DOES MEAT FROM CLENBUTEROL-FED HEIFERS TENDERISE?

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SUMMARY

Eight Charolais heifers weighing approximately 300 kg were fed a diet containing either 0 (control) or 1 ppm clenbuterol during 5 weeks. They were slaughtered after a week of withdrawal.

Calpastatin activity was higher in *Longissimus dorsi* muscle from clenbuterol-fed heifers at 0 and 1st days *post mortem*, while activity of μ-calpain was lower at 0 day for the treated animals. Activity of m-calpain was similar for clenbuterol-fed and control animals.

Meat from clenbuterol-fed heifers was significantly tougher than meat from control ones, at both 1st and 8th days *post mortem*, as evidentiated by the results of Warner-Bratzler shear force and the sensory evaluation. An additional finding was that meat from clenbuterol-fed heifers did not tenderise throughout aging.

According to our findings it seems possible to detect the administration of b-adrenergic agonist clenbuterol to animals by simply determining maximum stress and/or stress at yield in meat at 1st and 8th days *post mortem*.; furthermore, the parameter "slope after yield" is able to differentiate clenbuterol-fed and control animals at 1st day *post mortem*.

INTRODUCTION

Several b-adrenergic agonists (BAA) are being evaluated for safety and efficacy as repartitioning agents in a wide variety of meat animals. The b-adrenergic agonists will reduce the fat content of the carcass by 5 to 10% and will increase muscle mass to the same extent (Baker et al., 1984; Watkins et al., 1990). The response for poultry is not as large as that found in pork or beef (Gwartney et al., 1991).

Endogenous enzyme activity is affected by BAA. It is generally accepted that calpain/calpastatin system is modified by BAA. There are some discrepancies on the effect on calpains, several authors have confirmed that activity of μ-calpain is reduced in animals fed with BAA (Kretchmar et al., 1989; Pringle et al., 1993) while other authors indicate that activity of m-calpain increased with BAA administration (Higgins et al., 1988; Koohmaraie and Shackelford, 1991). But there is a general agreement that these compounds brought about an important increase in the inhibitory activity of calpastatin (Wheeler and Koohmaraie, 1992).

Consistent with the role of calpains in *post mortem* tenderisation (Koohmaraie, 1988, 1992), *post mortem* muscle proteolysis and meat tenderness were decreased with BAA feeding (Kretchmar et al., 1990; Koohmaraie et al., 1991a). Most of the studies with BAA reported increases in shear force of muscle evaluated (Gwartney et al., 1991; Koohmaraie et al., 1991; Pringle et al., 1993). Wheeler and Koohmaraie (1992) have demonstrated that muscles from steers fed with L644,969 did not tenderise during aging. Merkel (1988) suggested that all BAA did not affect tenderness to the same extent, and Jeremiah et al. (1994) have demonstrated that ractopamine can be administered to pigs in order to improve production efficiency and carcass composition without influencing palatability and cooking properties or consumer acceptance.

Several methods have been used for assesing clenbuterol. Thin layer chromatography and high performance liquid chromatography (HPLC) are the most frequently used (Hamann et al., 1985). Nowadays enzyme immunoassay screening and gas chromatography mass spectroscopy confirmation is the most suitable method for detection of clenbuterol (Elliott et al., 1993; García Regueiro et al., 1993).

Since it has been evidentiated that the use of BAA in general, and particularly clenbuterol , bring about tougher

meat, the aim of this research is to find out an objective method based on meat quality for demonstrating the use of clenbuterol or other beta-adrenergic agonists.

MATERIAL AND METHODS

Material

After a 7-days adaptation period, eight Charolais heifers of about 8 months of age and weighing aproximately 300 Kg were individually bucket-fed *ad libitum* a diet containing either 0 ppm (4 control animals) or 1ppm clenbuterol (4 treated animals) during 5 weeks. They were slaughtered after a week of withdrawal.

The life weight of the animals was measured at arrival and once a week during all the period until slaughter and Daily Live Weight Gain (DLWG, g/day) was calculated.

A portion of *M. Longissimus dorsi* was excised from animals after death for determining enzymatic activities as is described bellow. The rest of *M. Longissimus dorsi* was excised 24 h after death and stored under vacuum during 7 days for subsequent analysis.

pH of tissue. About 3 g of *M.Longissimus dorsi* were homogenised in 20 ml distilled water for 15 s. The measurement was carried out immediately using a Crison pH-meter with a combined glass electrode.

Cross-sectional area (cm^2) of *M. Longissimus dorsi* was measured at 12th rib.

Intramuscular Fat (IF) of *M. Longissimus dorsi* was analysed according to Soxlet method.

Preparation of muscle extracts. A portion of *M.Longissimus dorsi* was removed from each animal as soon as possible after slaughter (30-60 min) and processed immediately for determining calpain and calpastatin activities. Other portions of muscle were obtained the 1st and the 8th day *post-mortem*. Muscle was trimmed and minced.

Preparation of calpains. Partial purification of calpains and calpastatin from muscle tissue was performed according to the method described by Ducastaing et al. (1985) and adapted to HPLC. Muscle tissue (20 g) was homogenised with Ultraturrax in 3 vol. of 10 mM Tris-HCl buffer pH 7.5 containing 0.05 M NaCl, 4 mM EDTA, 2 mM 2-mercaptoethanol and 1 mM NaN_3. After 1h extraction under magnetic stirring, the homogenate was centrifuged at 30.000g for 30 min, the supernatant filtered through cheese cloth and then adjusted to pH 7.5. Precipitated material was eliminated by centrifugation at 50.000g for 50 min. All operations were performed at 0-4°C with pre-cooled solutions. Aliquots of 50.000g supernatant were filtered through 0.22 μm millipore membrane and loaded on a mono Q HR 10/10 column (Pharmacia) equilibrated in 5 mM Tris-HCl buffer pH 7.5, 0.1 mM EDTA, 0.05 M NaCl and 2 mM 2-mercaptoethanol. Protein elution by a non-linear NaCl gradient (0.05 - 0.5 M) was performed at a flow rate of 1 ml/min and fractions of 1 ml were collected.

Assay for calpain activity. Calcium-dependent proteolytic activity was assayed, according to the procedure described by Koohmaraie et al. in 1986, using casein (Hammerstein) as substrate at 25°C in 10 mM KCl, 50 mM tris-acetate, pH 7.5, 10 mM MCE, 2.5 mM Ca^{2+} and 5 mg/ml casein. Total reaction volume was 2 ml. Control for enzyme as substrate accompanied each assay. The reaction was initiated by addition of calpain and stopped by addition of 2 ml of 5% trichloroacetic acid (TCA).

The assay tubes were then centrifuged at 1000 x g for 15 min and the absorbance of the supernatant was measured at 278 nm.

Assay for inhibitor activity. The activity of the inhibitor was determined by pre-incubating appropriate amounts of inhibitor and enzyme at 25°C for 60 min in 1.5 ml reaction mixture (Koohmaraie et al., 1986).

Sensory evaluation. The 1st and the 8th days *post-mortem*, after aging at 4°C under vacuum, ribs were cut into 1.2 cm steaks and frozen for subsequent taste panel evaluation.

The steaks were thawed at 4°C for 24 h prior to cooking and serving. Steaks were placed in a preheated grill at 160°C and removed when internal temperature had reached 70°C. Muscle strips were served on preheated plates to be evaluated by a trained panel consisting of twelve members.

Tenderness at first chew, overall tenderness, juiciness, fibrousness, residue and overall acceptability were scored by placing a mark on an unstructured 100 mm line scale anchored at the ends with the terms "extremely tough", "extremely dry", "low quality" on the left and "extremely tender", "extremely juicy" and "high quality" on the right.

Objective texture measurements. The following texture parameters were investigated with an INSTRON mod. 4301 (Warner-Bratzler shear blade): stress at maximum, toughness, stress at yield, energy at yield and slope after yield point.

Meat samples were prepared as described previously for sensory evaluation, rectangular pieces of about 6 cm long x 1 cm high x 1 cm wide were placed inside the Warner-Bratzler shear blade to be sheared perpendicularly to the

longitudinal axis.

RESULTS AND DISCUSSION

Clenbuterol-fed heifers (table 1) had a higher Daily Live Weight Gain (DLWG, g/day), cross-sectional area (cm^2) of *M. Longissimus dorsi* was larger in treated animals although not significant, and there was not difference in Intramuscular Fat (IF) between control and treated animals. It is well documented that animals treated with BAA had less fat; most of the researchers have demonstrated a decrease in fat depots (McKeith, 1993) and others indicate that intramuscular fat is reduced in animals-fed BAA (Fiems et al., 1990; Berge et al., 1993). Final pH was not affected by clenbuterol treatment in good agreement with most of the researchers (Fiems et al., 1990; Berge et al., 1993); however, several authors have found higher final pH in pigs treated with salbutamol (Warris et al., 1990).

μ-calpain activity was lower in treated animals (31% on 3h pm); m-calpain activity was not significantly affected by clenbuterol treatment; and calpastatin inhibitory activity was increased as a consequence of feeding animals with clenbuterol (49% of increase on 3h pm). The rate μ-calpain/calpastatin was reduced in treated animals (53% on 3h pm). These results agree with most research done with BAA. The direct consequence is a reduced proteolytic activity during aging in muscles from animals treated with these agents (Kretchmar et al., 1989, 1990; Wheeler and Koohmaraie, 1992; Pringle et al., 1993).

Table 2 shows the results obtained in specific texture profile designed for this experiment (see Material and Methods). The most important differences were found on the 8th day pm, where these were significant ($p<0,05$) between controls and meat from clenbuterol-fed heifers in tenderness at first chew, overall tenderness and overall acceptability. It must be pointed out that meat from treated animals tenderised less than meat from the control ones, but the differences are not significant probably due to the reduced number of animals used. Control samples were the most tender and the best accepted. In contrast, treated samples were tougher and less accepted.

Results concerning the texture parameters investigated with an INSTRON (Warner-Bratzler cell) are shown in table 3. The most discriminate parameters for detecting meat from clenbuterol-fed heifers were: stress maximum, stress at yield and slope after yield point. Differences between control and treated samples obtained at 8th day pm are significant

In Figure 1 are represented the two first principal components after analysis of differences between 1st and 8th day *post-mortem* for some descriptors from sensory and objective analysis of texture. The component 1 (PC1) accounts for the 63% of the total variation, and is determined by overall acceptability and by some of the rest of the texture parameters studied {sensory parameters: toughness at first chew, overall toughness, residue, and shear force parameters: stress at maximum, toughness, stress at yield and slope after yield point} with the exception of energy to yield. The component 2 (PC2) accounts for the 15% and is determined by the opposition between the sensory and the objective parameters of texture.

It is very noticeable that control and treated animals are situated in differents areas. PC1 differentiates very well control from treated animals, while PC2 shows the individual variability for all the animals.

In summary, the texture parameters of meat studied allow to discriminate animals fed with clenbuterol from control animals. We can then conclude that it is possible to investigate the use of clenbuterol in animals by means of tstress maximum, stress at yield and/or slope after yield point at 1st day pm and 8th day pm. Furthermore, slope after yield point at 1st day pm differs significantly between control and treated samples, so that it is possible to detect meat from clenbuterol-fed heifers by this texture parameter.

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CAPTIONS OF THE TABLES AND FIGURE

Table 1.- Effect of clenbuterol administration on heifers performance, several meat quality characteristics and calpain/calpastatin activities in *M. Longissimus dorsi*..
Means on the same row differ significantly (* $p<0.05$, ** $p<0.01$).

Table 2.- Texture profile of meat from control and clenbuterol-treated heifers at 1 and 8 days *post-mortem*.
[a,b,c] Means on the same row differ significantly ($p<0.05$).

Table 3.- Effect of clenbuterol administration on several texture parameters investigated at 1 and 8 days *post-mortem* with a Warner-Bratzler shear blade.
[a,b,c,d] Means on the same row differ significantly ($p<0.05$).

Figure 1.- 1st and 2nd principal components analysis of differences between 1st and 8th day post-mortem of several meat texture parameters (sensory parameters: toughness at first chew, overall toughness, residue, overall acceptability and shear force parameters: stress at maximum, toughness, stress at yield, energy to yield and slope after yield point) from control (c1, c2, c3 y c4) and clenbuterol-treated (t1, t2, t3 y t4) heifers.
X-axis is principal component 1 (PC1). Range is from -4 to 4.
Y-axis is principal component 2 (PC2). Range is from -3 to 3.