age of the animals, or to the stress status, which has been shown to have an influence on cortisol concentrations in pigs (Shaw et al., 1995).

**Distinction Between Beef from Bulls and Beef from Steers.** An unequivocal distinction between bull and steer meat is not possible by considering only the concentrations of the principal androgen, testosterone, because some bulls show comparatively low testosterone concentrations (independent of age group). In 25% of the bull samples analyzed, testosterone concentrations were lower than  $.2 \mu\text{g/kg}$ . It is therefore advisable to consider other hormones that differ between bulls and steers.

The assessment of hormone profiles or ratios of hormones to their precursors or metabolites in urine is common for the detection of drug abuse or defects in

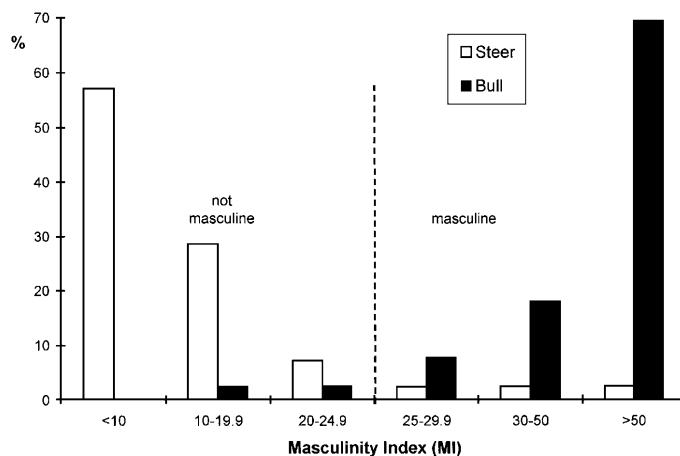


Figure 2. Frequency distribution of masculinity index.

steroid metabolism in humans and other animals (Gaskell, 1989; Bonnaire et al., 1995). Scippo et al. (1994) used plasma concentrations of hormone precursors and metabolites to set decision limits for distinguishing between untreated animals and bulls and heifers treated with natural hormones. Hartwig et al. (1997) could distinguish between beef from male and female cattle by considering the ratio of the female hormone progesterone to its precursor pregnenolone. The division by the precursor minimizes individual influences of the animals and influences of the fat content of the analyzed sample.

The results of the  $U$ -test show that the most important differences occurred in the concentrations of testosterone and its metabolite, epitestosterone ( $P < .001$ ), which were higher in bulls than in steers, followed by the concentrations of pregnenolone ( $P = .015$ ), which were higher in meat from steers than in meat from bulls. The performance of a discriminant analysis was not allowed, because the variances of most of the steroid concentrations differed significantly between bulls and steers ( $F$ -test). An empirical masculinity index (MI) was calculated, which consists of an addition of the testosterone (T) and epitestosterone (epiT) concentrations. Because T exerts a higher discriminating property than epiT, the influence of the latter was weighted by the factor .64. The androgen sum was divided by the square root of the pregnenolone (Preg) concentration (values in  $\mu\text{g/kg}$ ).

$$\text{MI} = \frac{\text{T} + \text{epiT} \times .64}{\sqrt{\text{Preg}}} \times 100$$

The frequency distribution of this MI among bulls and steers is illustrated in Figure 2. Thirty-nine of 42 steer samples had a MI  $< 25$  (median = 8). Thirty-seven of 39 bull samples had a MI  $> 25$  (median = 70). By using this quotient, 94% of the samples were correctly

classified. The five falsely classified samples were taken from varying muscles of Simmental (which was the main breed analyzed) cattle slaughtered at different ages.

The only researcher who determined T, epiT and Preg in cattle tissues was Hartwig (1993). He analyzed 11 beef samples from bulls, 10 from steers, and 3 from heifers for these three steroids. Nine of the 11 bull samples had a MI > 25. Two samples could not be classified unequivocally because one or both of the androgens were not determinable. The same was true for seven of the steer and two of the heifer samples. Of the remaining four samples, three were classified as not masculine and one steer as masculine.

We conclude from the present results that the MI does not seem to be restricted to a particular breed, slaughter age, or muscle, because it was applicable to each type of sample examined in a comparable manner. A further study that only considered animals with little genetic and environmental differences revealed no correlation of the MI with fat content ( $r = .11$ ) and age ( $r = .01$ ) in the case of bulls and only weak correlations (fat content:  $r = .19$ ; age:  $r = .35$ ) in the case of steers (Fritsche et al., unpublished data).

### Implications

A distinction between beef from bulls and beef from steers is possible by using a masculinity index (MI) that is calculated from the testosterone, epitestosterone, and pregnenolone concentrations of meat samples. By means of the MI, a classification of a beef sample of unknown origin can be performed for the first time, regardless of the cut or slaughter age of the animal. The MI was successfully applied to Simmental cattle and to Hereford  $\times$  Angus, Holstein, and Red Pied steers.

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